The Science Journal

LANDER COLLEGE OF ARTS & SCIENCES
A DIVISION OF TOURO COLLEGE IN FLATBUSH

Where Knowledge and Values Meet

The Science Journal of the Lander College of Arts and Sciences-Flatbush Vol. XIV • Number I • Fall 2020

Volume XIV • Number I • Fall 2020

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Vol. XIV · Number 1 · Fall 2020

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Cover picture: The cover picture was created by Professor Antony O'Hara of the Digital Multimedia Design Department, pertains to the article " Does Preimplantation Genetic Testing Increase the Risk of Adverse Clinical Outcomes?" by Robyn Weiss.

Does Preimplantation Genetic Testing Increase the Risk of Adverse Clinical Outcomes?

Robyn Weiss

Robyn Weiss graduated in June 2020 with a Bachelor of Science degree in Biology and is accepted into the Master of Science in Human Genetics program at Sarah Lawrence College.

Abstract

Before 1990, options were limited for couples who were at risk for transmitting a genetic disease or a structural chromosomal abnormality to their children. Couples traditionally underwent invasive procedures such as amniocentesis and chorionic villus sampling, after which termination was offered if the fetus was found to be affected. Many couples chose not to have children at all. Since then, technological advances have allowed preimplantation genetic testing (PGT) to be offered to these couples. Couples who choose PGT undergo in-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI), where the oocyte is injected by a single sperm and is then implanted into the mother a few days later. However, in PGT, a few cells are removed and genetically analyzed before implantation to determine whether the embryo has a specific genetic defect or aneuploidy. The purpose of this paper is to determine whether PGT causes adverse clinical outcomes by critically analyzing PGT research studies. Current research does not seem to show any major adverse clinical outcomes after PGT especially in cases of singleton pregnancies. It is important to continue to examine the effects of an embryo biopsy in terms of neonatal and obstetric outcomes, as well as future development.

Introduction

Preimplantation genetic testing (PGT) has become an integral part of assisted reproductive technology (ART) and over a third of ART Centers in the United States are utilizing PGT technology (Kuliev, Rechitsky, 2017). There are three kinds of PGT. The first, PGT-M, analyzes the embryo for monogenic diseases. This is generally used when one or both parents carry a mutation, such as those linked to Huntington's disease or cystic fibrosis. Testing is performed to ensure the single-gene trait has not been passed to the embryo. It is often used after a previous child has been diagnosed with a specific genetic condition. PGT-M may also be used for sex selection, such as when a parent is a carrier of an x-linked disorder (Pastore, et. al. 2019). The second, PGT-A, and third, PGT-SR, are not standard procedures and were developed to improve the success rate of IVF. PGT-A is used to look for embryonic aneuploidy and PGT-SR is used to look for chromosomal structural rearrangements such as inversions or translocations. PGT-A and PGT-SR are usually only recommended in cases of previous failed rounds of IVF, severe male infertility, recurrent pregnancy loss, in cases where one or both parents have a balanced chromosome structural rearrangement, or for patients at high risk for embryo aneuploidy, such as those of advanced maternal age. While all forms of PGT come with many ethical questions, in general PGT-M is considered more acceptable, especially when it is used to prevent severe genetic diseases with few treatment options. Genetic counseling is recommended before any form of PGT to ensure that the couple understands the risks and limitations of the procedure (Eskew, Jungheim, 2017).

There are multiple methods of performing PGT. Polar body biopsy (PBB) is a common method for genetically analyzing an embryo. Polar bodies are formed during meiosis of an oocyte and are not required for fertilization or embryo development. Therefore, they can be removed safely and screened without harming the embryo. Additionally, PBB avoids errors due to the presence of mosaicism that other methods of PGT incur. Mosaicism is when different cells have different genotypes within one organism and is not present at the zygote stage. PBB is considered a less invasive procedure and is a good option for patients who view more invasive procedures as unethical. However, PBB can only provide maternal genetic information. Because PBB does not include paternal genetic information and cannot be used to determine gender, this method can only work in certain cases (Schenk, et. al. 2018).

PGT can also be done through a blastomere biopsy during the cleavage stage. This is done three days after fertilization, when the embryo is between six to eight cells. A blastomere biopsy is an invasive procedure where cell-to-cell adhesions are loosened and one or two blastomeres are aspirated. The blastomeres are then genetically analyzed for either aneuploidy or specific genetic mutations (Kalma, et. al. 2018). A blastomere biopsy allows both maternal and paternal genetic information to be analyzed, which makes determining the gender of the embryo possible. However, given that this is an invasive procedure, this method may affect the growth and development of the embryo. While there is evidence that a day three embryo can tolerate and overcome the possible resultant damage, it is likely that embryos that would otherwise progress to implantation will be lost at this stage of embryo development. Additionally, a blastomere biopsy may not always be reliable since it is affected by both the technical and biological problems associated with single cell analysis. Specifically, mosaicism, which is at the highest level at this stage of development, can lead to false positive and false negative errors. In order to compensate, two blastomeres can be removed. While this may increase the accuracy of the genetic testing, around 25% of the embryonic mass is removed, which may impact clinical outcomes (Cimadomo, et. al. 2016).

A third method of PGT is a blastocyst biopsy. It is

usually done five to six days after fertilization, when the embryo is about one hundred cells. During a blastocyst biopsy, five to six cells of the trophectoderm are removed and analyzed. This method allows more cells to be tested, compared to the only one or two cells that can be removed during a blastomere biopsy, and allows for improved accuracy of the genetic testing. Additionally, this procedure removes a smaller proportion of embryo cell mass when compared to the day three biopsy and only removes cells from the trophectoderm, not the inner cell mass. However, blastocyst biopsies have limitations as well. Only 50% of IVF embryos develop to the blastocyst stage and waiting for a day five biopsy may result in no transfer at all. Additionally, following a day five biopsy, embryos typically need to be cryopreserved and then thawed which precludes the transfer of a fresh embryo. While there are many methods of performing PGT, each method has its own benefits and limitations (Wang, et. al. 2018). Once the cells are removed, they are genetically analyzed by either polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), array-comparative genomic hybridization, and more recently, next-generation sequencing, in order to determine if there are any genetic defects (Heijligers, et. al. 2018).

Because PGT is an invasive procedure, researchers have wondered if it increases the risk of adverse clinical outcomes. It is especially important to monitor the safety of PGT since the majority of the PGT couples, specifically couples undergoing PGT-M, have no fertility issues and have the alternative of a natural conception with or without invasive prenatal testing. This review is aimed at determining whether PGT increases the risk of adverse obstetric and neonatal clinical outcomes as well as future development.

Methods

The research discussed in this paper was collected using EBSCO, ProQuest, PubMed and Google Scholar with access provided by the Touro College Library. All articles included are original, peer reviewed research papers that were analyzed to ensure accurate data.

Discussion

Neonatal Outcomes

Malformation/ Perinatal Death

A study was aimed at evaluating the safety of PGT and focused on the rate of congenital malformations as well as other adverse perinatal outcomes. In this study, embryos for PGT analysis were produced by intracytoplasmic sperm injection (ICSI) and subjected to blastomere biopsy. Parents filled out a questionnaire regarding their pregnancy and the health of their child. Medical information, such as age of both parents at embryo transfer, gravidity, parity, number of previous in vitro fertilization (IVF)/ PGT cycles, whether the embryo(s) was fresh or frozen/ thawed, how many blastomeres had been removed, and how many embryos were transferred, was also obtained from their doctor. The largest proportion of couples in this study opted for PGT-M, in order to avoid passing an autosomal dominant disease to the child. In this study, more girls than boys were born after PGT with a ratio of 1.2. This may be due to sex-selection, where a female embryo is transferred to reduce the risk of inheriting an X-linked condition. Major congenital malformations were found in nine of the 364 live births (2.5%). Four of these children had multiple congenital anomalies and five children (1.4%) had minor malformations. Three pregnancies were terminated because of diagnoses of exencephaly, trisomy 18, and trisomy 21. The major malformation rate when including pregnancy terminations due to congenital malformations was 3.3%. A report by the European Surveillance of Congenital Anomalies stated a prevalence of 261.45 major and minor birth anomalies per 10,000 births (2.6%) between 2008 and 2012, which is similar to the rate in this study. According to these results, the risk of major malformations in children born after PGT does not seem to be increased when compared to the general population. The study also found that perinatal deaths were reported in 3 out of 364 PGT pregnancies studied. Two of the pregnancies were of a twin and a triplet. Additionally, at 37 weeks gestational age, a singleton was stillborn, after an uncomplicated pregnancy. With a perinatal mortality rate of 0.8%, no evidence for a potential increased risk in fetal or neonatal death was found after PGT (Heijligers, et. al. 2018).

Similar results were found in a study that looked at the health of 49 children conceived after PGT compared to 66 naturally conceived (NC) controls. Control children were matched for age, sex, ethnicity, maternal educational level and socioeconomic status. A majority of PGT subjects had undergone PGT-A, however the study did not distinguish between subjects who had undergone PGT-M, PGT-A, or PGT-SR. However, all PGT subjects were born after an embryo biopsy at the eight to ten cell stage. Pediatricians that assessed the children were blinded to the conception status of the children, strengthening the results of this study. The study found that two children born after PGT had congenital anomalies, one with a minor ear deformity and the other with mild hypospadias (Banerjee, et. al. 2008).

Another study examined whether PGT blastomere biopsies impacted the health of infants up to two months of age by comparing the data of 995 children born after PGT

and 1507 children born after IVF and ICSI. Twenty-three PGT children (2.3%) and 40 ICSI children (2.7%) presented major malformations. Major genital malformations were recorded in seven PGT children and 9 ICSI children. Four stillborns conceived after PGT and seven stillborns conceived after ICSI presented major malformations. The total major malformation rate, including stillborn and live born, was comparable in the PGT group (2.6%) and the ICSI group (3%). These results do not indicate that the added cleavage-stage biopsy procedure increases the risk of major birth defects compared to the ICSI procedure (Desmyttere, et. al. 2012).

In another study, data from the PGT pregnancies of 158 singletons, 42 pairs of twins, and 1 set of triplets was compared to data obtained from 242 children born after IVF/ ICSI and 733 randomly selected NC children born during the same time period. The mothers in all groups were matched for age, preconception body mass index, and parity. Data collected included parental demographic information, type of biopsy performed (polar body and/ or blastomere biopsy), number of embryos transferred, whether the embryo(s) were fresh or frozen/ thawed, gestational age and mode of delivery. At two to four months, parents also filled out a questionnaire regarding any malformations that had not been diagnosed at birth. In both single and multiple pregnancies, the type of embryo biopsy had no significant influence the neonatal outcome. Four of the PGT children (1.7%) presented congenital malformations. One intrauterine fetal death occurred at 33 weeks with a subsequent diagnosis of thrombophilia. The congenital malformation rate for PGT pregnancies is similar to the rates found in other studies (Eldar-Geva, et. al. 2014). Additionally, in another Israeli study completed around the same time of 213,288 NC births, the rate of congenital malformations was 1.9% which is similar to the rate of malformation after PGT in this study (Farhi, et. al. 2013).

Another study looked at the health of 581 children born after a blastomere biopsy. Questionnaires were sent to both physicians and parents at conception and delivery and children were examined at two months of age, usually by a clinical geneticist. The researchers followed 484 pregnancies, with three terminations for major malformations seen on prenatal ultrasounds. Of these, 385 were singleton pregnancies, 92 were twin pregnancies and four were triplet pregnancies leading to a total of 581 PGT children. There were no differences in any of the studied properties between biopsies done for PGT-M or PGT-A and the results were therefore combined. As seen in many other studies, sex distribution of live born children was uneven with 54% girls for 46% boys and is due to sex selection for X-linked diseases. Of the 581 children in the PGT cohort, eighteen were stillborn and nine died neonatally. Of these 27 perinatal deaths, four were in singleton pregnancies and 23 in multiple pregnancies. The rate of perinatal deaths in singletons is comparable to the ICSI cohort, however the ICSI multiple birth cohort had a higher perinatal death rate. Major malformations were seen in 17 PGT fetuses which led three to be terminated. This led to a malformation rate in born and unborn children of 2.9%. Of the fourteen children with malformations, two were stillborn and both were from a multiple pregnancy. This leaves one inherited and eleven sporadic mutations in the 563 PGT children born alive with a rate of 2.13%. The major malformation rate in the ICSI cohort was 3.13%. The main finding of this study is that a day three embryo biopsy does not seem to increase the risk of major malformations. When these results are compared with the data collected from IVF/ ICSI children born within the same timeframe, the rate of malformations is comparable. (Liebaers, et. al. 2010)

Gestational Age/ Birth Weight

Studies also looked at the gestational age and birthweight of children conceived through PGT. In the study by Heijligers et al. (2018), eighty percent of the PGT children were born full term. Eight children, all from twin pregnancies (2.2%), were born very premature (<32 weeks). The study distinguished very premature children from premature children to show that the very premature children were all from twin pregnancies. Less than 15% of the PGT children had a low birth weight and were either twins or triplets. Only one singleton had a very low birth weight. The child was born at 35 weeks through caesarian section because of HELLP syndrome in the mother. A z-score of +0.17 was calculated for the singletons which indicates a comparable birth weight between this cohort and the rest of the Dutch population. In concordance with other studies on PGT there is an evident increase in prematurity and low and very low birth weight in multiples when compared to singletons. This strongly supports the current Dutch single embryo transfer policy. Overall, data from this study on pregnancy duration and birth weight in the Dutch PGT population, especially in the singletons, seems similar to the published data on naturally conceived children.

In the study performed by Eldar-Geva et. al. (2014), the difference in mean birth weight for singleton pregnancies between the three groups was statistically significant. Singleton NC children had a significantly higher birth weight than those born after ICSI (P=.006) but not compared to the PGT singletons. Low birth weight was also more frequent in the ICSI group than in the PGT and

NC singletons. Also, significantly more ICSI twins (58%) presented with low birth weight compared to 41.0% of PGT twins and 44.2% of NC twins. Very low birth weight (<1,500 g) was rare in all groups. There was also a statistically significant difference among the groups when examining intrauterine growth for singleton pregnancies (P=.001). Intrauterine growth restriction was more frequent in ICSI pregnancies (9.5%) than in NC (5.5%) or PGT pregnancies (5.1%). Children born large for their gestational age was more frequent in the PGT group (16.5%) than the NC group (8.8%). The mean gestational age, rates of preterm birth and intrauterine growth restriction for twin and triplet pregnancies were similar for the three groups.

These results show that there are no increased risks of intrauterine growth restriction or low birth weight in singleton or twin pregnancies after PGT compared to NC. However, ICSI pregnancies did show an increased risk for both of these complications. These results remained true even after controlling for factors such as maternal age, parity, BMI, number of embryos transferred and whether the embryo was cryopreserved, which can affect pregnancy outcomes. The increased likelihood of adverse outcomes in ICSI pregnancies may be due to the fertility status of the parents. Infertile women are more prone to adverse outcomes even when conceiving naturally, indicating that infertility itself is what increases the risk of adverse outcomes such as low birth weight and preterm delivery (Basso, Baird, 2003). This may explain the similarity of the results in birth weight and intrauterine growth from PGT and NC pregnancies, since the majority of PGT couples usually do not struggle with fertility.

The difference in pregnancy duration for singleton pregnancies between the three cohorts was also statistically significant. NC pregnancies were longer, than both the PGT and the ICSI pregnancies. However, for the PGT group, these findings had no clinical significance because the frequency of preterm deliveries, both <37 (7.4%) and <34 weeks (1.3%) was comparable with NC pregnancies (5.7% and 2.0%, respectively). However, 11.4% of the ICSI cohort were born prematurely. PGT and ICSI pregnancies may have been shorter for different reasons. Women who undergo PGT are at high risk for autosomal recessive, X-linked, or dominant genetic disease and therefore have a higher incidence of previous pregnancy terminations for affected fetuses. Complications associated with induced abortions include premature delivery of future children and cervical incompetence. Additionally, some of the PGT women in the study had autosomal dominant diseases such as myotonic dystrophy, achondroplasia, neurofibromatosis and tuberous sclerosis and because of this chose to have a cesarean delivery at 37 to 38 weeks. In fact, the PGT cesarean delivery rate was more than double in PGT pregnancies. Additionally, some of the families had critically ill children which may have placed an emotional and physical burden on the family and pregnant mother (Eldar-Geva, et. al. 2014). The preterm birth rate for the IVF/ ICSI group is unsurprising. As discussed above many studies have found that preterm birth is associated with children conceived through IVF/ ICSI because of the parents underlying fertility issues (Wisborg, et. al. 2010).

Another study looked at the health of 49 PGT children and 66 NC children. The PGT cohort had a significantly lower gestational age at birth (P = 0.0001) and more preterm births than the NC group. The PGT group was also more likely to have a lower birth weight and a higher number of births with a birth weight of less than 2500 grams. Interestingly, this finding is consistent with other studies of assisted reproduction outcomes such as IVF/ ICSI. In most cases PGT conception is closest to natural conception and not assisted reproduction conception, with regard to the reproductive health of parents. Parents who opt to undergo the most common form of PGT, PGT-M, usually do not have fertility issues but are concerned with passing a genetic disease to their children. However, in this study, the majority of PGT patients had undergone PGT-A in which parents bear closer risk and resemblance to couples undergoing other assisted reproductive conception. PGT-A is usually used after failed IVF cycles or because of other fertility issues, such as advanced maternal age. It is therefore unsurprising that the age of the PGT mothers was significantly higher than the NC mothers, (P = 0.0001) as was the rate of preterm birth and low birth weight in the PGT group, which is commonly seen in other assisted reproduction outcome studies (Banrjee, et. al. 2008).

The study performed by Desmyttere et. al. (2012), found that the average birthweight for PGT singletons and PGT multiples with a very low birth weight (<1500 g) was comparable with the ICSI children. However, significantly more ICSI multiples presented a low birthweight (<2500 g), more specifically 268 (17.8%) ICSI compared to 161 (16.2%) PGT babies. Again, this may be due to the fertility status of the parents, since the ratio of infertile couples was higher in the ICSI cohort than in the PGT cohort. Measurements of height and head circumference showed no significant differences between the two groups. Mean gestational age at birth for PGT singletons, twins and triplets showed no difference compared to the ICSI group. Additionally, the number of PGT singletons and multiples born prematurely (<37 weeks) showed no differences compared with their ICSI counterparts

(all P-values > 0.05). Twenty-one ICSI and four PGT singletons versus 67 ICSI and 31 PGT multiples were born very prematurely (<32 weeks) which is not significant (P = 0.056 and P = 0.43 for singletons and multiples). Admission after delivery to the neonatal intensive care unit was comparable for both the PGT and ICSI groups for singletons and multiples. These results show that singletons and multiples born after a PGT embryo biopsy had similar neonatal outcomes in terms of auxological data, gestational age and neonatal hospital admission, to the control group of singletons and multiples with no embryo biopsy. Additionally, in this study, PGT multiples appear to be at a lower risk for low birthweight when compared to IVF/ ICSI multiples.

The study by Liebaers et al. (2010) found that in the PGT cohort, 11.5% of singletons and 65.7% of multiples were born premature. In the ICSI cohort, 8.4% of singletons and 57.9% of multiples were born premature. Low birthweight was observed in 7.4% of PGT singletons and very low birth weight in 3 (0.8%) PGT children. Multiple PGT births of a low birth weight (62.5%) were significantly lower than ICSI multiple births (49.4%). Very low birth weight was observed in 19 of the PGT multiples. These results suggest that a three day PGT biopsy does not seem to add significant health risks for singleton PGT children since when these results were compared to the data collected from IVF/ ICSI children born within the same years, gestational ages and birthweights were similar. However, PGT multiples appeared to be at an increased risk of low birthweight, preterm birth, and perinatal death compared to ICSI multiples. Multiple pregnancies should be avoided when possible and may potentially solve problems especially regarding perinatal death.

A similar study looked at whether women who conceived after PGT and their children have greater risks of adverse pregnancy and birth outcomes compared with children conceived spontaneously or after IVF with or without intracytoplasmic sperm injection (ICSI). The study looked at factors such as pre-eclampsia, preterm primary rupture of membranes, placenta previa, abruption of placenta, preterm birth, low birth weight, major malformations, and neonatal admission. It was found that compared to women conceiving spontaneously, women who had undergone PGT or IVF/ ICSI were older, more often uniparous, had a higher BMI and smoked less often during pregnancy. The children conceived after IVF/ ICSI had a lower birth weight, shorter gestational age, longer neonatal hospital admission and an increased risk of preterm birth and malformations. Children born after PGT had a comparable risk of the same complications, however, the results were just short of statistical significance for many of the outcomes. Nonetheless, PGT children were found to have a significant increased risk of preterm birth, shorter gestation, and longer neonatal hospital admission. The study also looked at the difference in outcomes between PGT-M, PGT-A and PGT-SR. When compared to NC children, PGT-SR and PGT-A children did not have an increased risk of adverse neonatal outcomes. When compared to IVF/ ICSI children, PGT-SR and PGT-A children had comparable neonatal outcomes and were found to have a higher mean birth weight. However, compared to NC children, children born after PGT-M had a significantly lower birth weight, shorter gestation and increased risk for longer neonatal hospital admission. These results show an increased risk of neonatal complications in PGT pregnancies when compared to spontaneous pregnancies. However, the risk of adverse outcomes was generally comparable to IVF/ ICSI pregnancies, indicating that the actual embryo biopsy does not add additional risks. Additionally, when separating PGT-SR and PGT-A pregnancies from PGT-M pregnancies, adverse neonatal outcomes were only found in children conceived through PGT due to a parental monogenetic disorder (PGT-M) and not in children born after PGT-SR and PGT-A. These results make it likely that the risk of adverse outcomes is not related to PGT itself, but to the underlying condition of the parents. These factors can include the known genetic disorder, associated comorbidities or any medications taken during pregnancy. (Bay, et. al. 2016)

A study compared the growth data at birth and two years for 70 singletons born after PGT, ICSI or natural conception. Children were matched for gender, language, birth order and maternal education level. At birth, height and head circumference data were comparable for the PGT, IVF/ ICSI and NC cohorts. While the PGT singletons tended to have a lower birthweight and gestational age compared with the NC children, these differences did not reach statistical significance. When comparing children born after a biopsy of one or two blastomeres, weight, height and head circumference measurements were comparable for the two groups. Additionally, admission to a neonatal ward was comparable in the three conception groups and PGT children did not experience more hospital stays for medical reasons than the ICSI and NC groups. PGT children were also reported to have undergone more complementary examinations (with normal results) compared with NC and ICSI babies. However, this is probably due to precautionary measures for 'specially conceived' children (Desmyttere, et. al. 2009).

Obstetric Outcomes

In the study performed by Eldar-Geva et. al. (2014), the incidence of pregnancy complications such as hypertension

and diabetes were similar in the PGT, IVF/ ICSI and NC groups. Of the PGT mothers 1% had hypertension and 2% had gestational diabetes. Of the ICSI and NC mothers, 1% had hypertension and 6% had diabetes, and 3% had hypertension and 4% had diabetes, respectively. Additionally, the differences in mode of delivery for singleton pregnancies was statistically significant. The cesarean delivery rates were 28.5% in the PGT group, 31.6% in the ICSI group, and 11.0% in the NC group (P<.005). As discussed earlier, the cesarean rate for the PGT cohort was more than double the rate of the NC group in this study and was probably due to the fact that women with autosomal dominant diseases in the PGT cohort opted to have a cesarean delivery at 37 to 38 weeks.

In the study performed by Desmyttere et. al. (2009), increased rates of cesarean births were found when PGT mothers were compared to IVF/ ICSI mothers. Results also showed that when compared to NC mothers, PGT mothers experienced more pregnancy complications such as gestational diabetes, thyroid pathology, pregnancy-induced hypertension, placental pathology and premature contractions. However, there were no differences regarding pregnancy complications when comparing PGT and IVF/ ICSI mothers.

In the study performed by Bay et. al. (2016) the IVF/ ICSI cohort showed an increased risk of placental disorders, including placenta previa, pre-eclampsia, placental abruption, preterm primary rupture of membranes, and induction of labor or cesarean section. The women who gave birth after PGT had a comparable risk for most of the same complications when compared to the NC cohort, although for most of the outcomes the results were just short of statistical significance. However, the PGT cohort did show a significant increased risk of placenta previa and cesarean section. Because the risk of adverse outcomes was generally comparable to IVF/ ICSI pregnancies in many of these studies, it seems like the actual embryo biopsy does not add additional risks.

When the study separated PGT-M subjects from PGT-A and PGT-SR subjects interesting results emerged. PGT-SR and PGT-A children did not have an increased risk of any adverse obstetric outcomes, except for a higher risk of placenta previa when compared to NC controls. When compared to IVF/ ICSI children, PGT-SR and PGT-A children had comparable obstetric outcomes. However, compared to NC children, children born after PGT-M had a significantly increased risk for preterm primary rupture of membranes, cesarean section and placenta previa. Again, these results make it likely that the risk of adverse outcomes is not related to PGT itself, but to the underlying condition of the parents. However, there was a consistent increased risk of placenta previa after both PGT and IVF/ICSI, which suggests that parental factors do not explain all the adverse outcomes.

Follow up Study

Growth

A study assessed whether PGT causes adverse outcomes by comparing findings at birth and at 2 years of age for singletons born after PGT, IVF/ ICSI, and NC. The study also investigated whether the body size of children born after biopsy of one blastomere was different from that of children born after biopsy of two blastomeres. Subjects in all groups were matched for gender, maternal educational level, mother tongue and birth order. A strength to this study is that all children were examined by the same pediatrician in a standardized way. At a two year follow up, weight, height, head circumference, and waist and arm measurements were comparable for the three cohorts. These results show that PGT singletons do not appear to be at a higher risk of growth retardation compared with IVF/ ICSI and NC singletons. In PGT children, the mean BMI was statistically significantly lower compared with NC children. Growth parameters of the PGT children born after biopsy of one blastomere were comparable to children born after a biopsy of two blastomeres. (Desmyttere, et. al. 2009)

The study performed by Banerjee et. al. (2008) found similar results. When assessed at the mean age of 18 months, growth parameters for all PGT children were within the normal range including the children who had been born preterm and/ or with a low birthweight. Furthermore, Desmyttere et. al. (2009) found that in their follow up study that rates of chronic disease and chronic use of medication were similar between PGT and NC children.

Socio-emotional and Language Development

A study was performed to assess the socio-emotional and language development of children at age two born after PGT, IVF/ ICSI, and NC, as well as parental wellbeing. A small number of children (n= 10) that were born before between 33-36 weeks gestations were included in the study and were equally distributed among the cohorts. Most of these children had a normal birth weight (<2500 g), and none of them had a very low birth weight or obtained an Apgar score of less than nine after ten minutes. Twins were excluded from the study because developmental outcome is affected by prematurity and low birth weight, which are known to be more common in twins and triplets. NC and ICSI controls were matched for gender, maternal education level, native language, and birth order. All members of the PGT cohort

had a blastomere biopsy at the eight-cell stage. Parents were asked to complete the Short Temperament Scale for Toddlers (STST) and the Child Behavioral Checklist (CBCL) in order to assess the child's socio-emotional development. The STST, placed children into one of three temperament categories, easy, average or difficult. The CBCL answers questions about the child's emotional and behavioral problems. Parents answered if the problem presented is 'not true', 'somewhat or sometimes true' or 'very true or often true' of their child, with item scores of 0, 1 or 2. A total score of 60 is at the bottom of the clinical range, and a score of 64 or more represents larger issues. Language comprehension and production were rated according to the McArthur Communicative Developmental Inventories.

The CBCL scores showed no difference in the proportion of children above the clinical threshold points according to mothers and fathers. After controlling for socio-demographic variables, PGT and ICSI mothers reported significantly fewer problems than the NC cohort. According to the STST scores, a similar proportion of parents from all three conception groups reported their child's temperament as easy, average or difficult. This remained true even after controlling for socio-demographic variables. Additionally, the mean Language Comprehension score and Language Production score did not differ significantly among the cohorts.

This study had some weaknesses. Firstly, results were obtained exclusively from parental reports and more valid reports could have been obtained from a multiple informant approach. Additionally, the PGT cohort had members that had undergone PGT-M and PGT-A. Since these procedures are usually done for different reasons, PGT-M and PGT-A populations have different medical histories and family backgrounds, which may influence socio-emotional and language results (Nekkebroeck, et. al. 2008a).

Additionally, the study by Banerjee et al. (2008) found that the PGT cohort had significantly higher scores on the Hearing and Language subscale, than the NC group. These studies suggest that PGT does not cause adverse neurodevelopmental outcomes.

Mental and Psychomotor Development

In the study by Banjeree et al. (2008), children up to age four were evaluated with a focus on neurodevelopmental screening which was measured using the Griffiths Scales of Mental Development. The mean Griffiths quotient for both the PGT and NC groups were in the normal range and did not differ significantly. The only significant differences were for the Locomotor subscale, where the PGT group was significantly lower than the NC group.

A similar study aimed at assessing the mental and psychomotor developmental outcomes in two-year-old children conceived through PGT compared to children born after IVF/ ICSI and natural conception (NC). ICSI and NC controls were matched for gender, maternal education, birth order, and native language. All PGT subjects had a blastomere biopsy at the eight-cell stage of the embryo. At two years of age, the children were all tested by a psychologist using the Dutch version of the Bayley Scales of Infant Development (BSID). The psychologist was blinded to the status of the subject's conception while conducting the evaluation. Parents were questioned regarding socio-demographic characteristics. The BSID consists of two major scales. The mental scale measures visual and auditory information procession, imitation, memory, hand-eye coordination, and problem solving. The motor scale appraises control of gross and fine motor skills.

There were no significant group differences regarding mental and motor scale scores. Additionally, equal numbers of PGT, ICSI, and NC subjects were represented in each level (accelerated, normal, delayed) of psychomotor and mental development. Interestingly, when compared across all three cohorts, psychomotor and mental development scores were very similar for males and females. However, when compared within each cohort, ICSI boys obtained lower scores on both scales than the ICSI girls ($P = 0.061$). The mode of delivery had no impact on psychomotor or mental development even after controlling for sociodemographic factors. (Nekkebroeck, et. al. 2008b).

From the results of these two studies, it can be concluded that the embryo biopsy done in PGT has no impact on the mental and psychomotor development of two-year-old children, compared to ICSI and NC children

Parent-Child Relationship

The study by Banerjee et. al. (2008), used the Parental Stress Index and the Parental Acceptance-Rejection Questionnaire to assess differences in the parent-child relationship. The Parental Stress Index, which asked parents about parental distress, parent–child dysfunctional interaction, and the difficulty of the child, showed no significant difference between the PGT and NC groups. In the Parental Acceptance-Rejection Questionnaire, the PGT group had significantly higher scores on the warmth-affection subscale, and significantly lower scores on the aggression-hostility and rejection subscales than the NC group.

In another study parental stress and health status were measured with the Parent Stress Index and the General Health Questionnaire (GHQ). No differences in parental stress were found for mothers and fathers among the

three groups. However, after controlling for socio-demographic variables, the ICSI mothers and fathers reported less stress from parenting (P = 0.048). These results are similar to findings in other studies and may be because greater efforts are made by ICSI parents to have a child compared to parents who conceive naturally. Another theory is that ICSI parents may be inclined to underreport behavioral issues because of their need to demonstrate their abilities as parents and move on from the issue of infertility where they struggled. On the other hand, there was an equal proportion from all three cohorts that experienced low, moderate or high levels of parenting stress. Scores on the GHQ measuring parental health were not significantly different, even after controlling for socio-demographic factors. Parents from all three conception groups obtained similar scores on the subscales: somatic symptoms, anxiety, social dysfunction and severe depression. The results from these studies imply that parents seem to cope with the extra stress of PGT without it affecting the parent-child relationship (Nekkebroeck, et. al. 2008a).

Conclusion

Overall, it does not seem that preimplantation genetic testing causes adverse clinical outcomes. This is especially important since the majority of couples who undergo PGT usually do not have fertility issues and have the option of natural conception with invasive prenatal testing. The results of these research studies show no significant increased risk of perinatal death or malformations, especially when compared to IVF/ ICSI births, indicating that the embryo is able to recover from the cells removed during the biopsy and it therefore adds no additional risk. Furthermore, children conceived through PGT seem to be on the same developmental level as their peers and show no growth retardation in follow up studies. While some studies show an increased risk of preterm delivery, low birth weight and some obstetric outcomes, it is important to determine whether this is because of the embryo biopsy or because of the underlying health condition of the parents such as the fertility issues or the genetic disease for which they chose to undergo PGT in the first place. However, since this technology is fairly new, there are few follow up studies that investigate the long-term effects of PGT. Additional follow up studies are necessary to ensure the long-term safety of this technology. Future studies can also investigate the specific outcomes for each method of PGT, since most of the research is either regarding a blastomere biopsy or combines all methods of PGT in the PGT cohort. Furthermore, future studies should focus on determining the outcome differences between PGT-M, PGT-A and PGT-SR since parents who undergo the different forms of PGT have different medical backgrounds which can affect the results of these studies. Couples considering PGT should consult their physician or a genetic counselor to determine whether PGT is the correct option as well as which method of PGT should be used.

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What Role does Age-Associated Neuroplasticity Play in the Efficacy of Cochlear Implantation?

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Abstract

Bilateral, profound-severe, congenital deafness causes widespread structural and functional changes of the auditory system. In humans, the consequences of these changes are extensive and often include detriments to language acquisition and auditory perception. Fortunately, early intervention methods, such as cochlear implantation, can significantly mitigate inevitable auditory deficiencies. This review begins by briefly addressing early stages of brain development and associated anatomical discrepancies observed in congenitally deaf subjects. Considering the deleterious effects of congenital deafness, neuroplasticity, the ability of the brain to rewire itself, is of paramount importance in reversing the auditory impairments. Hence, its incorporation into the methods required for successful auditory rehabilitation. Despite this phenomena, assistive devices such as the cochlear implant have shown a marked decrease in efficacy after a critical period has elapsed. Although the scientific community has made incredible gains in the understanding of neurogenesis and congenital deafness, additional research is required to concretize age-related limitations inherent in neural plasticity and provide further advances in congenital deafness intervention methods.

Introduction

Hearing loss is the third most common health problem in the United States. It is estimated that thirty million Americans struggle to hear. The disability is not only prevalent in America; it is estimated to affect 8.8 percent to 12.5 percent of the worldwide population (Burkey, 2015). The most common cause of this disability which affects approximately 2 out of every 1,000 children is sensorineural hearing loss (Sharma, Campbell, 2011). Sensorineural hearing loss is often caused by damage to the inner ear or as a result of non-functioning or missing sensory hair cells that normally operate within the cochlea. Without these cells, an individual is unable to detect and transmit auditory sound wave stimuli through the auditory nerve to the brain. As cortical development is contingent upon stimulus-driven learning, individuals born with sensorineural hearing loss are at risk for abnormal neurological development and brain connectivity needed for optimal auditory sensory function. In 1978, Dr. Graeme Clark introduced a revolutionary multi-channel cochlear implant that has developed into an incredibly effective and transformational neural prosthesis that allows severe and profoundly deaf individuals to achieve similar function to their unaffected peers. This device converts sound waves into patterns of electrical impulses that bypass the outer and middle ear, thereby directly stimulating Cranial Nerve VII fibers. The cranial nerve then carries the impulses to the brain, which converts and interprets these impulses as sound. Although different from typical acoustic stimulation, this electrical stimulation is able to mimic the coding of the cochlea and enable recipients to process speech and environmental stimuli (Hartmann, Kral, 2000).

As technology and implantation techniques improved in the 1990's, cochlear implant surgery gained FDA approval for use in younger subjects. As of 2010, approximately 80,000 of the 300,000 cochlear implant users worldwide were either infants or young children (Kral, O'Donoghue, 2010). Research studies quickly established that "when these children receive a cochlear implant at a relatively young age (for example, at 18 months) followed by intensive therapy, they tend to hear and speak better than those who received implants at an older age (fda.gov, 2017)." The success associated with such early cochlear implant intervention is often assumed to be related to a brain characteristic known as neuroplasticity. This fascinating neural capability allows neurons in the brain to compensate for injury or disease by restructuring and reorganizing neural pathways that affect function. It is the aim of the following analysis to explore the nature of age-related changes in neuroplasticity as they might specifically relate to the efficacy of cochlear implantation in subjects at various stages of development.

Methods

Critical analysis of the literature on age-related neuroplasticity in relation to cochlear implantation was conducted and compiled via access to the Touro College Library's online database, using PubMed and ProQuest search engines. In addition, Google Scholar was utilized in obtaining related research.

Discussion

Early Stages of Brain Development

Brain development begins around the eighteenth day after conception and continues into early adulthood. Approximately 2 weeks after conception, part of the ectoderm of the back of the embryo thickens and forms a neural plate. As the edges of this neural plate curl toward each other, eventual fusion occurs thereby forming the neural tube. The inner cells of this formation will comprise the central nervous system whereas the outer cells break away to create the autonomic nervous system. As the tube closes and matures, different areas become distinctive brain structures. In particular, the rostral end of the neural tube develops three interconnected chambers which become the three major parts of the brain: the

forebrain, midbrain, and the hindbrain (Carlson, Birkett, 2017). Any remaining cells will develop into the spinal cord. As the tube undergoes its metamorphosis, progenitor cells, descendants of stem cells, lead to the brain's multifaceted and sophisticated cellular network. Given the cerebral cortex's inside-out developmental pathway, the most recently formed neurons are more proximal to the cortical surface. The six layers of the cortex are formed by approximately 25 weeks after conception. The end of cortical development is observed when the progenitor cells receive a chemical signal which induces apoptosis. Once neurons have migrated to their proper locations, synaptogenesis occurs. The first synapses are usually detected around the 23rd week of gestation (Molliver et. al., 1973). Typically, synaptogenesis is followed by a gradual reduction of neurons known as pruning due to neural overabundance in the ventricular zones. Although this process occurs throughout an individual's lifespan, an initial explosion of synapse formations occurs during early brain development. Synaptic reduction is then significantly dependent on environmental exposure. Regions that are stimulated by these factors are strengthened and stabilized, whereas reduction occurs in synapses that are not sufficiently stimulated (Tierney, Nelson, 2009).

During the prenatal and early childhood years, the basic structure and functional capacity of the brain are formulated with refinement of neural networks persisting over time. Research suggests that brain development is hierarchical in nature. Higher level processes build on lower level processes. For example, language development depends on sensory and perceptual development. Infants are born with a brain wired for various types of experiences and abilities such as speech, language, and facial recognition. Newborns are initially programmed to perceive all languages, but with environmental experience, become focused and cognizant of their native language and lose the ability to perceive language that they are not exposed to. In this way, exposure drives subsequent learning (Kuhl, 2004). Research in 2004 supports the concept that healthy brain development requires adequate environmental exposure and that lack of these experiences could lead to underspecification and miswiring of brain circuits. This study found that children raised in Romanian institutions with a lack of stimulating experiences demonstrated underdeveloped brain and cognitive growth. Further research (Marshall et al., 2008) noted a critical time frame, suggesting that after age two the effects of decreased exposure on brain function worsen.

In early development, external stimulation is an important means through which significant neural connections and networks are created to facilitate behavioral growth and development. An absence of any one of the body's senses can have major implications on brain development. Animal studies have found that early deafness greatly affects auditory cortical development. Baker et al. (2010) performed a research study utilizing deaf cats to determine hearing loss related auditory brain stem pathology. Altricial animals, cats are born with closed ear canals that only open approximately 30 days after birth. The process of ear canal opening is the same in deaf and hearing cats; therefore, researchers hypothesized that abnormalities in the deaf cats would coincide with the development of hearing in typical-hearing cats. This would support the notion that lack of sound stimuli leads to pathological changes. Through the use of intracellular dyes, the Endbulb of Held in deaf white cats were examined. Large and complex synaptic endings, the Endbulbs of Held provide a coordinated release of neurotransmitters from presynaptic terminals onto the soma of bushy cells in the anteroventral cochlear nucleus (postsynaptic cell). They are considered to be centrally involved in the precise transmission of timing information from auditory stimuli. It was discovered at birth that the cochlea of the congenitally deaf white cats was void of abnormal morphology. The presence of a collapsed scala media and a degraded organ of corti appeared one week after birth. As time progressed, the deaf cats' endbulbs exhibited flattened and elongated postsynaptic densities (PSDs) and increased synaptic vesicle density. Cochlear abnormalities in cell synapses and circuitry as a byproduct of sound deprivation were exhibited. Human studies have subsequently arrived at similar findings. Using cortical auditory evoked potentials (CAEPs) with non-invasive EEGs on deaf children, these studies have found delayed or absent auditory responses supporting the theory that brain maturation is dependent on appropriate and adequate stimulation (Eggermont et al., 1997; Eggermont & Ponton, 2003).

Neuroplasticity and Developmental Periods

In addition to genetics, environmental factors also play an important role during the critical period of brain development. While genetics ostensibly play a larger and more significant role in prenatal development, environmental exposure is a key contributor to postnatal progression. Neural plasticity is the central nervous system's ability to attempt to support optimal performance by recovering functional abilities and enabling the body to adapt and learn in changing anatomical conditions. The nervous system's ability to reorganize its structure, connections, and functional abilities in response to intrinsic and extrinsic stimuli is complex. It can occur on a variety of levels from molecular to cellular during regular development and learning, or in response to disease or injury (Cramer et al., 2011). Plasticity of a brain region is affected by the area's peak synapse production.

This occurs at different times for various structures of the brain. For example, peak synaptogenesis for the visual and auditory cortices occurs between 4 and 12 months, whereas the prefrontal cortex that controls reasoning and planning increases more slowly and peaks at one year of age. The later the peak synapse production, the longer the area's plasticity (Goswami, 2004).

Neuroplasticity is an area of continuous research and hope in many clinical contexts. It is, for instance, widely researched in relation to stroke, trauma, and spinal cord injury. Associated studies have highlighted the brain's incredible ability to form representational maps with spontaneous intra-hemispheric and inter-hemispheric changes. For instance, when brain lesions of the left hemisphere damage important language centers, other areas in that hemisphere may be recruited for language function (Karbe et al., 1998b; Karbe et al., 1998a; Warburton et al., 1999). Moreover, in situations where severe impairment exists in the left hemisphere region, the right hemisphere appears to be capable of assuming some language functions (Warburton et al., 1999; Cramer et al., 2011).

The central nervous system's ability to adapt to pathology is affected by several parameters. One of the primary contributing factors is the age of onset, including critical developmental periods (Staudt, 2010). The greatest forms of neuroplasticity are available during early development. This is thought to relate to the overabundance of neuronal cells and synaptic connections present during early childhood which decrease through the pruning process with environmental exposure and aging. Additionally, other developmental events like inhibition and myelination can affect the developmental critical period. In the case of early neurological injury, research has found significant cross modal plasticity - the ability to reorganize and form new sensory maps and pathways. For example, successful changes in function from across brain hemispheres have led to highly successful behavioral advances for children (Cramer et al., 2011). Staudt's (2002) research supports this phenomenon showing that unlike adults, children demonstrate moderate to good right hemisphere control of language and movement following a significant injury to their dominant left hemisphere.

Data Defining Critical Periods for Cochlear Implantation

Adaptive plasticity and its relationship to age-dependent recovery of language is an active area of study. Research on children with a hemispherectomy showed a remarkable shift in motor and language function to the remaining hemisphere. Children under six years of age had the most significant level of reorganization (Chen et al., 2002b). Similar findings have been seen with congenitally deaf children. Cortical Auditory Evoked Potential (CAEP) testing - the time it takes for the brain to respond to auditory stimulation - was found to increase with age as a result of maturation and refinement of the central auditory pathways. These markers were tested in a variety of deaf children who received cochlear implants at different ages. In a study with a subject body of 245 congenitally deaf children with cochlear implants, researchers found that children implanted prior to 3.5 years of age had normal response times within 6 months of implant use. However, children whose initial stimulation occurred after age 7 demonstrated abnormal response times even after years of implant usage. Children who received cochlear implants between 3.5 and 7 years of age had variable responses (Sharma et al., 2002; Sharma & Campbell, 2011). These results have been supported by other studies utilizing PET scan brain imaging and behavioral measures. In addition, speech and language studies have demonstrated that children implanted under 3-4 years of age display significantly better speech and language skills as opposed to those children implanted at 6-7 years of age and older (Geers, 2006; Kirk et al., 2002). These results influenced the FDA to lower their age for approval of cochlear implantation for children to approximately 12 months.

Research reports that auditory cortex synaptogenesis begins in the first two months after birth with maximum density between 4 and 12 months followed by pruning (Goswami, 2004). This early synaptogenesis supports the need for early implantation and stimulation of the auditory nerve to allow maximal usage of the brain's regional plasticity and ability to learn to process auditory stimuli. Research has reinforced this theory. Electrical stimulation had a restorative effect on the Endbulb of Held synapse, and early electrical stimulation with a cochlear implant had significant positive results in congenitally deaf cats (Baker et al., 2010; Ryugo, 2015). Ryugo et al. (2005) reported decreased synaptic vesicle density and PSDs following cochlear implantation of congenitally deaf cats statistically similar to those of normal hearing cats. Auditory nerve activation at 3 months of age restored many key features of synaptic morphology, whereas less significant effects were seen at 6 months and on (Ryugo, 2015). With regard to humans, studies illustrate that those children who became deaf before the developmental onset of language and received early cochlear implant technology were successful in their acquisition of spoken language. However, those with late implantation displayed less benefit and ability to discriminate complex everyday sounds and speech (Svirsky, et al. 2004; McConkey, et al., 2004; Tong et al., 1988).

Cochlear Implant Considerations After Critical Periods

Early implantation, within the sensitive and critical period, is integral for speech and language development and necessary to avoid potentially deleterious re-organization of the cortex. Kral, 2007 found that in animal studies, the primary auditory cortex was partially or completely disconnected from the surrounding higher order cortex at the end of the sensitive period. This leaves the higher order auditory cortex at risk for recruitment from other sensory modalities. This has been seen in deaf adults where their visual processing may begin to take place in their auditory cortical areas. Although cross modal reorganization may allow for some enhanced processing, it could also result in significant deficits. For example, while deaf adults may have enhanced peripheral vision, they may suffer from severely impaired auditory processing and auditory-visual integration (Sharma & Campbell, 2011). Numerous studies have consistent data demonstrating notable improvement in speech perceptual skills in adolescents who received cochlear implants. However, adolescents with earlier implantation and shorter lengths of deafness exhibited significantly greater results in word and sentence testing. Children who were implanted after age 7 were found to demonstrate abnormal brain responses to auditory input and poorer language skills. Some relate these results to cortical plasticity where colonization of the auditory cortex occurs from other sensory modalities during critical periods of central nervous system development (Sharma et al., 2009; Zeitler et al., 2012). In post-lingual adults, studies relate that the duration of auditory deprivation has a negative impact on auditory performance with a cochlear implant, either due to cross modal plasticity or due to the limited capability of the superior temporal cortex (Anderson et al., 2017)

Cochlear Implant Benefits Before and After Critical Periods

Results from human studies report that uncorrected deafness results in fundamental change in the central auditory system so much so that benefit from a cochlear implant in later life is hindered. Adult recipients report cochlear implant benefits including increased environmental sound awareness, better quality of life, and increased psychological wellbeing. The area most variable is improvements in auditory speech perception. Specifically, the trajectory and rate of auditory performance vary across adult individuals (Anderson et al., 2017). Several abnormalities that arise in the auditory system include reduced number of spiral ganglion neurons, abnormal synaptic structure, ectopic projections in ascending pathways, and physiological alterations of auditory nerve responses in the cochlear nucleus. These affect synaptic transmission and result in decreased responsiveness in the inferior colliculus and auditory cortex. These fundamental changes inhibit older cochlear implant recipients from gaining true benefit. Although environmental sounds may be processed in adult recipients, language recognition is more difficult (Ryugo, 2015). Data from the Mayo Clinic's testing on 259 adults revealed that adult cochlear implant recipients had preoperative scores of 8% on tests of monosyllabic words and 7% on sentence recognition. After one year of implantation, these scores increased to 58% for word recognition and 75% for sentence recognition (Carlson, 2020). These results support the usefulness of cochlear implants in adulthood; however, when compared to the percentages and quality outcomes in children with early implantation they are markedly low.

Relevant Associated Neural Plasticity Research

For decades, scientists believed that neurogenesis was a process that existed in the brains of embryos and infants only to cease in adulthood. In the 1980's, this notion was challenged when researchers showed that neurogenesis occurs in the brains of certain adult animals. Further traction against the initial, misguided belief was made when signs of newly formed neurons in the adult human brain were observed. Alvarez-Buylla and colleagues studied the olfactory bulb in rodents and found continuous formation of new neurons. However, in humans the formation of new olfactory neurons occurs exclusively in infants. This dichotomy was also found in the frontal lobe where new neurons migrate during early childhood but cease migrating as age progresses (Pignatelli and Belluzzi, 2010). The most thorough study was done by Sorrells and colleagues on postmortem and postoperative hippocampal tissue from humans. The subjects ranged from fetuses at 14 gestational weeks to 77 years of age. Samples were stained with fluorescent marker antibodies to identify progenitor cells and young neurons. Definite signs of new neuronal formation in the hippocampus of infants and children were observed, whereas no such signs were exhibited in adult brains. Additionally, young neurons decreased in density as age level progressed (Sorrells et al., 2018).

In humans, it is theorized that neurogenesis occurs in the subgranular zone (SGZ) of the dentate gyrus of the hippocampus which maintains a neurogenic stem cell (NSC) niche. Some propose that the SGZ is an environment fit for NSC proliferation into granule cells from which migration to the granule cell layer occurs. Granular cells progress through the developmental

stages when specific protein markers are expressed, thereby revealing lineage specific cells in the neurogenic niche. This occurs before the cells integrate into the hippocampal circuitry and can influence the functions such as learning, memory, and spatio-motor performance (Kumar et al., 2019).

There is some research, albeit scarce and preliminary, that supports adult neurogenesis. One such study tested the brains of 5 cancer patients who had been injected with a chemical that incorporates into newly created DNA: Bromodeoxyuridine (BrdU). Traces of this chemical were found in the dentate gyrus of the hippocampus, thus supporting the theory that cells in this region are continuously dividing and creating new neurons (Eriksson et al., 1998). Another study also reported evidence of neurogenesis after identifying protein markers for various stages of neurogenesis in subjects 0 to 100 years of age (Knoth et al., 2010). In 2013, using carbon dating methods, Jonas Frisén's lab at the Karolinska Institute reported that up to 700 neurons are added each day to the dentate gyrus (Kumar et al., 2019). Although these are groundbreaking findings, many researchers question their validity. Asserting the possibility that BrdU can occasionally label dying cells instead of cells undergoing division, as well as the possibility that protein markers can accidentally label brain cells as glia instead of neurons, a body of researchers remains hesitant regarding claims of neurogenesis in adulthood. Nevertheless, the most robust study supporting adult human hippocampal neurogenesis was done by Boldrini and colleagues. Autopsying hippocampi of people ages 14 to 79, they found the production of intermediate neural progenitors, immature neurons, mature granule neurons, and glia to be similar between all age groups. Adhering to biological parameters and utilizing unbiased stereology, the researchers ensured that their samples were taken from healthy individuals (Boldrini et al., 2018). On the other hand, some studies have found results to be inconclusive. Kumar and colleagues used bioinformatic methods to study the differential expressions of neurogenesis signature markers in the hippocampi of prenatal to adult age subjects. Persistent but minimal hippocampal neurogenesis was observed. In addition, they initiated the criticism that newborn adult hippocampal cells could be glial cells (Kumar et al., 2019). The vast majority of research points to the stark difference between the human brains of infants and adults, with inadequate concrete knowledge and inconsistent evidence of adult neurogenesis. It is the hope of many scientists that future technology with the ability to provide imaging of new neuronal formation in the adult human brain will shed light on this debate.

Areas of Interest for Future Inquiry

Many factors limit the availability of research and tangible knowledge of adult neurogenesis, including sparse availability of ideal human brain tissue and limitations of study methods. A clearer understanding of the evidence surrounding adult human neurogenesis is crucial, as its presence or absence will have significant theoretical and practical effects on learning, age-related memory, pathology, and injury. Research and innovation are needed to produce safe investigatory methods to perform neurogenesis related research in living humans. Safe neuroimaging approaches to detect growth of newly formed cells in neurological niches and their integration into existing neural circuitry is needed. Possible stem cell methods of generating neural stem cells from the patient's own cells is another area of potential innovation.

Although the mechanisms of neurogenesis are not fully understood, there are a variety of avenues for further research and application. Some researchers have proposed a deeper investigation into the role of corticosteroids in reducing hippocampal neurogenesis. Others have suggested avenues related to trophic factors such as the brain derived neurotrophic factor, fibroblast growth factor, and epidermal growth factor, as well as the neurotransmitter serotonin, which have shown enhancement of neurogenesis. Additionally, studies have pointed to stress as the reason for increasing the production of glucocorticoids and decreasing trophic factors, thereby decreasing neurogenesis. On the other hand, environmental enrichment increases the secretion of trophic factors which, in turn, may facilitate neurogenesis (Kumar et al., 2019). These factors, along with further research, could conceivably be used as catalysts to promote adult neurogenesis and allow for greater recovery of learning and memory in the deaf population and beyond.

Limitations in conventional neuroimaging techniques to evaluate cortical plasticity pre- and post-implantation have hindered our ability to adequately study the effectiveness of cochlear implantation in the adult population. Given the application of powerful magnetic fields in MRI scanning, straightforward examination isn't feasible with the ostensible high-risk factor for the magnetic component of the cochlear implant. Surgically removing the magnet prior to MRI is risky and inconvenient. Removal would also impede auditory stimulation through the cochlear implant and would consequently distort the imaging of auditory cortical function. Unconventional neuroimaging techniques such as EEG and MEG are safe for cochlear implant users; however, they are unable to provide data about cortical processing of speech at the level of word identification and sentence comprehension.

In contrast, PET can be used to measure neural activity based on changes in cerebral blood flow and metabolism. Independent of electrical or magnetic cortical signaling, PET imaging allows for artefact free functional imaging in cochlear implant users. However, due to the use of a radioactive medium, testing repetition is limited and impedes on the adequate assessment of cortical changes that occur rapidly over a short period of time. The use of functional near infrared spectroscopy (fNIRS), a non-invasive optical imaging technique, is a possible area that, with further modifications, can be a promising means of data collection from cochlear implant recipients. This technique does not provide a direct measure of neuronal activation. Instead, it measures the consequential hemodynamic response seen in stimulus evoked changes in levels of oxygenated haemoglobin (HbO) and deoxygenated haemoglobin (HbR). Through the application of optodes, an optical sensor device with fiber-optic bundles, on the subject's scalp, the changes in HbO and HbR can be monitored and evaluated using a stimulus presentation paradigm. Although some limitations currently exist and further research is needed, multiple benefits of this technique include non-invasive and portable testing nature, high level of resilience to patient's head and body movement, and safe and flexible testing across a diverse population. Testing utilizing this technique has displayed an ability to measure cross-modal responses within the temporal lobes in cochlear implant recipients (Anderson et al., 2017).

Another area for further research is the therapeutic technique of speechreading prior to implantation. It is thought that speechreading in post-lingual deafness has the ability to maintain amodal linguistic functions and left hemisphere specialization for speech processing. Vision may facilitate the restoration of auditory function with modifications to the auditory cortex. This audio-visual synergy may enable adult cochlear implant users to capitalize on heightened levels of visual cortex activity to compensate for decreased auditory input from the implant. Consequently, sustainable close cooperation between the auditory and visual modality that post lingual deaf individuals can capitalize on during auditory rehabilitation is attainable. Evidence suggests that a synergy between modalities within the left temporal lobe may be a significant neural correlate in cochlear implant success (Anderson et al., 2017).

While cochlear implantation has opened a whole new world of hearing opportunities to the deaf population, the success rate is highly variable and still remains somewhat unpredictable. Although some basic markers for success are noted and understood, more sensitive prognostic tools are needed to accurately predict clinical outcomes. Growing research supports factors such as cortical plasticity within the temporal and temporo-occipital brain regions and synergistic relations between the auditory and visual modality and temporo-occipital interaction. Investigation on safe, sensitive, and thorough techniques to study brain changes pre- and post-implantation is an area of research that continues to expand.

Conclusion

Bypassing damaged peripheral structures, the multi-channel cochlear implant has provided profound to severe hearing-impaired individuals the ability to achieve similar auditory function to their unaffected peers. As anatomical and functional auditory integrity is of paramount importance, early cochlear implantation is a crucial determinant in the probability of a congenitally deaf individual attaining maximum auditory capacity. As supported by extensive data, implantation prior to the completion of the critical period plays an outsized role in neuroplasticity's ability to rewire one's neural circuitry while consequently preventing further recruitment of auditory cortical structures by other sensory modalities. As human sensory hair cells are incapable of regeneration, further research is needed to pursue avenues yet explored in the quest to further mitigate the deleterious side effects of congenital, early and late onset deafness.

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Should Advanced Maternal Age be a Deterrent for Attempting a Pregnancy?

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Abstract

In the developed world, the trend toward women of advanced age bearing children is very prevalent and seems likely to continue. For a variety of reasons, women are delaying pregnancy until 35 years and older when they suddenly seem keenly aware that their biological clocks are ticking. A review of various studies obtained from the Proquest and EBSCO databases indicates that there are definitely certain risks associated with advanced maternal age (AMA). As females age, the reduced number and quality of their remaining eggs contribute to reduced fertility. Congenital anomalies involving the number of chromosomes in the embryo increases as well. The risk of miscarriage gradually climbs with the mother's age and stillbirth is more likely than in younger women. The health of the older women prior to pregnancy is often compromised and birth-related complications are more common. Studies of mice have shown that maternal age influences the structure and functionality of the uterus. It has been suggested that delayed childbearing has an effect on the gene frequency in the general population since the zygotes best able to adapt to the Advanced Maternal Age (AMA) uterine environment were favored (Gloria-Bottini et al., 2005). Although women who postpone parenthood should take the increased risks into account, findings suggest that maternal age, per se, should not be a deterring factor when considering bearing a child. Individual health circumstances and behavioral choices are more important than age. With proper prenatal counselling and care, AMA women can hope to have normal pregnancies and deliver healthy babies.

Introduction

Advanced maternal age is defined as pregnancy in women equal to or greater than thirty-five years of age. It is becoming a growing trend, especially in high-income countries, to delay childbearing. Pregnancy in AMA mothers constitute 20% of pregnancies in the UK and 16% of pregnancies in the US as of 2014. Many women marry later, spend a decade or more building up their careers, pursue higher education, or enjoy the single life before deciding to have a family. Others may find themselves having their first child in their thirties because of previous fertility complications. Although better socio-economic conditions and developments in assisted reproductive technology are allowing women to have babies later in life, this new trend can possibly present clinical risks both to the mother and baby (Lean et al., 2017a).

Prenatal concerns for AMA mothers include fetal loss, chromosome anomalies such as Down's Syndrome, multiple births, hypertension, and gestational diabetes. Complications in labor and delivery might include placenta previa (when the placenta covers the opening in the cervix), caesarean birth, preterm birth, placental abruption (when the placenta detaches from the womb), low birth weight, trisomies, and other non-disjunction problems. Abnormal functioning of the placenta, due to aging, can result in fetal growth restriction and even stillbirths (Radhakrishnan, 2016).

It is important to point out that women who are pregnant and are older than 35 have some distinct advantages. They are more likely to follow the regimen outlined for them by their obstetrician, such as taking a folic acid supplement before conception and throughout their first trimester which helps decrease the risk of neural tube defects. These mothers are more likely to be diligent about keeping prenatal appointments. They are usually more established in their personal and professional lives and are more mature and knowledgeable, with a higher degree of personal control and coping strategies. Their higher income tends to contribute to more positive results in terms of their health. Lastly, AMA mothers tend to breastfeed more than younger mothers. Breastfeeding is considered the most optimal way to feed the infant. Its benefits include protecting the baby with passive immunity to infections of the respiratory and gastrointestinal tracts. Breastfeeding has maternal benefits as well; it reduces postpartum bleeding, returns the mother to pre-pregnancy weight faster, and decreases the risk of breast and ovarian cancers (Radhakrishnan, 2016).

Notwithstanding all of the above advantages, the ideal time for a woman to give birth remains up until the age of 35, during which time fertility is at its peak. Women who are older have a harder time conceiving. They usually also have longer work hours and stressful work environments and tend to drink more alcohol, putting the fetus at risk of Fetal Alcohol Spectrum Disorder, which can cause birth defects and neuro-developmental disorders. They have also been exposed to environmental toxins for a longer time. Most importantly though, their increased age makes medical conditions such as cancer, diabetes, hypertension, and arthritis more likely. These conditions, as well as their associated treatments and medications, can harm the fetus (Radhakrishnan, 2016).

Many studies have been done in various developed countries, investigating the impact AMA has on perinatal and neonatal outcomes in these women. These studies are important for both the mothers and the healthcare providers. The effects of AMA on maternal and fetal morbidity and mortality is of great concern.

Methods

The research was obtained from the online Touro College Library. Articles were collected from the Proquest and

EBSCO databases. The studies presented a comprehensive analysis of the topic and allowed for a conclusion to be reached regarding the research question.

Discussion

A systematic review and meta-analysis were done to determine the risk of stillbirth and other negative pregnancy outcomes in AMA women. It was the largest and most comprehensive review investigating pregnancy outcomes of AMA mothers and the only one specifically focusing on the increased risk of stillbirth. The studies in the review were significant considering that in 2013 the rate of births in England and Wales to women of 35 years and above was 20% as compared to a rate of 6% in 1980 (Lean et al., 2017a).

In the review, 63 cohort studies and 12 case control studies were included. The studies incorporated control groups of mothers younger than 35 years of age and AMA groups greater than or equal to 35 years. Primary outcomes studied were stillbirth and fetal growth restriction. Secondary outcomes were neonatal death, small for gestational age, neonatal intensive care unit (NICU) admissions, preeclampsia (blood pressure > 140/90 with significant amount of protein in the urine), placental abruption, preterm birth, and gestational diabetes mellitus. Studies that involved pregnancies of multiple fetuses or those that focused on chromosomal abnormalities or substance abuse were eliminated. Correlations between stillbirth rates and maternal obesity, diabetes, hypertension, and assisted reproductive therapies were recorded (Lean et al., 2017a).

AMA was found to increase the rate of stillbirth in the population to 4.7%. In addition, AMA increased the risk of fetal growth restriction, neonatal death, NICU admission and gestational diabetes mellitus. The relationship between AMA and stillbirth was not found to be associated with maternal morbidities, with the exception of hypertension which was positively correlated with AMA in women between the ages of 35 and 40. The use of assisted reproductive therapy (ART) showed no relationship to stillbirths in women equal to or under 35 years of age and was negatively correlated to stillbirth rates in women over 40. AMA only significantly increased the risk of fetal growth restriction in women over 40. The majority of secondary outcomes were more prevalent in AMA women but only NICU admissions and neonatal deaths had a substantial correlation to the increasing age of the mother (Lean et al., 2017a).

Stillbirths in advanced countries are presently thought to be related to placental dysfunctions. Accelerated aging of the placenta, altered vascular function and altered transport of nutrients compared to a control group have all been detected. Another theory for the cause of stillbirths is the diminished genetic quality of the aging oocyte (Lean et al., 2017a).

Another consideration that may contribute to fetal health with an AMA pregnancy is advanced paternal age. Research in this area is sparse. One study found a 24% increase in stillbirth rate with paternal age between 40-45 years old and a 50% increase with paternal age over 45, independent of maternal age. More studies are needed where paternal age is a co-variate to determine how it contributes to increased risk of stillbirth in AMA mothers (Lean et al., 2017a).

Future studies should be done to understand the mechanisms contributing to the increased risk of stillbirth in AMA, specifically in mothers over the age of 40, so that proper intervention can be applied to improve positive outcomes to those at greatest risk (Lean et al., 2017a).

The increased risks of fetal growth restriction and stillbirths in AMA mothers have been found to be independent of co-morbidities. These risks also do not seem to correlate with factors such as socio-economic status, nutritional supplementation prior to conception, good prenatal care, and the mothers being non-smokers. A hypothesis was offered that maternal aging was associated with utero-placental dysfunction which contributed to negative fetal outcomes (Lean et al., 2017b).

Dysfunction of the placenta is a major cause of fetal growth restriction and placental causes are noted in up to 65% of stillbirths. In both conditions, major changes in placental morphology were found, including lower placental weight, irregular shape to the placenta, and irregular insertion of the umbilical cord. Placentas from fetal growth restriction pregnancies had less vascular branching and fewer capillaries. These structural abnormalities were found even more in stillbirths related to fetal growth restriction (Lean et al., 2017b).

Abnormal function of the placenta was also apparent in fetal growth restriction and stillbirths. Among these were reduced amino acid transport in the placenta and impaired endocrine function (Lean et al., 2017b).

A study was done to test the hypothesis that AMA is associated with dysfunctions of the uterus and placenta. Women of AMA were studied along with young controls (20-30 years) with normal pregnancies. Investigations into these pregnancies identified multiple placental and utero-placental dysfunctions, potentially linking those mechanisms and increased fetal growth restriction and stillbirths in AMA women (Lean et al., 2017b).

The studies revealed increased placental weight but reduced placental efficiency in pregnancies of AMA women

with changes in placental morphology and function similar to the phenotype of placentas in fetal growth restriction and stillborn pregnancies. This phenotype was seen even in normal pregnancy outcomes which indicate both the negative effects of aging on the placenta and potential adaptation to achieve a normal birthweight. The abnormal placental phenotype was more evident in women >40 years of age who have a higher risk of adverse pregnancy outcomes (Lean et al., 2017b).

AMA mice displayed an even more exaggerated abnormality in placental phenotype than humans, with reduced number of offspring, late fetal deaths, and over half of offspring in the category of fetal growth restriction. Many similarities were found in the placental phenotype in the mice and human pregnancy models, including increased placental weight, decreased placental efficiency, and altered vascular function (Lean et al., 2017b).

All of these support the hypothesis that maternal aging is associated with placental dysfunction, which may result in higher rates of fetal growth restriction and stillbirths in AMA pregnancies. Abnormal cell turnover, abnormal nutrient transport, and increased relaxation of placental arteries all helped contribute to the less than ideal functioning of the placenta in AMA mothers (Lean et al., 2017b).

Another study was done to determine whether advanced maternal age in mice has a bearing on their pregnancy outcomes. The purpose of this study was to evaluate whether negative reproductive outcomes in aged female mice are a result of a dysregulation of methylation, and thus expression, of imprinted genes in their reproductive tissues. Tissues from the fetus, placenta, and ovaries were collected from young pregnant mice (4-5 weeks old) and aged mice (15 months old) and compared to matured oocyte and uterine tissue from non-pregnant ones (Paczkowski et al., 2015).

Results showed fetal growth restriction and overgrowth of the placenta in the older pregnant mice. Placental tissue showed aberration in methylation and transcript abundance of imprinted genes. Methylation and gene expression were severely dysregulated in the ovaries and in the uterus, including nutrient transport genes. There was also increased transcript abundance in oocytes obtained from older females, compared to younger ones (Paczkowski et al., 2015).

Major alterations in methylation and gene expression in the older ovary suggests that the environment in the follicle may not be optimal. This affects oocyte growth and quality, leading to compromised embryonic and fetal development. Aberrant methylation and expression of imprinted genes in the uterus obtained from aged mice may cause reduced implantation. Even though AMA mothers have an equal chance of becoming pregnant after ART cycles when using donor eggs, this study shows that the environment in the uterus may be compromised. If using their own eggs, the environment of the follicles may be compromised, indirectly effecting the development of the oocyte (Paczkowski et al., 2015).

Overall, AMA in mice changes methylation patterns of imprinted genes in reproductive tissue, which results in dysregulated gene expression associated with poor reproductive outcomes. This study is significant in that it suggests a possibility that the same factors in aged human females might cause negative outcomes in their pregnancies as well (Paczkowski et al., 2015).

A study was done where associations of maternal age at childbearing with gestational age (number of weeks fetus was in the womb) and fetal growth (birthweight adjusted for gestational age) were examined. Those features are important to study because these problems predict mortality and morbidity across the lifespan of the human, including psychological, health, academic, social, and economic difficulties. Preterm birth is associated with decreased cognitive functioning in childhood and the use of psychiatric medications in adults. Poor fetal growth has been found to be linked to psychiatric diagnoses in childhood (autism and ADHD) and adult health problems (cardiovascular disease and diabetes). Given these major health concerns, it is imperative to obtain a better understanding of the possible causes of these adverse perinatal outcomes (Sujan et al., 2016).

The study was designed in such a way as to rule out genetic and environmental factors that might contribute to the results. To assess potential familial confounding, two genetically informed designs were used comparing cousins and siblings. To be able to generalize results, data was taken from two samples with different cultures, races, ethnicities, economic backgrounds, and healthcare coverage. Some covariates were offspring birth order, and parental characteristics such as levels of education, history of severe psychiatric problems, history of substance abuse, household income, maternal race, and maternal age at first childbirth (Sujan et al., 2016).

There was strong evidence that AMA at childbirth was associated with a shorter gestational age. The gestational age of offspring born to 30-34 year old mothers, 35-39 year old mothers, and mothers of 40 years and above, were all less than the gestational age of offspring born to 25-29 year old mothers. Even after accounting for shared familial factors, the data pointed to a strong link between mothers who are older at childbirth with shorter gestational age. This did not hold true for lower fetal growth.

Results from the study supported a causal association between AMA at childbearing and shorter gestational age (Sujan et al., 2016).

Further research is needed to explore specific factors that might account for the findings. Some possibilities might be maternal diseases, such as hypertension, diabetes, and the quantity of oxygen and nutrients received during the prenatal period (Sujan et al., 2016).

Another study tested the hypothesis that reduced fetal weight and lower success of pregnancy outcomes in AMA rats may be attributed to sex-specific changes in the morphological development of the placenta and in its nutrient transport function. It was found that the sex of the fetus did indeed influence the placenta and offspring outcome in response to the adverse environment in the uterus during gestation (Napso et al., 2019).

Young (3-4 months old) and aged (9.5-10 months old) Sprague Dawley female rats were mated with young male Sprague Dawley rats (3-5 months old) and were evaluated on gestational day 20. The study demonstrated that advanced maternal age caused modifications in the phenotype of the placenta and in this way, altered its ability to support the growth of a fetus. In particular, it modified the expression of genes and proteins that are crucially important in placental growth, transfer of nutrients, endocrine control of maternal physiology, and control of exposure of the fetus to glucocorticoids. AMA causes oxidative stress and cell death in the placenta, in a manner that is partially dependent on the sex of the fetus. The study also indicated that gene expression changes in the placenta of female fetuses were mostly beneficial, with an up-regulation of genes that support the function of the placenta. Gene expression changes in the placenta of male fetuses, though, were mostly detrimental for placental growth and functional phenotype in older rats. There was similar growth restriction in both male and female fetuses, although the absolute weight of male fetal heart, brain, and liver were more reduced in fetuses of aged rats than younger ones (Napso et al., 2019). There were previous studies that demonstrated poorer cardiovascular outcomes for adult male rats born from aged mothers (Shah et al., 2018 as cited in Napso et al., 2019).

The sex-dependent changes in glucocorticoid handling in the placenta of AMA mothers affects health outcomes of offspring later in life. Elevated prenatal exposure to glucocorticoids affects development of the fetus and permanently alters the structure and functions of its organs, predisposing the offspring to certain diseases later in life such as hypertension (Napso et al., 2019). Even though the fetal weight for male and female fetuses of aged rats was compromised similarly, studies showed that adult male offspring of aged rats have a greater chance of developing cardiovascular dysfunction as adults, as compared to female offspring (Shah et al., 2018 as cited in Napso et al., 2019).

In summary, AMA affects the phenotype of the rat placenta in a sex-dependent manner. In female fetuses from aged rats, there were larger beneficial changes in structure and/or expression of genes related to placental transport of nutrients from mother to fetus and improved handling of glucocorticoids. In male fetuses of aged rats, there were no beneficial changes in the transport system and there was less protection from glucocorticoids, greater oxidative stress, and elevated levels of apoptosis. These sex-dependent changes in placental response to the environment in the uterus of the aged mother play a role in the future health of female and male offspring. The results of this study have implications for managing human pregnancies, especially in developed countries where women are becoming pregnant at an older age. Targeted interventions can be developed to improve placental development and function which, in turn, will aid fetal growth and development for mothers of AMA (Napso et al., 2019).

Although women of AMA are a growing population in all developed countries with greater obstetric risks, women who live in the Mediterranean have specific characteristics that are different than other areas. A study was done to establish an AMA cut-off age in a selected Mediterranean population in Barcelona. Although most studies define AMA as being above 35 years, this study defined AMA in Spain as being above 40 years. The average age of women delivering their first baby rose from 25.2 years in 1975 to 30.7 years in 2016. Models predict that by 2050, the average age of a first pregnancy will be 33 years. In 2016, 38.7% of deliveries in Spain were to mothers over 35 years, and 8.39% were to mothers over 40 years. The average age of women at childbirth in 2017 was 32.5. The scientific community is interested in gaining information about the impact of AMA on obstetrical outcomes so that they can better assess risks in this increasing population (Nieto et al., 2019).

The cohort in this study were women who gave birth between January 2007 to June 2017. A total of 25,054 pregnancies were included. The average maternal age was 34.7 +/- 4.2 years. In this study, 2,807 patients were between 40-44 years and 280 patients were >45 years. Of the total pregnancies, women above the age of 40 accounted for 12.3% of the deliveries. Confounding factors were considered, which included chronic hypertension, pregestational diabetes types I/II, use of ART, obesity, smoking, previous C-sections, and multiparity (having more than one child). Over 97% of the women were Caucasian and

99.8% were from the Mediterranean area. After adjusting for confounding factors, age proved to be a significant risk factor in developing gestational diabetes, especially in women >40. The risk of placenta previa increased as well in women >40 years. Preeclampsia and prolonged hospitalization were only associated with women >45 years. The risk of these negative outcomes increased as the age of the women increased (Nieto et al., 2019).

It was suggested that the reason for the increased risk of gestational diabetes in women >40 might be endothelial damage. Age is known to be a cardiovascular risk factor that produces structural and functional changes in the vascular system. Dysfunctional endothelium increases the risk of developing insulin resistance, which then increases the risk of hypertension, type II diabetes, and other metabolic syndromes (Nieto et al., 2019).

In summary, in this study, women >40 were the most positively associated with complications of gestational diabetes, placental previa, caesarian delivery, and prolonged hospitalization. They were the ones with the highest clinical risks. These patients were also at greatest risk for iatrogenia (effects from medical interventions) since they have high elective C-section rates. Future investigations should therefore address medical interventions and pregnancy surveillance to improve outcomes in AMA patients (Nieto et al., 2019).

Another cross-sectional study tested the hypothesis that there is an association between AMA and maternal and neonatal morbidity. The study was based on data obtained from 3,315 births. It compared the births of women who were 35 years and older, with a reference group of women between the ages 24-27 (Casteleiro et al., 2019).

Results of the study confirmed the hypothesis. Repeated spontaneous abortions were 2.2 times more frequent in the older age group compared to the younger reference group. There was a greater incidence of gestational diabetes in AMA women, particularly in primiparous women where the risk was eight times more prevalent. The possibility of giving birth with the help of instruments was multiplied by 1.6 and the possibility of a cesarean delivery was multiplied by 1.5 in both primiparous and multiparous AMA women. Lastly, there were correlations between preeclampsia, preterm birth (<37 weeks) and low birthweight in the older age group, although the numbers were not high enough to have statistical significance (Casteleiro et al., 2019).

The cause of repeated abortions in AMA women may be attributed to alterations in the chromosomes, which increase along with increasing maternal age. The cause of gestational diabetes is being continually researched. One theory is that diabetogenic hormones, such as the growth hormone that releases corticotropin, produce an increased insulin resistance which AMA women are not able to overcome (Casteleiro et al., 2019).

A study was done to ascertain the effect of advanced maternal age on singleton pregnancies in nulliparous women. Records of obstetric patients were reviewed from routine fetal ultrasonograms that were taken in the middle trimester of pregnancy. Three groups were included in this study: a control group of ages 18-34 years old, advanced maternal age women of 35-39 years, and very advanced maternal age women over 40 years of age. Altogether, 957 women met the criteria to be included in the study (Kahveci et al., 2018).

The study clearly demonstrated that AMA nulliparous women with no previous chronic diseases (including obesity) had higher rates of adverse perinatal and neonatal outcomes such as gestational diabetes, gestational hypertension, preeclampsia, small for gestational age, spontaneous late preterm delivery (between 34-37 weeks of gestation) and cesarean delivery. Gestational diabetes, gestational hypertension and cesarean delivery rates were more common in the VAMA group compared to the AMA and young age group. No increased risk was found for spontaneous preterm delivery before 34 weeks, prolonged rupture of membranes, placenta previa, large for gestational age, and operative vaginal delivery (Kahveci et al., 2018).

It has been suggested that poor oxygen exchange may be a factor in the association between AMA and small for gestational age. This stands to reason because when an unborn baby doesn't get enough oxygen during pregnancy, its organs don't grow as much as they should (Kahveci et al., 2018).

Concerning neonatal outcomes, admission to a NICU was more likely to occur in the AMA groups, but there was no major difference in APGAR scores, occurrences of low birth weight, and neonatal morbidity between the groups (Kahveci et al., 2018).

During the last few decades, there has been a significant increase in the amount of pregnancies involving multiple fetuses due to the use of ART. Even in singleton pregnancies, AMA poses a risk for more maternal and neonatal complications. Multiple gestation drives this risk up even more (Gluck et al., 2018).

A study was done with the goal of comparing obstetrical and neonatal outcomes between women older than 35 years and women younger than 35 with the same type of twin pregnancy. Records were reviewed of all dichorionic-diamniotic pregnancies (in which each fetus has its own placenta and amniotic sac) between 2009-2016 where the babies were delivered after 24 weeks gestation. Pregnancies with abnormalities in the chromosomes

and those with fetal malformations were excluded. The older women were divided into two groups: age 35-39, and those >40. The control group consisted of women younger than 35. Altogether, 716 women were studied. The rate of in-vitro fertilization for conception was higher in the study groups (older women) than in the control group (Gluck et al., 2018).

AMA was found to be independently associated with cesarean section after adjustments were made for nulliparity, in-vitro fertilization, and hypertensive disorders. The rate of hypertensive disorders was significantly higher in women over the age of 35 (Gluck et al., 2018).

Twins born to women over 35 also tended to stay longer in the NICU than those born to the younger women. Other neonatal outcomes in women age 35 or older who were carrying twins were similar to the younger group (Gluck et al., 2018).

A retrospective cohort study was done to evaluate whether AMA was independently associated with an increased risk of childhood cancer in the offspring. There were three maternal age groups studied: 35-39, 40-50, and a control group of 20-34. A comparison was made between the three age groups for incidences of malignant morbidity in the offspring. Pregnancies lacking prenatal care, gestations of multiple fetuses, pregnancies originating with fertility treatments, and pregnancies with fetuses displaying congenital malformations were not included in this study. The offspring were followed up to the age of 18 years. The three most common malignant forms of childhood cancer which were traced included leukemia, brain tumors, and lymphomas (Imterat et al., 2018).

Theoretically, advanced maternal age might correlate with childhood cancers due to DNA damage and decreased repair pathways in oocytes of older mothers. Several previous studies (Ross et al., 2005 as cited in Imterat et al., 2018) showed a connection between AMA and meiosis errors leading to trisomies (particularly trisomy 21 or Down's Syndrome) which are known to predispose for malignancies in childhood. Additionally, ART, which is increasingly used to offset infertility disorders in older women, is suggested as increasing the risk of childhood cancers such as retinoblastoma, by altering gene expression. Finally, changes in maternal hormones during pregnancy that are age-related, such as higher intrauterine exposure to endogenous estrogen and insulin growth factor-1, have been shown to help initiate cancers. Considering all the above hypothetical effects of AMA on childhood cancers, this study was important to conduct (Imterat et al., 2018).

In the study, 201,738 deliveries were included. Results showed that advanced maternal age did not increase the risk of future malignancies in the offspring up to 18 years old. There was some association, though, between maternal age and leukemia of the offspring which warrants further investigation (Imterat et al., 2018). However, overall, childhood cancer of offspring does not seem to be a major concern for AMA women planning to conceive.

A study was done to investigate what effect AMA has separately in nulliparous and multiparous women on obstetric and neonatal outcomes in singleton pregnancies. The study was a hospital-based analysis of 6,619 births between January 2004 and May 2007. AMA was defined as 35 years and older. Nulliparity was defined as women who had not delivered a viable fetus (greater than 24 weeks of gestation in the past) and multiparity was defined as women who had at least one pregnancy in the past that progressed beyond 24 weeks of gestation. The actual parity number wasn't considered. Of the study participants, 42.7% were nulliparous and 57.3% were multiparous. Of the nulliparous, 21.8% were of advanced maternal age and of the multiparous, 42.1% were of advanced maternal age (Wang et al., 2011).

Among nulliparous women, AMA was associated with higher rates of cesarean sections both before and during labor, as well as vaginal deliveries assisted by instruments. Among multiparous women, however, AMA was only associated with a higher rate of cesarean sections before labor. This might be due to a greater percentage of multiparous women requesting cesareans (Wang et al., 2011).

A study was done in order to assess how pre-pregnancy weight (or body mass index) and gestational weight gain affect pregnancy outcomes in AMA women. This retrospective analysis was conducted on postpartum and hospital delivery data in China. A total of 1,015 women were included in the study who gave birth from January-June 2017. In China, 60% of all pregnancies are in women greater than 35 years of age and half of these women are above 40 years of age. AMA women are often overweight before pregnancy and are more likely to have internal diseases. They are also more likely to be multiparas having had at least one previous child. Age is also an independent risk factor for adverse pregnancy outcomes (Lin et al., 2019).

The women were divided into an advanced age group (35-40 years) and a super advanced age group (>40 years). Body mass indices prior to pregnancy were divided into underweight, normal weight, overweight and obese groups. Gestational weight gain was subdivided into three groups (Lin et al., 2019).

Results showed that being overweight prior to pregnancy increases gestational diabetes mellitus, hypertensive disorders complicating pregnancy, and fetal macrosomia where a newborn is significantly larger than average.

Poor weight gain during pregnancy increased the risk of preterm births, but excessive weight gain during pregnancy increased macrosomia in the babies of AMA women (Lin et al., 2019).

The women in the super advanced maternal age group had higher incidences of both being overweight and obese than those in the AMA group. The super advanced maternal age group had more incidences as well of gestational diabetes mellitus, hypertensive disorders, and macrosomia than in the advanced age group (Lin et al., 2019).

Overall, controlling weight gain both prior to and during pregnancy reduced adverse pregnancy outcomes in AMA women (Lin et al., 2019). It is good advice for older women who are planning to conceive to get their weight under control beforehand.

A study was conducted with the objective of determining whether there was an association between AMA and race on pathology of the placenta in very low birthweight infants. A retrospective cohort analysis was done on placental pathology on very low birthweight singleton infants born between July 2002 and June 2009. There were 739 cases included in this study (de Jongh et al., 2015).

The placenta is the area where maternal-fetal oxygen and nutrients get exchanged and so it has an important influence on birthweight and is necessary for a successful outcome to a pregnancy. Placental growth, which can be measured in terms of placental weight, is a good indicator of how well the placenta is functioning. The placenta weight/birthweight ratio is used to indicate neonatal outcomes (de Jongh et al., 2015). Both high and low ratios have been associated with an increased risk for stillborn and other negative neonatal outcomes (McNamara et al., 1998 as cited in de Jongh et al., 2015).

The subjects of the study were divided into two groups: maternal age <35 and maternal age >35. Mean placental weights, mean birthweights, and placental weight/birthweight ratios were calculated for each age group (de Jongh et al., 2015).

In the final analysis, AMA was seen to be associated with a decrease in placental weight and placental weight/birthweight ratio. AMA, and not race or ethnicity, remained independently associated with placental weight/birthweight ratio. It could not be determined, however, if older maternal age directly causes reduced placental weight/birthweight or whether it represents confounding variables not tested for. Further research is necessary to discover possible causal mechanisms that can explain the relationship between AMA and lower placental weights in very low birthweight babies (de Jongh et al., 2015).

In Denmark, the rate of cesarean sections increased by 49% between the years 1998-2015 and occurred in 21% of all births. This is a cause for concern since cesarean sections can have short and long term consequences for the mother and child and can present risks in future pregnancy. A possible cause of the increase may be the delaying of pregnancy until advanced maternal age. AMA at childbirth has been found to be linked to pre-pregnancy morbidity that might account for the increased risk of cesarean section. The goal of this study that included one million Danish women was to examine the relationship between AMA and cesarean section, taking demographics, health and obstetric factors into the equation. There were three age groups (30-34, 35-39, >40 years) with a reference group of maternal age less than 30 (Rydahl et al., 2019).

A positive association was found between AMA and cesarean section. When adjusting for confounders, there were only minor changes in the risk factor. When compared to the reference group, nulliparous women aged 35-39 had twice the risk of cesarean section, while women of 40 and above had three times the risk. For multiparous women, the risk was more moderate (Rydahl et al., 2019).

Cesarean sections are one of the most intrusive obstetric interventions and is becoming much more common in industrialized countries. The increase in cesarean sections is mainly a result of AMA, especially in nulliparous women. Morbidity tends to increase as age increases, so AMA women will include more individuals with prenatal risk factors such as hypertension, diabetes mellitus, and higher body mass index than younger pregnant women. More women of advanced age will also develop pregnancy related complications, including gestational diabetes, preeclampsia and placenta previa. There is also evidence that there is a decline in the physical ability to maintain uterine contractions which can cause labor dystocia. Future studies on the possibility of advanced age affecting the ability to maintain progression of labor is recommended. Placenta previa in AMA women, as well as non-vertex fetal presentations might also necessitate a cesarean delivery (Rydahl et al., 2019).

The higher cesarean section rate may be a result of these comorbidities related to AMA. It is possible, though, that the lower threshold for cesareans may be influenced by the attitudes of the healthcare professionals treating AMA women who may view them as high-risk patients or by the mothers themselves who choose a cesarean birth because they are more anxious about the health of the fetus (Rydahl et al., 2019).

A study was done regarding the association between maternal age and offspring adult health. There is increasing documentation associating AMA with negative health outcomes in their offspring. This association is considered to be a reflection of aging physiological processes of the

mother, such as the decreasing quality of her placenta or eggs (Myrskyla & Fenelon, 2012).

In this study, the role of socioeconomic selection and lifespan overlap between the generations is examined, as they are related to the association between maternal age and offspring adult health. It was found that offspring born to mothers younger than 25 or older than 35 have the worst outcomes regarding health, height, obesity, and even mortality. The reason for the young maternal age-offspring health link may be due to the physiological immaturity and socioeconomic disadvantage of young parenthood. Regarding the older age group, the results of the study showed that negative outcomes are not due to the mother leaving a physiological imprint in the offspring that causes a predisposition to diseases in adulthood. Rather, maternal education (which is strongly connected to socioeconomic status) and the age at which the child loses the parent are two important factors that affect the correlation between AMA and offspring adult health (Myrskyla & Fenelon, 2012).

Mothers of advanced age have generally had the opportunity to obtain a higher level of education which translates into greater affluence. Childhood socioeconomic status has been found to be strongly connected to adult health and mortality (Myrskyla & Fenelon, 2012).

The age at which a child loses a parent is directly related to maternal age. If all other factors are held constant, a child born to a younger mother will have many more years of lifespan overlap with the mother than a child born to an older mother. The psychological shock of losing a parent at a younger age is one factor that may affect the adult child's health. Another factor might be the loss of parental involvement with the offspring. Thirdly, a shorter lifespan overlap might indicate a genetic frailty that runs in the family (Myrskyla & Fenelon, 2012).

Maternal age, therefore, is related to the mother's education, socioeconomic status, and lifespan overlap with the offspring, all of which were found to be confounders in the maternal age-offspring health association. These factors all help explain the mechanism behind the association of AMA and negative health outcomes in the offspring and challenge the standard interpretation for that association. The reason this study is important is because today's parents, even if they reproduce at an older age, will likely live longer due to medical advances and therefore have a longer lifespan overlap with their offspring. If biological aging is indeed not the factor to influence the adult health of offspring but rather lifespan overlap is, AMA women can be reassured going forward that their late childbearing will not adversely affect their offspring (Myrskyla & Fenelon, 2012).

In the latter half of the last century, the delay of childbearing in Western cultures has affected the biology of the mother-fetus relationship. Data obtained from experiments, as well as clinical observations, have ascertained this change. As an example, there has been an increase in type 1 diabetes in developed countries during this time. This trend parallels the tendency to give birth at a later age which might suggest a causal relationship between the two events. Delayed childbearing might therefore have a detrimental effect not only on the development of the fetus in the uterus but may possibly be causing a predisposition to certain severe disorders. The change to the reproductive organs due to age, along with changes in hormone activity, may be the causes of this negative relationship (Gloria-Bottini et al., 2005).

A study done in Italy might also suggest that delayed childbearing is causing a change in the genetic composition of the population. Gene frequency refers to the proportion of a population that carries one type of allele, or gene variation, at a particular point on a chromosome. Certain phenotypes either increase or decrease with maternal age. These changes in the genetic composition of AMA women were found to be similar in the mothers and newborns studied (Gloria-Bottini et al., 2005).

The hypothesis of the study, which included over 600 women giving birth, was that advanced maternal age may influence intrauterine selection by favoring genotypes that are best able to adapt to the intrauterine environment of the older mothers. Zygotes that are more resistant to the changes in the maternal environment of older women will be the ones to survive. Maternal and neonatal genotypes were found to be similar in the AMA women studied. Maternal age was, therefore, having an effect on the gene frequency in the offspring (Gloria-Bottini et al., 2005).

Very Advanced Maternal Age (VAMA)

In Australia, as in most other high-income countries, childbearing age has been rising to 35 years and above. A new trend is emerging of pregnancies in women even 45 years and older which comprise 0.1% of all births in the country and is continually rising. This is driven partly by assisted reproductive technologies such as oocyte donation. There were few studies examining perinatal outcomes in this age group. A state-wide population-based study was done in Victoria, Australia covering the years 2005-2006 to determine maternal health and the outcomes of pregnancies of women in this age group compared to women aged 30-34 years (Carolan et al., 2013).

The study revealed prenatal complications of at least one of the following: gestational diabetes, antepartum hemorrhage (bleeding from or in the genital tract),

placenta previa, and multiple births. There was weak evidence for increased risk for preeclampsia. Women >45 were also more likely to have caesarian sections. They were not found to be more likely of having a postpartum hemorrhage (Carolan et al., 2013).

Pre-existing medical conditions of the mothers, as well as parity and use of ART, were taken into account. Women aged 45 and older were most likely to have pre-existing hypertension, but there was weak evidence of pre-existing diabetes. Women in the group were 12 times more likely to have used ART (Carolan et al., 2013).

Babies born to these VAMA (very advanced maternal age) mothers were at a higher risk for preterm birth (between 32-36 weeks gestation) regardless of parity, low birth weight (less than 2,500 grams) for primiparous women and had higher odds of being small for gestational age. Rates of preterm birth were very high for multiple births and for those who used ART (Carolan et al., 2013).

The findings in this study have ramifications for the women as well as for their healthcare providers. Although results displayed higher rates of pregnancy and perinatal negative outcomes for the women aged 45 and older in Victoria, Australia, nevertheless, the findings were reassuring as the vast majority of these women of very advanced age gave birth to healthy babies. The rate of perinatal death was low (less than 10 per 1,000 births). This suggests that with good prenatal care, most women in this group can achieve a live birth. Future studies should include investigations into the future health of these children who were preterm birth or were of low birth weight or small for gestational age (Carolan et al., 2013).

A study was done to investigate the interplay between very advanced maternal age (>43 years) and assisted reproductive technology on adverse perinatal outcomes. Data was taken from a cohort of women who delivered babies in Ontario, Canada between 2012-2015. In Canada, the number of births in women over 40 years of age tripled from 2005-2014. Even though fertility declines as a women ages, ART has allowed many more women of VAMA the chance to become pregnant. ART, though, has been considered a risk factor for adverse pregnancy outcomes as compared to spontaneous conceptions. This particular study aimed to assess the pregnancy outcomes of women who were both of very advanced age and who used ART to conceive. Singleton pregnancies of women >20 years who delivered at 20 weeks of gestation and above and whose offspring's birthweight exceeded 500 grams were included. The women were put into three categories of age: 20-34 years, 35-42 years, and >43 years of age. VAMA was defined as >43. All the subjects used were noted as either having used ART or having spontaneously conceived (Wu et al., 2019).

The mothers age was the independent variable. The type of conception (ART or spontaneous conception) was the main covariate. ART conceptions included intrauterine insemination, intrauterine insemination with ovulation induction but without in-vitro fertilization, in-vitro fertilization, and vaginal insemination. Many potential confounders for adverse maternal and neonatal outcomes were included, such as parity, income, education, body-mass index, drug/alcohol use, and maternal preexisting health issues (Wu et al., 2019).

Women at VAMA had a higher risk of a composite outcome which included preeclampsia, intrauterine growth retardation, stillbirth, and placental abruption than the mothers in a younger age bracket. The fact that ART was used did not add to the adverse effect of VAMA, even though ART may play an independent role for adverse perinatal outcomes. These outcomes were found in 10.41% of women under 35 and in 13.35% in women of VAMA. Regardless of the conception method, women of VAMA had a higher incidence of adverse outcomes (Wu et al., 2019).

Another study was done to evaluate if the adverse pregnancy outcomes in women of very advanced age differed by parity and by conception method. According to a national perinatal database in Japan, women of VAMA giving birth rose to 28.1% of all births. A total of 365,417 women over the age of 30 were included. The women were divided into four age groups: 30-34, 35-39,40-44, and >45. Pregnancies involving multiples, as well as fetuses with congenital abnormalities, were excluded (Ogawa et al., 2017).

Compared with the 30-34 year reference group, women aged 45 or older showed a higher risk for cesarean sections, preeclampsia, placenta previa and preterm birth. Placental abruption, very preterm birth, low APGAR score and perinatal death were not seen as being of increased risk in VAMA. The effects of older age on the negative outcomes were significantly higher among those women who conceived naturally, compared to those who used ART to conceive. However, the possibility that abnormal embryos were removed during ART could have skewed the results. The effect of advanced age on cesarean deliveries was stronger among primiparous women, but the risk for preeclampsia was significantly stronger among multiparous women (Ogawa et al., 2017).

The effects of advanced age on preterm birth were significantly greater among women who conceived without ART than among those who used ART to conceive. To clarify, while the risk of preterm birth generally increased with age in women who conceived without ART, it decreased in women who conceived with ART. Therefore,

younger women who conceived through ART, may have a greater risk of preterm birth than women who are older and conceive through ART (Ogawa et al., 2017).

The greater risk of cesareans in primiparous women of advanced age may be due to higher rates of elective cesarean sections requested by primiparous older women. It might also be due to prolonged labor or compromised fetal status requiring emergency cesarean section with increasing age, but these hypotheses were not tested (Ogawa et al., 2017).

While the greater risk of preeclampsia in multiparous older women in this study contrasted to a previous study (Bianco et al., 1996 as cited in Ogawa et., 2017) which found no change in risk of preeclampsia in both primiparous and multiparous women of age, this might be explained by the recent use of low dose aspirin in women with a higher risk of preeclampsia. Since both primiparity and advanced age are both strong risk factors for preeclampsia, these women may be more likely to receive the medication than multiparous women (Ogawa et al., 2017).

Regardless of the way VAMA women conceive, the need for preconception counselling, and greater prenatal care in these women should be a priority. Regular prenatal visits and testing can help address potential concerns early on and reduce the risk of pregnancy and birth complications which can be even more devastating, both emotionally and financially.

Conclusion

Many studies have been done in various developed countries investigating what impact AMA has on perinatal and neonatal outcomes. The consensus is that advanced maternal age increases the mother's risk for developing medical conditions such as cancer, diabetes, hypertension, and arthritis. The probability of pregnancy complications increases as well. Among these complications are chromosome anomalies, placenta previa, placental abruption, caesarean birth, preterm labor and low birthweight. Abnormal functioning of the placenta and impairment in oocyte development potential, both due to aging, can result in fetal growth restriction and even stillbirth. Women who are of very advanced maternal age (over 40 and especially over 45 years of age) seem to be at even greater risk of pregnancy and perinatal difficulties, although risks differ by parity and conception method.

Results of studies have been conflicting at times. The reason for this might be differences in homogeneity in study groups or failure to adjust for potential confounders such as maternal diseases, obesity, assisted conception, pregnancies of multiples, and parity. Differences in definitions of pregnancy outcomes might also be a reason for incompatible conclusions. It is vital to determine if age effects are direct and have a causal relationship with adverse outcomes or if they are indirect since other factors are at play. In order to accomplish this, future studies need to have control groups for age-dependent confounders. Inconclusive results concerning the ramifications of delayed childbirth must be resolved so that women of advanced age can make informed choices when deciding whether to bear children.

Information regarding chromosome anomalies and the availability of prenatal screening tests are vital for any women over the age of 35 who are planning to become pregnant. Those who receive positive results for a screening test can be sent for genetic counselling. Other emotionally supportive forms of help might come in the form of bereavement counselors, adoption services, or information on support groups for parents of multiples or for those who suffered a perinatal loss.

Preconception care is especially important for women of advanced age planning a pregnancy. Fertility concerns, pre-existing health conditions and risks for chromosome anomalies can be discussed. The importance of folic acid during pregnancy to decrease the risk of neural tube defects, reducing workplace risk and avoiding alcohol use and medications are all important topics to address. Older first-time mothers may need counselling regarding their transition into parenthood which may be more difficult for them as opposed to younger, first-time mothers. Most importantly, pregnancy surveillance in this older age group must be stressed in order to improve outcomes for both mother and baby.

The trend of increased advanced maternal age is expected to continue and thus the effects of AMA on maternal and fetal morbidity and mortality is, and will be, of great concern. The unique needs of this population must be addressed not only to benefit the mothers and babies themselves but for the implications that it has for the healthcare system and for service providers. Promoting the health of these women and their offspring and preventing negative outcomes reduces healthcare costs overall and will have an impact, going forward, on employment policies which will, hopefully, become more pregnancy-friendly.

Overall, older women who are in generally good health can hope to have favorable outcomes to their pregnancies with good prenatal care. Advanced technologies in perinatology have improved their chances of giving birth to a healthy child. If the needs for preconception counselling, targeted surveillance and early medical intervention are met, then there is no definite medical reason for excluding AMA women from attempting a pregnancy on the basis of age alone.

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Human Organ/Limb Regeneration: A Dream or Reality?

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Abstract

Organ and limb regeneration might seem like something out of science fiction, but research has been ongoing since the late 1960s and has greatly increased at the turn of the century. It is an understatement to say that this has the potential to be life changing. The need for donor transplant organs and transplant waiting lists can become obsolete and the use of immunosuppressants post-transplant will become unnecessary (leading to higher survival rates). Should this happen, trauma patients will be able to achieve complete recoveries and the reign of some congenital disorders will come to an end. Nature has provided several opportunities for us to study this subject. Many species have a natural ability to regenerate complete organs. Human fetuses display a tremendous power of regeneration and healing in utero. The struggle has been in determining how and why this ability disappears after birth as well as applying the lessons we have learned from other species to humans. (However, great progress has been made and this paper will discuss where science is holding in terms of being able to give a human the ability to regenerate complete organs and limbs.) This paper will discuss whether science has been able to determine which lessons to learn from nature and how and when to apply it.

Introduction

In 2008, nearly 2 million amputees have been reported in the United States and its prevalence is estimated to escalate more than 3-fold by 2050 (Ziegler-Graham, et al., 2008). Although great strides have been made in treatments for amputees which have in turn greatly increased their ability to lead productive lives, there are many side effects and frequent compromises that affect the quality of life (i.e. the adverse effects of long-term immunosuppression) need to be accepted. In addition, current therapeutic approaches, such as allogeneic hand transplantation, suffer from a limited donor supply (Schneeberger, et al., 2007). Besides for injuries and congenital diseases, regenerative medicine therapies have the potential to allow us the ability to treat (or even replace) failing organs which have begun to decline due to age (Heidary Rouchi, Mahdavi-Mazdeh, 2015; Ranjeet Singh, 2016). This will allow for a great increase the general quality of life, especially for the elderly. Regenerative medicine has the potential to provide treatment for a tremendous variety of currently intractable diseases and ailments (Upadhyay, 2015).

Methods

Data was collected using Google and PubMed databases through Touro College's online library. Among the key-phrases used were "regeneration", "limb regeneration", "human regeneration", and "regenerative medicine."

Discussion

To begin the discussion of regeneration we must first gain a clear understanding of the conditions and processes that are required for it to occur. There are a few species that have a natural ability to regenerate organs and limbs. All known livings things can be classified into three groups concerning their natural ability to regenerate. This paper will refer to the three groups as "complete", "partial", and "minimal". "Complete" refers to a lifelong, absolute ability to regenerate complete organs and limbs. Examples include urodeles such as newts and axolotls such as salamanders (Bensoussan-Trigano, et al., 2011; Dinsmore, 1996; Brockes, Kumar, 2002; Roy, Lévesque, 2006). "Partial" refers to an absolute ability to regenerate but only for a portion of the lifespan, after which the capacity is lost. For example, anurans such as frogs and toads can completely regenerate a limb in the larval stage, however, once passed metamorphosis they lose this ability (Satoh, et al., 2005; Suzuki, et al., 2006). "Minimal" refers to a very limited capacity for regeneration (only very simple organs and/or simple portions of complex organs) and only for a small portion of the lifespan. Neonatal mammals for example, have been shown to be able to regenerate the tips of digits, however, this ability fades with aging (Sánchez Alvarado, Tsonis, 2006; Farah, et al., 2016).

Differences

Wound healing is a necessary component of regeneration and is comprised of four stages (a) hemostasis (blood clotting), (b) inflammation, (c) proliferation (growth of new tissue including the formation of wound epidermis (WE)), and (d) maturation (remodeling) (Fernando, et al., 2011; Simkin, et al., 2013; Yokoyama, 2008). Wound healing occurs in all groups, regardless of their ability to regenerate (Raz, Mahabaleshwar, 2009). However, there are important variances within the exact mechanisms for each of the groups (Borgens, 1982; Han, et al., 2008; Takeo, et al., 2013). Variances include the duration of wound closure (Mu, et al., 2013; Stocum, 2011), inflammatory response (Ferguson, et al., 1996; Wulff, et al., 2012), and wound maturation (remodeling) (Bellayr, et al., 2009; Ravanti, Kähäri, 2000; Xue, et al., 2006). Understanding these variances is essential for developing regenerative capacity in humans (Mu, et al., 2013). The differences will be highlighted here, for a more thorough review of the mechanism for limb regeneration refer to "New Insight into Functional Limb Regeneration: A to Z Approaches" (Taghiyar, et al., 2018)

Wound Closure

Unlike adult anurans and mammals (postnatal) who form scar tissue during wound healing, urodeles, embryonic and fetal anurans, and neonatal mammals do not (Dang, et al., 2003). One difference is the length of time it takes for wound closure in each of these groups. In adult mammals, epidermal closure takes between 8- and 12-days post amputation (DPA) (Reginelli, et al., 1995; Simkin, et al., 2013). Embryonic mammals, however, take much shorter time. A study found it takes only up to 24 hours for an embryonic mouse (McCluskey, Martin, 1995). In addition, urodeles take only between 10 and 24 hours (Campbell, Crews, 2007; Murawala, et al., 2012) and a study conducted on early post-metamorphic anurans found the wound closure time to be between 2 and 3 days (Goode, 1967). Last, a study on embryonic tadpoles (which are anurans) found wound closure took only 30 mins though the paper does admit that this rate seems "extremely high" (Yoshii, et al., 2005). It logically follows that scar tissue formation is related that the length of time required for complete wound closure (Manuel, Gawronska-Kozak, 2006; Wilgus, 2007). Though it seems the differences between those with scar formation and those without must manifest sometime after 24 hours, this is not the case. The first 24 hours of each of the groups are not the same. In fact, in (adult) mammals, it can take more than 24 hours for the first migration of epithelia to begin to cover the wound. There seems to be a fundamental difference in the wound healing process that must be explored..

Immune System

One such fundamental difference seems to be the immune system. Urodeles have a weak immune system compared to anurans and mammals (G. Chen, Robert, 2011; Kaufman, et al., 1995) who seem to share the same immune system in terms of complexity, specificity, and memory (du Pasquier, et al., 1989; Mescher, Neff, 2005). This isn't the only place where anurans and mammals are grouped together. Interestingly, the changes that occur in mammals as they mature and shift from fetal scar-free repair to adult scar-based repair have a close resemblance to the changes that occur in anurans as they begin metamorphosize and lose their regenerative ability and in both cases immune signaling has been identified as a key regulator. In mammals specifically, scar-free healing is associated with an immature immune system. (Kishi, et al., 2012; Wilgus, 2007). Also, note that although most of what we know about urodeles immunity has been obtained from studies on axolotls specifically, it appears it can be inferred to the many different species and genre of salamanders (Cohen, 1971).

Past research indicates that there is a correlation between the level of maturity of an immune system and the regenerative capacity. As an anuran undergoes metamorphosis (a developmental period referred to as "refractory period"), we find an inverse relationship between the maturation of their immune system and the loss of scarless healing (Bertolotti, et al., 2013; Godwin, Brockes, 2006; Mescher, Neff, 2005). In fact, the suppression of the more potent immune response that develops during the refractory period restores a metamorphosizing anuran's regenerative ability (Fukazawa, et al., 2009). This is backed up by another study that found that the decrease in regenerative capacity that an anuran experiences as it matures is negatively correlated with the intensity of the inflammatory response as well as structural modifications in the thymus (Franchini, Bertolotti, 2012).

Comparison of Systems

The immune system of adult anurans and mammals is intricate with a wide range of adaptive immune responses in addition to a complete innate immune response. By comparison, urodeles are considered immunodeficient relative to adult anurans and mammals though they have a strong innate immune system (G. Chen, Robert, 2011). This is because, despite their reasonable B-cell and sizable T-cell reserves, their humoral response is extremely slow (60 days), not able to facilitate anamnestic responses, and only has one unique IgM class (Kaufman, et al., 1995; Tournefier, et al., 1998). In addition, immunization with soluble antigens gives negative results and its B cells are not triggered by T-helper cells; in fact, thymectomy, X-ray irradiation or corticosteroid treatment has shown to improve the humoral response (Charlemagne, 1979; 1981; Tournefier, 1982).

A urodeles cytotoxic immune response is very slow as well (21 days) and shows weak mixed lymphocyte reactions (MLRs) (Kaufman, et al., 1990; Koniski, Cohen, 1992) causing there to be no acute xenograft rejection reactions. However, since they have reasonable B-cell and sizable T-cell reserves they have a large diversity of B and T cell antigen receptors which, over time, causes rejection to ultimately occur. Therefore, xenograft rejection appears to be dependent on the thymus (Tournefier, et al., 1998). Due to the weak adaptive immune response of urodeles they are extremely susceptible to viral infections relative to anurans. Although they display a complex immune response, they fail to generate adequate T cell proliferation in the spleen early on. By comparison, anurans are able to generate adequate T cell proliferation in the spleen early on and therefore are capable to clear viral infections (Cotter, et al., 2008).

There are further differences, specifically in terms of innate vs. adaptive immunities (Godwin, Rosenthal, 2014) that are beyond the scope of this paper. However, clearly there are differences that seem to correlate with the ability for regeneration. The specific aspect(s) of the immune system that is/are responsible is still not known, but it appears that the more sophisticated the immune system is, the less of a regenerative ability there is. Perhaps this is the way species have evolved; prioritizing survival (by prevention of infection) over function and aesthetics of damaged organs and limbs. However, now that we have antibiotics, perhaps both can be achieved.

Wound Maturation

Analogous to the cytoskeleton in cells the extracellular matrix (ECM) allows for individual cells to come together and create tissues and eventually organs by providing a non-cellular structural platform upon which the cells adhere to. It is made up of macromolecular network composed of collagens, proteoglycans/glycosaminoglycans, elastin, fibronectin, laminins, and several other glycoproteins (Bonnans, et al., 2014; Michel, et al., 2010; Theocharis, et al., 2016). For organ or limb regeneration to occur, the proper ECM form must be created for the cells to have a place to go. In addition, the interaction between the cells and the ECM allows for the control of growth by providing negative feedback when a sufficient number of cells have been produced preventing an overgrowth.

Matrix Metalloproteinase (MMP)

During regeneration, for the proper structure to be formed the use of matrix metalloproteinases (MMPs) are employed. The main function of these molecules is to degrade the matrix strategically and help sculp the proper structure needed. There are other functions that have been discovered but they are beyond the scope of this paper. To keep things in control, tissue inhibitors of metalloproteinases (TIMPs) keep the protease activity of MMPs in check and therefore are the regulators of wound closure, tissue regeneration and scar formation (Mu, et al., 2013).

In adult mammals a severe inflammatory response and high fibroblast activity results in collagen fiber accumulation between the epidermal layer and the transected bone (Satoh, et al., 2012; Seifert, et al., 2012). The collagen deposition hinders a reciprocal interaction between the surface layer and most underlying mesenchymal tissues preventing normal skin restoration and causing scar formation to occur (Satoh, et al., 2008). The discrepancy between scar formation and epimorphic regeneration is most probably attributed to the histolysis phase of regeneration in which MMPs are absent for ECM remodeling (W. Chen, et al., 2007).

In urodeles, pre-morphologic anurans, and fetal mammals, higher ratios of MMPs/TIMP have been observed relative to those who do not have a regenerative capacity (Parks, 1999; Ravanti, Kähäri, 2000). Studies have shown that MMP1 specifically has a beneficial impact on muscle healing (note: these studies were completed on those that do not have a natural regenerative capacity) (Bedair, et al., 2007; X. Chen, Li, 2009; Kaar, et al., 2008; Wang, et al., 2009). In fetal mice, who are not able to regenerate complete limbs, introduction of MMP1 has been able to cause complete regeneration (Muneoka, et al., 2008). There have been many additional benefits observed when the use of exogenous MMP1 has been employed. Building on the results of the study by Chen et. al. (2007) stated above, results of a study from 2013 showed that in adult mice who underwent MMP1 treatment achieve an increase in the formation of capillary blood vessels, peripheral nerve fibers and neuromuscular junctions, as well as a decrease in the formation of fibrotic scar tissues in the amputated digits. However, the healing of skeletal tissue and digit elongation was not significantly improved (Mu, et al., 2013). A possible solution may be stem cells which are able to form the segmented pattern of bone and cartilage crucial needed for regeneration (more on this later on) (Masaki, Ide, 2007).

Additional Hypotheses

Land vs. Sea

An anuran loses its regenerative ability as it transitions from living in the water to living, at least partially, on land. As noted above the changes to the immune system of anurans as they metamorphosize and transition from water to land leave it with a much more powerful weapon, which makes sense as terrestrial conditions require a more effective immune system (Alibardi, 2018). However, a study on Xenopus laevis (anuran) embryos found that the rate of wound closure tends to decrease as the osmotic pressure approaches isotonicity (Yoshii, et al., 2005). The authors suggest that perhaps the extremely rapid rate of wound closure is a result of the stimulation of the osmotic pressure regulation system, something which amniote embryos do not require and therefore why they have a much slower rate of wound closure. This begs the question; why do urodeles have an increased wound closure rate if they are terrestrial organisms? While it may be true that osmotic pressure can affect an organism's ability to regenerate, it is unlikely for urodeles and pre-metamorphic anurans to have developed two completely distinct methods of regeneration, especially since anurans and

urodeles are part of the monophyletic Lissamphibians. In addition, of the three orders included, anurans and urodeles are more closely related to each other than to the third order (the legless caecilians) (Elinson, del Pino, 2012). More likely, once an organism has the ability to regenerate, the increased wound closure rate due to osmotic pressure helps speed up the process. However, research into this is unnecessary since we are looking for a way to replicate the mechanism of regeneration in humans and since humans do not have an osmotic pressure regulation system, increasing osmotic pressure to induce regeneration would be futile. Though, perhaps once we are able to regenerate maybe we can use the principles of osmotic pressure to help speed up the process without compromising on the regenerative ability.

Intrinsic and Extrinsic Cellular Properties

If there are fundamental cellular differences between those with the ability to regenerate and those without, then the potential for humans to achieve this capacity will be considerably more challenging. In that case it would require edits to the genetic code which we are a long way from being able to do at this point. Fortunately, although some evidence indicates that regenerative capacity stems from intrinsic cellular properties, it is not much. For example, transplantation of limb blastemas from post-metamorphic (regeneration incompetent) to larval (regeneration competent) stages failed to regenerate despite the conducive host environment. This seems to indicate that there may be an intrinsic property of those post-metamorphic cells that prevents regeneration from occurring (Sessions, Bryant, 1988). However, this conclusion is only theoretical and evidence for manipulation of extrinsic (specifically immunological) properties have yielded some interesting results in mammals (Leavitt, et al., 2016; Satish, Kathju, 2010). Genetic deletion of the anti-inflammatory cytokine IL-10 induces scar tissue formation in fetal mice (recall embryonic mammals heal scarlessly) (Liechty, et al., 2000). Though, in all fairness, these results were achieved by genetic manipulation. In order to be certain that there is no intrinsic cellular property that is responsible for regeneration, this study must be performed without any manipulation of any intrinsic cellular properties (i.e. no genetic deletion). For all we know, the deletion IL-10 caused some other effect, and which may very well be the actual reason for the results that were achieved.

Treatment Proposals

With the above in mind we will now explore some of the therapeutic approaches that been hypothesized to enable humans to be able to regenerate organs and limbs. These hypotheses include modulating the host environment, manipulation of the host immune system, and gene manipulation. However, we must keep in mind that the best possible solution might be to combine multiple approaches simultaneously. We will try to determine which discoveries discussed above form the basis for the proposed treatments to build upon.

Cell-Based Approaches

Many of the concepts discussed above describe differences that develop because species are inherently different down to the cellular level. This is true for all living things on an individual level as well. For example, the liver contains two types of epithelial cells named hepatocytes and cholangiocytes. However, both those cells originate from a single cell type called hepatoblasts (which are fetal liver stem/progenitor cells) during development (Oertel, et al., 2003; Tanimizu, et al., 2003). To overcome the lack of regenerative capacity of differentiated tissues, many have hypothesized harnessing the power of stem cells. Since stem cells are inherently able to develop into multiple cell types, we can theoretically achieve an environment that is similar to a fetus (recall that mammal fetuses do possess an ability to regenerate to some extent). However, due to ethical concerns related to the use of embryonic stem (ES) cells as well as the desire to move away from the use of immunosuppressant drugs commonly used nowadays post allogeneic or even xenogeneic transplantations due to rejection, many have turned to the use of the patients own cells and then "reprogramming" them into a stem cell that behaves like an embryonic one. ES cells are pluripotent, meaning they can form tissues from all three primary germ layers (ectoderm, endoderm, and mesoderm). Somatic cells that have been reprogrammed to behave like ES cells are called induced pluripotent (iPS) cells. This is achieved through a number of techniques which artificially turn on expression of specific pluripotency genes (Hackett, Fortier, 2011). Last year, a study to trace the origin of adult intestinal stem cells provided a direct link between the observed plasticity and cellular reprogramming of differentiating cells in adult tissues following damage (Tetteh, et al., 2016; van Es, et al., 2012; Buczacki, et al., 2013; Yui, et al., 2018; Nusse, et al., 2018). This indicates that stem-cell identity is an induced rather than a hard-wired property (Guiu, et al., 2019).

Amongst the various candidates for reprogramming, mesenchymal stem (MS) cells are of central importance for several reasons. MS cells are found in a majority of adult tissues including, bone marrow, adipose, cartilage, and dental pulp (Eslaminejad, et al., 2006; Karamzadeh, et

al., 2012; Zomorodian, Baghaban Eslaminejad, 2012). The benefit of being able to use cells that are derived from a patient's own body is that transplantation will not elicit a host immune response. In addition, several studies suggest that MS cells induce immunomodulatory effects, which suggests that even allogeneic transplantation of these cells would not trigger the host immune response (Shi, et al., 2011).

In the past MSC transplantation has been performed for different diseased tissues (Emadedin, et al., 2012; R. Fekrazad, et al., 2015; Reza Fekrazad, et al., 2016). However, in 2007 Masaki et al. experimented with neonatal mice and compared the application of bone marrow mesenchymal stem cells (BM-MSCs) and limb bud transplantation into amputated limbs. They demonstrated that both BM-MSCs and limb bud transplantation form the segmented pattern of bone and cartilage which is crucial to regeneration (Masaki, Ide, 2007).

One issue with the stem cell approach is that stem cells lack the positional information needed for regeneration to occur. It doesn't help to just have the cells grow; they need to know where to grow. Without going into too much detail, there have been a few studies that attempted to help guide the stem cells to where they belong through introducing different factors into the mix. One such study showed they can induce the formation of multi-digit frog limbs in post-metamorphic specimens (Lin, et al., 2013). Another study attempted to replicate the in-vivo niche of a multifaceted limb through genetic modification of BM-MSCs to produce blastemal-like cells. After injecting the blastemal-like cells they noticed the presence of digit patterning and they achieved complete regeneration of an amputated digit tip (Taghiyar, et al., 2017). According to this author that cell sources with BC qualities are able to provide a the highly complex signals required for regeneration (Taghiyar, et al., 2018).

Immune-Based Approach

Immune cells such as monocytes and tissue resident macrophages seem to be an important element in the regulation of tissue repair, regeneration, and fibrosis. Post injury, these cells begin to function significantly different. They begin to produce inflammatory mediators and growth factors that enable the regeneration process (Taghiyar, et al., 2018). In 2013 a study showed the important part that macrophages play in the successful development of new limbs in amphibians (Godwin, et al., 2013).

Although we noted above that it seems the more sophisticated the immune system is the less of a regenerative ability there will be, there still seems to be a role for at least some immune cells to play. Systemic macrophage depletion has been shown to prevent limb regeneration in axolotls during the first 24 hours after amputation. A study found that the depletion of macrophages caused an increase in inflammatory factors and a decrease in anti-inflammatory cytokines. In addition, certain growth factor levels decreased significantly causing dedifferentiation markers to become dysregulated and disrupted blastema formation (Godwin, Rosenthal, 2014). Another study found that a wide arity of proinflammatory and anti-inflammatory chemokines and cytokines can be found immediately after an injury in axolotl limb tissue (Godwin, Rosenthal, 2014). In addition, similar to mammalian wounds, various leukocytes travel to the site of an injury and many of them persist throughout the beginning of blastema formation. Despite this we do know that a severe inflammatory response promotes fibrosis and therefore obstructs the successful patterning needed to regenerate a new organ (Eming, et al., 2009) so it seems a proper balance needs to be achieved.

While immune-based approaches will not be able to induce a regenerative capacity in humans they may help improve other approaches when used simultaneously. To achieve the proper balance mentioned above we need to be able to distinguish subpopulations of immune cells. However, due to the lack of reliable markers we have not been able to make good progress thus far.

Genetic-Based Approach

Although there has been a "proof-of-concept" studies that indicates there is a link between genes and cell therapy in terms of regeneration as well as tremendous progress in gene-based strategies, I have not been able to find any clinical trials that have used this approach. The general idea of this approach is to genetically modify specific cells so that the cell can then regulate cell differentiation. It could be the reason why we have not seen any clinical trial is because of the lack of safe and efficient methods of doing so. On one hand, viral vectors achieve high transfection rate, but they have significant safety concerns. On the other hand, although non-viral vectors are relatively safe, they do not achieve efficient transfection rates (Taghiyar, et al., 2018). However, as new technological innovations, specifically CRISPR/Cas9, begin to show promise they may allow for clinical trials to begin. A review of gene-based therapies noted the opportunities that CRISPR/Cas9 holds because of its widely acclaimed abilities and relative ease of use (Janssen, et al., 2016). Today the vast majority of CRISPR/Cas9's use case has been in basic research (i.e. knockout mice) but that is slowly starting to change.

Conclusion

It seems probable that the future will see regeneration of complete organs and limbs. Perhaps it will be in vitro at first but eventually in vivo as well. New breakthroughs have been able to apply many of the techniques employed by species that have a natural ability to regenerate to ourselves. Whether it is the biodome or the 3D printing or tissue engineering, it seems that whatever final solution we come up with will require us to employ multiple tactics. It also seems that different organs and limbs will require different approaches a well.

Acknowledgements

Thank you do Dr. Robert S. Bressler, Ph. D., Professor and Undergraduate Chairman of Biology at Touro College for his input throughout this project. Thank you to Dr. Robert P. Goode for his insight as well.

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Treating Carcinomas through Integrin α6β4 Modification and Inhibition

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Abstract

Integrin α6β4 is a membrane protein which is expressed in both normal and cancerous epithelia. In normal cells, it is involved in adhesion to the basal membrane. In carcinomas, however, it takes on cell signaling roles related to survival and migration. Due to its key role in cancer progression, it is now being considered as a therapeutic target. This paper examines how we can affect integrins, with a specific focus on integrin α6β4, to reinforce their ability to prevent migration of cancer cells and progress toward metastasis.

Methods

Data was collected using ProQuest, PubMed databases, and the National Library of Medicine. The images and diagrams can be found in the research articles referenced.

Introduction

Integrin α6β4 is a membrane protein found on the basal side of epithelial cells. It is a member of the integrin family of proteins. Integrins are heterodimers which are imbedded in cell membranes and signal allosterically; ligand binding to extracellular binding sites causes conformational changes in the integrin which ultimately affect their cytoplasmic domains. These domains link to the cell's cytoskeleton and thereby perform signal transduction. As much as their signaling works outward-inward, their signaling also works inward-outward. Integrins' position in the cell makes them a key component in signaling, cell migration, and cell adhesions. Each integrin has a distinct, specific function, and, therefore, they have diverse ligands. Many integrins, including α6β4, can be turned on or off and need to be activated by tyrosine kinases or growth factors in order to bind to their ligands.

Integrin α6β4 is different from other integrins because it has a unique β subunit, with an atypically long cytoplasmic domain which connects to the keratin intermediate filament system, as opposed to the microfilament system. The α subunit binds to its ligand, laminin 332, in the extracellular matrix. Through mediating the interactions with laminin and keratin, integrin α6β4 is the core component of the hemidesmosome, a protein complex which attaches basal epithelial cells to the basement membrane.

Normally, integrin α6β4 is involved in hemidesmosomes and contributes to tissue stability. Cancerous cells, however, lack hemidesmosomes and integrin α6β4 is localized at the leading edges of metastasized carcinoma cells. Integrin α6β4 interacts with many cancer-related growth factors such as epidermal growth factor receptor (EGFR). Significantly, integrin α6β4 activates phosphoinositide 3-OH kinase (PI3K), a key signaling molecule for carcinoma invasions.

Because of integrins' unique positioning in the cell and in cell signaling, they are highly involved in development of cancers. At times, integrins can inhibit pathways, and at times they can activate pathways instead. Therefore, integrins should be considered as a possible target for treating cancers. Integrin α6β4 can be considered as a model, as it attaches cells to the basement membrane and can contribute to invasion and metastasis of carcinomas. However, before examining cancerous cells, it is necessary to understand the behavior of integrins in normal epithelial tissue.

Integrin α6β4 in Regular Cells

In healthy epithelial cells, integrin α6β4 occupies a central place in the hemidesmosome, a protein complex which connects epithelial cells to the basement membrane. There are two kinds of hemidesmosomes: type 1, which is found in stratified and pseudostratified epithelia (such as in the epidermis) and type 2, which is found in simple epithelia (such as in the intestine). In the more complex type 1 hemidesmosomes, integrin α6β4 facilitates the interactions with most of the proteins involved, including the intracellular proteins plectin and BP180, the transmembrane proteins BP230 and tetraspanin CD151, and the extracellular protein laminin 332, which is part of the basal lamina (Walko et al, 2015). In the simpler type 2 hemidesmosomes, the only components are plectin, laminin, and integrin α6β4, which mediates the interactions between the other two. Integrin α6β4's presence even in these simplified hemidesmosome proves that it is essential for the adhesion of epithelial cells to the basement membrane below (de Pereda et al, 2009). Additionally, mice missing β4 exhibited pathology similar to junctional epidermolysis bullosa, a human disease characterized by fragile skin that blisters quickly due to the basal layer's separation from the basement membrane. The epithelial tissues of the mice, both simple and stratified, lacked hemidesmosomes and did not adhere normally (Dowling et al, 1996).

The α6 subunit is homologous to other integrin α subunits. α6, like many other integrin protomers, associates with more than one β subunit. For instance, α6β1 is an integrin found in the retina and cortex, and is linked to disorders of those tissues (Hynes, 2002). When it is found in epithelial cells, however, it is exclusively associated with β4 and binds to laminin as its ligand. α6 is made up of

1,055 amino acids, with the first 1,011 outside the cell, 36 in the transmembrane region, and 19 in the cytoplasm. (Tamura et al, 1990). The amino domain has 7 homologous repeats, which were found to make up a 7-bladed β propeller (Kamata et al, 2001). It is made up of two polypeptides, a heavy chain and a light chain, which originated from one polypeptide but which were cleaved using post-translational modification. They are joined by a disulfide bridge, similar to α5, αv, α11b, and αP52. However, it does not contain the insert domain near the amino terminus that other α subunits possess (Tamura et al, 1990).

β4 has 710 amino acids in its extracellular region, with cysteine conservation of that region the same as that of other β subunits. It has a 23 amino acids in its transmembrane region and has 1089 amino acids in its cytoplasmic region (Tamura et al, 1990). The cytoplasmic domain is made up of 5 globular domains. From the membrane toward the inside of the cell, they are: a Na+- Ca+ Calx-β exchange motif, a pair of fibronectin type III domains (FnIII-1,2), and another pair of fibronectin domains (FnIII-3,4). Between the two pairs of FnIII domains, there is a flexible connecting segment (CS), and after the second set, there is a c-terminal tail (Walko et al, 2015). There are 5 possible N-glycosylation sites, all of which are glycosylated (Tamura et al, 1990).

Over time, a clearer picture of α6β4 has been formed. Scanning electron microscopy revealed that both subunits have a large globular head and a stalk portion which then traverses the cell membrane. The two protomers complex by binding their heads together (Luo, 2007). α6's amino domain contains a 7-bladed β propeller domain, which is made up of 7 four-strand β sheets similar to those found in other integrins, which is topped by a ligand binding site. However, it lacks the "insert" domain, otherwise known as a Von Willebrand Factor A domain, which has a MIDAS (a cation coordination site), that is found imbedded in the propeller of the majority of integrins (Kamata et al, 2001). This makes sense because only integrins that are exclusive to chordates have this domain, and α6β4 is homologous to that of more primitive species. When an integrin has this insert domain, cation coordination to the MIDAS site causes a conformational change in the protein, pulling part of the α6 head further down and allowing the ligand to bind to its binding site.

Ligand binding occurs differently in integrins lacking this insert domain like α6β4. In this integrin, β4 has its own insert domain with a MIDAS motif, which, when inactive, is in contact with α6's propeller. When the C-terminus of β4 moves downward, or when a cation binds to the MIDAS, it pulls the rest of the protein, exposing more of the propeller surface to allow for a greater affinity for ligand binding (Hynes, 2002). When priming and ligand binding occur, a large-scale conformational change overcomes both subunits. In their resting, low-affinity phase, both subunits' stalks are bent downward in the middle in a genuflected position, with their intracellular domains touching. When ligand binds, the subunits straighten in a switchblade-like motion and the legs separate, exposing binding surfaces. This is one instance of allosteric signaling in integrins (Luo, 2007).

The majority of connections in the hemidesmosome occur with the large intracellular domain of β4. The four FnIII domains have a conserved hydrophobic core, with 9 out of 11 identical residues, but their outer surfaces, which are exposed to solvent, are entirely different, so they can bind to different groups (Alonso-Garcia et al, 2015). The critical interaction within the hemidesmosome is between β4 and plectin. Plectin binds to the IFs in the cell, while α6's interaction with β4 allows it bind to its ligand, laminin. The two proteins join at β4's second Fn-III domain and at plectin's actin-binding domain (ABD). This complex is formed by three sets of ionic interactions: between R1225 of β4 and D151 of plectin, between R1281 of β4 and E95 of plectin, and between E1242 of β4 and R98 of plectin. Missense mutations in one of the residues involved in these salt bridges disturbs the interaction with plectin. The first 7 residues of the connecting segment also play a part in this interaction by extending the binding surface of the Fn-III domain. The first FnIII domain also contributes to the interaction through a hydrophobic pocket made up of I1163, Y1187 and C1190, which interacts with an R-side chain in plectin. When β4 binds to plectin, a conformational change is triggered in β4; in unbound β4, part of the CS is folded over FnIII-2 and engages in antiparallel H-bonding with it. In bound β4, that part of the CS is unbent and may reveal binding sites for other proteins in the hemidesmosome, like BP230 and BP180 (de Pereda et al, 2009).

Integrin α6β4 in Wounded Cells

Although integrin α6β4 is associated with cell adhesion, it is also involved in signaling pathways associated with cell migration, differentiation, and proliferation. These processes assist in healing wounds. This is indicated by its localization to the front edge of cells migrating into a wound. Additional proof can be seen in an experiment involving A549 (adenocarcinomic human alveolar basal epithelial) cells. When the β4 gene was knocked down, the cells migrated less processively than the controls. When the α6 gene was knocked down, the cells migrated both less processively and more slowly. Examination of keratinocytes has revealed that wild-type keratinocytes

move on a straight line along their matrix of laminin, while β4-deficient keratinocytes move in a circular fashion. All of this shows that integrin α6β4 plays an essential role in healing wounds (Colburn, Jones, 2017).

In order for cells to migrate toward a wound bed, the hemidesmosomes need to disassemble, a process that begins from the cytoplasmic domain and then continues in the extracellular domain. β4 serine residues are first phosphorylated by tyrosine kinases to separate β4 from its binding partner, plectin; this is stimulated by epidermal growth factor (EGF) (Mainero et al, 1996), as well as protein kinase C (Rabinovitz, et. al. 1999). After β4's phosphorylation, the rest of the connections in the hemidesmosome are then severed (Walko et al, 2015). At this point, α6β4 detaches from the intermediate filament (keratin) cytoskeleton and instead associates with the actin cytoskeleton. It becomes involved in the formation of lamellae and filopodia, which are protrusions of the cell membrane that give the cell motility.

Epithelial cells involved in wound healing are similar to metastasizing carcinoma cells, allowing comparisons to be drawn between them. In both cases, there is a lack of polarization in the cells and an induction of migration. (Rabinovitz et al, 2015). Importantly, many of the same mechanisms are involved, including the coordination with growth factors, the disassociation from the hemidesmosome, and the formation of lamellae and filopodia.

Integrin α6β4 in Carcinoma

In many types of carcinomas, high levels of integrin α6β4 correlate with the invasiveness of the cancer, as well as the patient prognosis. For example, in basal-type breast cancer, which is a highly aggressive form of breast cancer, a β4 "gene signature" was associated with decreased time to tumor recurrance and a smaller chance of patient survival (Lu, 2008). Other research shows similar correlation with elevated β4 expression in other invasive phenotypes of squamous cell carcinomas, including some kinds of bladder (Grossman et al, 2000), cervical (Jeffers et al, 1997), head and neck (Kurokawa et al, 2008), lung (Stewart et al, 2016), and pancreatic (Cruz-Montseratte et al, 2007) carcinomas. Interestingly, although thyroid cells do not normally express α6β4, it has been found in thyroid carcinomas (Kitajiri et al, 2002). In addition to overexpression of α6β4, it is also located throughout multiple layers of cells in tumors, as opposed to its normal concentration of the basal side of the epithelial cells (van Waes et al, 1995).

However, in some varieties of cancer, integrin α6β4 expression is correlated with tumor suppression. For example, in prostate carcinomas, α6β4 is downregulated (Nagle et al, 1995). Also, when α6β4 was added to cells from the RKO cell line, a colon carcinoma cell line which is missing β4, the result was partial G1 arrest and apoptosis (Clarke et al, 1995). Because this integrin is associated with both growth and migration, but with suppression as well, it may be hard to target as a treatment for cancer. In order to examine treatment options, an in-depth look at the mechanism of action of integrin-implicated signalling pathways must take place.

Integrin α6β4 Signalling Mechanism

When integrin α6β4 is released from hemidesmosomes, it leads to altered signals promoting tumor cell growth, invasion and metastasis (Lipscomb, Mercurio, 2005). Once free from the hemidesmosome interaction, integrin α6β4 can bind or cooperate with many different molecules or growth factor receptors to activate various cell signalling pathways. Integrin α6β4 can bind directly to its regular ligand, laminin, activating both phosphatidylinositol 3-kinases (PI3K) and RhoA small GTPases. Alternatively, it can cooperate with various growth factor receptors, including those in the EGFR (epidermal growth factor receptor) family, such as ErbB-1,2, and 3 (Guo et al, 2006), as well as c-Met (Chung et al, 2004), Ron, LPA1 and LPA2 (O'Connor et al, 2012). This leads to intensification of signaling through PI3K, AKT, MAPK and the Rho small GTPases, as depicted in Figure 1. Integrin α6β4 can also promote tumor progression through transcriptional regulation. It has been shown to increase the expression of invasive and metastatic proteins such as the epithelial to mesenchymal transition (EMT)-associated protein S100A4 (Stewart, O'Connor, 2015).

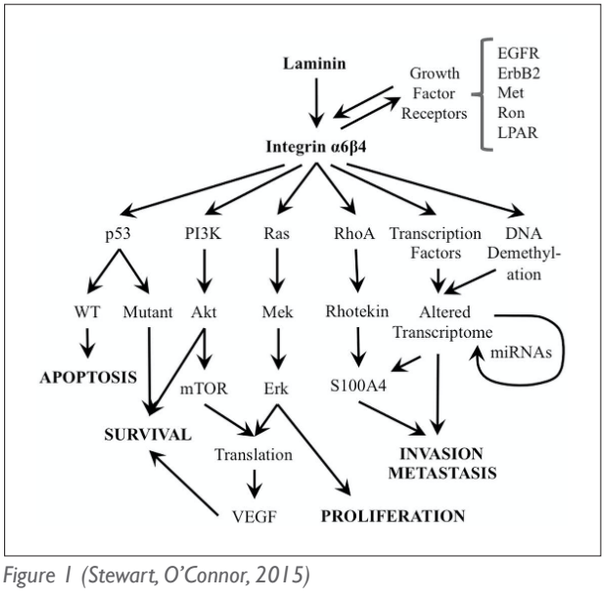

Figure 1 (Stewart, O'Connor, 2015)

Integrin α6β4 is involved in the PI3K pathway, a signaling pathway known for promoting carcinoma progression (Shaw et al, 1997). However, the cytoplasmic domain of the integrin β4 subunit does not have a consensus-binding motif for the regulatory subunit of PI3K , so direct activation of the pathway does not seem possible. One possible mechanism for integrin β4-mediated activation of PI3K involves insulin receptor substrate-1 and –2 (IRS-1 and IRS-2), which act as signaling intermediates facilitating integrin α6β4-mediated PI3K activation. Ligation of integrin α6β4 promotes phosphorylation of IRS-1 and IRS-2, and then activation of PI3K (Shaw, 2001). Additionally, integrin α6β4 cooperates with ErbB-2 to promote PI3K-dependent invasion (Gambaletta et al, 2000). Integrin α6β4 has also been shown to localize to lipid rafts in the membrane, allowing it to recruit other receptor tyrosine kinases, and thus allowing it to activate PI3K (Gagnoux-Palacios et al, 2003).

ErbB-2 is one of the members of the EFGR family which signals for invasion and aggression in breast carcinoma. Integrin α6β4 has been shown to associate with ErbB-2 in multiple breast carcinoma cell lines (Falcioni et al, 1997). Although ErbB-2 is implicated upstream of PI3K, ErbB-2 does not have a binding site for the regulatory subunit of PI3K. ErbB-2, as a receptor tyrosine kinase, must dimerize with another receptor to function. In this case, it dimerizes with an ErbB-3, a different EGFR receptor. Erb-B3 has a binding site for PI3K's regulatory subunit, and this heterodimer is a strong activator of PI3K. There is a positive association between integrin α6β4 and ErbB-3 expression in patient-derived tumors (Folgiero et al, 2008).

Other receptor tyrosine kinases that cooperate with integrin α6β4 include c-Met, which is activated by HGF (hepatocyte growth factor); a complex of α6β4 and c-Met was shown to promote HGF dependent invasion (Trusolino et al, 2001). Ron, a tyrosine kinase receptor closely related to c-Met, has been shown to form a complex with integrin α6β4 that induces hemidesmosome disassembly and the relocation of integrin α6β4 to motility structures. Ron activation is important in pancreatic carcinoma progression, and it interacts with the integrin β4 subunit in this setting to disrupt the association between integrin β4 and plectin (Yu et al, 2012).

Integrin α6β4 also upregulates the Rho family of small GTPases. The Rho family of small GTPases control the reorganization of the actin cytoskeleton required for cell motility (Machacek, et. al. 2009). The activation of RhoA leads to lamellae formation, as well as the generation of contraction forces that enable cell migration (O'Connor et al, 2000). Significantly, RhoA generally controls the generation of stress fibers rather than lamellae formation, suggesting that integrin α6β4 changes RhoA's function to facilitate tumor invasion (Stewart, O'Connor, 2015).

Integrin α6β4 can also stimulate angiogenesis by enhancing signaling through the protein kinase ERK and transcription factor NF-κB. Studies using knockout mice carrying a deletion in the signaling domain of the integrin β4 subunit displayed reduced angiogenesis in a retinal neovascularization model, and developed smaller and less vascularized tumors after subcutaneous implantation. The same study demonstrated that the integrin β4 subunit could promote both bFGF (basic fibroblast growth factor)- and VEGF (vascular endothelial growth factor)-induced angiogenesis by enhancing signaling through ERK and NF-κB (Nikolopoulos et al, 2004).

Cell Survival and Tumor Suppression

Returning to the paradox above, integrin α6β4 promotes either cell survival or apoptosis, depending on context. For instance, in normal epithelial cells, integrins promote survival when in contact with the extracellular matrix. However, once separated, the loss of integrin signaling can inhibit cell growth and promote anoikis, a form of apoptosis (Ruoslahti, Reed, 1994). Similarly, in cancer, at times integrin α6β4 acts as a promoter of cell survival, while at times it acts as a tumor suppressant.

To discover why these two different scenarios exist, a group studied two cell lines. In the RKO cell line, β4 led to increased apoptosis, while in the MDA-MB-435 cell line, β4 did not induce apoptosis. They discovered that the difference lay in the cells' p53, a protein which is often implicated in cancer when mutated. RKO cells have wild-type p53, while MDA-MB-435 cells have mutant p53 (Bachelder et al, 1999). Integrin α6β4 can trigger apoptosis through p53 activation in cells harboring wild-type p53; however, in carcinoma cells deficient in p53, integrin α6β4 promotes cell survival by activating the PI3K pathway and through growth factors such as VEGF (vascular endothelial growth factor). This discovery suggests that tumors expressing high levels of integrin α6β4 in conjunction with mutated p53 are resistant to apoptosis and will therefore display a more aggressive clinical course. Interestingly, an association between p53 mutations and integrin α6β4 overexpression is present in a number of aggressive human malignancies, including basal-like breast cancer, head and neck squamous cell carcinoma, and pancreatic ductal adenocarcinoma (Stewart, O'Connor, 2015).

Treating Carcinomas Through Integrin Modification

Since integrins were discovered as molecules involved in

cancer, there has been a move to target them in cancer treatment. However, drugs developed to impact integrins have largely been unsuccessful. It's possible that it is because the integrin-targeting drugs may only have moderate affinity in vivo for integrin receptors, as shown by the doses used in clinical trials. Another reason may be that integrin antagonists work best when inhibiting early metastatic spread and not in advanced cancer stages, when cancer cells are already widespread (Paolillo, Schinelli, 2017)

Another issue may be that the wrong integrins have been targeted. Not all integrins have been thoroughly explored as therapeutic targets, including integrin α6β4. That said, a common issue for all the integrins is that cells express multiple integrins at the same time, and if one would be targeted in treatment, the others might compensate for it. That explains why the drugs given were well tolerated with few ill effects, but also did not impact the cancer. The solution would be to try and impact multiple integrins at once, but that might be difficult to achieve in a patient (Alday-Parejo et al, 2019).

The issue may also not be the stage or affinity, but the drug target itself. Most drugs designed for integrins target the integrin-ligand interaction; in this case, they would try to competitively inhibit the interaction between the integrin's extracellular side and laminin 332. This could be a problem because integrins signal allosterically, so if an inhibitor would bind to the integrin's ligand binding pocket, it could actually turn on the integrin and the signalling cascades could begin. The cytoplasmic domain may be a better option (Alday-Parejo et al, 2019).

Thus far, one group has worked on an inhibitor targeting the cytoplasmic side of integrin α6β4. They found that curcumin, a yellow pigment derived from turmeric, inhibited the interaction between the cytoplasmic tail and EGFR, which is implicated in the PI3K pathway related to survival, invasion, and metastasis. EGFR is activated when the residues Y1068 and Y1045 are phosphorylated; when α6β4 was knocked out, there was significantly less phosphorylation, showing a connection between the two structures. They added curcumin to other cells and found that it had a similar effect on EGFR phosphorylation as knocking down α6β4. The hemidesmosomes in cells with curcumin remained stable upon stimulation with EGF (Soung, Chung, 2011).

Discussion/Conclusion

Integrin α6β4 plays an essential part in both regular and cancerous cells. Significantly, it contributes to the PI3K pathway, related to cancer aggression and survival. Integrin α6β4 should definitely be studied, both as a diagnostic tool and as a target for therapeutic intervention. As discussed above, it is a predictor of the cancer's severity and can be used in a diagnostic setting. More research should be put into targeting its intracellular interaction with growth factors so that these pathways can be inhibited.

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Uterine Transplantation: A Review of Some of the Factors that Account for the Success or Failure of this Experimental Procedure

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Abstract

Those who suffer from infertility, either due to a congenital or acquired cause and cannot or are opposed to other means, such as surrogacy or adoption, to have a child, are potential candidates for uterine transplantation. Uterine transplantation is a form of allograft transplantation, meaning that it is a transplant of tissue from a donor who is genetically different from the recipient. Additionally, it is a vascularized transplant, including donor blood vessels that will be sutured to the vasculature of the recipient (Testa et al. 2017). This procedure has been attempted around the globe with varying levels of success ranging from delivery of a healthy child to necrosis of the graft and rejection of the transplanted tissue. While there does not seem to be one specific reason that can account for the rejection of a uterus transplant, the main factor responsible for failure is problems with arterial inflow or venous outflow in the recipient. Other potential factors that may contribute include cervix-vaginal size differences between donor uterus and recipient, pathology of the donated uterus, immunosuppressants, and wait time from transplant to embryo transfer. Advancements in robotic assisted hysterectomy to procure the donor uterus, using ovarian veins instead of uterine veins for venous outflow, and deceased donor uteri instead of living donor uteri are all promising ways that can streamline the transplant process and help transition the experimental procedure of uterine transplantation to an accepted clinical one.

Introduction

Uterine transplantation is a procedure where a uterus from either a live or deceased donor is surgically implanted temporarily in a recipient, in order that the recipient can carry her own child in the transplanted uterus. The transplanted uterus is subsequently removed, either when a baby is born, if the pregnancy is terminated due to complications, or due to rejection of the transplant. Uterine Transplantation is an innovative treatment for those who are unable to carry a fetus in their own respective uteri, either due to a congenital disorder, such as the absence of or an underdeveloped uterus, hysterectomy, due to disease, cancer, endometriosis, elective hysterectomy, or for transgender individuals who do not have a uterus (Testa et al., 2017). Since uterine transplantation is not considered a life-saving procedure, many are ethically opposed to the promotion and investment into such an experimental and costly surgery.

In this paper we will try to determine if the uterus can be transplanted successfully with childbearing results, and which factors are responsible for the rejection of a uterus transplant.

Materials and Methods

The Information contained in this review was found by searching the Touro online library general search, Ebsco, and Proquest databases using key terms such as "uterine transplantation." Pubmed and Google Scholar were also searched utilizing similar key words and phrases.

Candidates for Uterine Transplantation

People who express interest in uterus transplants generally suffer from what is known as uterine factor infertility, UFI (Arian et al., 2017), also known as absolute uterine factor infertility, AUI (Branstrom et al., 2020), This can be due to a congenital cause, such as a condition known as Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH), or Mullerian agenesis, which is caused by the "underdevelopment of the Mullerian duct, with resultant agenesis or atresia of the vagina, uterus, or both" (Oelschlager, 2018). Agenesis refers to the failure during embryological development of an organ and atresia is where there is either an obstruction to the lumen, or an opening is abnormally narrowed. Since the Mullerian duct in utero is responsible for the development of the uterus, fallopian tubes, cervix, and the upper part of the vagina, when it fails to develop in MRKH, all those structures, aside from the ovaries, are affected, and this often results in infertility. Patients with MRKH are good candidates for uterine transplantation because they have functioning ovaries, which are not included in the uterus transplant. Their oocytes can be extracted for in vitro fertilization, and they can have a biological child of their own genetics. Another class of those who are interested in uterine transplants include those who have absolute uterine factor infertility due to an acquired cause which resulted in hysterectomy, such as fibroid tumors, endometriosis, chronic pain, abnormal bleeding, malignancy, and obstetric complications. In one study, out of the 239 people screened, one third sought treatment due to congenital UFI, while the other two thirds had acquired UFI. Of the acquired group, half were due to benign conditions, 25.3% were due to gynecologic malignancies, and 24.7% had had prior obstetric complications that resulted in hysterectomy. Although there were five transgender and one intersex individual, they were not included because they did not fit the inclusion criteria of the screening (Arian et al., 2017). In another 2017 study, candidates similarly included those with MRKH, acquired conditions such as irreversible intrauterine adhesions, fibromas that could not be operated

on, and those who had lost their uterus from either benign or malignant pathologies or postpartum complications (Testa et al., 2017).

Donor Eligibility

Different studies had varied qualifications included in their inclusion criteria. Firstly, age was a consideration. In one study, age was determined to be from 40-60, though those under the age of forty were considered if a candidate had successful pregnancies in the past and did not want to have another pregnancy in the future (Testa et al., 2017). Similarly, in other studies, with successful live-birth outcomes, a 32 year old uterus was used, having borne 2 children prior to transplantation (Testa et al., 2018), and a 24 year old nulligravid uterus was also used in successful transplantation (Chmel et al., 2019). However, in the first successful transplant in 2014 that resulted in childbirth, a 62 year old uterus was used, and another a successful live birth resulted from a 63 year-old uterus at the time of birth, indicating that although older uteri are not necessarily considered ideal, they have yielded live births. (Brannstrom et al., 2020). Since often enough recipient's mothers want to be donors for their daughters, in some studies an older upper limit is given at 55-65 years of age (Kisu et al., 2018).

Additional donor requirements in some studies included criteria such as testing negative for gonorrhea, chlamydia, syphilis and HPV, or previous vaccination against HPV. Previous history of HSV-2 was considered if there were no current symptoms, and previous HPV was considered as long as a negative history was shown since the case of HPV. Last, one prior full-term live birth was a qualification (Testa et al., 2017).

Other requirements included normal-shaped uteri, proper perfusion through uterine vasculature, and the absence of uterine damage or disease (Chmel et al., 2019).

Nulliparous, Nulligravid, or Multiparous Donor Uterus

In choosing between a nulligravid uterus, one that has never carried a fetus, a nulliparous uterus, a uterus that has never born offspring, and a multiparous uterus, one that has born offspring, different studies included multiparity in the inclusion criteria while others did not. According to a 2019 study using a nulliparous deceased donor, nulliparity was considered an advantage in terms of graft recovery because the uterine veins and arteries were straight and not varicose, as opposed to what they noted with multiparous donors (Chmel et al., 2019). However, that was an observation made using few subjects, and would need to be further investigated to determine if a significant difference between the straightness of vasculature exists between nulliparous and multiparous uteri.

Live vs. Deceased Donor

Before investigating the differences between a living as opposed to a deceased donor, it is important to define who is considered "deceased." In this paper, deceased is used to mean brain-dead.

There are certain advantages to using a deceased donor over a live donor. Firstly, using a live donor to procure a graft includes certain risks associated with any surgical procedure, such as the use of anesthesia, and surgery, and specifically possible urological, psychological or sexual dysfunction that may result, which are not considerations that need to be taken with a deceased donor. Secondly more radical surgical dissection can be done using a deceased donor than a live donor, which enables surgeons to procure larger vessels, decreasing the risk of graft thrombosis. Additionally, a longer vaginal cuff can be procured from a deceased donor, allowing a better vaginal–vaginal anastomosis between the donated uterus and the recipient's vagina (Chmel et al., 2019).

A clear disadvantage of using a deceased donor is the practicality; the nature by which deceased donor uteri become available is unpredictable. Also, multidisciplinary teams and the recipient must go to the location of the donor. Moreover, in many countries, physicians have less access to deceased vascularized composite allografts, and there is more restricted access to deceased donor uteri due to the complicated nature of surrogate consent if the deceased has not expressly stated the desire to donate before death (Chmel et al., 2019).

However, new innovations in procurement of the donor uterus, such as using robotically-assisted minimally invasive procedures, can likely decrease risks to live donors by minimizing tissue trauma and bleeding compared to open surgery, which would favor using live donor uteri (Brannstrom et al., 2020). In one minimally invasive, robotic surgery, the estimated blood loss of the donor was 400 mL, compared to the researchers previous surgeries using laparotomy in nine cases, which ranged from estimated blood loss values between 300-2400 mL, with an average of 920 mL (Brannstrom et al., 2020). Using robotic technology can also provide more dexterity and seven degrees of freedom for the surgeon, not to mention micro-suturing can be done more efficiently through laparoscopic ports (Wei et al., 2017). Also, minimally invasive robotic procedures can minimize both surgery and recovery time for the donor (Carbonnel et al., 2020). Surgery time in one robotic assisted surgery took 6 hours, which is the shortest surgery duration time recorded for uterus procurement for transplant. That may be partially due to the choice of vasculature that the researchers procured with the graft, but nonetheless they were able to drastically reduce

surgical time (Wei et al., 2017). In addition to a reduction in bleeding and trauma, minimally invasive surgery yields better cosmetic results, which may influence a candidate's readiness to donate (Carbonnel et al., 2020).

Steps involved in Uterine Transplantation

The process of uterine transplantation can be summarized briefly, according to Testa, et al., as follows. First, the successful transplantation of the graft, including perfusion, or blood flow through the grafted vasculature, and the assessment of vital tissue present on the cervix in cervical biopsy to determine if the tissue is accepted by the recipient. Next, a normal menstrual cycle should result, which means that the grafted uterus is responding to the recipient's hormones. Stable immunosuppression, suppression of the recipient's immune system toward the foreign uterus should be maintained. Once immunosuppression is achieved, a fertilized ovum is implanted. After implantation that results in pregnancy, the last step is the successful delivery of a hopefully healthy child. At any of these steps, complications can occur that would result in the failure of the uterine transplant (Testa et al., 2017).

Removing the donor uterus can be done through either laparotomy or laparoscopy. In a 2017 clinical trial on five individuals, the laparotomic removal of the donor uterus is detailed. The hysterectomy performed on the donor, however, is more complicated than a regular hysterectomy, due to the vasculature that needs to be connected to the recipient to allow graft inflow and outflow in the recipient. To allow that, the vascular pedicles, tissues containing the arteries and veins of the donor uterus, were dissected completely. The arterial vascular pedicle of the graft included the uterine artery, which supplies blood flow to the uterus, and a part of the internal iliac artery. The graft uterus was drained by the uterine and utero-ovarian veins, located between the uterus and ovary. A transverse cut was made below the cervix, in order to obtain a cuff that could sufficiently be anastomosed to the upper vagina of the recipient. After removal, arteries were reconstructed, when needed, through microvascular surgery (Testa et al., 2017).

As for the recipient, the external iliac artery and the external iliac vein were dissected and the internal iliac artery patch of the donor was grafted to the external iliac artery of the recipient. For venous outflow, the uterine or utero-ovarian vein, depending on which provided better venous outflow, was sutured to the external iliac vein of the recipient. Once blood flow was reperfused, the cervix of the new uterus was connected to the vaginal vault of the recipient. Blood flow was subsequently monitored by a doppler blood flow monitor to assess arterial inflow to the transplanted uterus. Postoperatively, for the first five days Doppler ultrasounds were performed, cervical biopsy was done on the fifth day, MRI of the uterus was performed to evaluate blood flow, and cervical examination under anesthesia was done to determine if rejection of the transplant tissue occurred. To suppress the immune response of the host to the allograft, immunosuppressive drugs such as thymoglobulin, tacrolimus, mycophenolic acid, and a steroid taper were administered (Testa et al., 2017).

Vasculature Involved

The common iliac artery bifurcates into the internal and external iliac arteries. A branch of the internal iliac artery is the uterine artery, which supplies blood flow to the uterus. Blood flow from the uterus is via the uterine veins, internal iliac veins, inferior vena cava, and to the heart. Figure 1 shows a diagram of the vasculature where blood vessels attached to the allograft are connected slightly differently in the recipient than expected. At point A, the uterine artery, with a branch of the internal iliac from the donor is anastomosed to the external iliac artery, instead of the recipient's own internal iliac artery. At point B, the uterine vein of the donor uterus is attached to the external iliac vein of the recipient, instead of the internal iliac vein.

One of the most difficult parts of uterus transplantation is securing a good venous outflow. In most cases, the uterine veins are used, which often have thin walls, can vary in number, and often are not shown sufficiently in preoperative imaging. During surgery, their dissection can be tedious, can increase the risk of bleeding, and increases surgery time (Testa et al., 2018). Moreover, the uterine vein has multiple branches and is close to the

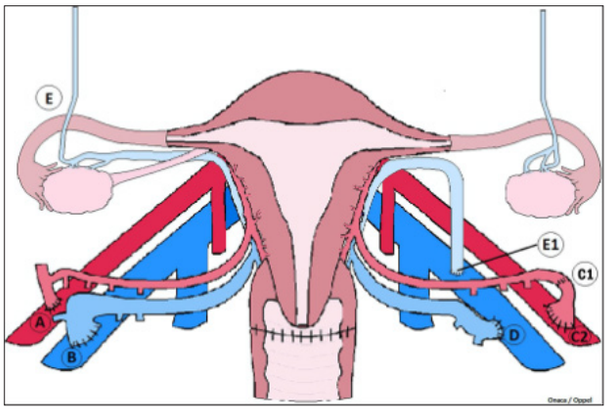

Figure 1. This schematic diagram portrays the blood vessels supplying and draining the allograft uterus. Point A is the anastomosis of the uterine artery to the external iliac artery. Point B is the anastomosis of the donor uterine vein to the external iliac vein (Testa et al., 2017).

ureter, which could result in accidental bleeding or injury to the ureter (Carbonnel et al., 2020). In one 2018 successful trial, researchers sustained an allograft through the utero-ovarian veins alone, without uterine veins. The researchers recommended using the utero-ovarian veins instead of the uterine veins, which are easier to identify, dissect, and provide for safer surgery on the part of the donor. In another clinical trial, the proximal portions of the utero-ovarian veins were dissected as potential extra venous outflow, and ended up being used along with the uterine veins as well, due to the thinness of the uterine veins of the donor (Brannstrom et al., 2020). Further, by using the utero-ovarian veins instead of uterine veins, a robotic laparoscopic approach is more feasible, which will again increase the safety and ease for the donor (Testa et al., 2018). Also, the ovarian veins are more distant from the ureter, so surgeons will not be concerned about inadvertent damage to them (Carbonnel et al., 2020). There can, though, be negative repercussions for the donor associated with using the ovarian veins as venous outflow. In transplant procedures done in both India and China, removal of the ovarian veins required bilateral oophorectomy, removal of the ovaries. The donor women were premenopausal, which increases their risk of morbidity and mortality (Brannstrom et al., 2020). However, using ovarian veins for venous outflow still need to be further researched in the future to determine whether uterus outflow can in all cases be sufficiently maintained using the ovarian veins alone (Carbonnel et al., 2020).

Wait Time Between Transplant and Surgery

Common convention for many of the different uterus transplant trials has been to wait one year between transplantation and attempt at pregnancy, similar to other solid organ transplants. In a 2019 study, for example, embryo transfers were done starting at least 12 months post-transplant, and were done in months 13, 16, 19, and 23, until the last transfer resulted in clinical pregnancy (Chmel et al., 2019). This convention arose out of a recommendation from the American Society of Transplantation which suggested that recipients of organ transplants wait one year between the transplant and conception to decrease any risks that may result, such as acute rejection to the recipient, infection that could harm the fetus, immunotherapy that could potentially be toxic to the fetus, and to be sure that the allograft is functioning properly (Johanesson et al., 2019).

However, women who receive uterus transplants are generally healthy individuals whose surgical recovery should resemble that of any surgical intervention. There is no reason to believe that tissue healing would be impaired, and immunosuppression does not seem to hinder tissue healing (Testa et al., 2018). Further, a uterine transplant, unlike other organ transplants, is not meant to be a long-term functioning transplant. Its longevity is for about five years and for a maximum of two pregnancies (Johanesson et al., 2019). Some suggest that there is no scientific basis for waiting a year between transplant and embryo transfer, especially because immunosuppression must be terminated as soon as possible, to avoid renal failure, which could be a risk of long-term immunosuppression. Decreased time on immunosuppressants also reduces costs and decreases waiting time for the recipient to give birth to her baby. In their clinical trial, embryo transfer was done before the six month post surgery mark, and yielded successful results (Testa et al., 2018).
To provide a groundwork for the amount of time to be waited, researchers laid down conditions to be met in the recipient, which if achieved, should be a signal to begin embryo transfer, as opposed to giving a generalized time recommendation for all uterine transplant procedures. The patient criteria are given in order as follows. First, graft function should be stable. An indication of graft stability would be menstruation, as it is a sign that the foreign uterus is responding to the recipient's hormones. Next, the absence of any acute rejection, which is determined via cervical biopsy. Finally, stable immunosuppression achieved with low teratogenic, or carcinogenic, risk, and the recipient is at low risk for opportunistic infection, which is generally associated with those who undergo transplantation. Once these conditions are met, embryo transfer should begin as soon as possible, even as early as three months post-transplant if the recipient is ready (Johanesson et al., 2019).

Complications in Pregnancy and Post-Operatively

Generally, pregnancies resulting from uterine transplantation do not reach full-term. However, many studies have been successful in which participants have carried close to term. In the first successful clinical study in Sweden, mean delivery for the six patients was at 35 weeks gestation. Besides the difficulty in reaching full term, there are some other pregnancy complications that have arisen. Some patients from the previously mentioned study experienced preeclampsia. It is possible though, that the preeclampsia, preterm delivery, and delivery by cesarean section may be related to the original infertility; MRKH sometimes presents with renal defects, such as having a solitary kidney, which could cause extra stress to the recipient during pregnancy, and has been generally associated with higher risk for preterm delivery, preeclampsia, and cesarean section (Kisu et al., 2018).

Other major complications that have arisen post-operatively in recipients include urinary tract infections, thrombosis, and hematoma. The risk of thrombosis is because the vessels that are anastomosed are generally narrower and can get obstructed more easily than vessels attached in other organ transplants. Hematoma could be the result of inadequate hemostatic processing, or cessation of bleeding. Care must be taken with re-bleeding from capillaries that are unligated or bleeding from the vaginal cuff (Kisu et al., 2018). Also, in one particular clinical study, vaginal stenosis, vesicovaginal fistula, herpes, and cytomegalovirus were complications experienced by some of the recipients. Vesicovaginal fistulae can occur more frequently in patients with MRKH who have a neovagina, which is constructed due to an underdeveloped or absent vagina, and which needs to be separated from the bladder during surgery. This could lead to a fistula, or an opening between the bladder and vagina (Kisu et al., 2018).

Immunosuppressants During Pregnancy

Since the transplanted uterus is from a foreign donor, the recipient needs to be on immunosuppressive drugs to prevent her own immune system from mounting a response against the uterus. There have been different recommendations as to the type of drug, based on trials from both pregnant women on immunosuppressants due to uterus transplants and other organ transplants. The importance here is that the immunosuppressive drug should not be toxic to the developing fetus. Therefore care must be taken to either prescribe immunosuppressants that are not fetotoxic, or at least halt fetotoxic immunosuppressants when the uterus recipient begins embryo transfer. One immunosuppressive drug that has been implicated in spontaneous abortion in the first trimester and congenital abnormalities of the fetus is mycophenolate salts, which come in two different prodrugs, mycophenolate mofetil and mycophenolate sodium, that release the compound mycophenolic acid. The FDA classifies this drug as a Category D drug, meaning that there is evidence that it can cause risk to the fetus during pregnancy (Ponticelli et al., 2018). Therefore, if it is used following the transplant, there must be a waiting period afterward before embryo transplant begins. In one uterine transplant trial, mycophenolate mofetil was taken out entirely from the maintenance immunosuppression to decrease the time exposed to toxic medications for the fetus. They were thus able to expedite the waiting time before embryo transfer. Another drug, thymoglobulin, is considered a category C drug, meaning it is currently unknown if risk exists to the fetus. Therefore, there should also be a wait time between pregnancy and usage of thymoglobulin in immunosuppressive therapy. Other common immunosuppressants such as corticosteroids, azathioprine, and calcineurin inhibitors appear to be safe in doses prescribed for transplant recipients and are not associated with increased risk of congenital defects (Johanesson et al., 2019).

Reasons for Rejection of Transplanted Uterus

Although in some cases there is not a clear reason why the graft failed and rejection occurred, different studies have hypothesized reasons for the difference in outcome. A conclusion was that uterus pathology and venous outflow were largely responsible for three graft failures that occurred. The authors explained that assessment of vasculature preoperatively could be improved. Thus, imaging could be used to eliminate uteri with vasculature that is unsuitable for a uterus transplant. They explain that using a CT angiogram, while helpful in showing arterial blood flow, was not sufficient in their study to tell the length and diameter of veins. This was significant because a large part of the donor hysterectomy surgery time was spent in dissection of the uterine vein, which can taper into thinner tributaries where it joins with the internal iliac vein, even though it may appear satisfactory near the uterine body. The researchers concluded that MRI with venous phase would have been a better choice in imaging to view the uterine veins (Testa et al., 2017). Similarly, the researchers explained that the arterial vasculature was not of the expected quality; in some patients arterial pathology was present. In the preoperative imaging, the vessels appeared patent, however; in the three grafts that failed in their study, there was severe arterial disease present. In one donor specifically there was thickening that resulted in over a fifty percent decrease in luminal size. Additionally, the researchers noted that in the first two of the five patients studied there was a downward kinking of the vein if the anastomosis was to the superior aspect of the host external iliac vein. In the other three patients they switched the venotomy position of the external iliac vein to the medial aspect to avoid kinking. Further, the researchers observed that procuring a patch of the internal iliac with the uterine vein provided a vessel with thicker and stronger walls.

Another potential reason that contributed to the failure of one of the transplants was the variation in size between the large cervix size of the donor and small size of the vaginal vault of the recipient, which made it difficult for vaginal anastomosis. The researchers believed that the limited space, due to the larger cervix size contributed to the graft congestion, poor venous outflow, and pathologies present ultimately resulted in transplant rejection (Testa et al., 2017).

As far as immune-system induced rejection, ostensibly, since the uterus carries a non-self, it would appear that pregnant uterus should have some immune tolerance. However, cervical biopsies have shown rejection in some cases post-operatively. In one transplant study, 13 out of 163 cervical samples taken from seven patients showed mild rejection after transplantation. Steroid therapy helped patients reach remission, yet some developed rejection complications again during pregnancy, which is more difficult to manage and measures need to be developed for the future for treatment-resistant rejection. Some suggest that the contact that the uterus has with the external environment, through the vagina, is responsible for the prospect of rejection of the allograft (Kisu et al., 2018).

Ethical Implications

The practice of uterine transplantation is a subject of great ethical controversy. As a non-life-saving procedure, it differs from other forms of organ transplantation, which are considered essential. However, there are those who categorize it similarly to face, hand, or other vascularized transplants, which though not life-saving transplants, are nonetheless justifiable organ transplants for improving the quality of life of recipients. Additionally, although uterine transplantation is considered an elective treatment, it stands as the only medical treatment for absolute uterine factor infertility. Surrogacy and adoption are options for women with infertility, but for some women they are not adequate substitutes for the experience of pregnancy (Bayefsky and Berkman, 2016).

Once uterine transplantation transitions from an experimental procedure to a common clinical one, there are multiple points that will need to be taken into consideration. There will likely be a shortage of uteri for donation. While there are those who argue that uteri from women who have had hysterectomies should be used to supply organs for uterine transplants, often hysterectomies are done to remove unhealthy uteri, and are done in a way that does not leave the organ fit for transplantation, so those would not be feasible sources of uteri. Uterine transplants must also come with sufficient vasculature, which is generally not removed in an average hysterectomy. Given that there will likely be a greater demand than the supply of uteri, there will need to be a way to prioritize some individuals over others. As far as for other organ transplants, such as heart, kidneys, or lungs, pediatric or younger individuals are generally prioritized over older adults, because adults have experienced childhood, and pediatric patients, if not given the transplant, may never have that experience. In the same vein, it is sensible to say that when prioritizing women for uterine transplants, women who are of childbearing age should be given precedence over both women who are older and beyond reproductive age and adolescents who have not yet reached childbearing age. However, in choosing between younger and older individuals within the span of child-bearing, women who are nearing the end of their childearing years should be given priority over younger individuals so that they can have their chance to have children before they age out of their childbearing years, while the younger individuals still have time. "Normal childbearing age" is considered between the ages of 15-49 according to the World Health Organization, and a standard for recipients should be within those limits. A national standard, in limiting the age of recipients, should reflect medical assessment of the surgical and obstetric risks incurred to women of different ages, the risk to the fetus, and the probability of successful pregnancy.

Further, there must also be some sort of child-rearing capacity standard created that potential candidates will demonstrate before allocating a uterus, similar to how women who wish to adopt must meet certain criteria.

In differentiating between women with uterine infertility, different candidates have different needs with regard to treatment. Some need only a transplant, while others may need IVF, egg donation, or sperm donation. It is reasonable to suggest that those with the least interventions necessary to achieve pregnancy should be given priority, in the same vein as choosing candidates for transplantation who are more likely to have successful transplantation (Bayefsky and Berkman, 2016).

Putting aside the controversy associated with candidates for treatment, there is also dispute about the source for donor uteri. Procuring a uterus from a live donor can incur risk to the individual. While there are innovations to the process of securing a uterus, through laparoscopic robotic procedures, as opposed to open laparotomy, advancements will need to be made to decrease the risks for the donor. Also, the advancement of using cadaveric or nonliving donor uteri will be important in fueling the future of uterine transplantation, though it will be a source of contention as to whether a person who has lost all brain function and is considered "brain dead," will be categorized as deceased along with those whose circulatory and respiratory systems have failed.

Conclusion

Uterine Transplantation is a possible radical and innovative surgical option for those who suffer from absolute uterine factor infertility. Many studies have been done world-wide, which have contributed to the available data on the risks and ways to improve the process. While

there is no one factor that contributes to the rejection or success of a uterine transplant, certain factors have been implicated in the reasons for transplant failure or success. Vasculature problems can account for some reasons for rejection, such as sclerotic vessels, or using thin-walled uterine veins that are difficult to dissect and may not provide adequate venous outflow. Adequate imaging should be done to identify vessel pathology and the acceptability of the donor vasculature. The possibility of using the utero-ovarian veins might be a good alternative to the uterine veins, though it can cause the onset of menopause and may increase morbidity in premenopausal women, due to oophorectomy. Also, mismatch in vaginal-cervix size between the donor and recipient may be problematic. Wait time between transplant and embryo transfer and immunosuppressants are other factors to take into account. Advances in using deceased donor uteri, which allows harvesting longer length of vessels, or using live donor uteri procured through laparoscopic robotic assisted hysterectomy can streamline the process. Although a promising treatment for those with absolute infertility, more research needs to be conducted to ensure its safety and to standardize the procedure.

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What is the Future of Organoids?

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Abstract

The term organoid refers to a miniature version of any organ of the body. An organoid is artificially produced in vitro from stem cells or tissue that possess the ability to recapitulate and form the three-dimensional structure of the organ they were once a part of. Scientists have learned to create a culture for the organoid that mimics its original micro cellular environment, allowing the 3-D structure to self-organize and develop into miniature organs. The purpose of this paper is to explore the many advancements of organoid technology, and how this progress has benefited the medical field. Organoid technology allows for the observation of human organ development and disease, while also providing scientists with the opportunity to test drug interactions with these "mini-organs" (Barbuzano, 2017). Organoids also shed light on the future of artificial transplantation, providing replacements for dysfunctional organs and tissue. The advancements of organoid technology can potentially revolutionize the field of medicine, contributing to the progress of modern biology.

Introduction

The human body is composed of a variety of tissue-specific adult stem cell populations. Adult stem cells of each organ in the body possess the ability to self-renew and generate all the different cell types present in that specific organ. Recent studies allow scientists to apply this knowledge of stem cells by culturing them in vitro. Through growing these cells under the right conditions, the cells can assemble miniature versions of the organs they were once a part of (Drost & Clevers, 2017). Through the development of new 3-D technologies and these ideas, scientists were successful in producing artificial organs like the liver, placenta, stomach, lymph nodes, and brain. (John D. Loike and Robert Pollack, 2019).

These miniaturized models that mimic real organs have been termed 'organoids' (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017). Cells of an organoid can be derived from tissue stem cells, embryonic stem cells or induced pluripotent stem cells (iPSCs). These cells possess the ability to regenerate, allowing the tissue to perform its original function (Prior, Inacio , & Huch, 2019).

The ability to create and develop human tissue in a lab offers a major breakthrough in modern biology. The advancement of organoid formation provides scientists with the ability to study human development and disease and test therapeutic medicine through a personal approach. Organoids also offer hope for transplantation of functioning organs, eliminating the need for organ donations (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017). Organoid research can also provide improvement when it comes to animal experimentation, bioethics, and gene therapy (Xinaris, 2019).

Method

This study was written by researching peer reviewed scholarly articles and medical journals to obtain the most accurate information on the advancements of organoid technology. The data was analyzed and evaluated from many different angles, using Touro College's access to online publications. Additional searches were done through Google Scholar, PubMed, and the Science Journal to accumulate relevant information for the study.

Discussion

In 1998, the first successful organoid culture system derived from adult stem cells was the small intestinal organoids of a mouse. This development in organoid research led to many discoveries, among them the knowledge that Wnt signaling is crucial for maintaining the stem cell compartment of the mouse small intestine. Further observation brought about the awareness that ectopic expression of R-spondin, which communicates with Wnt and acts as a Lgr5 ligand, is necessary to include in a culture of intestinal stem cells as well, for ultimate function of the organoid (Drost & Clevers, 2017).

Following these observations and through his own research, Toshiro Sato of Keio University confirmed that the artificial inclusion of growth factors present in the organ's original stem cell niche is crucial for optimum efficiency and function of the organoid. Sato too researched and studied intestine stem cells. He observed that the 3D organoid forms from single Lgr5-positive Intestine Stem Cells. He retained the architecture of the authentic intestine, and included Wnt/R-spondin, epidermal growth factor (EGF), noggin (BMP inhibitor) and an artificial laminin-rich extracellular matrix in his in vitro culture. (Drost & Clevers, 2017).

In the body, cells are exposed to intricate and complex surroundings where they are involved in many chemical interactions (Prior, Inacio, & Huch, 2019). When creating an organoid derived from adult stem cells in vitro, the stem cell niche of the specific tissue must be duplicated. Common niche factors necessary when dealing with healthy human tissue include, but are not limited to, Wnt, R-spondin, noggin and EGF (Drost & Clevers, 2017). Inclusion of the precise concentrations of growth factors within the culture, and maintaining identical micro-architecture, allows stem cells to mature into organized tissues, healthy or diseased (Dumont, Heremans, Jan et.al. 2019). Incorporating these factors in the culture will ensure natural interactions among stem cells. It is crucial to mimic these interactions when creating in vitro phenotypes to maintain the functions of the cells. This is a major advancement from 2D monolayer technology to 3D technology.

To begin creating an organoid, stem cells must be isolated from embryonic stages or adult tissues and placed into a media with these natural growth factors. Development begins from a totipotent cell undergoing rapid cell division. The divided cells become more restricted as they evolve. At the blastocyst stage, the outer cells mature into extraembryonic cells, while the inner cell mass of the blastocyst (ICM) is composed of pluripotent cells which possess the ability to evolve into tissue of the embryo proper. These pluripotent cells are isolated to form the embryonic stem cells. The next step in the developmental process is gastrulation. During this period, cells of the ICM mix together and perform morphogenetic movements that are controlled by signaling factors like Wnt, fibroblast growth factor (FGF) and transforming growth factor-beta ligands. These signaling factors activate transcription and differentiation into the three germ layers: endoderm, mesoderm and ectoderm. After forming the three germ layers, the progenitor cells become more specific and form the primitive structures of organs, thus developing into any tissues and organs of the body (Prior, Inacio, & Huch, 2019).

Transplantation and Xenotransplantation

The development of organ transplantation (OT) is "one of the most successful advances in modern medicine." For struggling patients with end stage disease, transplantation is usually their last hope to survive. One major problem we face today regarding OT is the lack of organ availability. We are unable to meet the exceptionally high organ demand (Bezinover & Saner, 2019). Arthur Caplan of NYU Langone Medical Center reports that the space between supply and demand of transplanted organs is "worse than it appears to be" (Caplan, 2014).

The phenomenon of creating human 3D cultures that duplicate authentic organs allows for extensive use of these organoids for many cell therapies, and as a potential substitute for whole-organ transplantation (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017). Organoids have been successfully transplanted both orthotopically, in the organs, in vivo location, and ectopically, outside of the organ's natural environment. Through both methods of transplantation, organoids were successful in undergoing cell maturation. These advancements show that organoid models can effectively mature and develop when in contact with natural signals provided by the in vivo environment, a crucial understanding for developing organoid transplantation. An abundance of research in the field of organoid transplantation is done to further advance our knowledge of its capabilities. The use of immunocompromised mice in many experiments has paved the way for great progress. When transplanting organoids into these mice for maturation, the organoids were positioned at highly vascularized sites, making it easier to control organoid engraftment and growth. This allows the organoid to develop for several months, without interfering with the mouse's required organ function. Some of these sites include epididymal fat pads and underneath kidney capsules (Holloway, Capeling, & Spence, 2019).

More recently, scientists were successful in orthotopically transplanting colonic epithelial organoids derived from human primary tissue into the murine colon. This study was supported by the Research Center Network for Realization of Regenerative Medicine project from the Japan Agency for Medical Research and Development. The experiment was done by removal of epithelial tissue from the mice. Results were most efficient when only one side of the colon mucosa was removed, to prevent obstruction of the murine colon. Following the removal of the epithelial cells, human colon organoids were transplanted. The xenografted organoids were observed by fluorescent endoscopic imaging. After many trials, long-term engraftment of the human colon organoids was eventually successful and was monitored and observed by endoscopy for over 6 months. Immunohistochemistry of human-specific cytokeratin (KRT8/7) confirmed the presence of the human donated epithelium in the colon of the mouse. This study broadened our understanding of transplantation through organoids in general, but also suggested that the location of the injury must be significantly large to successfully create a niche for the transplanted organoid. Small injuries requiring engrafted tissue, although also allowing for engraftment, proved to be more complicated. The tissue did not last as long as the engrafted tissue of the case of the larger injury, and eventually disappeared over time (Sugimoto, et al., 2017).

Personalized Medicine

Of the many exciting advancements brought about through organoid research, the ability to model diseases of specific patients in vitro can be of great benefit to medicine (Drost & Clevers, 2017). Scientists begin to predict a future in which organoid cultures derived from a patient can provide correct and effective therapeutic options for that particular patient. Induced pluripotent stem cells (iPSCs) are skin or blood cells that have been placed back into an embryonic-like pluripotent state. This allows the cells to mature and develop into any kind of human cell and is commonly used for therapeutic purposes. (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017).

In regard to oncological patients, these organoids could allow testing of drug response, minimizing the load of

therapy. Organoids created from tumor-derived cells are obtained from human tumor biopsies. Induced pluripotent stem cells or adult stem cells are taken from the biopsy and develop into organoids that mimic the cancerous organs. These organoids display identical tumor heterogeneity to their in vivo counterparts (Dumont, Heremans, Jan et.al. 2019).

Marc van de Wetering, a senior researcher dedicated to pediatric oncology, created a biobank of 20 tumor organoids derived from patients with colorectal cancer (CRC). He characterized the organoids by genes and performed drug testing to observe the correlation of specific mutations with drug responsiveness. For example, only one of the tumor organoid cultures reacted sensitively to LGK974, an inhibitor of Wnt secretion. The sensitivity toward this inhibitor was observed to be connected to a mutation that was found in the negative Wnt regulator. This study indicated that characteristics of tumors, histological and genetic, were mimicked precisely in the organoid. These findings can offer a promising future in understanding the correlation between tumor genetics and drug response (Drost & Clevers, 2017).

Additional studies have also been done to create organoid models from normal and neoplastic murine and human pancreas tissues. Pancreatic cancer is of the most fatal malignancies because of its lack of improvement from treatment and late diagnosis. Pancreatic organoids can develop quickly from cells derived from resected tumors and biopsies. These organoids display characteristics that are helpful in understanding drug and therapy response of the pancreas. Neoplastic organoids that are transplanted back into the body, reperform the whole tumor development. The organoids develop from early-grade neoplasms into "locally invasive and metastatic carcinomas." Organoids are an ideal model for genetic analysis because of their ability to be genetically duplicated. Close observation of murine pancreatic organoids presented altered genes and pathways during the onset and progression of the disease. After confirming these characteristics in human tissue, organoids have been deemed as an effective model system for discovering the nature of this deadly malignancy (Boj, et al., 2015).

Gene Therapy

Incorporating organoids into gene-editing through the use of tools like CRISPR/Cas9 technology offers the possibility of correcting gene mutations of patients. These repaired and healthy genes can then be transplanted into the patient (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017). CRISPR/Cas9 also allows for manipulation of genes in organoid cultures, causing drug testing for specific genes to be more definitive and reliable (Dumont, Heremans, Jan et.al. 2019). Comparing a variety of organoids that are all derived from the same iPSC line allows scientists to observe mutations in specific genes, and the effects on tissue specification and development in organs when the genes of a patient carry a specific congenital allele. The ability to create organoids from the diseased tissue offers various methods for clinicians to explore, like computerized drug screening tests, or mirroring antibiogram tests to determine antibiotic susceptibilities (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017).

Studies were done to prove the benefits that can be gained from adult stem cell-derived organoids with regard to hereditary diseases (Drost & Clevers, 2017). Recently, advancements in organoids have shown that CRISPR/Cas9 technology can repair cystic fibrosis gene mutations (Huch, Knoblich, Lutolf, & Martinez-Arias, 2017). Dr. J.M. Beekman of the Regenerative Medicine Center Utrecht, along with some colleagues, created a modeled cystic fibrosis (CF) in vitro culture. The model was composed of cells obtained from CF patients through rectal biopsies, creating intestinal organoids. The cystic fibrosis transmembrane conductance regulator (CFTR) gene is responsible for encoding an anion channel that is expressed on the surface of epithelial cells. CFTR maintains fluid and electrolyte homeostasis. Cystic fibrosis is the result of a mutation in the CFTR gene. As a result of this mutation, epithelial ion transport is weak, and a build-up of viscous fluid becomes present in the respiratory and gastrointestinal tract. CFTR is activated by forskolin, which increases intracellular cyclic AMP levels. In a healthy sample of intestinal organoids swelling was rapid. However, organoids derived from cystic fibrosis patients presented minimal swelling in comparison. It is now possible for researchers to test various drugs on different CF patient-derived organoids, depending on specific CFTR mutations. These findings shed light on a future of patient-specific treatment strategies (Drost & Clevers, 2017).

The Liver

There are many diseases associated with the liver, and over two million deaths a year worldwide are related to liver complications like cirrhosis, viral hepatitis and hepatocellular carcinoma (Asrani, Devarbhavi, Eaton, & Kamath, 2018). The amount of deaths associated with liver disease calls for the advancement of liver organoids as treatments for struggling patients. Researchers like Meritxell Huch of Gurdon Institute and Takanori Takebe from Yokohama City University present their progress in the field of liver organoids. They were successful in creating ductal structures from adult liver and went further

to activate these structures to differentiate into hepatocytes capable of producing bile acid and maintaining cytochrome activity (Muthuswamy, 2017).

The creation of the liver organoid has been further developed through the use of co-culture systems. Many studies reported that liver hepatocyte-like cells derived from human pluripotent stem cells (hPSCs) in 2D resulted in the cells developing immaturely and did not perform all functions of mature hepatocytes. Additionally, after implantation of these hPSC derived hepatocyte-like cells into immunocompromised mice, the cells operated with little effectiveness. Due to these poor results, research has been dedicated to creating more functional hPSC-derived hepatocytes, to produce a liver organoid model that will function more efficiently. The use of co-cultures is thought to be the answer to this progress. When observing mice, researchers found endothelial cells to be a crucial cell type in the process of

liver organogenesis. Due to this finding, a hypothesis was formed that placing endothelial cells and mesenchymal precursors with the hepatic endoderm in a co-culture will provide an environment that closely mimics in vivo development of the liver. This will give rise to efficient hepatocyte differentiation, ultimately generating a complex human liver bud organoid (Holloway, Capeling, & Spence, 2019).

The liver is made up of epithelial, stromal, endothelial and mesenchymal cells that all work together to carry out its functions. Hepatoblasts are the embryonic progenitor cells of the liver. At the time of organogenesis, these cells are set aside from the posterior foregut endoderm. Hepatoblasts communicate with surrounding signaling factors found within the mesenchyme, like FGF, BMP, hepatocyte growth factor (HGF) and Wnt. These communications cause the hepatoblasts to evolve and modify until they can migrate into the adjacent mesoderm to form the liver bud (Prior, Inacio, & Huch, 2019). After creation of the hPSC-liver buds, transplantation of the liver buds into immunocompromised mice tested the functionality of these artificial structures. The hepatocytes of the liver bud were proved to be functional due to the presence of high sustained levels of albumin within the liver bud (Holloway, Capeling, & Spence, 2019). After the liver bud is formed, the hepatoblasts expand and differentiate to form hepatocytes and biliary epithelium. Meanwhile, the mesenchyme generates liver fibroblasts and stellate cells. The structure of the liver is formed completely when the hepatocytes and cholangiocytes mature further and the endothelium and mesenchyme are integrated (Tremblay & Zaret , 2005).

Artificial Ovaries

Of all gynecological cancers, epithelial ovarian cancer (EOC) is the fifth largest cause of cancer-related deaths in European women. EOC patients frequently relapse, with an expanding resistance to chemotherapy and targeted therapy. Consequently, further research is crucial for the advancement of therapy in EOC patients, and organoid development seems promising. Models of EOC organoids can contribute a constructive platform for drug testing and screening, and can ultimately be utilized as a personalized in vitro prototype for an individual patient.

High-grade serous ovarian carcinoma (HGSOC) is a tumor that arises from the serous tubal intraepithelial carcinoma (Dumont, Heremans, Jan et.al. 2019). The fallopian tube epithelium (FTE) has been observed as the site where HGSOC originates (Yucer, et al., 2017). Therefore, observation of fallopian tube tissue can be very enlightening in discovering the nature of EOC. Mirjana Kessler of the Department of Infectious Diseases and Respiratory Medicine, through experimentation, created differentiated ovarian organoids from a single fallopian epithelial stem cell. These organoid models maintained their stability for over several months. This experiment uncovered the existence of fallopian tube stem cells for the first time. The creation of an ovarian organoid requires growth factors and microcellular substances, particularly Wnt and Notch pathways. The inclusion of Notch is especially important because it primarily regulates and maintains the stemness of these fallopian epithelial cells (Dumont, et al., 2019).

The urogenital system arises from the intermediate mesoderm (IM) of a developing embryo. The Müllerian duct within the urogenital system gives rise to the entire female reproductive tract (Yucer, et al., 2017). Following Kessler's research, Nur Yucer of the Regenerative Medicine Institute at Cedars-Sinai Medical Center was successful in constructing Fallopian tube organoids (Dumont et al., 2019). He created an in vitro culture of the Müllerian duct from induced pluripotent stem cells. Yucer then generated IM differentiation by adding pro-Mullerian growth factors, causing the fallopian tube epithelium to develop. The differentiation of the cells was monitored by the addition of cell-related markers such as PAX2, GATA3, OSR1, WT1, and OVGP1. A 3D growth platform was set up for further development and maturation of FTE, which successfully caused the tissue to self-organize into an FTE organoid structure. When forming the FTE organoid, the canonical WNT signaling pathway proved to be crucial. Sub- set of WNT gene family members were also included in the culture, like WNT3a and WNT4. These genes were necessary for generating the different phases of development and maturation of the Müllerian duct. WNT3a and WNT4

are also responsible for inhibiting male differentiation pathways. After three days of forming the culture, epithelial buds formed from the flat cell sheets. The buds then became spheroids, which were put in Matrigel beads with pro-mullerian growth factors. The intermediate mesoderm matured most at day 4, so spheroids were observed on days 3 to 6. Inclusion of a weak estrogen, phenol red, in Matrigel caused the complete organoid structure to form (Yucer, et al., 2017). This finding highlights the necessity of estrogen for the differentiating and maturing of the Fallopian tube. To maintain and stabilize fully developed organoids, both estrogen and progesterone must be present. (Dumont, et al., 2019) This is because estrogen alone did not maintain the organoids for long periods of time.

More progress has been made in regard to EOC organoid research. Dr. Oded Kopper of NewStem efficiently produced organoids with epithelial ovarian cancer characteristics. He obtained cells from primary tumors or metastatic lesions. The organoids he created successfully retained identical genomic landscapes as the tumors they were derived from. Histological aspects and heterogeneity of the tumors were also maintained. (Dumont, et al., 2019)

Ovarian cancer organoids can be utilized to observe various tumor responses to chemotherapy and for drug-screening assays. Xenografting OC organoids also allows clinicians to test drug susceptibility in vivo. These are some of the many advancements and applications of ovarian cancer organoids (Kopper, et al., 2019).

Another major benefit of an artificial ovary is to preserve fertility in patients suffering from cancer. An artificial ovary can allow women to fall pregnant after the damaging effects of chemotherapy. (University of Erlangen-Nuremberg, 2019) Ovarian tissue cryopreservation is the process of preserving reproductive potential in oncological patients after being exposed to gonadotoxic therapeutic agents. (Liverani, et al., 2019) Ovarian tissue cryopreservation involves the removal of a section or of the complete tissue of an ovary. This tissue is then preserved for use in the future. (Ben-Aharon, et al., 2016) Cryopreservation can be utilized by patients with aggressive malignancies who must receive instant gonadotoxic treatment. These women do not have the time to receive ovulation induction, and cryopreservation can be a great benefit. Although ovarian tissue cryopreservation can be of great assistance for many, cancer patients with moderate-to-high risk of ovarian metastasis are at too high of a risk. The process of cryopreservation introduces the possibility of malignant cells being reimplanted back into the patient.

A promising approach for these high-risk patients is the use of artificial ovaries. Isolating follicles from a patient's ovarian tissue and reimplanting the follicles into an artificial ovary can guarantee that no malignant cells will be reintroduced to the patient. (Liverani, et al., 2019) Because follicles are isolated in a basement membrane, there is no interaction between capillaries, follicular cells, white blood cells and nerves. Therefore, there is no threat that malignant cells will be introduced back to patients. Artificial ovaries make it possible to transplant primordial and primary follicles that were isolated to survive and could mature into follicles after transplantation. This permits sex hormones to be secreted, regulating oocyte maturation to the point where they are capable of fertilization. In the artificial ovary, it is essential for the follicles to be supplied with an environment that mimics the natural human ovary. In order for the follicles to survive and develop, interaction with the ovarian extracellular matrix (ECM), as well as surrounding ovarian cells must be present. For example, ovarian stromal cells are required to activate primordial follicles, while endothelial cells are necessary for vascularization, providing transportation of oxygen and nutrients, paracrine factors, and removal of metabolic waste. The presence of the ovarian ECM is responsible for maintaining 3D follicle structure, as well as other important roles. Therefore, all these factors must be present. (Dolmans & Amorim, 2019)

Drs. Teresa Woodruff and Ramille Shah of Northwestern University led a team of scientists in creating artificial ovary structures. The process utilized a 3D printer to create the structure that would hold the follicles. A stainless steel nozzle was used for the printer, which was around the width of a strand of hair. Five layers of gelatin filaments were printed at various angles. Tiny cylinders were punched through the sheets, creating pores to hold mice follicles. Nine mice were involved in the study, and all ovaries were removed. Seven of the mice received the structures with the follicles, and two received the artificial structures without follicles. Following the mating of these mice with male mice, three mice with artificial ovaries produced litters. The artificial ovaries also joined with the blood vessels of the mice, allowing for female hormones involved in milk production to be made. The hormones successfully traveled the mouse bloodstream to the breast tissue where it stimulated milk production (Piazza, 2018). The ability of an artificial ovary to restore endocrine function and provide an environment for follicular development can be extremely useful for oncological patients in the future (Cho, Kim, Noh, & Ku, 2019).

Virology

The coronavirus disease-19 (COVID-19), caused by the SARS-CoV-2 virus is responsible for over 250,000 deaths globally. (Han, et al., 2020) The COVID-19 pandemic

proved to the world just how threatening newly surfacing viruses can be. Understanding the nature of these viruses rests in the use of in vitro cultures capable of viral replication. The use of organoids is now demonstrating its practicality when experimenting the tendencies of these deadly viruses.

The novel coronavirus first infected humans in December of 2019. Various research groups have been experimenting with organoids to better understand the nature and tissue tropism of SARS-CoV-2. Josef Penninger of the Institute of Molecular Biotechnology, along with his colleagues, created capillary and kidney organoids, both derived from human iPSCs. Through this research, Penninger proved that SARS-CoV-2 can infect both sites in the body. These observations help explain the ability of the virus to spread throughout the body and the damaging effects on kidney function in infected individuals.

Multiple studies were done to establish whether the virus can infect the gastrointestinal tract. Intestinal organoids derived from adult stem cells were examined and presented high levels of angiotensin converting enzyme 2 (ACE2), a receptor for SARS. The studies displayed that enterocytes, the most common intestinal epithelium cell type, were infected with the virus. These findings verified that the intestine is a potential site for SARS-CoV-2 infection (Clevers, 2020).

Mart Lamers of Erasmus Medical Center, along with his colleagues, further investigated the ability of SARS-CoV-2 to infect the intestines. The human small intestine epithelium is composed of multiple cell types. The experiment consisted of 3D structures that displayed all cell types, grown in four separate cultures. Each culture displayed different amounts of ACE2, proving their ability to be infected with SARS-CoV-2. Through the use of electron microscopy, Lamers and his colleagues observed that the virus caused infection in mature and progenitor enterocytes alike. This study suggests that the use of human organoid models can provide researchers with beneficial resources for examining the nature of SARS-CoV-2 and other coronaviruses. (Aaas, 2020)

Since the primary infection site for SARS-CoV-2 is in the respiratory organs, research was done to create a lung organoid derived from human pluripotent stem cells (hPSCs). Development began with the differentiation of the hPSCs into definitive endoderm. The cells then further progressed into the anterior foregut endoderm (AFE) and formed AFE/lung progenitor cells. Further specification and maturation of these cells eventually formed the lung organoids. The ability of these lung organoids to provide a platform for modeling COVID-19 was then put to the test. The cultures were introduced to the SARS-CoV-2 virus, and after 24 hours viral RNA was detected. The use of immunostaining proved the presence of SARS-S protein in the lung organoids. Based on this research, scientists can utilize these organoids to observe the effects of various drugs on the coronavirus, by observing any decrease in the amount of markers present from the injected virus. (Han, et al., 2020)

Conclusion

Organoids possess the ability to model tissue structure and function with great accuracy, but more research is required to take full advantage of organoids. Organoids still lack vasculature, immune cells, and other cellular components that tissues communicate with and depend on in the body (Iakobachvili & Peters, 2017). Without all the essential cellular components of an organ, the use of organoid in human transplantation will be limited. Studying processes where these components are necessary is therefore limited. (Souza, 2018) Efforts to improve the vasculature of organoids will provide nutrient supply, excretion of toxins and signaling that is present in the body. This research is presently making progress, though it is demanding and yet to be fully incorporated into standard tissue culture facilities. (Iakobachvili & Peters, 2017) Organoid technology brings up many ethical questions as well. For example, what might happen if scientists can successfully create a brain organoid? Utilizing a brain organoid for drug testing would be helpful, but it may pose an ethical issue to transplant this organoid into a patient. These matters must be addressed in the future. However, we do know that organoid technology is adding a whole new dimension to the medical field.

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The Effects of Fetal Microchimerism on Maternal Health

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Abstract

Maternal-fetal cellular trafficking is the bidirectional passage of cells between the fetus and mother during pregnancy. The presence of fetal cells in maternal circulation, brain, and muscles is known as fetal microchimerism. During pregnancy, fetal cells can be obtained from the mother's blood for prenatal diagnosis of the fetus. Furthermore, it has been confirmed that fetal microchimerism persists decades later in women who have been pregnant. Investigation of the long-term consequences of fetal microchimerism is a frontier of active study, with preliminary results pointing to both beneficial and adverse effects. This review will examine the relevant literary information on fetal microchimerism during pregnancy and provide current knowledge regarding the long-term effects of naturally acquired fetal microchimerism.

Introduction

Microchimerism is the presence of two genetically distinct cell populations in the same individual. Foreign cells and DNA are found in an individual's plasma or tissues that originated from a genetically different individual. Naturally acquired microchimerism is commonly observed and results from the maternal-fetal exchange of cells and DNA during pregnancy. (Gammill & Nelson 2010) In pregnancy, there is transplacental two-way trafficking of living fetal cells from the fetus into the maternal circulation, and cells from maternal circulation impart in the fetus. (Naik, Shrivastava, Suryawanshi, & Gupta 2019) This paper provides an overview of the role of fetal cell microchimerism in autoimmune and cancerous diseases. The mechanisms by which fetal microchimerism is believed to modulate the protection against cancer or tumor progression will be discussed, along with future research directions.

Methods

This document was written by researching peer-reviewed scholarly articles and medical journals to assess the newest research and methods in fetal microchimerism. The information and data were gathered from numerous sources, including databases such as Google Scholar, Touro Library, The National Center for Biotechnology Information, and The National Institute of Health. Keyphrases searched included, "fetal microchimerism," "the effects of fetal microchimerism," and "fetal microchimerism in autoimmune disorders." All the content was carefully selected, compared, and analyzed to assure its validity and determine the articles' standpoint.

Fetal Microchimerism

Microchimerism is the fetus's cell legacy after pregnancy. Fetal stem cells can leave the fetus and migrate across the placenta to engraft in the maternal bone marrow and other tissues. The transfer of fetal progenitor stem cells begins four or five weeks after fertilization and continues throughout the pregnancy. This form of microchimerism occurs in both male and female embryos, but the detection of male microchimerism in maternal tissue is easier to detect due to the unique presence of Y-chromosome in the women. (Miech, 2010)

Fetal microchimerism has been reported in the peripheral blood of women in cellular subsets, including lymphoid-lineage and myeloid-lineage cells. An experiment was done to evaluate microchimerism in CD66b sorted granulocytes. Granulocytes were isolated from the peripheral blood of healthy women. CD66b+ cells were then isolated by fluorescence-activated cell sorting, and a panel of polymorphism-specific quantitative assays was employed to investigate the presence of fetal and maternal microchimerism. One-third of the women tested positive for at least one form of microchimerism. 40% had maternal microchimerism compared to 15% who had fetal microchimerism. The maternal and fetal origin CD66b+ cells are strong evidence for an active microchimeric hematopoietic stem and progenitor cell niche. Higher proportions of women might test positive for even larger quantities of microchimerism if more sensitive assays were available. (Sunku, Gadi, Lacoste, Guthrie, & Nelson, 2010)

Fetal DNA in Maternal Plasma During Pregnancy

Another study was undertaken to evaluate fetal DNA in maternal plasma and urine. DNA was isolated from plasma and urine samples of 80 pregnant women, ranging from 7 to 40 weeks of gestation. Their DNA underwent amplification for Y specific chromosome DNA via a nested polymerase chain reaction. The postpartum analysis of fetal gender showed that 25 women carried female fetuses and 55 male fetuses. Among the 55 women bearing male fetuses, Y chromosome-specific signals were detected in 96% of their plasma and 38% of their urine samples. (Al-Yatama et al., 2001) The detection of fetal sex chromosomes with Y-chromosome-PCR has a specificity of 100% and a sensitivity of 96%, which increases along with gestational age. This minor lack of sensitivity explains why Y-chromosome signals were only located in 96% of the plasma of pregnant women. (Rijnders et al.) The analysis of their results with respect to gestational age showed no significant difference in the Y chromosome-specific DNA detection. These results showed that fetus-specific DNA was detected in the maternal plasma by nested PCR. (Al-Yatama et al., 2001)

Fetal DNA is present in low concentration in maternal plasma increasing throughout pregnancy with a 0.1% increase each week from 10 to 20 weeks' gestation, followed

by a 1% increase each week from the 21st week until term. (Taglauer et al.) Clinical tests have tried to capitalize on this DNA to identify the baby's sex, determine whether the child and mother have Rh incompatibility, and identify chromosomal disorders. (LeslieDec, et al. 2017)

Many early investigations of fetomaternal cell trafficking sought to develop methods whereby noninvasive prenatal diagnosis of genetic disorders could be achieved. (Gammill & Nelson 2010) Noninvasive testing would ameliorate the risk of fetal miscarriage associated with current invasive procedures such as amniocentesis. This non-invasive prenatal test is a blood test, and between 25 and 42.5 milliliters are collected. (Bianchi, Williams, Pelletier, Klinger, & Shuber 1996) The main conditions for which prenatal diagnosis is considered are monogenic diseases, when the causation of the disease results from one gene, and therefore, is easier to detect and obtains conclusive results. Fetal DNA circulates in a high background of maternal DNA and is isolated and amplified using polymerase chain reaction technology. However, even after that, maternally inherited fetal alleles are very difficult to identify from maternal plasma, therefore, scientists have focused on the detection of paternally inherited fetal alleles that are not present in the maternal genome. The diagnosis of autosomal dominant diseases transmitted by the father could be made noninvasively. Also, by detecting the paternally inherited fetal alleles, one can exclude the fetal inheritance of autosomal recessive diseases. The discovery of free cellular fetal DNA in maternal plasma has offered new approaches for noninvasive diagnosis, and fetal isolation analysis is now possible. (Lun et al., 2008)

Fetal DNA in Maternal Plasma After Pregnancy

Microchimerism occurs as a result of the fetomaternal transfusion of blood cells across the placenta during pregnancy. After delivery, once the placenta has been expelled, fetal-origin cells should hypothetically depart from the maternal circulation. Nevertheless, studies of women who had given birth to a male child showed a high incidence of male cells in their blood after delivery. Additionally, some women have displayed male cells in their tissues up to 27 years postpartum after having birthed a male child. This event can be interpreted to mean that fetal cells transferred from the fetus to the mother during pregnancy established permanent microchimerism in the maternal body. This permanency was reported in 30–50% of women who gave birth to a male child. (Sato, Fujimori, Sato, & Ohto, 2008)

There is an increased fetal-to-maternal transfer of progenitor cells during an abortion procedure as the placenta is being destroyed. Maximized fetal cell trafficking following a medical abortion was confirmed in an in vivo model. Since the embryonic circulatory system is established in the first trimester of pregnancy, there is a greater chance for the transfer of a larger number of progenitor T cells during the first-trimester termination of pregnancy. Therefore, women who had an elective abortion in their first or second trimester have a greater tendency towards fetal microchimerism. (Miech, 2010)

Male microchimerism in women without sons and the correlation of microchimerism to prior pregnancy history was researched. Y-chromosome-specific quantitative PCR was used to test peripheral blood mononuclear cells of 120 women. Women were categorized into four groups based on their pregnancy histories. Group A was nulligravid and displayed 10% male microchimerism, Group B had induced abortions and displayed 57%, Group C had spontaneous abortions and displayed 22%, and Group D had only daughters and displayed 8%. Male microchimerism prevalence was the greatest by a significant amount in those with induced abortions. Other possible sources of male microchimerism, such as those obtained in Groups A and D, include unrecognized spontaneous abortion or a vanished male twin. (Yan et al., 2005)

Autoimmune Effects on The Mother

It has been theorized that the persistence and abundance of fetal microchimerism are dependent on numerous factors, including the immunogenetic relationships between mother and fetus, and perhaps between and preexisting inhabitants of the maternal system, from her mother or previous children. (Gammill & Nelson 2010)

Fetal microchimerism, a form of trans-placental stem cell transplant, helps to explain the etiology, diversity of tissue pathology, predilection for females, and increase in the annual incidence of autoimmune diseases in women. (Miech, 2010) Fetal stem cells can differentiate into mature and competent cells, such as lymphocytes and monocytes. Fetal cells in maternal circulation or tissues may mediate a graft vs. host disease, which can lead to the development of autoimmune diseases. Women comprise 80% of people with autoimmune diseases. Researchers have begun investigating the association of micro-chimerism with autoimmune diseases that predominately affect women. (Naik, Shrivastava, Suryawanshi, & Gupta 2019)

Fetal microchimeric cells in maternal tissues led to the discovery of a positive correlation between fetal microchimerism and autoimmune diseases in women. Progenitor cells in the fetal immune system, such as T cells, monocytes, macrophages, lymphocytes, and NK cells, are among the many fetal cell types that can be transferred to maternal tissues. In maternal tissues, the fetal microchimeric

progenitor immature T cells are capable of self-renewal, proliferation, and differentiation. Progenitor cell activation can result in the production of autocrine and paracrine inflammatory responses in autoimmune diseases. Triggering agents that activate these fetal microchimeric immune cells to attack the maternal host cells resulting in autoimmune disease have not yet been identified. Suspected triggers include viral or bacterial agents, abnormal localized tissue protein, and drugs. Microchimerism in affected tissues is more likely to be abundant in women with autoimmune diseases than in women with non-autoimmune diseases. (Miech, 2010)

Pregnancy alters symptoms of autoimmune diseases. The maternal immune system develops a tolerance to the fetus, and the suppression of the maternal response is lifted postpartum. Fetal tolerance may explain why some autoimmune disease symptoms decrease during pregnancy in some women. (Boddy, Fortunato, Sayres, & Aktipis, 2015) Autoimmune diseases are often repressed during pregnancy and exasperate postpartum. It was then hypothesized that fetal cells in maternal circulation might be important in influencing autoimmune disease in pregnancy and postpartum. (Ando, Imaizumi, Graves, Unger, & Davies, 2002) Systemic sclerosis provided the first evidence for the involvement of fetal microchimerism with autoimmune diseases. Patients frequently had fetal cells not only in peripheral blood, but also in skin lesions. The hyperthyroidism of Graves' disease frequently abates during pregnancy and exacerbates after childbearing. (Ando, Imaizumi, Graves, Unger, & Davies, 2002) Between 43 and 75% of patients with rheumatoid arthritis exhibit amelioration of some or all their rheumatoid arthritis symptoms during pregnancy. Additionally, in rheumatoid arthritis patients, symptoms tend not only to show improvement during pregnancy, but an exacerbation of symptoms postpartum. Correspondingly, the rate of relapse declines with pregnancy in women diagnosed with multiple sclerosis, with the lowest rate in the third trimester. Relapse rates return to pre-pregnancy levels postpartum. This framework suggests that a contributor to autoimmune disease development may be the maternal immune response to the presence of fetal cells that invade maternal tissues. (Boddy, Fortunato, Sayres, & Aktipis, 2015)

The risk of developing an autoimmune disease in parous women is higher after the first year postpartum. Fetal cells in the thyroid may contribute to thyroid cancer risk or susceptibility to other thyroid diseases. Current research suggests an association with postpartum thyroid tissue and thyroid diseases. Fetal cells are more abundant in the thyroid tissue and blood of women with Graves' disease and Hashimoto's thyroiditis, compared to healthy controls. Also, the risk of autoimmune disease in parous women is significantly higher after the first year postpartum. This knowledge suggests that the presence of fetal cells in the thyroid is associated with a maternal disease rather than health. (Boddy, Fortunato, Sayres, & Aktipis, 2015)

Autoimmune thyroid disease affects reproductive-aged women. It commonly initiates or exacerbates postpartum, and so the involvement of fetal microchimerism was suspected. Autoimmune thyroid disease does have e a profound relationship with pregnancy. Autoimmune thyroid disease involves autoimmune antigens, such as the TSHR, Tg, and TPO. Placental immune suppression during pregnancy lessens the activity of autoimmune thyroid disease, as seen in the remission of Graves' disease. However, exacerbation of preexisting autoimmune thyroid diseases or initiation of autoimmune thyroid disease is also common postpartum. Increased titers of thyroid autoantibodies have been observed in the postpartum. Graves' disease has also been shown to be most markedly suppressed by pregnancy itself, but; however, up to 60% of Graves' disease patients of childbearing age have been reported to develop this disease within one year of delivery. (Ando & Davies, 2003)

Cancerous Effects on the Mother

During pregnancy, fetal cells enter the maternal body, cross the placenta, and are found frequently in the maternal lung. It was generally accepted that the fetal cells in the lung were passing through the mother's pulmonary circulation; however, the presence of fetal cells in the lung shows an association with cancer. A study reported higher levels of male DNA and significantly more fetal cells in the lung and thymus tissue in the diseased lung compared to healthy bone marrow from the same person. (Boddy, Fortunato, Sayres, & Aktipis, 2015) Fetal cells have also been detected as clusters in lung tumors in women decades after pregnancy. Their tumor frequency was higher in lung tumors than in the healthy surrounding lung tissue. The fetal cells may be recruited from the bone marrow to the tumor sites where they assumed their role in immunosurveillance and tissue repair. (Naik, Shrivastava, Suryawanshi, & Gupta 2019)

Fetal cells are frequently found in normal breast tissue postpartum women. The research presents a complicated picture of fetal cell's role in breast cancer. Fetal cells are found less the tissue and blood of women with breast cancer compared to healthy controls. (Boddy, Fortunato, Sayres, & Aktipis, 2015) It was reasoned that naturally acquired allogeneic immune cells in the form of fetal microchimerism might correlate with protection from the development of breast cancer. Fetal microchimerism was found in higher quantities in healthy women compared

to breast cancer patients, 43% to 14%, respectfully. This shows a correlation between the role of fetal allogeneic cells as malignant cell immune surveillance and protection from breast cancer. (Gadi & Nelson, 2007)

Reduced risk of breast cancer is recognized among parous compared to nulliparous women. Eighty-two women were tested for male DNA in peripheral blood, presumed from a prior pregnancy with a male fetus. Forty-seven of the subjects were healthy, and 35 had breast cancer. The levels of male DNA were determined by real-time PCR for a Y chromosome-specific gene in DNA extracted from peripheral blood mononuclear cells. Fetal microchimerism was found in 43% of healthy women and 14% in women with breast cancer. These findings suggest that allogeneic fetal microchimerism may contribute to a reduction in the risk of breast cancer. (Gadi & Nelson, 2007)

Through the evolutionary history of fetal microchimerism in the maternal body, it is possible that it now has a role in normal breast tissue physiology. The maternal mammary gland hosts stem cells that contribute to the development of normal breast tissue and can be transferred to the neonate during lactation. Mouse fibroblast fetal cells have been shown to differentiate into mammary epithelioid cells when exposed to lactation hormones in vitro, and a functional mammary gland has been generated from a single stem cell in a pregnancy mouse model. This suggests that fetal progenitor cells play a role in breast morphology and maternal milk supply. (Boddy, Fortunato, Sayres, & Aktipis, 2015)

Pregnancy at older ages has been linked to risk for ovarian cancer. Given the data that microchimeric cells in parous women decline overtime after pregnancy, and that ovarian cancer develops most commonly in postmenopausal women, fetal microchimerism may play a protective role in ovarian cancer as well. (Naik, Shrivastava, Suryawanshi, & Gupta 2019)

Neurological Effects on the Mother

It was unknown whether fetal cells, which are capable of crossing the placental barrier to enter maternal blood, could also cross the blood-brain barrier and enter the maternal brain. To determine if fetal cells could enter the maternal brain during pregnancy, female wild-type mice were crossed with green mice. Their offspring ubiquitously expressing the enhanced green fluorescent protein. Green mouse fetal cells were found in the maternal brain, and quantitative real-time PCR of genomic DNA for the enhanced green fluorescent protein gene showed that more fetal cells were present in the maternal brain for weeks postpartum than on the day of birth. After an excitotoxic lesion to the brain, more fetal cells were detected in the injured region. For weeks postpartum, enhanced green fluorescent protein-positive green mouse fetal cells in the maternal brain were found to adopt locations, morphologies, and expression of immunohistochemical markers indicative of astrocytes, neurons, oligodendrocyte, and macrophage-like cell types. The expression of morphological and histochemical characteristics of these cells was confirmed on the identification of fetal cells in the maternal brain by Y chromosome fluorescence hybridization. The characterization of these cells that allow them to cross both the placental and blood-brain barriers and to target the injured brain may improve selection procedures for isolation of stem or progenitor cells for brain repair by infusions. (Tan et al., 2005)

Several studies have found male DNA in human and maternal mouse brains, but the function of these cells is not entirely known. One study found that fetal cell DNA in numerous regions of the maternal brain, even decades after the woman had birthed a son, suggesting that fetal microchimerism in the human maternal brain may be long-lasting. (Boddy, Fortunato, Sayres, & Aktipis, 2015) A team led by autoimmunity researcher, J. Lee Nelson sought to discover how leftover fetal cells affect the brain. They took samples from autopsied brains of 59 women. Male DNA evidence was found in the brains of 63% of the participants. The male DNA was scattered across several brain regions. Studies have hypothesized that the risk of Alzheimer's disease increases with an increasing number of pregnancies, so the team also examined the brains for signs of the disease. Of the 59 women, 33 had Alzheimer's disease. Compared to healthy controls, fetal cells were found to be less common in the brains of women who had Alzheimer's disease. That correlation suggests that the presence of fetal cells in the maternal brains helps protect women against Alzheimer's disease. (PhillipsSep, et al. 2017)

Conclusion

The placenta is unique because it allows for intimate contact between maternal and fetal cells at the maternal-fetal interface throughout pregnancy. (Than et al.) During pregnancy, the semi-allogeneic fetus is protected from rejection by the maternal immune system. (Trowsdale and Betz) It was hypothesized that the semi allogeneic fetus could survive due to the regulation of the immunologic interactions between mother and fetus. Such regulation can be caused by functional suppression of the maternal immune response or a lack of fetal antigen expression. (Morelli et al.)

In the human placenta, fetal trophoblast cells do not express MHC HLA-A and B molecules, which are responsible for allograft rejection in humans. (Chen et al.)

Mixed hematopoietic chimerism, the state in which bone marrow hematopoietic stem cells from two genetically different animals coexist, remains the most robust tool for tolerance induction. Unfortunately, bone marrow or hematopoietic stem cell transplantation for patients awaiting solid organ transplantation remains at high-risk, due to the dangers of graft-versus-host disease. (Zavazava) Human embryonic stem cells express low levels of (MHC)-I antigens and lack expression of MHC-II antigens. These stem cells must engraft in the transplant recipient, inducing tolerance to foreign cells, and reducing the immune targeting of the transplant. Upon injection into immunocompetent mice, human embryonic stem cells are unable to produce an immune response. Human embryonic stem cells may provide a potential tool for the induction of immunotolerance. Further studies in human embryonic stem cells immunobiology are warranted and may reveal unique mechanisms that account for the immunological properties of human embryonic stem cells. (Menendez et al.)

Fetal cells and DNA go beyond the womb and into maternal blood and tissues during pregnancy and can last long after birth. In some instances, fetal microchimerism contributes to perturbations in maternal immunity that may contribute to autoimmune diseases. In other cases, these interactions seem to benefit long-term maternal health by allowing fetal microchimerism cells to act as allogeneic surveyors in her system. Fetal microchimerism has potential implications for the understanding of women's health and disease pathology postpartum. The pregnant mother mshould never be viewed as merely an incubator. Rather, microchimerism expands the biological bonds that are established between the pregnant mother and her fetus.

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Advancements in Vaccine Development: Measles vs. COVID-19

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Abstract

As an ever-progressing field of study, vaccine development has made headway over the past decades. By doing a comparative study between development of the measles vaccine in the mid-20th century, and the ongoing development of the COVID-19 vaccine in 2020, many of the insights and advancements in the field can be easily highlighted. First, the knowledge and experience gained over those decades has helped the vaccine developing process become more efficient. With more vaccines in production, or at least under study, it is more likely to have a related precedent to build upon, as opposed to relying on a successful vaccine of some unrelated disease. More to the point, different vaccine types have altered the way researchers attempt to formulate future vaccines. No longer are inactivated and attenuated vaccines the only option; subunit vaccines, as well as innovative, (though yet to be proven), nucleic acid vaccines are now additional approaches. These advancements have opened doors for researchers in their quest to fight diseases. This paper will explore the advancements and their impact on the present-day COVID-19 vaccine development.

Keywords: Vaccine, Measles, COVID-19, mRNA

Advancements in Vaccine Development: Measles vs. COVID-19

Though the majority of successful vaccines were developed in the 1900s, the challenge to develop vaccines still exists today, with both known diseases, such as cancer, as well as with novel infections that sweep the world. Measles used to be a common childhood disease; one which everyone contracted and with the majority of patients recovering completely. It was practically inevitable until the late 1900s, when a measles vaccine was introduced to the public. In the mid-1950s, after weeks of trying, John Enders's lab successfully isolated the measles virus. Eventually, through implementation of routine measles vaccine—one dose at 12-15 months, and a second at 4-6 years—measles was successfully eradicated from the U.S. in 2000, and from the Americas in 2016 (The College of Physician of Philadelphia, n.d.). However, that milestone of eliminating measles was not yet to be reached, as it was another ten years from the isolation of the virus until the measles vaccine officially debuted.

Contrastingly, it took Chinese researchers weeks to sequence the genome of SARS-CoV-2, a feat way beyond the simplicity of merely isolating the virus. Even more so, a projected release date for a safe and effective COVID-19 vaccine is only 12-18 months from the start of development, and one can only hope that the vaccine will eradicate the virus. Normally, vaccine development with FDA approval can take anywhere from 5-10 years. Obviously, vaccine technology has critically advanced in the decades since the measles vaccine, thus allowing researchers to expedite the vaccine development process for COVID-19 and all future pandemics. This paper will explore some of these advancements and their potential impact on the COVID-19 vaccine development.

Methods

All of the information discussed in the paper was obtained through the PubMed database, as well as via Google searches which led to government sites, like the CDC and NIH, and also to known and established medical sciences related sites, like CHOP and The College of Physicians of Philadelphia.

The majority of the amassed information pertained to the techniques involved in developing both the measles vaccine and the potential COVID-19 vaccine, so that I could compare and contrast the respective availability of resources.

One study explored in the paper included vaccine trial studies in which the efficacy of various measles vaccines were tested either alone, or in a series with other forms of developing measles vaccines. At a designated interval after each trial, measles titers were measured and eventually, the most effective vaccine was sought out.

There are currently over one hundred biotechnology companies and universities developing vaccine candidates for COVID-19. For the vaccine studies that are not yet ready for human test trials, i.e. majority of the COVID-19 studies, this paper analyzed selected studies which elaborated on the numerous approaches towards an effective COVID-19 vaccine.

Discussion

An Overview of Vaccines

How vaccines work

The purpose of a vaccine is to trigger the immune system to stimulate an initial immune response and ultimately create a cellular memory mechanism to fight future attacks by the pathogen. This way, when the body is exposed to the actual disease in the future, the body will produce an immediate secondary immune response and not a primary response (Clem, 2011). Secondary immune responses are quicker and more specific, and are therefore better than a primary immune response.

Classically, vaccines are created by deactivating or attenuating the virulent part of the pathogen, while leaving the antigenic portion of the pathogen intact so that the vaccine can induce an immune response without causing the disease itself (The College of Physicians of Philadelphia, 2018). When the body detects a foreign antigen, the innate immune system goes into action first (Clem, 2011). The

innate system includes non-specific white blood cells (i.e., macrophages and natural killer cells), which can either destroy the invader or process the pathogen and present its antigen to aid in the adaptive immune response. Both the humoral and cell-mediated immune responses are part of the adaptive immune response. The humoral response consists of B-cells, which detect the foreign antigen and then self-mutate to find the antibody that best fits the antigen. After that is successfully accomplished, the B-cell that is able to produce the most effective neutralizing antibodies replicates and becomes either plasma cells, which secrete antibodies to help fight the current infection, or it creates memory B-cells specific to that antigen, so that upon future infection the body will have an antibody that targets and destroys the specific disease pathogens. The cell-mediated immune response contains two types of cells, T-killer and T-helper cells, which are either activated by major histocompatibility complex I (MHC I) or major histocompatibility complex II (MHC II). MHC proteins are expressed on the surface of all bodily cells to signal that the cells belong in the body. However, when the cells become infected with a pathogen the MHC will take the processed antigen and present it on the surface of the cell, so that the T cells of the cell-mediated immune system can respond. T-killer cells recognize the antigen presented by MHC I molecules and subsequently advance to kill the invading cell. On the other hand, T-helper cells recognize the antigen presented by MHC II molecules, and T-helper cells aid in the activation of B cells as well as T-killer cells. Regardless of their function, either T cell can replicate and form T-memory cells in preparation of future infections (Clem, 2011). In all, the job of a vaccine is to prime the body for a future encounter with a specific pathogenic agent.

Stages of Vaccine Production

Before any vaccine can be mass produced for public use, it must go through a series of developmental phases to assess first and foremost its safety, in addition to the vaccine's efficacy, required dosage and dose frequency, and screenings for any harmful side effects (The College of Physicians of Philadelphia, 2018). Prior to these phases though, researchers study the specific disease and attempt to identify its immunogenic parts. Once that has been done, researchers can either isolate the antigenic portion, or inactivate the virulent part, depending on what type of vaccine they propose to create.

Following those preliminary steps, the candidate vaccine can then enter the preclinical phase of study. In this stage, researchers test the vaccine safety and immunogenicity on animals (The College of Physicians of Philadelphia, 2018). Also during this preclinical stage, researchers may conduct what are known as challenge trials. In these tests, researchers inject their candidate vaccine into the lab animals or human volunteers, wait an amount of time so that the vaccine can elicit an immune response and create memory cells, and then finally, they inject the targeted virus into the subjects to determine if the vaccine can do its job. Based on the challenge trial results, researchers will adjust their vaccine development accordingly. During this phase, there may be a bit of trial and error in determining a safe starting dose, i.e. how many viral particles are needed to elicit an immune reaction, and method of delivery for human subjects of the next phase (The College of Physicians of Philadelphia, 2018). For the COVID-19 mRNA vaccine trials conducted by Moderna and the NIAID, three trial groups have been set up each with a different dosage. One group is testing the low dose of 25mcg, a second group is receiving the midrage amount of 100mcg, and the third group is receiving the highest dose, 250mcg (National Institute for Allergy and Infectious Disease [NIAID], 2020).

In order to advance from the preclinical stage to the clinical phases, the research group must submit an investigational new drug (IND) application to the FDA for approval to further their studies (The College of Physicians of Philadelphia, 2018). Once the researchers receive authorization, they can proceed to phase I clinical trials. At this juncture, researchers experiment with human subjects once again to test safety and immunogenicity of the proposed vaccine. This step starts with healthy adult subjects and if the vaccine is intended for younger or older age groups, the trials gradually trend towards the desired group. During this phase, researchers may conduct human challenge trials as well (The College of Physicians of Philadelphia, 2018). With regard to COVID-19, Moderna has reported that after two doses of their potential mRNA COVID-19 vaccine, participants in the low and mid groups have expressed immunity at or above the level in which someone who was naturally infected would have. This seems to be a promising result for phase I trials, especially since it seems that once someone contracts COVID-19 they do not get it again, and so the natural amount of antibodies should be sufficient for vaccine induced protection (CBS News, 2020).

After successful phase I trials, the vaccine can move on to phase II clinical trials. Here, researchers study a larger test group including a control group and the main point of this phase is to further characterize dosage, frequency of immunization, and method of delivery (The College of Physicians of Philadelphia, 2018). In this phase, the clinical studies will also ascertain whether the vaccine shows any

efficacy. Safety of the vaccine is monitored as well.

Next, there are phase III clinical trials. This is where the potential vaccine is determined to be effective by recruiting hundreds or thousands of volunteers to help quantitate how effective the vaccine is in a large cohort (The College of Physicians of Philadelphia, 2018). The large subject group also allows for the detection of rare side effects. During this phase, vaccine safety and dosage are still monitored for necessary corrections. Vaccines are never 100% effective. Measle vaccine is thought to be about 85% effective. Therefore, phase III trials must assess vaccine effectiveness.

Finally, after the vaccine has successfully passed through all phases of clinical testing, the researchers can submit a biologics license application to the FDA for licensure of their candidate vaccine (The College of Physicians of Philadelphia, 2018). If the FDA approves, and the CDC subsequently recommends this vaccine for routine administration, the researchers will be able to manufacture their vaccine and enable mass population immunization.

At this point, researchers can conduct an optional phase IV clinical trial to further monitor the vaccine's safety and efficacy (The College of Physicians of Philadelphia, 2018). Studies can also be done to learn if the vaccine has potential for any alternate uses (i.e. other than for protection of the targeted pathogen).

Post licensure, the FDA and CDC continue to monitor the vaccine (The College of Physicians of Philadelphia, 2018). In 1990, the FDA and CDC established the vaccine adverse event reporting system (VAERS). The VAERS allows for anyone to report adverse side effects, which seem to be caused by a particular vaccine. The CDC then analyzes all of the input data to determine several conclusions. Among those conclusions are whether a new adverse side effect has indeed been detected, and whether certain health conditions place a patient at a greater risk of developing an adverse side effect. Also in 1990, the CDC established the vaccine safety datalink (VSD). This system provides access to numerous databases listing which vaccines were given to a particular patient on the reported date. The VSD conducts vaccine safety studies based on questions raised in medical literature or reports from the VAERS. In addition, the VSD monitors the safety of new vaccines.

Advances in Vaccine Development

In the sixty years since the development of the measles vaccine, the technologies used have improved as well as increased. For example, while Enders, Hilleman, and all the other researchers of that time were experimenting with either inactivated (dead virus) or live-attenuated vaccines, researchers of today can work with a greater variety of vaccine types ranging from pathogen subunit vaccines to nucleic acid vaccines (NIAID, n.d.). Both forms of the newer vaccine methods aim at inoculating with only the antigenic portion of the microbe as opposed to injecting the whole pathogen, which is what is done with inactivated and attenuated vaccines. In an inactivated vaccine, the entire pathogen is killed and then subsequently administered to the patient (Clem, 2011). Attenuated vaccines, on the other hand, leave the pathogen partially alive, and then upon administration, induce a stronger immune response than inactivated vaccines. However, because the attenuated vaccine is slightly live, it does present a greater risk to the immunocompromised community (Clem, 2011). The challenge in designing inactivated viruses or attenuated viral particles is that there is no set formula for how to kill or attenuate the virus. Each virus is different. That is why it can take months, or even years, to generate an effective candidate vaccine.

Over the past decade, new innovative technologies have been instituted to develop effective vaccines. Subunit vaccines are one example where a mere pathogenic unit—a protein, or sugars on the microbe's outer coat—are administered into the patient to elicit an antibody response, thus allowing a person to attain immunity (NIAID, n.d.). However, subunits vaccines are often not effective enough on their own and require an adjuvant to enhance their immune response (NIAID, n.d.). While historically composed of only aluminum salts, adjuvants today come in many more varieties like MF59 (oil in water emulsion composed of scalene), CpG 1018 (cytosine phosphoguanine, a synthetic form of DNA that mimics bacterial and viral genetic material), and others (Centers for Disease Control and Prevention [CDC], n.d.-a). All of these adjuvants serve to aid in the immunogenicity of the vaccine, hopefully providing a stronger response and longer lasting protection. The inclusion of adjuvants in vaccine formulation requires researchers to carefully assess where these added compounds elicit unwanted side effects.

Contrary to the aforementioned vaccines, nucleic acid vaccines do not inject any physical part of the pathogen into the body. Rather, these vaccines inject a lab synthesized DNA or mRNA sequence that codes for one or more antigenic proteins (NIAID, n.d.). Once inside the body, the nucleic acid is taken up by the virally targeted cells and instructs those cells to synthesize and secrete the desired protein. Only then, after the protein is in the body, will the body generate an immune response and acquire antibodies to that specific disease (NIAID, n.d.). With this method of inoculation, the body itself is an integral part in creating the immunogenic portion of the

vaccine. An mRNA strand alone will do nothing. However, when acted upon in vivo, the body completes the last step in the vaccine synthesis, creating the protein to which it needs to attain immunity.

Nucleic acid vaccines have a quicker production time than traditional whole-pathogen vaccines (NIAID, n.d.). This is because a pathogenic specimen does not need to be grown for the creation of the vaccine (Park, 2020). Under normal circumstances, the microbe can take a few months to grow to the desired quantity. Thus, nucleic acid vaccines may be favored over whole-pathogen vaccines, especially in situations where time is a consideration.

Additionally, though nucleic acid vaccines can be injected directly into the body, another vaccine delivery mode has developed over the years. Instead of injecting the DNA or mRNA directly into the body, options now exist to use a vector for introduction of the vaccine (NIAID, n.d.). A vector is a small particle that acts as a vehicle for vaccine delivery. A viral vector, such as adeno-associated virus, can incorporate the vaccine's genetic material by replacing some of its viral genes with the vaccine's desired sequence (Robert-Guroff, 2007). Studies have proven that viral vector administration is both safe and effective, though the quantitative values vary amongst the viruses (Robert-Guroff, 2007). There are also non-viral vectors, for example, liposomes and lipid nanoparticles (LNP), which can be used to deliver nucleic acid vaccines as well. One advantage of non-viral vectors, is that they are less immunogenic than their viral counterparts. With both forms of vectors, however, the target site in the body can be specified with the help of specific receptor molecules. Like this, researchers can guide and control the vector's integration and vaccine delivery within the body.

The Measles Vaccine

In 1954, just a year before the introduction of the inactivated polio vaccine, Thomas Peebles, MD, working in John Enders's lab at Boston Children's Hospital, succeeded in isolating the measles virus from the blood of 13-year-old David Edmonston (The College of Physicians of Philadelphia, n.d.). After isolating the virus, Enders's goal was to formulate a vaccine. It took until 1960 to prove that the isolated strain could be formulated into an effective attenuated measles vaccine; it just needed additional tweaking to attenuate it further (Hendriks & Blume, 2013). Wanting other researchers to also attempt to create a measles vaccine, Enders shared the Edmonston strain. Most researchers of that time were inspired by the recent success of the polio vaccine, and tried to mimic that development in their construction of a measles vaccine (Hendriks & Blume, 2013). In countries where Salk's inactivated polio vaccine (IPV) took credit for controlling the disease, like in Sweden and Netherlands, researchers worked at creating an effective inactivated measles vaccine. In other countries, such as the U.S. and U.K., where Sabin's recently released oral polio vaccine (OPV) was used to combat polio, researchers preferred to attempt an attenuated version of the measles vaccine.

Regardless of the method chosen, subsequent patient trials proved the attenuated measles vaccine to be more effective than the inactive vaccine (Hendriks & Blume, 2013). Some studies created several groups, each receiving a different vaccine regimen. While one group received only inactivated doses, another group received some inactive doses followed by a live dose, while a third group received one dose of the attenuated vaccine. Though several studies tried this method, all pointed to the same results: the inactivated vaccine initiated a lesser immune response and it was not known how long those antibody titers would last. The attenuated vaccine however, generated a substantial response, making it the vaccine of choice for elimination of measles (Hendriks & Blume, 2013).

It was in 1963 that John Enders and his associates received FDA licensure for their live-attenuated measles vaccine and mass measles vaccination began (CDC, n.d.-b). However, the vaccine was not attenuated enough and thus required coadministration of gamma globulins to prevent children from developing fever and a rash following inoculation (Hendriks & Blume, 2013). Approximately five years later, in 1968, Maurice Hilleman, working at Merck labs, developed Moraten—more attenuated Enders—which eliminated the need to inject the vaccine along with gamma globulins (Hendriks & Blume, 2013). Since licensure, Moraten, a descendant of the original Edmonston strain, has been the only measles vaccine administered in the U.S. (The College of Physicians of Philadelphia, n.d.). Even today, when the monovalent vaccine is no longer on the market, the Edmonston strain is still used to create the measles component of the MMR vaccine routinely given to children (CDC, n.d.-c; The College of Physicians of Philadelphia, n.d.).

Potential COVID-19 Vaccines

The COVID-19 pandemic, rampant now in early 2020, and possibly beyond, has killed hundreds of thousands worldwide in a matter of months (World Health Organization [WHO], 2020a). As of June 1, 2020, the WHO reports that there have been 371,166 deaths globally. Researchers all over the world are racing to develop a vaccine to combat the virus and stop the ever-rising death toll.

Luckily, there is an extensive history of vaccine development, allowing researchers to base their COVID-19

vaccine developments on the experiences and accumulated information from the past. More so, unlike the measles vaccine development, COVID-19 vaccine development has a related precedent. Coronaviruses SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome), while claiming no marketed vaccine, have documented research and experimentation on vaccine development. Current researchers are using these semi-constructed vaccines and adapting them for further development towards the current strain of coronavirus, SARS-CoV-2. However, regardless of the starting inspiration, all potential COVID-19 vaccines will have to go through all phases of vaccine production before being offered on the market.

As of June 15, 2020, there are scores of COVID-19 vaccine candidates starting the preclinical stage or clinical studies phase of vaccine development. These attempts include vaccine designs containing mRNA, DNA plasmid, protein subunit, non-replicating viral vectors, or inactivated COVID-19 viral particles (WHO, 2020b). Just the breadth of these alone shows how many more resources and technologies are available now versus the 1960s, when the measles vaccine was in development.

The more recent vaccine technologies, namely nucleic acid vaccines, demonstrates how the updated and increased methods are truly playing a role in COVID-19 vaccine development. In this paper, I would like to highlight one specific mRNA candidate vaccine. (An LNP-encapsulated mRNA vaccine, co-developed by Moderna and the NIAID.) As an RNA virus with RNA-dependent RNA polymerase, SARS-CoV-2 replicates RNA from an RNA template as opposed to transcribing RNA from a DNA template (using DNA-dependent RNA polymerase). This can result in high levels of RNA present within the virus (Wang, et al., 2020). Thus, using RNA as a method of priming the body towards an RNA-rich pathogen makes sense in the overall picture of vaccine development (Wang, et al., 2020).

Also, when developing this RNA vaccine, researchers opted to use the spike protein as the vaccine's target sequence. Though there are other targetable proteins on SARS-CoV-2, such as the envelope, nucleocapsid, and membrane proteins, the spike (S) protein is the subunit of the virus that binds with the body's ACE2 (angiotensin converting enzyme 2) receptor, and therefore the S protein comes across as a more effective vaccine target (Wang, et al., 2020; Zhang, et al., 2020). Figure 1 shows the location of all four proteins on the virus as well as the binding of the spike protein to the ACE2 receptor.

After injection of the vaccine which contains the mRNA sequence for the S protein, a cell in the body should translate the genetic code into the functional S protein that can be secreted to allow the immune system to respond. Figure 2 depicts the process by which this COVID-19 mRNA vaccine ultimately yields memory cells in the body.

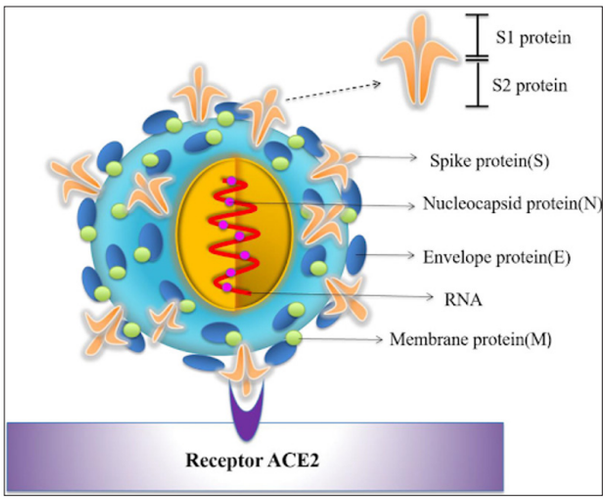

Figure 1 Proteins and Binding Site of SARS-CoV-2 Source. Zhang, et al., 2020

As of May 30, 2020, this candidate vaccine has advanced to phase 2 clinical trials (WHO, 2020b). Though mRNA vaccines have great theoretical potential, there are currently none on the market. However, this mRNA vaccine may successfully proceed through all phases of vaccine development, and should that happen, the Moderna/NIAID mRNA vaccine will be the first of its kind on the market

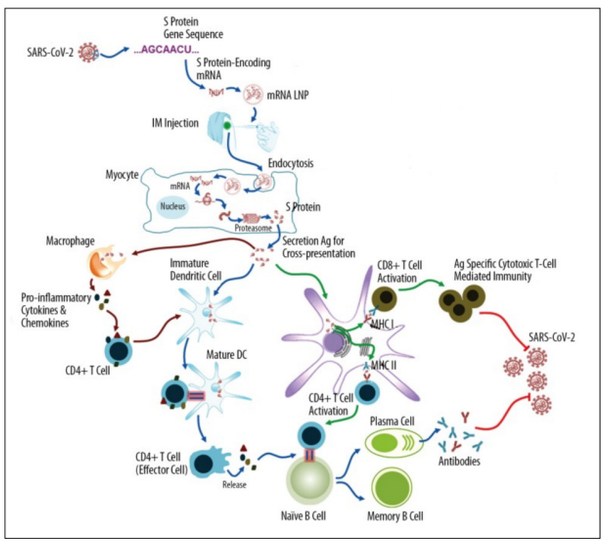

Figure 2 COVID-19 mRNA Vaccine Response

Conclusion

For the ongoing developments for a COVID-19 vaccine, it is unknown whether the vaccine's immunity will be lifelong. Currently, it is unknown if natural immunity is long

lasting, though whatever that case is, I would hypothesize that the artificially induced immunity would follow suit. In my opinion, though vaccine type does play a role in duration of immunity (as illustrated in measles vaccine development), a larger portion depends upon the properties of the virus itself. Since that is yet to be determined, it is too soon to draw a conclusion with regards to any COVID-19 vaccine. Also, because all vaccines are at most in early phase II clinical trials, one cannot determine which candidate vaccine will be more effective than the others. Currently, the mRNA vaccine from Moderna is on a road to success, though results are still too incipient to make a final decision.

Finally, although the essence of a vaccine is unchanged—the goal to elicit an effective immune response still drives development—advancements in vaccines development as well as accumulation of scientific knowledge have broadened our minds when attempting to develop a new vaccine. We now have more options, some of them with more precise targeting than ever, and it is the hope that with our newfound tools, we can go on to create better vaccines and continue saving lives.

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The Formation and Manifestation of Kidney Stones

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Kidney Stones

Nephrolithiasis is the process of the aggregation of certain minerals in the urine due to super saturation, thereby causing the formation of kidney stones. What complicates matters, is, that there is no single cause that can be attributed to Nephrolithiasis. In fact, as research progresses, an abundance of causative agents are being linked. Outlined below is a comprehensive summary of the occurrence, recurrence, treatment and causative factors of kidney stones.

Kidney stones usually form in the renal pelvis and pass through the urine unnoticed. However, at times these stones can grow up to several centimeters. These large stones can cause blockages in different locations in the urinary system and can be very painful. Stones that do not obstruct passages cause no symptoms besides hematuria (blood in the urine). Passing a stone causes renal colic. The pain intensifies as the stone progresses downward. Pain is felt as the stone passes through the ureter. When a stone reaches the uretero-vesicular junction it may cause dysuria and urinary frequency. Unfortunately, colic is felt regardless of body position and motion. Stone size is a big determinant of treatment option. Stones smaller than five mm in diameter have a high possibility of passing. Those that are between five to seven mm have a 50% chance of passage. Stones that are greater than seven mm almost always require medical intervention (Coe, Evan and Worcester, 2005).

The prevalence of kidney stones is dependent on many different factors, including gender. About 5% of American women will develop a kidney stone at some point in their life. By contrast, about 12% of American men are likely to develop a stone. The peak age in men is 30 years old. In women stones are most common between the ages of 35 and 55 with peaks at both 35 and 55. Previous stone formers have a 50% chance of forming a second stone within five to seven years of the first.(Malvinder,2004).

In recent years there has been an increase in the incidence of kidney stones. This has been associated with many different factors. For example; BMI, race, ethnicity and region of residence are strongly associated with stone formation. A seasonal disparity is also seen. Men have high urinary calcium oxalate saturation during the summer and women during the early winter. High levels of calcium oxalate in the urine are prone to solidify into stones. (Malvinder, 2004)

In the majority of cases, stones are passed without medical intervention. Even so, physicians will still prescribe strong pain killers to combat the typical pain that is experienced. In addition, anti-nausea medications as well as antibiotics may also be prescribed. Medicinal treatment is also an option. Alpha blockers may be prescribed, which relax the muscles of the ureter, allowing the stone to pass thereby mitigating the pain. If a stone is too large to pass, it may require a medical procedure to either break it up so that it can pass, or to completely remove it. In some situations, although the stone is small, it still requires medical intervention to remove it if it has lodged itself somewhere between the kidney and the urethra; usually in the ureters (Frassetto & Kohlstadt, 2011).

One method of treatment is using sound waves to break up stones. This is done through a procedure known as extracorporeal shock wave Lithotripsy (ESWL). ESWL shatters stones into very small pieces by use of shock (sound) waves that that create powerful vibrations. These stones can now pass more easily in the urine. This procedure is done under sedation or light anesthesia. It lasts around 45 minutes to an hour. Possible side effects include bruising on the back or abdomen and bleeding around the kidney and other nearby organs (Mayo Clinic, 2020).

Another method involves using a scope to remove stones. This is often done when the stones are smaller but have been lodged and can no longer pass on their own. The physician will pass a ureteroscope equipped with a camera through the urethra to the ureter. The stone will then be removed or broken into pieces. This is done under general anesthesia. Often a stent is placed in the ureter to relieve some of the swelling. The stent is left inside the ureter for a few days following the ureteroscopy to allow for further healing (Mayo Clinic, 2020).

When all other therapeutic options have been exhausted, a surgical procedure will be necessary. Smaller stones that did not respond to the aforementioned treatments and larger stones require surgery. This procedure is called percutaneous nephrolithotomy. It is the process of removing the kidney stone using small telescopes and other tools that are inserted through a small incision in the patient's back. The patient will be under general anesthesia at the time of the surgery and will remain hospitalized for a few days to recover (Mayo Clinic, 2020)

The Composition of Kidney Stones

Kidney stones are composed of different minerals that are found in urine. Calcium oxalate and Calcium phosphate comprise the composition of approximately 80% of stones. 19% are composed of struvite and uric acid. The remaining one percent are composed of cystine or ammonium acid urate. Kidney stones arise from the unwanted phase change of these substances from the liquid state to the solid state. The substances listed above exist in the urine as dissolved salts. However, at times, these salts can become super saturated. When a substance is super saturated no more of this solute can dissolve in solution and it will consequently precipitate out as a solid, forming hard granules. Super saturation is approximated by the ratio of

a substance's concentration in the urine to its solubility. At super saturation levels less than one, crystals of a substance will dissolve. But at super saturation levels greater than one, crystals can grow and form. The composition of stones correlates with the super saturation values from the urine produced (Coe, Evan and Worcester, 2005).

Calcium Oxalate Stones:
How/Why do they form?

The vast majority of Calcium Oxalate stone formers do not suffer from a systemic disease and are therefore described as idiopathic stone formers. In other words, they form stones spontaneously; the cause is unknown. The commonality found in many CaOx stone formers are disorders of calcium metabolism, resulting in abnormal levels of calcium. In addition, low urine citrate levels have been shown to permit CaOx stones. This is because urine citrate binds urine calcium in a soluble calcium citrate complex which reduces calcium super saturation (Coe, date unknown). However, when levels are low, calcium levels rise and ultimately stones may form. Furthermore, high urine uric acid excretion in men and women (above 750-800mg/d) is associated with idiopathic CaOx stones as well. These dissolved uric acid salts appear to reduce the solubility of calcium oxalate thereby promoting the formation of stones.

Disorders of Calcium metabolism range in severity and in the scope of their consequences. The most common cause of calcium metabolic irregularities is Primary Hyperparathyroidism, a systemic disorder in which an excess of Parathyroid hormone causes increased levels of calcium in the blood. In a healthy body, the kidneys lose calcium in the urine every day. In response, calcium receptors on the surface of the parathyroid glands cause the parathyroid glands to release PTH, a hormone that increases blood calcium levels. However, because of abnormalities, there is excess calcium and less reabsorption by the kidney leading to stone formation (Primary hyperparathyroidism).(Coe, date unknown). High calcium levels in the urine are also found in people with Idiopathic Hypercalciuria. Hypercalciuria results in increased intestinal absorption of calcium. High intestinal calcium absorption raises the amount of filtered calcium presented to the renal tubules in the kidney. It can also result in the reduction of the reabsorption of the renal tubules. Both of these mechanisms can increase the urinary calcium level. In certain instances, the excess calcium may solidify.

Other diseases linked to Hypercalciuria are of genetic natures. Dent disease is an X-lined hypercalciuric disorder found almost exclusively in males. In affected individuals, kidney problems result from damage to proximal tubules. The disorder is due to mutations in the gene (CLCN5) that codes for the voltage-gated endosomal chloride channel 5 (Heller & Pak 2002). Another type of genetic disorder is Barter Syndrome – specifically types I and II. In this syndrome renal calcifications are found in the cortex and the medulla. These calcifications come from problems in transport that reduce the capacity of the thick ascending limb of the kidney tubules which consequently reduces salt and calcium reabsorption. This results in a higher salt and calcium concentration leading to hypercalciuria (Heller & Pak 2002).

High Oxalate concentrations also lead to the formation of stones. Hyperoxaluria occurs when there is too much oxalate in one's urine. Hyperoxaluria can be caused by inherited-genetic disorders, because of intestinal disease or because of a diet of oxalate rich foods (Mayo Clinic, 2019). Primary hyperoxaluria is an autosomal recessive disorder caused by the overproduction of oxalate. This overproduction leads to calcium oxalate precipitation in the kidney and eventually to end stage renal disease (Ther, 2018). Enteric hyperoxaluria is caused by an increase in absorption of oxalate from foods. Several intestinal diseases including Crohn's disease and irritable bowel syndrome increase the absorption of oxalate from foods. This in turn increases the amount of oxalate excreted from the urine (Mayo Clinic, 2019). There are also many oxalate rich foods that can contribute to an overabundance of oxalate in the urine. These foods include beans, beer, beets, berries, chocolate, coffee, cranberries, dark green vegetables, nuts, oranges, sweet potatoes and tea. It has been proven that avoiding high oxalate foods can substantially help lower oxalate levels (Thomson, Husney, Romito, & Vachharajani, 2019)

Treatment

A new and developing form of treatment for CaOx stones is using a drug called Stiripentol. Stiripoentol is an antieplipetic drug used to treat children affected by Dravet syndrome. It has been shown to inhibit neuronal lactate dehydrogenase 5 enzyme. This isoenzyme is the last step in hepatic oxalate production and therefore can potentially reduce hepatic oxalate production and urine oxalate excretion. A young child affected by type I hyperoxaluria received this drug for several weeks, and her urine oxalate excretion decreased 66% (Dudal… Letavernier, 2019). Studies are still being conducted to ascertain the long term affects and the possibly harmful side effects that are accompanied by this drug.

In addition, a study was performed to see if there was an association between gut microbiome alterations and renal calcium oxalate stones. It was found that short chain fatty

acids may prevent stone formation. The research proposed that by regulating gut microbiome composition using short chain fatty acids, it may be possible to prevent kidney stones. Microbiome analysis was performed on 160 Chinese individuals some with recurring stones, some with one incidence of stone formation, and some non- stone formers. The results identified that recurrent stone formers and single time stone formers both exhibited higher fecal microbial diversity than the controls. While the mechanism of using short chain fatty acids to regulate gut microbiome is still unknown, the research has shown promising signs that this may be a cogent method to preventing and treating kidney stones (Wang…Li, 2019).

Recurrence

While there are many methods of treatment for kidney stones already in practice and many more in the development stages, recurrence of kidney stones reduces the effectiveness of many therapeutic methods. Following an initial stone event, the spontaneous five-year recurrence rate is 35 to 50 percent. As such it is imperative to institute a treatment plan that will change the whole nature of the kidney's function in order to be sure that renal calculi will not form yet again.

One such method is to alter the pH of the urine. Recently, the administration of potassium citrate has been considered as a therapeutic and preventative measure specifically for calcium oxalate nephrolithiasis. In a study conducted last year patients who underwent urolithiasis surgery were divided into subgroups. One subgroup in each category were given potassium citrate 40 mEq per day orally. The results were conclusive. The stone recurrence rate for the treatment subgroup was 1.72%, while it was 25.86% in the control subgroup. The difference was statistically significant. The treatment works because citrate inhibits the aggregation of calcium oxalate crystals by increasing the solubility by a mechanism mentioned above. It also inhibits urinary calcium stone formation due to change in urine pH. There are many other methods of prevention as well (Hosseini, Alipour, Omidbakhsh, and Ashraf, 2019).

Calcium Phosphate Stones:
Why/how do they form?

Calcium phosphate stones are not as common as calcium oxalate stones. Calcium phosphate stones may be caused by hyperparathyroidism, renal tubular acidosis, and urinary tract infections. Calcium Phosphate is present in urinary stones as either apatite, or brushite. What drives the development of brushite versus apatite stones is unknown. In patients of calcium phosphate stones, plugs of apatite fill the lumens of the terminal collecting duct. Epithelial cells are damaged and destroyed and around affected tubules, the interstitial area is inflamed and scarred. Besides for being hypercalciuric because of idiopathic hypercalciuria Calcium phosphate stone formers have a distinctive feature- their urine has a higher pH. This favors calcium phosphate crystallization by increasing the abundance of urine mono-hydrogen phosphate, the ion that combines with calcium. Urine pH rises progressively with increasing calcium phosphate percentage in stones. The mechanism of increased urinary pH in Calcium phosphate stone formers is unknown (Frassetto & Kohlstadt, 2011).

Remarkably, Pregnant women have a double than average chance to form calcium phosphate stones compared with age-matched nonpregnant women. They are two to three times more likely to have calcium phosphate stones over oxalate stones. This can be attributed to the fact that women have an increased glomerular filtration rate and have higher urinary calcium during pregnancy. In late pregnancy, the urine tends to have a higher pH which can predispose women to calcium phosphate stones. Kidney stones during pregnancy increase the risk of urinary tract infections. Also, pregnant women with renal colic have a 50% higher risk of early delivery compared to women who do not have kidney stones (Frassetto & Kohlstadt, 2011).

Treatment

Thiazide diuretics such as hydrochlorothiazide can help the kidney absorb more calcium, leaving less of it in the urine where it can form stones. Thiazide diuretics are a type of diuretic drug. Diuretic drugs increase urine flow. They act directly on the kidney and promote urine flow by inhibiting the sodium/chloride cotransporter located in the distal convoluted tubule of a nephron. Thiazide decreases sodium reabsorption which increases fluid loss in urine. Potassium citrate is another medication that can bind to calcium and help keep calcium phosphate in the urine from forming stones (Mayo Clinic, 2019).

Brushite stones (calcium phosphate stones) are among the hardest to break, which, combined with the large size of the stones, results in poor outcomes with shock wave lithotripsy (SWL). In an experiment conducted, several shock waves were needed to break brushite stones. It was also discovered that pure brushite stones are harder to break than brushite stones that contain a mixed composition. Shock wave lithotripsy is not the optimal treatment for brushite stones. However, invasive imaging is needed to determine a stones composition and so it remains a challenge in deciding the best treatment plan (Williams… McAteer, 2012).

Uric Acid Stones:
Why/how do they form?
Uric acid stones form because of abnormally low-acidic pH of urine a pH below 5.5. low urinary volume and hyperuricosuria are also associated with the formation of uric acid stones, (Van Hattum, de Bie, and Somani, 2019). Low urine pH can be partly ascribed to low ammonia excretion. Low urinary pH and uric acid stones are common in patients with an assortment of disorders. Patients with high BMI, gout, diabetes mellitus, and the metabolic syndrome, may have reduced renal ammonia excretion. The reduced ammonia excretion may be due to insulin resistance which is common in these diseases. In addition, urinary pH is inversely proportional to body weight. As body mass increases, urinary pH falls and becomes more acidic. Chronic diarrhea also lowers urinary pH and causes uric acid stones. Uric acid gravel can obstruct ureters and produce acute anuric renal failure. Uric acid stones can fill the entire renal collecting system. Uric acid pebbles and stones are often orange or red, as they have absorbed uricine, a pigment of bilirubin breakdown. (Van Hattum, de Bie, and Somani, 2019).

Treatment/Recurrence
Prevention and even dissolution depend on the three main components of uric acid stone formation; an increase of urinary volume, prevention of hyperuricosuria and increase in urinary pH. By increasing daily fluid intake the urinary volume increases thereby decreasing the concentration of uric acid. There are different medications that can increase the pH of the urine thereby minimizing the risk of supersaturation and consequently stone recurrences. Medications such as potassium citrate, sodium citrate and sodium bicarbonate have been proven to increase the pH of urine (Van Hattum, de Bie, and Somani, 2019). Allopurinol is not usually required; however it can be used if one of the above three medications does not suffice (Coe, Evan, and Worcester, 2011).

Cystine Stones:
How/ why do they form?
Cystine stones are the most rare form of kidney stones. They constitute less than 1% of urinary tract stones. Cystine stones are often caused by Cystinuria. About 1 in 7000 people worldwide have cystinuria. Most people with cystinuria get their first stones in their twenties or thirties, but 30% get them in their teens. According to some research, 8-10% of kidney stones in children are cystine stones. Cystinuria is a genetic condition causing mutations in renal epithelial cell transporters that result in reduced reabsorption. This leads to an increased urine excretion of the dibasic amino acids, including cystine. Because of the high super saturation of cystine, stones form (Coe, Evan and Worcester, 2005).

Treatment
Cystine stones can be removed in the same manner as other kidney stones. They can be removed by nephrolithotomy, a procedure where an instrument is inserted through the skin and into the kidney to either take the stone out or break it apart. Ureteroscopy is another method. It involves sticking a tiny instrument into the bladder and then up the ureter to remove the stone. A third method, is Extracorporeal shock wave lithotripsy (ESWL). This is a procedure that uses shock waves to break up larger stones into smaller pieces so that they are passable. This method however, does not work as well for cystine stones as it does on other types of stones (Frassetto & Kohlstadt, 2011).

Recurrence
Being that Cystinuria is a lifelong condition, the recurrence of cystine stones is quite high. To combat this, the patient must be sure that the super saturation concentration of cystine is below one. To begin with, patients try to attain a urine volume of three to five liters a day which can dissolve the cystine that is excreted. Some also require a medication to raise the pH of the urine. In addition, reduction of sodium and protein intake reduces cystine excretion in the urine. It is for this reason that attention to diet is an essential part of the treatment process. If increasing fluid levels and lowering the pH of the urine by diet does not work, there are medications that can be used. D-penicillamine, α- mercaptopropionylglycine, or captopril, all form soluble heterodimers with cysteine and so they can be used. However, these medications may have severe side effects such as loss of taste, fever and proteinuria, and so the former mentioned methods are more desirable (National Kidney Foundation, 2016).

The Effect of the Seasons
When attempting to connect seasonal changes with occurrence of kidney stones a huge number of factors must be taken into account. There have been many studies done on this topic and the results have been confusing. In one study, research has shown no difference in urine volume from the summer to the winter. In another study, during the winter months when vitamin D intake is limited, urinary calcium excretion was found to be much higher. This being the case, it would seem that kidney stones would more likely form in the winter (Attalla, De, Sarkissian, and Monga, 2018) .

Dietary patterns may fluctuate as well with the changing

seasons. With a reliance on packaged and processed food during the winter, urinary sodium may be increased. With an increase in urinary sodium, urinary calcium will subsequently increase as well. Also, weight fluctuations may complicate the analysis further. With less opportunities for physical activity during the winter months, coupled with the indulgent eating during the holiday season, urinary calcium may be increased. Also, lower levels of vitamin D have been associated with weight gain (Attalla, De, Sarkissian, and Monga, 2018) . All these factors point to the winter months having a high propensity for stone formation.

In contrast to the research above, William Haley, a Mayo Clinic nephrologist, has found that heat, humidity, and lack of proper hydration all lead to a higher prevalence of kidney stones in the summer. In the summer there is a greater likelihood of dehydration and thereby the possibility of a decrease in urine volume. This would consequently raise the possibility of nephrolithiasis (Sparks, 2015).

Socioeconomic Status

A study was done to ascertain if socioeconomic status can affect nephrolithiasis. The study used the Distressed Communities Index (DCI) which takes into account employment status, education level, poverty rate, median income, and business growth. Diabetes and hypertension were more prevalent in the group of people from severely distressed communities. Patients from these communities needed more invasive treatment and 13% needed staged surgery versus 9% from the non-severely distressed communities. Men from the severely distressed community had a significantly larger stone size; 12.5mm vs 9.7mm. The men also had a higher prevalence of stones greater than 20 mm. Interestingly, there was no difference in stone size between the two different groups of women. Socioeconomic status seems to correlate with stone size, but appears to have more of an effect in men than in women(Quarrier, Li, Best, Hedican, Penniston, and Nakada, 2020)

Obesity

Obesity contributes to the risk of kidney stones more than dietary factors. Maladaptive changes associated with obesity are disturbed thermogenesis and dehydration. This is because body fat is hydrophobic, so the proportion of body water decreases with increasing obesity and this can lead to dehydration. Also, the decrease in body surface area compared to body volume complicates heat exchange and metabolic functions. Obesity is often associated with electrolyte imbalances and altered urine chemistry. All evidence points to obesity being a great risk factor for kidney stones.

It would seem logical and apparent that weight loss would be the solution to the abovementioned complications. However, it is not that simple. Weight loss has to be done in a healthy and reasonable fashion. A high animal protein diet, quick loss of lean tissue, or insufficient hydration can actually increase the likelihood of kidney stone formation. Diets high in acid can increase the risk of uric acid stones. The patient must make his or her dietary choices in an informed manner taking into account, his specific propensities to stone formation (Frasseto & Kohlstadt, 2011)

Serial soda drinkers may be at an increased risk for kidney stones. An experiment was done with a sample of males who ingested 200 grams of fructose daily for two weeks. Research showed that fructose intake greatly increases the chances of kidney stones. This is because fructose affects urate metabolism, urinary pH and affects oxalate. Since fructose is present in so many of the foods that the average American consumes, it can play a major role in increasing the risk of forming stones. Moreover, a randomized trial stated that a 10% reduction in stone recurrence for those who could reduce their soft drink intake to less than 24 ounces a week (Johnson, Perez-Pozo Lillo, 2018)

Occupation-"You are what you do"

Studies have shown that people with certain occupations have a higher probability of developing kidney stones. Some forms of work don't allow for proper fluid intake leading to a saturated urine concentration. Individuals who are more active when they work, especially if they work outdoors, perspire more, leading to more concentrated urine. However, those who have more sedentary jobs are also in danger. Since they have a higher chance of metabolic syndrome, they also have a higher risk of forming stones. Since Astronauts work in environments without gravity, they assemble calcium from their bones, leading to greater calcium levels and consequently a higher risk of stone disease (Malieckal & Goldfarb, 2020).

Removal of Kidney Stones- A Wild Ride

In 2016 two physicians came up with a rather unique method to dislodge a kidney stone after hearing about this interesting phenomenon from their patients. One patient reported passing renal calculi after each of 3 consecutive rides on the roller coaster. Many other patients reported passing renal calculi within hours of leaving the amusement park, and all of them rode the same roller-coaster during their visit. They used three renal calculi, based on real life measurements. The renal calculi were suspended in the urine model and taken for 20 rides

on the Big Thunder Mountain Railroad roller coaster at Disney World in Orlando. When the model was sitting in the front seat of the roller coaster, there was a passage rate of 4 out of 24. When sitting in the back there was a passage rate of 23 of 36. Sitting in the rear, different kidney stone location had different passage rates. An upper calyceal calculi passage rate of 100%, a middle calyceal passage rate of 55.6% and a lower calyceal passage rate of 4%. Although the data written above is encouraging, physicians must determine if a roller coaster ride is the correct form of treatment for their patients, as size and location may complicate the situation (Mitchell &Wartinger, 2016).

Corona Virus and the Kidney:

There has been emerging connections between corona virus and kidney failure but why this happens is still unclear. Many doctors have proposed different theories. One theory is that the virus directly attacks the kidneys by its way of infiltration. A study published in March shows that Corona virus infiltrates the body by binding to the ACE2 cell receptors. The kidney contains these ACE2 receptors, and in this way is under direct attack. Yet another theory states that the kidneys are affected secondary to the virus. Because the lungs are so hard hit, the body is unable to deliver adequate oxygen to the body. In turn organs are damaged and functionality is reduced. The virus also effects the blood, which can lead to clots. The kidney, being the biggest blood filter in the body is not susceptible to blood clots within its thousands of capillaries and vessels of filtration. Also blood clotting has an effect on the body's immune system possibly triggering a cytokine storm in which the body attacks its own cells and tissues. Furthering the potential damage to the kidneys and other essential organs (Edwards, 2020)

Conclusion

As more research is done on kidney stones it becomes abundantly clear that even more research is needed. There are an innumerable amount of factors to take into account when determining the best course of treatment for persons with renal calculi. The patient's occupation, ethnicity, BMI, family history, and region of residence are all contributing aspects. Stone composition may be very varied from case to case. Treatment that was successful for one may be detrimental for the other. There is no one-size-fits-all solution. Each case is unique and requires its own analysis and diagnosis. One thing is universal though, sufficient hydration is essential for the prevention of kidney stones.

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Is Fecal Microbiota Transplantation a Safe and Effective Treatment for Gastrointestinal Diseases?

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Abstract

Fecal microbiota transplantation (FMT) is a method of transferring feces from an individual with a healthy microbiome to a patient whose healthy gut bacteria is deficient. While this method is not a new one, it is constantly being explored and studied to determine if it can be an effective way to treat patients with different bowel diseases. The main target of most of these studies are patients with recurrent Clostridium difficile infections. Many studies were done to determine if the method is safe, and which method is most effective, as well as who can be a good donor or recipient of the treatment.

Introduction

The large intestine is comprised of the cecum, the colon, the rectum and the anal canal. The colon is the last step in the digestive tract before the feces are expelled through the anus in a bowel movement. It is a tube-like structure containing the usual four layers; the mucosa, submucosa, muscularis and serosa. Problems can occur if the mucosa becomes inflamed or infected. Bacteria colonize the large intestine and aid in the digestion of proteins and other food particles to ready them for defecation. Bacteria also play a role in the homeostasis of the gut (Tortora, 2014).

The colon is packed with bacteria that make up the human microbiome. These are symbiotic organisms that perform different functions in the human body. Research has shown that many are important for metabolism and epithelial cell growth, doing jobs that are similar to those of the endocrine system. It seems that the microbial diversity turns out to be significantly higher in adults than children, and even more so in that of the elderly. Infants are found to have an extremely low diversity. (Blaut, et al., 2002)

The many commensal bacteria in the gut influence immune function. There are a few different ways this could be true. One of these is that the bacteria can promote the health of the epithelial lining in the intestines. This strengthens the primary barrier, so that pathogens cannot pass through the lining. In addition, some of these good bacteria can help produce and secrete an anti-inflammatory response to some inflammatory cytokines. Other mechanisms include the production of secondary bile acids, and the competition for nutrients with the pathogenic bacteria (Zeng et al., 2019).

In people with a healthy gut composition, there are a different kind of bacteria that contribute to the symbiosis of the gut. These bacteria serve as an anatomical barrier to the different pathogens that enter the intestines through various ways such as with food that is ingested. The four main phyla that are found are Firmicutes, Bacteroides, Proteobacteria and Actinobacteria. (Lopetuso et al., 2013).

In a normal bacterial gut composition, there is a symbiosis in which the bacteria work together to perform different functions. Dysbiosis occurs when there is an imbalance of the different gut bacteria, often resulting in disease such as inflammatory bowel diseases (IBD), and other gastrointestinal disorders. One common disease is infectious colitis caused by C. difficile. Others include gastritis, peptic ulcer, irritable bowel syndrome (IBS) and even gastric and colon cancer. (Lopetuso et al., 2013).

Methods

Research for this paper included using databases such as the Touro College Library, PubMed and Google Scholar. These were used to find peer reviewed articles on studies related to gastrointestinal diseases and FMT as a treatment.

What is C. Difficile Infection?

As a result of taking antibiotics, many patients are left with a decrease in gut bacteria diversity, namely of the commensal Bacteroidetes and Firmicutes variety. At the same time, there tends to be a proliferation of proteobacteria which include pathogenic organisms such as C. difficile and shigella (Staley et al., 2016). C. difficile, an opportunistic organism can now cause infection because it is not outcompeted by good bacteria for nutrients and other factors that help bacteria thrive. In addition, as mentioned above, there is a reduction in the bacteria that normally prevent the proliferation of these pathogens by different mechanisms. In the majority of cases, C. difficile is treated with the antibiotic vancomycin or other similar drugs. Often, this course of antibiotic therapy is ineffective, resulting in recurrent C. difficile infections. Fecal microbiota transplantation attempts to address the problem by allowing the feces of the healthy donor to aid in replenishing the depleted gut bacteria of the patient. This leads to the patient's stool bacteria becoming very similar to that of the healthy donor (Kelly et al., 2015).

Secondary Bile Acid Production by Bacteria

There is evidence that suggests that secondary bile acid production is a crucial factor in preventing C. difficile infections. There appears to be a difference in the bile acid composition in the colon of patients with C. difficile infections in comparison to those with a healthy gut composition. A bile acid analysis study was done on fecal extracts of both donors and recipients of FMT. The results demonstrated that the pre-FMT samples contained mainly primary bile acids and bile acid salts and did not contain any secondary bile acids. In contrast, the post-FMT samples, as well as the

donor samples contained a high abundance of secondary bile acids, indicating that the bacteria from the healthy donor had an effect on secondary bile acid production. These factors seem to indicate that it is indeed the metabolic function of these bacteria that aid in preventing C. difficile infections (Weingarden et al., 2014).

Primary bile acids are produced in the liver. The main ones are cholic acid and chenodeoxycholic acid. Before being secreted into bile, the bile acids are chemically attached to the amino acids taurine and glycine to form bile salts. These are then secreted into bile and then used for lipid digestion in the small intestines. While most of these are reabsorbed, about 5% of them reach the large intestine where they subsequently become deoxycholic and lithocholic acids which are secondary bile acids. As mentioned before, post FMT and donor samples contained mainly secondary bile acids and few primary bile acids. Some studies have inferred that the primary bile acid taurocholic acid (cholic acid combined with taurine), is actually a requirement for C. difficile growth and proliferation, and is used in growth media for this organism. It was also noted that the secondary bile acids lithocholic and ursodeoxycholic acids (from chenodeoxycholic acids), inhibit the growth of C. difficile (Weingarden et al., 2014).

In a study done, it became apparent that the Clostridium scindens, of the firmicutes phylum, is the bacteria that is likely responsible for preventing infection due to its role in secondary bile acid production (Staley et al., 2016).

The bile acid experiment was done in conjunction with the bacterial diversity survey. There was a correlation between the two studies, showing that an increase of secondary bile acids came along with an increase in Bacteroides and Firmicutes. These experiments indicate that it is likely these commensal bacteria play a metabolic role in bile acid production, inhibition of the pathogenic C. difficile, and overall gut health (Weingarden et al., 2014).

Who is a Good Fecal Donor?

It is not completely clear who makes a good donor for FMT. It is assumed that it would greatly depend on the donor's lifestyle and diet. Though it was noted during one study that there were good outcomes from one donor in particular, it was hard to trace exactly what made that donor so effective. In addition, many FMT trials are done with multi-donor stools for increased diversity. This would cause the researchers to be unable to isolate which donor made it most effective (Quraishi et al, 2017).

There is indication that relatives are beneficial as FMT donors because they share genetics, and are likely to have a similar microbiome to the patient. Others say that it is beneficial for a patient to receive FMT from his or her spouse because they likely have been in contact with similar pathogens and therefore may be protected from harmful effects of introducing many new bacteria to the gut. Some think that an unrelated donor is better, because they feel that someone who is not related would be more honest in answering questions that may rule them out from donating. There is not enough conclusive evidence to determine if any of these are indeed true (Kelly et al, 2015).

In gathering the donors, there is a list of exclusion factors that can rule out those who should not donate stool. These include those who have been on antibiotics in the past 3 months, have had any history of a disease that is normally transmitted by stool, or who are known to have IBD, IBS or other gastrointestinal complications or conditions. In addition, those who have had a history of autoimmune diseases, malignant diseases, chronic pain or metabolic syndromes cannot donate feces. The reason for this is because it is assumed that people who have these conditions may have an altered microbiome which might be detrimental to introduce to a patient who is already compromised (Kelly et al, 2015). In addition, someone who has an autoimmune disease is likely being treated with immunosuppressants which suppresses the body's immune system. If this was introduced into the recipient, this could be especially harmful because there is already an infection in the patient that needs to be treated, and they must have an active immune system. Additionally, it is possible that certain DNA which causes these conditions would be present in the stool and it would be very risky to introduce to the patient (Petrov, M. E., 2011). Once they have fulfilled the above requirements, they are screened for infections within a month of donating and can then donate stool (Kelly et al, 2015).

Screening Before Donations

The specific tests done to screen a donor include those that play a role in metabolic function and the digestive system in particular. Some of these include a complete blood count, as well as a c-reactive protein test which checks for inflammation within the body. In addition, they are tested for levels of creatinine and liver enzymes. The stool itself is screened for ova and parasites and C. difficile in particular, among other intestinal pathogens (Satokari et al, 2015).

Methods of Preparing Feces for Transplantation

The transferring of feces can be done by different procedures. Those include transplantation with an oral capsule, colonoscopy or by enema. (Kao et al, 2017). There is also debate about whether it makes a difference if the feces

were previously frozen, or if fresh stool is used. Results of trials show that there is little difference in effectiveness between these methods. Therefore, it is suggested that frozen samples be used simply because they are easier to produce and obtain (Satokari et al, 2015).

In addition to what was mentioned above, using frozen stool allows for universal donors. That means that when a potential donor is screened and found to be a good candidate, his feces can be used for multiple patients. This would also allow clinics to store the frozen feces and use them as needed (Satokari et al, 2015).

Fresh Stool for FMT

When using fresh stool for FMT, the stool must be used within a 6-hour window of time, from defecation to transplantation. With this method, approximately 30 grams of stool is mixed with 150 ml of tap water and then administered to the patient within 15 minutes of preparation. This method is very limiting, and not as readily available as frozen stool (Satokari et al, 2015).

Preparing Fecal Slurry for Colonoscopy with Frozen Stool

There are slight variations in methods of preparing frozen stool for FMT. The following is one of them:

Fresh stool is collected and stored in units of 80-100 g. To each collection, 200 ml of .9% saline is added. The resulting mixture is filtered through a stomacher bag, designed to keep samples uncontaminated, yielding 180 ml of fecal slurry. Mixing the slurry with 20 ml of 100% glycerol allows it to be frozen at -70 degrees Celsius for 2 months, until needed. For use, the slurry is defrosted overnight at 4 degrees Celsius and then reconstituted with 160 ml of .9% saline. (Kao et al, 2017)

In using fresh or frozen stool, one method of administering it is through a biopsy channel, a piece of flexible tubing in the cecum (Satokari et al, 2015).

Preparing Feces for Capsule Manufacturing

Forty ml of 100% glycerol is added to 200 ml of prepared fecal slurry and centrifuged for 20 minutes at room temperature and 400 G (gravitational force). After decanting the supernatant, the sample is then centrifuged at 4-8 degrees Celsius and 10,000 G by high speed centrifuge. The remainder of the sample is mixed to yield about 12 ml of sample containing 10^13 microbes by estimation. This is then pipetted into size No. 1 gelatin capsules which are then encapsulated twice by size 0 and then 00, yielding 40 capsules. These capsules are then flash frozen at -55 degrees Celsius on dry ice to preserve them. Just like the fecal slurry, the capsules can remain stored at -70 degrees Celsius for 2 months. To administer FMT, the patient swallows approximately 40 capsules within a short period of time (Kao et al, 2017).

Synthetic FMT

Vos W.M writes that synthetic FMT is likely a good alternative to the common use of donor stool. Synthetic FMT is a lab produced combination of the needed strains of bacteria, without the actual stool of the donor. The reason for this is because by the time the donor stool reaches the patient, many of the organisms are no longer viable. In addition, stool contains other wastes, mucus and pathogens. Vos suggests that administering synthetic microbiota which was cultured to get the right composition of bacteria is not only more effective, but less likely to have adverse effects as well. Synthetic FMT would not contain all the parts of the stool that are unnecessary as well as detrimental to the patient. In addition, it would allow for greater viability and microbial diversity as well as being able to be manufactured and reproduced (Vos, 2013).

Effectiveness in Treating C. difficile Infections

In order to determine effectiveness, a DNA sequencing test is performed to determine which bacteria were colonized in the gut of the patient post FMT treatment. In studies that were done, the patients who were treated with heterologous stool samples- that of a donor showed a greater diversity in stool microbiology than those who were treated with autologous samples- their own, i.e. the placebo group. In the heterologous stool samples, there was a significantly higher presence of Bacteroidetes and firmicutes, indicating that these are the phyla that should be present in healthy stool. In contrast, those who were treated by placebo did not contain an abundance of these bacteria. Instead, they presented with more of the Clostridium XIVa clade and Holdemania bacteria that were thought to take part in causing the C. difficile infection. Results from this study seem to imply that a complete engraftment- proliferation of all the bacteria, is not necessary so long as the needed bacteria are present. (Staley, et all ,2016)

In a systematic review by Cammarota, Laniro, and Gasbarrini, A. indicates an 87% success rate when comparing the data of numerous studies. The rates of diarrhea resolution varied depending on the site of the fecal transplantation. The data indicated a rate of 81% in the stomach, 86% in the duodenum-jejunum, 93% in the cecum-ascending colon, and 84% in the distal colon. (Cammarota, et al, 2014)

A randomized trial was performed on 46 patients with 3 recurring episodes of C. difficile. All of these patients

were treated with vancomycin without success. During the study, all the patients were treated by colonoscopy. Some of the patients were treated with donor stool, while others were treated with their own autologous stool. Those treated with autologous stool were checked to determine if the donor stool was indeed more effective, or similar to the placebo effect. Of the 22 who were treated with donor stool, 20 had a full recovery from C. difficile, accounting for a 90.9% recovery rate. In contrast, of the 24 who were treated with their own stool, only 15 recovered, which is a 62.5% recovery rate. The patients who contracted another C. difficile infection following FMT with autologous stool were subsequently treated with donor stool, with success. The outcome of this study seems to indicate that FMT is a safe, effective way to treat recurrent C. difficile infections (Kelley et al, 2016)

A study was done to determine whether transplantation by colonoscopy or by oral capsule was more efficient. The outcome of the study seems to indicate that neither one is preferable and both were effective. The factors to consider were those that an oral capsule is both less invasive and cheaper to administer. (Kao et al, 2017)

FMT for Ulcerative Colitis

Ulcerative colitis is a condition in which the mucosal layer of the colon is inflamed. Patients with this condition present with bloody stool, anemia and abdominal pain (Costello et al, 2019). A trial was done in 3 hospitals, treating 85 patients to see if FMT could work to cure ulcerative colitis in addition to the known effects of treating C. difficile infections. In this trial, patients were treated with stool from multiple donors to increase the biodiversity. There was also a placebo group. In both groups color and odor was added so that the patients would not know if they were receiving the real transplant. The original infusion was administered by colonoscopy, directly into the terminal ileum and caecum. The patients were monitored periodically for 8 weeks (Paramsothy et al., 2017).

Though there was not a significant majority, 27% of people who received donor FMT saw a relief of symptoms, while only 8% of placebo patients saw these effects. After the 8 weeks however, the numbers on both sides increased. It was observed that a particular bacterium of the Fusobacterium variety was present in those who did not have a remission of symptoms after FMT. It is important to note that majority of the patients in the placebo group had milder symptoms to begin with. The outcome of the study indicated that FMT may be a good alternative to usual steroidal therapies used for ulcerative colitis, though more research would need to be done to determine if that is indeed the case (Paramsothy et al., 2017).

A study was done to determine if anaerobically prepared stool would be as effective as aerobically prepared FMT for ulcerative colitis. The purpose of the trial was to determine if the organisms in the stool would be more viable if they were prepared anaerobically. This study was done after the study mentioned above by Paramsothy. Based on data that was collected from the above study, researchers were hopeful that FMT could be very effective in ulcerative colitis patients. This study was a variation of the first one. The outcome of the study indicated that 32% of those who received donor FMT saw an initial relief of symptoms. Nine percent of those who received autologous stool saw an initial relief of symptoms as well. (Costello et al, 2019). This data seems to be very similar to that of the aerobically prepared FMT. More trials would be needed to determine if anaerobically prepared stool is actually more effective. This study adds to the research that FMT is helpful in some cases for ulcerative colitis.

FMT for IBD

IBD is a general term for different inflammatory bowel diseases. Included in these are ulcerative colitis and Crohn's disease. As mentioned above, these diseases cause chronic inflammation of the bowel. Either of these conditions can cause colorectal cancer, and other medical problems in the individual. There is evidence that suggests that it is the microbiome gut composition that plays a role in these diseases. After seeing great success in treating recurrent C. difficile with FMT, researchers are hopeful that it can work as a therapeutic treatment for other conditions as well (Quraishi et al., 2017).

According to studies, it seems that IBD can be an abnormal reaction to having microbiota in the gut. Patients with this condition possess genes that view these enteric bacteria as pathogens, even those that are considered symbiotic in a healthy person. Evidence to support this study included testing on germ-free animal models that were predisposed to IBD. In addition, there were studies done in which the fecal stream was diverted. In both of these studies, it appeared that not having any bacteria present in the gut led to having no inflammatory symptoms. The activation of innate and adaptive immunity by bacteria that is normally nonpathogenic can lead to an inflammation in the gut in the absence of pathogenic bacteria (Quraishi et al., 2017).

It is noted by Quraishi et al., that though it seems that any presence of bacteria induces IBD in those that are predisposed, there are studies done that suggest that there is indeed a difference in microbial diversity in those with IBD in comparison to those with a healthy gut (Quraishi et al, 2017.) The composition is characterized by an increase

in bacteria which include Enterobacteriaceae, Pasteurella, Veillonella, and Fusobacteria, and also contains less Firmicutes, Bacteroidetes, and Clostridia (Gevers et al., 2014). It is also noted that there is an increase of Proteobacteria in those with IBD. Using FMT with IBD has been met with very limited success in the past. There has been more evidence of success with ulcerative colitis patients, than in those with Crohn's disease (Quraishi et al, 2017).

FMT for Hepatic Myelopathy

Hepatic myelopathy is a neurological complication that comes from severe liver disease. Usually, it can only be treated by a liver transplant. There was a case study done, however, that indicated that FMT may indeed be a solution to this illness. The study was done on one 45-year-old woman in china. She was admitted to the hospital numerous times, and underwent various procedures. The reasons for these included different complications such as splenomegaly, vomiting blood and other internal bleeding. The last visit was due to a weakness and stiffening in her legs. Interestingly, after being treated with 3 courses of FMT, she reported a relief of symptoms. There is no conclusive evidence that this can work in all cases. However, due to the known correlation between gut symbiosis and neurological health, the researchers say that FMT may be a good therapeutic alternative to a liver transplant as it is more available and less invasive (Sun et al, 2019).

Adverse Effects of FMT

In a randomized trial done, it was noted that there were no serious adverse effects following fecal microbiota transplantation. The study did not include anyone who was immunocompromised, or above age 75. Following FMT the patients were monitored periodically for 6 months, and none exhibited any serious symptoms in relation to FMT (Kelley et al, 2016).

Kao et al reports that two patients died following FMT, though it was not likely due to the procedure. Both had significant cardio-pulmonary disorders and both were elderly (Kao et al, 2017).

While FMT has seems to have many beneficial uses, as with any therapy, there are downsides as well. Dr. Schwartz, Gluck and Koon say that a reason for this may be because though fecal donors go through extensive screening, it is possible that they were carrying diseases that they were unaware of. They could have been asymptomatic and not have been tested for that specific pathogen. There were two cases noted of norovirus in Virginia Mason Medical Center in Seattle. Norovirus is a common contagious virus that causes gastroenteritis in many people. In those cases, the donors were asymptomatic and it was unknown whether the virus was transmitted through the feces as there was a window of time between the collection of the feces and the actual transplantation. The most significant effect of these cases was that both patients presented with a relapse of C. difficile infections. It was speculated that it is possible that the norovirus altered the bacterial community present in healthy stool resulting in an ineffective FMT (Brandt, L. J., 2013).

In an FMT trial on ulcerative colitis patients, a significant amount reported adverse effects. Approximately 78% of the donor stool recipients and 83% of the placebo recipients reported gastrointestinal upset. The complaints were self-limiting, resolving on their own and having no long-term effects. In addition, 2 patients who received donor stool, and 1 who received autologous stool as a placebo presented with serious adverse effects (Paramsothy et al, 2017). The study did not mention what they were.

Quraishi et al. note that FMT should not be administered to those who have Crohn's disease with deep patch ulceration in the gut. The reason for this is because it is likely that the bacteria may translocate. That is, the bacteria may cross the organ barrier and enter into the bloodstream where it does not belong. Administering FMT in those patients could cause many harmful effects. It is different however for ulcerative colitis because in that condition the inflammation is contained to the epithelium and risk of bacterial translocation is not high (Quraishi et al., 2017).

In a letter written to the editor of The American Journal of Gastroenterology, Dr. Brandt presents his observations that there are effects of C. difficile that are not yet known. He noted however that it is reasonable to assume that down the line there would be adverse effects. Those may include short term affects like allergic reactions or transmitted infections. He speculates that it is likely that FMT patients may suffer long term effects such as conditions that result from an altering the recipient's microbiome to be similar to that of the donor (Brandt, L. J., 2013). It is interesting to note, however, that this was written in 2013, and articles written in later years did not report these predictions.

Why Not Oral Probiotics?

People think that probiotics are helpful for the gut microbiome. The reason for this assumption is that if some bacteria are considered good, an increase in those bacteria should be considered helpful to a person's overall wellbeing. Research has shown, however, that this may not necessarily be the case. It was noticed that certain cancer patients were not responding to immunotherapy and that majority of those patients were taking probiotic

supplements. There is evidence that taking a probiotic supplement reduces the biodiversity in the gut. Though it does cause an increase in certain essential bacteria, those bacteria in large quantities may be harmful to other systems. For example, an increase in Bacteroides is said to increase metabolism, but seems to be detrimental to the immune system (Hardy, L., 2019).

There is also research that indicates that taking probiotics after a course of antibiotics actually slowed the body's response in replenishing the gut. In this study, three groups of patients were given antibiotics. One group was not subsequently treated, another was given probiotics, and the third was given autologous FMT with stool collected before they were given antibiotics. Evidence from this study indicated that those who were given probiotic supplements took significantly longer to recover than the others. This study also seems to show that FMT is indeed a good way to replenish the gut bacteria after treatment with antibiotics (Hardy, L., 2019).

Conclusion

In conclusion, there is hope that FMT can effectively treat dysbiosis of the gut. This is especially true for the case of a recurrent Clostridium difficile infection, where the majority of cases were cured with few or no adverse effects noted. There are studies that imply that other gastrointestinal diseases could be treated effectively as well. It was also noted that there is little difference in outcome of treatment when different methods of transplantation are used. Other than for recurrent C. difficile infections for which FMT has been deemed effective, more research should be done to determine if it is effective for the other mentioned diseases. Overall, FMT is a promising and innovative therapy for gastrointestinal problems.

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Effective Treatments for Nicotine Addiction

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Abstract

Nicotine dependence as a result of smoking is a chronically relapsing disorder with detrimental effects. However, fortunately for smokers, an armamentarium of smoking cessation aids is available in the forms of nicotine replacement therapy (NRT), non-nicotinic drugs (namely varenicline and bupropion), and the novel nicotine vaccines, each with their own mode of action to moderate nicotine addiction. This paper analyzes the mechanism of action associated with nicotine addiction and the various methods of combat, or at the very least, attenuation of the addiction.

Introduction

Though incognito, nicotine addiction has been proven to be the true killer in the seemingly innocent activity of smoking, for it reinforces the desire to smoke. To prevent the myriad adverse effects of smoking, smokers need to work very hard to break nicotine addiction through smoking cessation. Nonetheless, many smokers find themselves dealing with many ups and downs in the quitting process. It comes as no surprise that nicotine addiction is therefore identified as a "chronic condition" by The US Clinical Practice Guideline, as many smokers need to make several attempts before they successfully wean themselves off completely (Fagerström & Hughes, 2008). Smoking is the second most expensive chronic health condition in the United States, with an estimated economic cost of 300 billion dollars per year (Jordan & Xi, 2018). Yet, stopping smoking can reverse the biological and economical damage caused by smoking (Benowitz, 2010).

Understandably, 70% of smokers admit that they would like to quit. Every year, about 40% quit for at least a day. However, due to the extreme difficulty to abstain, about 45 million Americans currently smoke tobacco. Moreover, the 80% who attempt to quit on their own return to smoking within a month. Each year, only 3% of smokers quit successfully and remain abstinent one year later, highlighting the critical need for effective long-term smoking treatments (Benowitz, 2010).

Methods

This comprehensive review is based on critical analyses of literature obtained using various databases available through The Touro College Library online, such as PubMed and ProQuest. The National Center for Biotechnology (NCBI) website was also useful in providing additional source material.

Nicotine Addiction

Cigarette smoking is a major cause of death, cardiovascular disease, and pulmonary disease. It also presents the risk for various infections, osteoporosis, reproductive disorders, adverse postoperative events, delayed wound healing, duodenal and gastric ulcers, and diabetes (Benowitz, 2010). Although nicotine itself plays a minor role, if any, in causing smoking-induced diseases, the addiction to nicotine, which leads to sustained smoking use, is the proximate cause of these diseases (Onor et al., 2017).

Nicotine ($C_{10}H_{14}N_2$) is a plant alkaloid found in the tobacco plant (Onor et al., 2017) that consists of a pyridine and pyrrolidine ring, each one possessing a tertiary amine (Escobar-Chávez et al., 2011). The pKa of the pyridine nitrogen is 3.04 and the pKa of the pyrrolidine nitrogen is 7.84 under standard conditions. Based on these characteristics, nicotine's distribution exists among three forms, depending on the pH of the solution. An increase in acidity of solution increases the fraction of protonated molecules; conversely, a more basic environment increases the fraction of the unprotonated, or free base, form (Figure 1). Although all forms of nicotine are highly soluble in water and can easily dissolve in lung fluids and blood, the unprotonated nicotine smoke particles are volatile, whereas the protonated form is not. Conventionally, a sample of particulate matter from cigarette smoke is not acidic enough to cause the protonated form to dominate. Thus, a higher percentage of unprotonated nicotine can rapidly cross biological lipid membranes and be deposited in the respiratory tract (Centers for Disease Control and Prevention, 2010). Nicotine begins to reach the brain ten seconds after inhalation and its concentration continues to increase gradually (Dani et al, 2011).

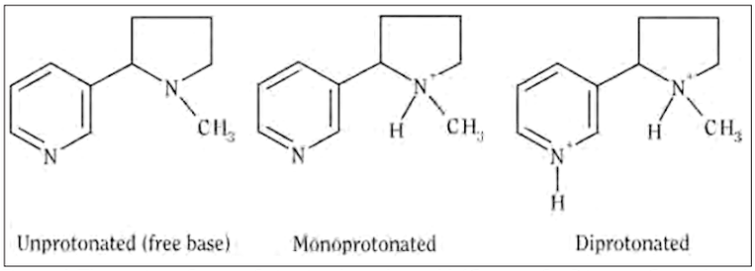

Figure 1. The various forms of nicotine based on pH (Centers for Disease Control and Prevention, 2010).

Blood concentrations of nicotine rise rapidly and peak at the completion of smoking. The rapid absorption of nicotine is attributed to the broad surface area of the alveoli and small airways. This rapid rise allows the smoker to titrate and manipulate the level of nicotine during smoking, which makes smoking the most reinforcing and dependence-producing form of nicotine administration (Benowitz et al., 2009).

Essentially, pharmacologic feedback, learned factors, genetics, and environmental factors (including tobacco product design and marketing, stress, smoking cues, or peers who smoke) contribute to nicotine addiction. Other factors include sex, age, mental illness, and substance abuse. Although each of these features contributes, the one that will be discussed with percipience is the pharmacological interplay with nicotine addiction (Benowitz et al., 2009).

The pharmacological basis for nicotine use is enhancement of mood and augmentation of mental and physical functions. Inhalation of smoke from a cigarette allows nicotine from the smoke particles to diffuse through the lungs, where it is rapidly absorbed into the pulmonary venous circulation. From there, it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and brain. Based on human autopsy samples from smokers, the liver, kidney, spleen, and lung have the highest affinity for nicotine (Benowitz et al., 2009). Subsequently, the nicotine enters arterial circulation to be moved from the lungs to the brain with high affinity, where it binds to nicotinic acetylcholine receptors (nAchRs), ligand-gated ion channels that normally bind a neurotransmitter acetylcholine (Benowitz, 2010).

NAchRs are pentameric structures consisting of a combination of five different subunits, including nine α subunits (α2 through α10) and three β subunits (β2 through β4), resulting in at least 12 unique nAchR subtypes that have been identified thus far. The α4β2 receptor, though, is the prime mediator of nicotine dependence. As seen in positron emission tomography studies in humans, smoking a full nicotine cigarette nearly saturated α4β2 receptor occupancy. In fact, when disruption of the β2 subunit gene was tested in mice, the behavioral effects of nicotine were eliminated. Similarly, the α4 subunit is an important determinant of sensitivity to nicotine. This was confirmed when a mutation affecting a single nucleotide in the pore-forming region of the receptor gene in mice made it hypersensitive to the effects of nicotine. These observations strongly implicate α4β2 nAchRs in nicotine addiction and illustrate the α4β2 receptor as a potentially attractive medicinal target for treatment of the addiction (Jordan & Xi, 2018).

The smoker craves nicotine to propagate dopamine overflow in the pleasure-seeking areas of the brain. The α4β2 are located on the dopamine (DA) cells of the mesolimbic system. The system is comprised of projections from DA neurons in the ventral tegmental area (VTA) to the nucleus accumbens (NAc), the part of the brain responsible for reward, pleasure, laughter, aggression, and fear, and the prefrontal cortex. Nicotine binding to α4β2 receptors on VTA DA cells increases neuronal excitability and neurotransmitter release, opening the ligand-gated ion channel, and allowing Ca2+ and Na+ to cascade intracellularly, which stimulates DA release to NAc (Figure 2). This is the underlying effect of nicotine's reward cascade, as dopamine serves as a pleasure signal and mood modulator and is critical for reinforcing nicotinic effects (Jordan & Xi, 2018).

When studied under laboratory conditions, nicotine elicits classic addictive responses (Dani et al, 2011). In order to reap the rewarding feeling associated with nicotine and avoid withdrawal symptoms, smokers must maintain a certain nicotine level. Repetitive exposure to nicotine leads to neuroadaptation and tolerance to nicotine's

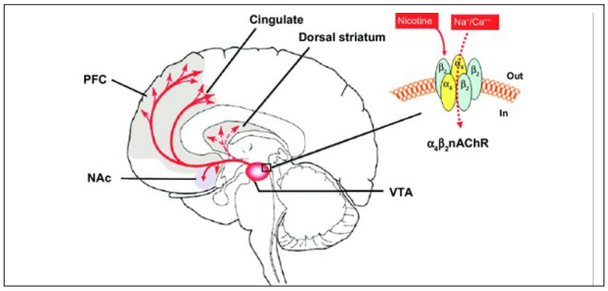

Figure 2. Schematic diagram of the mesolimbic DA projection pathway in the human brain. Nicotine activates a4b2 nAChRs located on DA neurons located in the VTA, as illustrated (Xi, 2010).

effects, and accumulation of nicotine in the body leads to a more substantial withdrawal reaction if cessation is attempted. Common withdrawal symptoms include anxiety, difficulty concentrating, and irritability, all of which can last for days, weeks, or longer (Onor et al., 2017).

As neuroadaptation occurs, the number of binding sites on the nicotinic cholinergic receptors in the brain increases. This causes desensitization, wherein a ligand-induced closure and unresponsiveness of the receptor occurs due to the profusion of ligand infiltration. Thus, the feelings of craving and withdrawal are exacerbated during periods of abstinence due to the mitigated levels of dopamine and other neurotransmitters. However, during a smoking period, binding to the α4β2 cholinergic receptors alleviates the need for nicotine. To circumvent withdrawal symptoms, smokers will sustain sufficient levels of plasma nicotine (Benowitz, 2010).

It comes as no surprise that nicotine withdrawal is extremely taxing on the smoker. Such repercussions are powerful incentives to take up smoking again (Benowitz, 2010). Fortunately for smokers, there is an expansive market of nicotine treatments that promote smoking cessation, some of them in the form of nicotine replacement therapy (NRT), non-nicotinic drugs such as bupropion and varenicline, and finally, the emergence of nicotine vaccines.

Nicotine Replacement Therapy (NRT)

Nicotine's rapid rate of absorption and entry to the brain are key factors responsible for the high abuse potential. Unlike cigarettes, nicotine replacement therapy (NRT) products such as gums, inhalers, and transdermal patches can help relieve the physical withdrawal symptoms by providing gradual increments of nicotine without the damaging chemicals found in cigarette smoke (Jordan & Xi, 2018). The gradual distribution of nicotine results in low abuse liability of NRTs. Although NRT doesn't completely eliminate withdrawal symptoms since it does not provide rapid and high levels of nicotine, NRT may provide a coping mechanism, making cigarettes less enticing to smoke (Molyneux, 2004) and increasing the rate of quitting by 50 to 70% (Stead et al., 2012). NRTs are well tolerated and have minimal adverse effects, but are most effective when used in conjunction with intense behavioral support (Molyneux, 2004).

In 1984, transmucosally delivered nicotine polacrilex, or nicotine gum, was introduced as the first effective NRT to serve as a smoking cessation aid, as approved by the US Food and Drug Administration (FDA). It is not chewed like ordinary confectionary gum, as it must be intermittently chewed and held in the mouth for over 30 minutes to achieve optimal release of nicotine. The absolute dose of nicotine absorbed systemically is much less than the nicotine content of the gum, in part because considerable nicotine is swallowed with first-pass metabolism (Benowitz et al., 2009), where it gets metabolized in a specific location other than the location of interest, reducing the concentration that enters systemic circulation (Herman &Santos, 2019). The dosage is slowly decreased until it is no longer required (Wadgave & Nagesh, 2016).

To satisfy the behavioral hand-to-mouth ritual of smoking, the nicotine oral inhaler was introduced to the NRT market. Contrary to its label, the inhaler is mainly delivered to the oral cavity, esophagus, and stomach, and negligibly to the lungs. Because absorption is mainly through the oral mucosa, a slow absorption rate of nicotine is achieved, akin to that of nicotine gum (Wadgave & Nagesh, 2016).

In a parallel fashion, nicotine patches deliver nicotine at a relatively steady rate when applied to and readily absorbed through the skin. In fact, the patch is the form that delivers nicotine at the slowest rate when compared to the other forms of NRT. A chief advantage of nicotine patches is the simplicity of user compliance, since the patch can be placed on the skin in the morning and worn for the duration of the day. The patches are available in a range of doses, allowing users to gradually decrease their nicotine intake over the span of several weeks or longer to ensure a proper adjustment to lower nicotine levels until they can attain a nicotine-free state. The rate of nicotine release is controlled by the permeability of the skin, rate of diffusion through a polymer matrix, and rate of passage through membranes in the various patches on the market. In all cases, there is an initial lag time of 1 hour before nicotine enters the bloodstream, followed by continued systemic absorption once the patch is removed, the latter due to the vestiges of nicotine in the skin (Benowitz et al., 2009). Current evidence supports the safety of long-term use of nicotine patches for nicotine treatment (Wadgave & Nagesh, 2016).

Non-Nicotinic Drugs

Bupropion (Wellbutrin)

The first non-nicotine drug to treat nicotine addiction was introduced in 1997. Bupropion (amfebutamone), marketed as Wellbutrin and Zyban among others (Fava, et al., 2005), an amphetamine-based drug, is a reuptake inhibitor of dopamine into neuronal synaptic vesicles and a blocker of nicotine's activation of several neuronal nAChRs. Bupropion undergoes metabolic transformation to an active metabolite, 4-hydroxybuproprion, through hepatic cytochrome CYP2B6, (Foley et al., 2006). Bupropion's structure is akin to nicotine, rendering it a compatible competitor (Figure 3). Originally developed as an antidepressant, a systematic review of 44 clinical trials found that sole therapy with bupropion significantly increased long-term (≥6 months) smoking abstinence, affirming its efficacy as an anti-smoking agent (Onor et al., 2017). It should be noted that the antismoking effect does not seem to correlate with its antidepressant effect, as bupropion is equally as effective for smoking cessation for individuals with and without depression (Roddy, 2004).

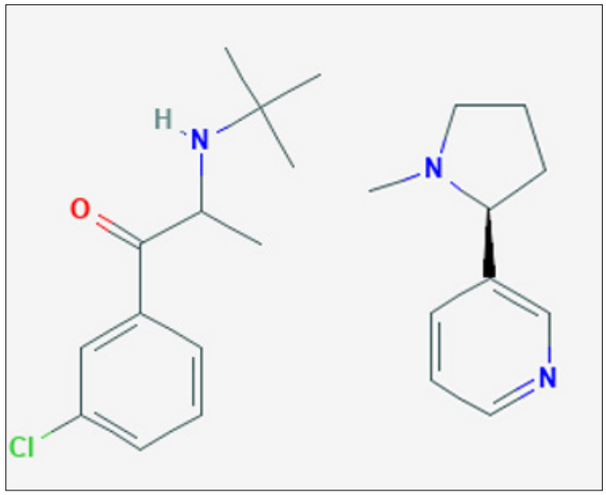

Figure 3. The chemical structures of bupropion (left) and nicotine (right) (National Center for Biotechnology Information).

When nicotine infiltrates the blood and crosses the blood brain barrier, there is a release of dopamine into the synaptic cleft of neurons in the dopaminergic pathways. After nicotine levels subside, dopamine reuptake into the axon terminal vesicles occurs. Bupropion is thought to inhibit this dopamine reuptake. In vivo studies have also shown that bupropion antagonizes the effects of nicotine at the postsynaptic acetylcholine nicotinic receptor (Wilkes, 2008). During withdrawal, bupropion may attenuate symptoms by mimicking the effects of nicotine on dopamine (Warner & Shoaib, 2005). These effects may explain how bupropion inhibits the reinforcing effects of nicotine, though it is still unclear whether bupropion offers any long-term relapse prevention following termination of treatment. Nonetheless, both pragmatic and observational trials of bupropion have shown that approximately 1 in 5 smokers will successfully remain abstinent for at least a year post-treatment (Wilkes, 2008).

Varenicline (CHANTIX)

Cytisine is a naturally occurring insecticide found in the leaves and seeds of Cytisus laburnum (golden rain tree). During World War II, soldiers smoked leaves of this tree in lieu of tobacco. Both varenicline and cytisine target the α4β2 receptor, where varenicline was developed to improve binding to the receptor to enhance efficacy of smoking cessation. However, the cytisine structure did not lead to a viable drug candidate. In due course, a series of efforts based on analgesic bicyclic benzazepines, one of which was unveiled as a potent α4β2 nAchR antagonist, served as a novel template that led to the development of varenicline, branded as CHANTIX. FDA approval was based on randomized clinical trials conducted in 3659 subjects in the United States. The subjects, all of whom were chronic smokers, averaged 43 years of age and reported smoking an average of 21 cigarettes per day for the previous 25 years. The primary outcome measured abstinence from smoking, which came in at a 44% rate, a significant improvement over bupropion (30%) and placebo (18%). Secondary outcomes, such as the urge to smoke and withdrawal symptoms, were likewise improved in varenicline-treated subjects over placebo (Jordan & Xi, 2018). In 2006, varenicline received FDA approval, and it was highly touted as an aid to quit smoking (Fagerström & Hughes, 2008).

Like bupropion, varenicline has a somewhat parallel configuration to its nicotine competitor in order to operate as an appropriate replacement. In fact, in vitro binding assays indicate that varenicline's affinity for the α4β2 receptor (Ki = .15 nM) is higher than that of nicotine (Ki = 1.6 nM) and cytisine (Ki = 0.23 nM) (Jordan & Xi, 2018). Varenicline has the following chemical name: 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3- h] [3]benzazepine, (2R,3R)-2,3-dihydroxybutanedioate (1:1) (Figure 4). Varenicline has a molecular weight of 361.35 Daltons and is highly soluble in water (Pfizer Labs, 2016).

With varenicline, dopamine is still released, but less so than with nicotine. Since the α4β2 had been identified to have the highest sensitivity to nicotine, it had become a potential target for the smoking cessation drug. Varenicline was developed to have a high affinity for the α4β2 neuronal nicotinic acetylcholine receptors in the mesolimbic dopamine system and stimulate receptor-mediated activity, but at a significantly lower level than nicotine. Varenicline's highly selective nature ensures that it will bind more potently to α4β2 receptors than to other common nicotinic or non-nicotinic receptors (Pfizer Labs, 2016).

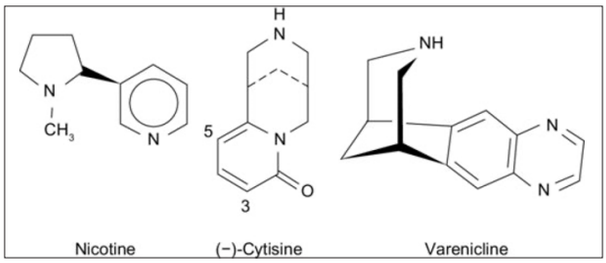

Figure 4. The chemical structures of nicotine (left), cytisine (middle), and varenicline (right) (Xi, 2010).

As a result of being a partial agonist, varenicline displays both agonist and antagonist effects. Partial agonists have been reviewed thoroughly as a method of attenuating nicotine addiction. Partial agonists bind to nAchRs but do not elicit the maximum response of a full agonist, and instead depend on receptor occupancy by other ligands. For instance, in the presence of a full agonist like nicotine, a partial agonist would behave as an antagonist by occupying the receptor site, thereby minimizing nicotine's effects at the receptor. However, in the absence of nicotine, a partial agonist would behave as an agonist by mitigating nicotine withdrawal symptoms through triggering a degree of dopamine release (Jordan & Xi, 2018).

Given that continuous abstinence rates across studies remain low (18-30% with varenicline; 4-10% with placebo), novel and more effective treatments may be required. However, since FDA approval in 2006, incoming reports have been continuing to demonstrate varenicline's efficacy for smoking cessation over the alternatives. For example, in a randomized trial involving 376 participants over the span of 52 weeks, varenicline resulted in higher abstinence rates from smoking (55.9%) when compared to transdermal NRT (43.2%), highlighting varenicline's progress (Jordan & Xi, 2018).

Nicotine Vaccines

With the advent of the novel nicotine vaccines, smokers have an alternative course of action through which they can quit smoking. Currently undergoing clinical trials, the goal of the vaccine is to generate antibodies that sequester nicotine in the blood and hinder the pharmacological effects by preventing access into the brain. Thus, the vaccine brims with potential for treatment of nicotine addiction and relapse prevention (Goniewicz & Delijewski, 2013).

Based on the assumption that a rapid increase in brain nicotine levels induces feelings of reward, preventing nicotine from entering the brain is an intriguing idea with precedent in other, similar treatments. Using antibodies to bind a drug and thus disabling it from crossing the blood-brain barrier was first tested in the realm of heroin addiction and extended to nicotine and cocaine addiction (Raupach et al., 2012).

Since nicotine is too small to elicit a response from the immune system, nicotine is not immunogenic. In order to elicit an immune response, nicotine or a structurally similar hapten needs to be paired with a larger carrier protein, thus producing a conjugate vaccine (Goniewicz & Delijewski, 2013). Vaccination administers an immunogenic substrate that activates T and B cells, leading to the formation of specific antibodies within the individual, imprinting the response in immunological memory. By virtue of this mechanism, this approach has been shown to yield longer lasting protection (Raupach et al., 2012).

When nicotine enters the body, the vaccine causes it to bind to the nicotine-specific antibodies, forming a complex too large to cross the blood-brain barrier. Thus, there is no nicotine-induced cerebral stimulation for the smoker and the impression received by the smoker is comparable to smoking a cigarette without nicotine (Escobar-Chávez et al., 2011).

The success of this immunological strategy hinges on immunogenicity of the vaccine, affinity of antibodies, and specificity of antibodies (Raupach et al., 2012). Immunogenicity refers to the antibody serum concentration. A vaccine must elicit and maintain a high antibody serum concentration throughout the period of interest in order to be maximally effective (Escobar-Chávez et al., 2011).

The primary measure of antibody affinity to the target drug can be measured by the binding equilibrium constant, Ka. The Ka is defined by Ka = [NicAb]/[Nic][Ab]. [NicAb] represents the plasma volume concentration of bound nicotine-antibody complexes, and [Nic] and [Ab] denote the volume concentrations of unbound drug and unbound antibody, respectively. Hence, in order to calculate the percentage of bound nicotine, data regarding the amount of antibody present in circulation must be obtained beforehand. The Ka should be high enough to bind to nicotine and low enough to allow for unbound nicotine release and elimination (Goniewicz & Delijewski, 2013). However, extremely high affinity may be disadvantageous, as saturation of all antibodies compromises efficacy for subsequent nicotine doses (Fahim et al., 2011).

Interestingly, the interaction of antibodies with nicotine is reversible and each antibody binds to and releases nicotine many times, much like a juggler catches and releases multiple sticks many times. Thus, it is observed that the binding capacity of the antibodies for nicotine is far in excess than the expected stoichiometric calculation (Escobar-Chávez et al., 2011).

Specificity refers to the extent to which the elicited antibodies bind to nicotine in preference to other molecules (Raupach et al., 2012). Greater specificity reduces competition from other molecules, thus improving safety and minimizing the likelihood of adverse side effects (Escobar-Chávez et al., 2011). This has practical applications for the design of conjugate vaccines. For example, one recent study showed that using longer rather than shorter linkers, amino acid sequences used to separate multiple domains in a protein (Reddy Chichili et al., 2013), increases antibody selectivity to nicotine. Additionally, linker position influences specificity. Linkers that are distant from the prime sites of metabolism (i.e. attached to the 6- rather than 5- position of the pyridine ring) help enhance antibody selectivity (Raupach et al., 2012).

In order to maintain an ideal serum antibody concentration, repeated administration of the nicotine-conjugate system in the form of the vaccine is required. The first vaccination administered causes a primary immune response, comparable to when the organism had its initial encounter with an infectious antigen. Each subsequent administration acts as a "planned infection," which uses memory about the antigens during the production of antibodies. Therefore, a faster and more effective response to the subsequent vaccinations is anticipated (Goniewicz & Delijewski, 2013).

Due to the prolonged effect that nicotine vaccines provide, they have an advantage over the existing pharmacotherapies (Shen et al., 2012, as cited in Goniewicz & Delijewski, 2013) and are a critical addition to the pharmacological smoking cessation aids. The relapse rate is minimal since only bimonthly booster shots are required to achieve a high level of antibodies. Thus, patient adherence to the necessary protocol can significantly improve. Nevertheless, early clinical trials have casted some doubts in that many patients may not elicit a sufficient antibody response. To circumvent this issue, novel carriers and/

or adjuvants with immunogenic properties can be introduced to stimulate a more potent immune response (Cerny et al., 2009, as cited in Goniewicz & Delijewski, 2013).

For those who attain high levels of antibodies, vaccination has been shown to be effective in achieving and maintaining abstinence (Goniewicz & Delijewski, 2013). Vaccines against nicotine are at an advanced stage of clinical trials but have not yet been approved for treatment of individuals (Escobar-Chávez et al., 2011). Future strategies for enhanced specificity that the vaccine can provide in conjunction with a high affinity to nicotine and increased antibody level offer an effective avenue for smoking-cessation (Goniewicz & Delijewski, 2013).

Discussion and Conclusion:

After reviewing the various smoking cessation techniques, it seems that a combination of a few would be the most viable option. The smoker can implement preliminary arrangements with the use of NRT. NRT may be useful for those who want to attenuate the smoking habit but do not want to put a halt to it completely, known as quitting "cold turkey." The choice of NRT can be guided by the patient's preference, though it may be wise to have a first-line agent in conjunction with NRT; however, healthcare professionals must learn the benefits and potential detriments of different types of NRT before guiding patients in its potential use (Wadgave & Nagesh, 2016).

Smokers who find that they are unsuccessful with NRT can choose an alternative method. Although nicotine vaccines have an advantage over existing pharmacotherapies in that they have a prolonged effect and require substantially less cooperation from patients with bimonthly booster shots, data from clinical trials suggest that many patients may not produce sufficient antibody response (Goniewicz & Delijewski, 2013), suggesting that it may not be the most pragmatic approach.

Since nicotine addiction is primarily responsible in impeding smoking cessation and long-term abstinence, it seems that the most prudent option would be a modality that targets the activity at the α4β2 receptor, the prime mediator of nicotine dependence. Where bupropion therapy aims to alleviate the withdrawal symptoms experienced during the transition state to a steady state of neurotransmitter activity, as does NRT, varenicline was developed to selectively target nicotine activity at the receptor that leads to the addiction, a seemingly more robust approach. Though varenicline presents a surfeit of undesirable side effects, some in the forms of nausea, abnormal dreams, taste perversion, and headaches (Burke et al., 2016), these effects may prove manageable and worthwhile under a cost-benefit analysis. The strong rationale for targeting the α4β2 receptor with a partial agonist, coupled with promising findings from clinical studies, reinforce varenicline's efficacy and safety as a reliable smoking cessation aid. Upregulation of these receptors and adaptation lead to the compulsive use of nicotine to maintain homeostasis, both of which render the α4β2 receptor an effective candidate for pharmacologic intervention. However, patients and providers should determine whether to use varenicline only after an assessment of the potential risks and benefits. The efficacy of varenicline can be improved in combination with NRT and bupropion, especially for smokers who are more heavily dependent on nicotine (Burke et al., 2016). Greater understanding of the exact mechanisms of these drugs, particularly bupropion, could lead to the development of drugs that are more effective in promoting smoking abstinence (Warner & Shoaib, 2005).

All things considered, nota bene that relapse is often prominent in a patient's attempt to quit smoking. The average patient will quit four or five times before reaching complete cessation, an important point to convey to patients to prevent disillusionment and hopelessness during recovery (Woody et al., 2008).

Essentially, the adverse health effects associated with cigarette smoking are numerous and continual efforts to reduce the prevalence of smoking are imperative (Onor et al., 2017). Nevertheless, due to futile attempts to quit, many smokers feel demoralized and incapable of taking action towards quitting. However, there are options available to the smoker. Whether in the form of NRT, non-nicotinic drugs (namely varenicline and bupropion), or the novel nicotine vaccines, nicotine addiction can be mitigated to aid the journey towards recovery.

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Parkinson's Disease: Causes, Symptoms, Research, and Interventions

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Abstract

This paper covers several pathogenic theories of Parkinson's disease (PD); the physiology and biological pathways involved. This includes a mitochondrial DNA (mtDNA) route, a nuclear DNA route, and other hypotheses about idiopathic PD. The subsequent discussion of PD symptoms utilizes a neurological perspective, analyzing the neuroanatomical systems involved, and how they are treated. This includes medications and surgical techniques that are employed in an effort to manage symptomatology and increase health-related quality of life.

Keywords

Parkinson's disease, mitochondria, alpha-synuclein, dopamine agonists, pallidotomy, DBS

Introduction

Neurodegenerative diseases are characterized by progressive and selective loss of anatomically or physiologically related neuron systems. The clinical syndromes associated with particular neuroanatomical patterns of cell loss and dysfunction are typically categorized by whether they initially affect cognition, movement coordination, sensation, vision, or autonomic control. Typical examples of such neurodegenerative diseases include Alzheimer's disease, Huntington's and Parkinson's disease (Lezi and Swedlow, 2012). Cholinergic neurons are implicated in Alzheimer's, and a degeneration of neurons in the dopaminergic system is responsible for Parkinson's disease.

Parkinson's disease (PD) is the second most common neurodegenerative disease, afflicting 1-3% of the 65+ age group. It is characterized by accumulation of abnormal protein (Lewy bodies) in the dopaminergic neurons of the substantia nigra pars compacta (SNc) and their subsequent degeneration. These are abnormal circular structures with a dense protein core and a halo of radiating fibers. They consist of aggregations of misfolded α-synuclein along with neurofilaments and other proteins. This neuron loss leads to a difficulty controlling movement. The motor symptoms include bradykinesia, postural instability, muscle rigidity, and tremors. Some of the non-motor symptoms (NMS) include depression, insomnia, anxiety, apathy, psychosis, incomplete bowel emptying, impulse control disorders, and dementia.

The disease involves progressive degeneration which, as of now, cannot be stopped or slowed. There are effective medications that can be used to compensate for dopaminergic neuron loss, such as L-dopa, and other types of dopamine (DA) agonists. These work by increasing the potency of the surviving neurons and synapses, but only temporarily. Eventually, the disease will wipe out these pathways entirely. Furthermore, the disease spreads to other regions of the brain and causes other symptoms. For example, when neurodegeneration spreads to cholinergic neuronal pathways, the patient will start to show signs of dementia. For now, treatment options are limited to symptom management. This paper discusses some of those treatment options, but first it is important to consider the PD pathogenesis and symptom origin. Then, this paper will delve into the innovations available for those suffering from PD, and explain how they work.

Mitochondrial Pathogenesis of PD

Mitochondria are the site of bioenergetics and biosynthesis in the cell. Hans Krebs, for whom the tricarboxylic acid (TCA) cycle is named, said of his discovery "in some micro-organisms the cycle primarily supplies intermediates rather than energy, whilst in the animal and most other organisms it supplies both energy and intermediates". The energy supplied by the TCA cycle is in the form of NADH and FADH2, whose electrons are then fed into the electron transport chain (ETC) to pump protons into the inner membrane space of the mitochondrion creating a pH gradient. This gradient is then used to power the conversion of ADP into ATP. A high ATP/ADP ratio is required to catalyze the chemical reactions that comprise many of the metabolic operations of the cell.

Additionally, many of the building blocks that a cell needs to form its assortment of macromolecules are made using the intermediates of glycolysis and the citric acid cycle. For example, about half of the 20 amino acids found in human protein can be synthesized in vivo through the modification of Krebs Cycle intermediates (Reece et al., 2011). Also fatty acids are synthesized from acetyl CoA, which is produced by the conversion of pyruvate within the mitochondria. It is important to note that when the mitochondria are employed in an anabolic capacity (the building of larger molecules and utilization of TCA intermediates) they are no longer producing ATP but consuming this molecule.

There is some evidence implicating mutations in mitochondrial DNA (mtDNA) or nuclear genes coding for mitochondrial protein in the pathogenesis of PD. Several research teams working in 1989 reported a reduction of activity of Complex I of the ETC in the substantia nigra in patients with idiopathic PD (Lezi and Swedlow, 2012). This study was based on reports of healthy individuals developing Parkinson's like symptoms after consuming the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). It was determined that the active metabolite

of MPTP is taken up by the cell via the dopamine transporters, allowing them to cluster and wreak havoc in dopaminergic neurons. This particular metabolite inhibits Complex I of the electron transport chain, which was assumed to have led to the exhibition of Parkinson's symptomatology.

The suggested hypothesis is that mutations within genes coding for mitochondrial DNA or in mtDNA itself, specifically Complex I genes, can be implicated in PD pathogenesis. Complex I contains 46 protein subunits, seven of which are encoded by mtDNA. A mutation causing a decrease in Complex I activity would lead to a drop in ATP production and increased oxidative stress and cellular deterioration from the accumulation of free radicals (Lezi and Swedlow, 2012).

It is unclear why dopaminergic neurons of the substantia nigra would be uniquely susceptible. However, what is clear is that the failure of these nigral dopaminergic systems are responsible for the symptoms of PD. Based on the research done on mitochondrial involvement in dopaminergic deterioration in PD or induced PD symptoms, there is sufficient justification to investigate treatment options targeting the mitochondria, which work to encourage proper functionality and cellular respiration (Lezi and Swedlow, 2012).

α-synuclein and PD

Researchers have discovered that a mutation on chromosome 4 will produce PD (Polymeropoulos, et al., 1996), the gene that codes for α-synuclein. This protein can be found in the axon terminals and is involved in synaptic transmission in dopaminergic neurons. A mutation in this gene can result in a mistranslated and subsequently misfolded α-synuclein that is toxic to the cell. These proteins group together in large aggregates called Lewy bodies which devastate normal cell function.

The majority of PD cases are sporadic. They occur without any family history or hereditary basis for the disorder. Some researchers suggest that an unknown toxin in the environment, faulty metabolism, or infection may be the culprit in these cases. There are two insecticides that are known to cause PD, and presumably there may be more. These toxins might interfere with mitochondrial signaling which, when impaired, could cause these aggregations of α-synuclein to resist degradation and persist in the cytoplasm of nigral neuron systems.

Symptoms and Their Physiology

The loss of the brain's most important dopamine suppliers, the neurons of the SNc, leads to a variety of symptoms. Resultant motor symptoms include tremors, muscle rigidity, bradykinesia, and postural imbalance - the symptoms that normally come to mind when people think of Parkinson's disease. These symptoms arise directly from neuron loss in the motor regulation centers of the brain (Carlson & Birkett, 2017). Additionally, many other neural pathways are affected by damage to the substantia nigra, such as the areas where these dopaminergic neurons project, namely, the basal ganglia and the nucleus accumbens. The basal ganglia are involved in controlling movement, but they also project to the frontal lobes of the brain and play a role in thinking and executive functions. The nucleus accumbens has been linked to behavioral regulation, Thus, dopamine irregularities in this region can lead to changes in personality (Carlson & Birkett, 2017).

The causes for some of the non-motor symptoms are pretty clear, while others are more complicated and debatable. Some symptoms arise directly from a decrease in dopamine (DA) production in the substantia nigra. Other symptoms are a result of the DA agonist prescribed to the patient. This might be due to the dramatic fluctuations of DA in the brain owing to the medication schedule. Still other symptoms might be due to an increased potency in certain dopaminergic systems that have not been impacted by the disease. When the patient takes L-dopa, these functioning dopaminergic neurons release too much DA. This DA flood triggers a deficit in executive functions, according to the Dopamine Overdose Hypothesis (Dirnberger & Jahanshahi, 2013).

Other symptoms result from unrelated pathways that happen to be proximal to damaged areas of the brain. Lewy bodies are often present in the dopaminergic neurons of those with PD. Many of these misfolded proteins that cause neurodegeneration can be transferred from cell to cell (Lee, et al., 2011). This perhaps explains how the serotonergic, noradrenergic, and cholinergic systems become impacted in later stages of PD.

Treatments

Since there is no cure for PD, the standard treatment is symptom management, by way of DA agonists. The most common one in use is L-dopa, a neurotransmitter (NT) precursor which dopaminergic neurons can convert to dopamine. This maximizes its potency through increasing the amount of NT present in the synaptic cleft with each firing of the surviving neurons. When other systems are involved in degeneration, such as the serotonergic, noradrenergic, and cholinergic systems, agonists for each of those NTs can be used to alleviate symptoms. Some symptoms arise from too much dopamine in the healthier dopamine pathways, or fluctuations of dopamine based

on medication schedule (Dirnberger & Jahanshahi, 2013). These symptoms can be treated by using different DA agonists at different times and by changing doses. The reason for the variety of dopamine agonists is to reduce fluctuations and mitigate some symptoms that may arise from any one particular DA agonist.

Another possible medication is Deprenyl, which was initially discovered to be effective after the outbreak of PD among users of the drug MPTP. The idea behind it was to inhibit the activity of the monoamine oxidase-B enzyme and hopefully block the toxins from attacking and damaging the neurons. Although the drug does alleviate symptoms, it does not reverse, stop, or slow the progression of disease (Williams, 2010).

One surgical technique involved the grafting of nigral neurons taken from aborted fetuses, to replace the ones lost to PD. This was shown to work particularly well in patients who responded well to L-dopa earlier in the disease. Presumably, these patients had enough healthy neurons in the basal ganglia to process and secrete dopamine, whether intrinsic or from grafted tissue. Unfortunately, many of these patients later developed debilitating dyskinesias and the surgery is no longer recommended (Olanow, et al., 2003).

Upon closer inspection, it seemed as though the fetal tissue had been successful in making the proper connections with the basal ganglia. However, with time, the aggregate α-synuclein deposits made their way from damaged host cells to transplanted healthy cells (Kordower, et al., 2011). Any misfolded proteins responsible for neurodegeneration can be transferred from cell to a cell close by (Lee, et al., 2011). It seems that adding healthy cells to the basal ganglia will not work unless a way is found to prevent the α-synuclein from aggregating in the first place and spreading to neighboring cells.

GPi and STN Related Therapies

The main output of the basal ganglia is from the internal division of the globus pallidus (GPi) and it is inhibitory. Researchers found that whenever there is a decrease of activity to the putamen and the caudate nucleus (which is a byproduct of SNc neuron death due to PD) there is an increase in inhibitory signaling from GPi to the motor cortex. It was suggested that destroying this area might help mitigate some of the motor symptoms. This strategy worked well and was a pretty good option but the surgery was quite risky. The optic tract is located quite proximal to the GPi and some patients were blinded by the surgery. Due to advancements in imaging and surgical techniques, this option has become safer, and can be recommended for younger patients who no longer respond to L-dopa. Neurosurgeons can get similar results by destroying the subthalamic nucleus (STN) (Guridi, J & Obeso, 2001), which has an excitatory effect on GPi.

Another option that is growing in popularity due to refined surgical techniques is deep brain stimulation (DBS). Here, instead of destroying GPi and STN, microelectrodes are placed in these regions for the patient to stimulate as needed. This technique is as effective as brain lesions in suppressing tremors but with fewer risks (Esselink, et al., 2009). DBS might also be effective against depression and cognitive impairment in PD.

Conclusion

There are some innovative techniques for treating PD and a lot of promising research on the horizon. Through pursuit of the various pathogenesis hypotheses, we are getting closer to understanding the mechanisms of neurodegeneration. This information is crucial to finding a strategy to reverse the disease progression. In the meantime, there are many interventions available to those suffering from PD that can dramatically improve quality of life. It is a devastating diagnosis to receive but there is plenty of hope. It is crucial that those diagnosed with PD are taught about the potential symptoms and how they can be treated. Specifically the many non-motor symptoms not directly caused by degeneration of dopaminergic neurons. Treatment of these symptoms is achieved through some of the more creative and innovative treatments discussed in this paper. These non-motor symptoms are less known by the patients but were found to be more injurious to the patient's quality of life than the classic symptoms (Duncan, et al., 2013). As the research into PD genesis, pathways and mechanisms develops, more therapies are being discovered to treat this complex and multifaceted disease.

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