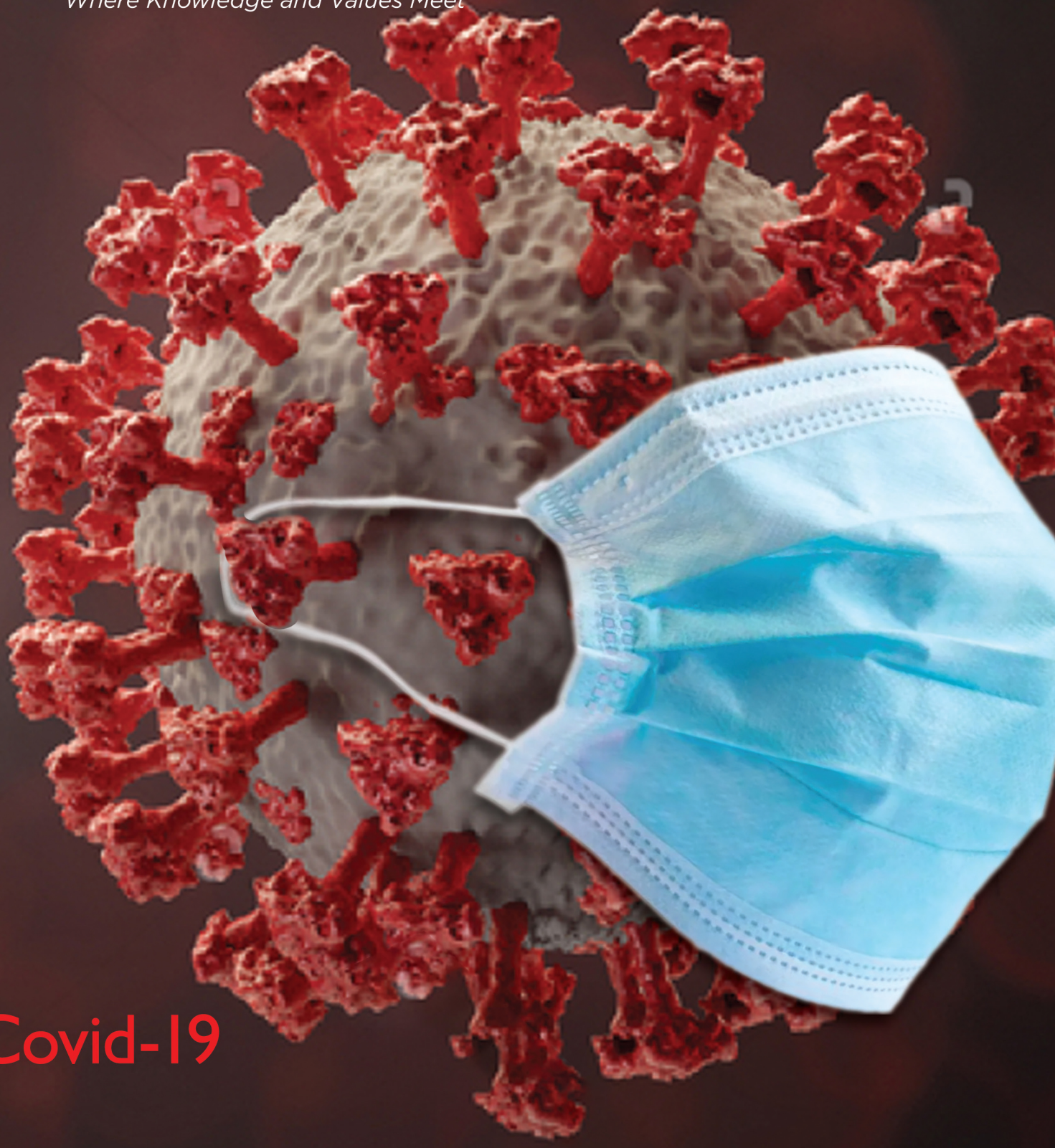
The Science Journal

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

Where Knowledge and Values Meet



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LANDER COLLEGE OF ARTS & SCIENCES
A DIVISION OF TOURO COLLEGE IN FLATBUSH
Where Knowledge and Values Meet

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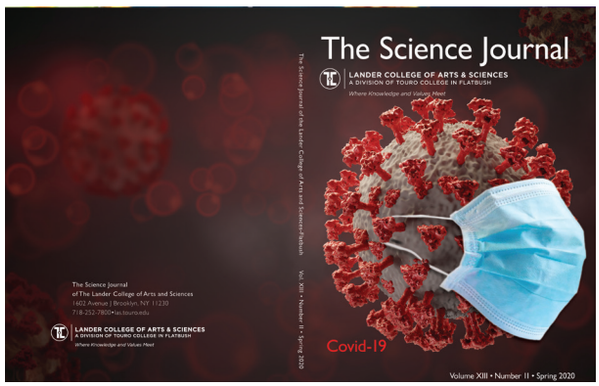

Cover picture: The cover picture was created by Professor Antony O'Hara of the Digital Multimedia Design Department. Although there are no papers in this issue dealing with viruses, it seemed appropriate to mark this historical semester with note of this predator.

Which Hypothesis Best Explains the Development of Cancer?

Ariel M. Mayer

Ariel M. Mayer Graduated with a Bachelor of Science Degree in Biology in June 2020

Abstract

There are three theories of cancer development analyzed in this review. The first theory is the immunological theory, which states that cancer is a result of the immune system failing to detect a cancerous cell in which results in uncontrolled cell growth. The second theory is the somatic mutation theory, which states that genetic mutations are a direct cause cancer. The third theory is the stem cell theory which states that cancer results from an uncontrolled stem cell. The difference in each theory helps guide a clinician's judgment in how to treat cancer. If a clinician believes in the immunological theory, he/she will view the best route of treatment as being by targeting the patient's immune system. One who believes somatic mutation theory would say that the patient's genetic makeup of the patient is the ideal target for treatment. One who believes in the stem cell theory would say that the only method of treatment is to remove the cancer entirely as any residual cancer will return. Based on all the evidence it appears that there is not one individual factor that causes cancer development, rather it is a combination of several factors that result in cancer.

Introduction

In 2013 cancer was reported to affect over 1.6 million people (Siegel, et al., 2013). Despite cancer being such a highly discussed and researched disease there are many things that remain unknown. One of the major debates is how cancer develops; there are three major schools of thought: the immunological theory, the somatic mutation theory, and the stem cell theory.

The immunological theory postulates that cancer develops as a result of the immune system failing to detect the cancerous cell. As a result of an undetected cell the cancer grows at an uncontrolled and dangerous rate, thereby damaging the host. If this is the sole etiology of cancer, the immune system could be harnessed to treat a person who had developed cancer. For example, a new cancer vaccine has been developed for prostate cancer patients. This vaccine was developed for two reasons. Firstly, prostate cancer cells have several tumor-associated antigens. Secondly, because the prostate is a nonessential organ, annihilation of any normal prostate tissue that comes about because of the immune response has no clinical consequence (Singh & Gulley, 2014). While not all cancers have these qualifications that make them susceptible to a vaccine, other forms of immunotherapies are in development to try and take advantage of the specifics of each cancer to attack it immunologically.

The somatic mutation theory postulates cancer develops due to a genetic mutation leading to uncontrolled growth. A study showed that there are specific gene mutations involved in breast and ovarian cancer. These are mutations of either the BRCA1 or BRCA2 gene (Miki, et al., 1994). More specifically, it has been shown that women with BRCA1 mutation by age 80 have a 72 percent chance of developing breast cancer. While women who have the BRCA 2 mutation have a 69 percent chance of developing breast cancer (Kuchenbaecker, et al., 2017). Treatments based on this theory focuses more on the genetics of a person and using that field as a method to control or treat the cancer.

The stem cell theory of cancer assumes that cancer develops because there is an uncontrolled stem cell in the body. All humans develop from stem cells that multiply repeatedly. However, during human development stem cells become specialized and eventually stop multiplying. This theory of cancer development says that cancer results from a stem cell that has not "turned off", resulting in uncontrolled growth to the point of being detrimental to the host. This theory has many implications. Some clinicians evaluate a cancer treatment based on how much it causes the tumor to shrink, but without removing the source the tumor will come back very quickly because the stem cells are still there. Physicians and Oncologists who subscribe to the stem cell theory may treat cancers differently than those who believe in the immunological theory or the somatic mutation theory.

The significance of each theory is that the information regarding how the disease develops can help determine the best course of treatment. Evidence seems to point to all three of these theories, hence there is no conclusive explanation. This review will examine all the evidence in order to determine which hypothesis best explains the development of cancer.

Methods

The articles and journals used in this review were found on PubMed, Ebsco, and Google Scholar. These articles were carefully reviewed in order to determine their relevance to the thesis.

Discussion

Immunological Theory of Cancer

This theory has been associated with three general steps the body constantly goes through: elimination, equilibrium and escape. The elimination step consists of the immune system surveying for all cancerous cells and destroying them. However, there are times when tumorous cells remain undetected while remaining dormant. This period is defined as equilibrium because there are cancerous cells in the body, but they are not doing any harm. The final step is escape in which the tumor gains dominance over the immune system and starts spreading (Lopez, et al., 2016). This implies that the immune system's activity and

ability to detect cancerous cells is important before the cancer gets out of hand.

To try to determine how immune activity affects cancer, residents of Japan, mostly above 40, were given a questionnaire that covered 90 lifestyle factors. People who participated in the study gave a peripheral blood sample after fasting for over 12 hours. A follow-up was performed 11 years later where cancer incidence and death totals were gathered. Based on the questionnaire, each patient was assessed for cancer risk at one of three levels, low, medium, or high. Of the 8552 individuals who took the survey, 211 of the 3625 whose blood was sampled were identified as cancer cases. A number of the cases had to be excluded based on age, blood samples being inaccessible, etc. The total number of cases remaining was 154, 92 men and 62 women. Accounting for age, it was found that patients with lower cytotoxicity activity, were at a significantly higher risk for developing cancer when compared to those with medium or high activity (Imai, et al., 2000). This study points to the fact that cytotoxic activity can help a person fight cancer, further illustrating that the immune system plays a role in cancer treatment.

A specific type of immunotherapy is currently being developed in which some of the patient's T-cells are extracted and modified to become chimeric antigen receptor t-Cells (CAR-T cells). These T-cells are then given artificial receptors that are from monoclonal antibodies which allow the CAR-T cell to bind to the cancer cells. The patient is then treated with chemotherapy to eliminate any immunosuppressant activity in the body. The CAR-T cells are then injected and are free to attack the cancer cells. This treatment sounds perfect but there are some complications that arise. Cytokine release syndrome (CRS) is a life-threatening complication in which the immune system goes into a state of being overly active and releases an excessive number of cytokines which results in organ toxicity. CRS is manageable but one has to be conscious of it when treating a patient with CAR-T. Another complication that can arise is CAR-T cell related encephalopathy syndrome. This syndrome can result in some patients feeling slightly disoriented while others can have seizures (Graham, et al., 2018). This methodology of cancer treatment is new but is showing great promise. It shows that the immune system, under careful monitoring, can be harnessed to fight cancers.

A person's immune system doesn't allow cancer cells to grow because the natural killer cells, or NK cells detect the cancers and kill them. These cells can identify a cancerous cell because they identify which cells are "not self" cells by searching for specific receptors that only one's own cells have. Therefore, they find the cancer cell and kill it before it starts growing. Although some cancer cells go undetected, there are researchers who believe that the NK cells can be used to kill an active cancer. A new treatment is being studied in which NK cells are being combined with the concept of CAR-T cells creating CAR-NK cells. These cells have several advantages over CAR-T cells. Firstly, because NK cells have natural receptors for tumors, they have an easier time identifying cancer cells even if the CAR portion of the cell is downregulated by the cancer. Secondly, CAR-NK cells do not undergo clonal expansion or immune rejection thus eliminating the issue of cytokine release syndrome that is present with CAR-T cells. Lastly, HLA matching is not necessary for CAR-NK cells, which means the graph-versus-host disease is not an issue when it comes to CAR-NK cell treatment. Currently there is not enough clinical data to fully implement the treatment, however, there are studies being performed to help investigate new treatment options. For example, CAR-NK cells are being researched to help determine their ability to fight hematological and solid tumors, including glioblastoma, prostate cancer, and ovarian cancer (Hu, et al., 2019). While this treatment is in very early stages it has had great results thus far. If this treatment can prove to be effective it can have massive implications in the successful treatment of cancers because there are few side-effects.

Dendritic cells are another one of the immune cells being used to fight cancer. Dendritic cells function as an antigen presenting cell meaning, when it recognizes an antigen it alerts the body to produce antibodies. There are vaccines that have been proven to work that are prepared by removing a patient's dendritic cells and "teaching" them to recognize cancer cells, then reinjecting them into the patient. Once these dendritic cells are in the bloodstream, they can identify the cancer cells and initiate a T-Cell response to them. However, there were several issues when the dendritic cells were taken from ex vivo and put inside patients. Firstly, some patients experienced a T-cell response from the injection, but it did not result in significant improvement. This alteration in functionality could be a result of the cells being transferred from an in vivo environment to an ex vivo environment, then returned to in vivo. Another issue with the vaccine is that the process is very time-consuming and expensive. Lastly, the dendritic cell vaccine is reliant on the patient's immune system and its function. Because of this, in vivo preparation of the dendritic cell is still being perfected (Le Gall, et al., 2018). This technique can be very helpful to cancer patients as often the cancer proliferates because the immune system fails to recognize the cancer cells. With this treatment the incidents of cancer cells going

undetected can be virtually eliminated resulting in the body halting the cancer cells growth.

Another form of immunotherapy using dendritic cells is called dendritic-cell cytokine induced killer cells (DC-CIK). In the study, there were several criteria that had to be met when determining who was an appropriate candidate. The first requirement was that the patients had to have advanced cancer (stages 3-5). Secondly, prior to this study, patients had to have received first-line treatment, including surgery, adjuvant chemotherapy and/or radiotherapy. The study examined a total of 142 patients with histologically confirmed colorectal carcinoma, 71 were treated with DC-CIK and 71 were not. Patients were examined at three separate times, after 1, 3 and 5 years. The retrospective study found that patients who were treated with DC-CIK had 1,3 and 5-year prolonged progression free survival and overall survival versus those who were not treated. Minor side-effects were experienced by the patients treated with DC-CIK such as mild fever, chills, fatigue, while three also developed a headache and one developed chest tightness and hypotension. While these symptoms cannot be ignored, in the grand scheme of cancer treatment these side-effects are very mild. This study shows the effectiveness of DC-CIK and its ability to combat colorectal cancer in patients who were treated with first-line treatments (Xie, et al., 2017). While this study has its limitations because it required first line treatment such as surgery, it does provide a powerful tool for patients who have already gone through or may require first line treatments.

The examples listed above all seem to point to the accuracy of the immunological theory of cancer. Perhaps the most indicative proof that cancer prevention is a function of the immune system is from a study performed several years ago which analyzed 12 patients with glioblastoma who received regular course of immunotherapy (a dendritic cell vaccine). However, a few patients were also given a tetanus vaccine. The patients who were given the immunotherapy and the tetanus vaccine lived between four to eight years after their treatment. The patients who received the immunotherapy with another placebo drug lived only 11 months following their treatment (Mitchell, et al., 2015). The reason the tetanus shot impacts the cancer defense is because once given, the body begins to create t-cells as a typical reaction to a vaccine. Due to this additional t-cell production the body was more prepared to fight the cancer as well (Haelle, 2015). While this study was performed on a small sample size the results point to the same conclusion as the previous studies; it illustrates the role the immune system plays when fighting cancer.

These are just some of the treatments in development, all working under the assumption of the immunological theory of cancer being correct. As it stands, the treatments appear promising and with further research they can hopefully be perfected and cancer patients can be cured with minimal side effects. All the studies cited point to the immunological theory of cancer as a very real and likely explanation for cancer development.

Somatic Mutation Theory of Cancer

The somatic mutation theory analyzes the development of cancer by looking at genetic factors. The specific genetic variances in squamous cell carcinoma (SCC), adenocarcinoma (AC), and adenosquamous carcinoma (ASC), 3 types of cervical cancers, were analyzed. Three hundred and one patients with SCC, AC, or ASC who were all treated with radical hysterectomy with lymphadenectomy as their primary treatment were analyzed. All follow-up data was obtained 5 years after primary treatment. One hundred and sixty-six (55%) of the patients had SCC, fifty-five (18%) had AC and eighty (27%) had ASC. In 103 of the tumors there were 123 somatic mutations detected as roughly 4% of the tumors had multiple mutations. The specific mutations were identified for each cancer. The PIK3CA was most common in SCC and ASC while KRAS was most common in AC. However, in each type of cervical cancer there was not only one mutation rather there were several mutations found across multiple genes such as, PTEN, PPP2R1A, CTNNB1, CDKN2A, FBXW7, FGFR3, NRAS, and HRAS. The KRAS mutation is not exclusive to cervical cancers as it has also been linked to colorectal, lung and pancreatic cancers. KRAS and PIK3CA mutations were both not associated with survival; however a clear trend of lower survival rate was in patients carrying a PIK3CA mutation (Spaans, et al., 2015). This study points to a connection between specific cancers and mutations that result in them. If an unknown cancer's genetic sequence could be inspected it could give a clinician a clear idea of what cancer he/she is dealing with and how to properly treat that patient.

Cervical cancers are not the only cancers to have a gene associated with them as prostate cancers were also found to have specific genes connected to them. These genes were identified by looking at a prostate specific antigen (PSA) a protease produced in the prostate, as well as prostatic acid phosphatase (PAP). By looking at these two markers the researchers used the technique of guilt-by-association which works based on combinatoric measure of association. Forty-thousand different genes were analyzed, and each gene was determined to be present in prostate cancers if the cDNA corresponding to that gene is detected in the library. Upon analysis of all

40,000 genes most of them were determined to not be related to prostate cancer. However, eight of the genes were identified as being associated with it (Walker, et al., 1999). The extent of their association is difficult to prove because correlation is more provable than causality, but the association is significant none the less.

Certain genes have been identified as being related to breast cancer that is metastatic to bone. One hundred and seven breast cancer patients who had developed metastasis had their negative-lymph nodes biopsied. Sixty-nine of the relapses were categorized as bone while 38 were labeled as not bone. Upon analysis, 69 significantly unique gene sets were discovered across bone and non-bone samples. The five most common genes in bone cancer were TFF1, TFF3, AGR2, NAT1, and CRIP1. TFF1 was studied in 122 independent breast tumors in node-negative patients which indicates it is the most prevalent mutation. The researches then attempted to predict a correlation between a cancer's gene sets and that cancer spreading to the bone. The samples were divided into a test set and a training set. Five hundred and eighty-eight genes were selected and subjected to PAM analysis. A 31-gene predictor was selected after 10-fold cross validation that could identify relapse to the bone at 100% sensitivity and 50% specificity. The predictor showed 79.3% positive predictive value and TIFF1 was present in the gene list. Two random gene sets of 50 and 100 genes were analyzed to test the validity of this method. Twenty-nine of the 50 random genes showed 100% sensitivity and of these 29, the average specificity is 13.2% which indicates that the 50% specificity found by the earlier gene list is significantly higher than a random gene set. Based on SAM sets the researchers determined that the most common genes associated with the cancer metastases were TFF1 and TFF3. TFF3 was also found as overexpressed in some metastatic prostate cancers, while TFF1 was found to induce cellular invasion of kidney and colon cancer (Smid, et al., 2006). This further proves that there is a strong tie between cancer and genetics.

In high-grade serous ovarian cancers, triple-negative breast cancers, esophageal cancers, small-cell lung cancers, and squamous cell lung cancers, the p53 gene is mutated in at least 80% of patients' tissue samples (Duffy, et al., 2017). P53 has two major functions in the cell cycle thereby linking it to cancer. P53 serves as a cell cycle regulator, so if the cell no longer needs to go through the cell cycle p53 will stop the cycle. Another function is that a normal p53 protein stops a cell that has already grown too much and is technically cancerous by initiating apoptosis for that cell (Zilfou & Lowe, 2009). When p53 is mutated the cells experience uncontrolled growth either because its cell cycle is not arrested or because a cell that is already out of control is not sent to apoptosis.

Because of the prevalence of mutant p53 in cancer patient's researchers have looked into a way to stabilize these mutant forms of p53. They analyzed mice whose p53 proteins were removed by one of several methods. One method was that the mice were injected with an anti-p53 antibody in which the p53 proteins in the mice underwent ubiquitination. Another method was by using an adenovirus, either Ad/GFP (green fluorescent protein) or Ad/His. They found that when these mice were exposed to a drug called CP-31398, not only was the DNA binding activity of mutant p53 restored thus allowing cell cycle arrest and induction of apoptosis, but it can also increase the steady-state levels of wild type p53 (Takimoto, et al., 2002). Another study showed that CP-31398 also inhibited the ubiquitination of p53 proteins. A non-small-cell lung carcinoma cell, H460, was exposed to CP-31398 for an hour, it was then exposed to ALLN, a proteasome inhibitor, for four hours. Another group of cells was exposed to ALLN alone and both were analyzed. The results showed that the cells that had just been exposed to ALLN showed a typical pattern of ubiquitinated p53 ladders. However, the cells treated with a mixture of CP-31398 and ALLN or just CP-31398 alone showed no ubiquitinated p53 ladders (Wang, et al., 2003). These two applications of CP-31398 demonstrate how useful it can be in preventing cancer because of the prevalence of p53 mutations in cancer patients.

Another protein that has been a target for treatment is p21, a cell cycle inhibitor, cell proliferation effector, and apoptosis regulator. As with p53, many cancer patients have been shown to have their p21 proteins mutated. Gene editing techniques such as CRISPR, TALEN's, ZFN's and rAAV have all been used to change p21 expression and have been shown to suppress tumorigenesis phenotypes and reduce drug resistance. Several chemical treatments target p21 as well. Histone deacetylase inhibitors help increase the expression of p21. Trichostatin A, PAC-320, and HDAC inhibitor combined with bortezomib or doxorubicin, have shown to enhance p21 expression in pancreatic cancer, prostate cancer and ovarian cancer respectively (Shamloo & Usluer, 2019). All these treatments targeting p21 can have massive ramifications because of p21's effect on cell cycle arrest.

Specific genetic mutations are directly linked to specific cancers which seemingly indicates that certain genetic mutations are linked to specific cancers, thus

legitimizing the claim that cancers have genetic components to them.

Stem Cell Theory of Cancer

This theory assumes that cancer is a result of a stem cell that is unregulated and grows uncontrollably. Because of several unique characteristics of prostate cancer such as histological heterogeneity, metastatic growth, drug resistance, and distant relapse after effective primary treatment, the stem cell theory was proposed as a possible explanation for prostate cancer. It was tested by searching for stem cell markers in the cancerous tissue. While there is not one marker specific for prostate cancer stem cells, there are a few methods used to help identify the stem cells such as: β1 integrins, CD133, CD44, stem cell antigen 1 and the ABCG2-associated drug-resistance proteins. β1 is a marker for "stemness" because it is essential for sustaining a functional stem cell population and establishing asymmetric division. β1 is also important to stem cell maintenance. CD133 is a marker because it is generally found in progenitor stem cells, linking it to a stem cell. CD133+ prostate cells use their stem cell like features developing prostatic-like acini in immunocompromised male mice. CD44 is a cell surface protein involved in cell-to-cell interaction, migration and adhesion. Stem cell antigen 1 is expressed in the tissue of several stem and progenitor stem cells including, cardiac mammary, hematopoietic, testicular, integumentary and muscular. These markers have been found in prostate cancers thus giving credence to this theory (Tu & Lin, 2012). It was also found that cancers that had CD44 were much more tumorigenic and metastatic than cancers without CD44. This further proves that these markers have relevance when it comes to identifying cancers (Patrawala, et al., 2007). These two studies indicate these classic stem cell markers are present in cancers and furthermore they enhance the growth and metastasis of the cancer when they're present.

Breast cancer was also examined for these markers. The researchers analyzed surgically removed breast cancer tissue in 47 cases of only invasive duct carcinoma (IDC), 135 cases of IDC with ductal carcinoma in situ (DCIS), 35 cases of DCIS with microinvasion, 58 cases of pure DCIS and 73 cases of IDCs with adjacent DCIS. Four major subtypes of breast cancer were looked for in this analysis: luminal A, luminal B, HER2+, and basal-like. Each subtype was defined based on certain characters, luminal A (ER+ and/or PR+, HER2–), luminal B (ER+ and/or PR+, HER2+), HER2+ (ER–, PR–, HER2+), basal-like (ER–, PR–, HER2–, basal cytokeratin+, and EGFR+/–). CD44+/CD24– and CD44–/CD24+ cells were found by way of double immunohistochemistry. It was found that luminal A tumors were least common in DCIS with microinvasion, luminal B tumors were least common in IDC alone, and basal-like tumors were least common in pure DCIS groups. CD44, CD24 and ALDH-1 markers were analyzed in normal breast tissue they were then compared to cancerous breast tissue. In normal breast tissue CD44 was localized to the basal, myoepithelial, and a subset of luminal epithelial cells. CD24 was found on the apical membranes of luminal cells while ALDH1 was heterogeneously expressed in luminal and basal cells. CD44 was expressed in 57% of IDC only samples, 59% IDCs with DCIS samples, 62% of DCIS with microinvasion samples, and 85% of DCIS samples. CD24 was not nearly as prevalent as it was present in 24% IDC only tumors, 38% of IDC with DCIS tumors, 59% of DCIS with microinvasion tumors and 62% of DICS tumors. While ALDH1 was not commonlyf ound in any of the four subtypes, it was far more common in IDC alone and IDC with DCIS than the other two subtypes of DCIS with microinvasion and DCIS alone, 9%, 6%, 3%, 3%, respectively (Park, et al., 2010). These figures point to the fact that there is an association between stem cell markers and breast cancer, further legitimizing the stem cell cancer theory.

Head and neck squamous cell carcinoma (HNSCC) such as cancers of the oral cavity, pharynx, larynx, paranasal sinuses, nasal cavity, salivary glands, or head and neck lymph nodes were studied to aid in further proving stem cell theory. Multi-modality therapy including surgery, has been emphasized as the method of treatment, yet the five-year survival rate for HNSCC is 0-40%, with no significant improvements in the past 30 years. A study was done using tissue specimens of cervical lymph nodes, primary tumors, and normal mucosa of patients undergoing surgical treatment of squamous cell carcinomas of multiple sites in the upper aerodigestive tract. A total of 82 primary tumors and 24 metastatic lymph nodes from 82 patients were analyzed. Several variants of CD44 were tested for: CD44s, CD44 v3, CD44 v6, and CD44 v10. It was found that a majority of the cells from both the primary tumors and lymph nodes presented strong expression of all 4 variants. CD44v3, v6 and v10 had a higher proportion of being strongly expressed in lymph nodes than they did in primary tumors. Strong expression of CD44 v3 in primary tumors was also proven to have association with lymph node metastasis. Strong expression of CD44 v10 in primary tumors showed association with radiation failure and with distant metastasis. The expression of CD44 v6 was significantly associated with perineural invasion. Next, an analysis was performed with regard to disease free survival and overall survival. Overall tumor stage was

found to be correlated with shorter disease-free interval. Non-oropharyngeal primary site, positive cervical lymph nodes, distant metastasis, CD44 v6 and CD44 v10 expression, in primary tumors, were all significantly associated with worse disease-free survival. Prior radiation therapy, expression of CD44s and, both the standard and variant form of CD44, in metastatic lymph nodes, were all not significantly associated with disease free interval or overall survival. Expression of CD44s, v3, v6, v10 were all associated with advanced primary tumor stage, treatment failure, reduced disease-free interval, and metastasis (Wang, et al., 2009). These stem cell markers are all indicative of very poor prognosis in HNCSS patients.

Researchers analyzed two cell groups of one patient who suffered from colon cancer, the primary tumor cell (SW480) and the metastatic lymph node (SW620). These cells were analyzed by phase contrast microscopy and were revealed as having two separate morphologies. SW480 was shown to have 80% of its fully adhered cells to be irregularly shaped, while 20% were spindle-like. SW620 displayed a more mixed morphology amongst its cells with 53% of its adhered cells as elongated spindle-shaped and the remaining 47% were either more rounded spindles or irregularly shaped. The two cell lines displayed similar measures of cell growth normal to their respective cell line's. When measuring migratory potential, SW480 cells were found to have a significantly higher migratory potential than SW620 cells. Several stem cell markers were analyzed in each cell line including, CD338, CD44, CD133, CD24 and CD49f. SW620 revealed a 50.6% proportion of CD44+/CD133+ cells and SW480 displayed 28.6%. But the proportion of CD44+/CD133- cells favored SW480 over SW620 with 54.3% and 20.7% respectively. CD44+/CD24- cells were abundant in both cell lines with SW480 having 62.7% and SW620 having 75.3%. The presence of CD49f+/CD338- was the highest with SW480 and SW620 expressing it at 98 and 99%. Because of the prevalence in both groups of cell lines, it was deemed that these stem cell markers were not significant enough to differentiate between SW480 and SW620 in vitro cells (Slater, et al., 2018). While utilizing stem cells did not prove to be a method to differentiate between colon and metastatic tumors, it does prove the presence of these stem cell markers in cancers.

Utilizing the stem cell theory, researchers have developed a new treatment for leukemic stem cells (LSCs) that reside mainly in the bone marrow. Chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) were analyzed. CML is categorized as a clonal hematopoietic stem cell that is caused by a translocation of a fusion of two parts of two separate chromosomes, ALB1 (chromosome 9) and BCR (chromosome 22). AML is the most common form of adult leukemia and is categorized by infiltration of leukemic cells into the bone marrow and blood. The current therapies have an overall survival of roughly 40% in patients under 60 and decline by 5-15% in older patients. AML is very difficult to treat because of how drug resistant the disease is. While CML and AML are both considered to have leukemia stem cells, the environment of the bone marrow that contains many growth factors only expedites the growth process. The markers used to indicate CML LSCs are CD25 IL-1 and CD26 have been suggested as markers that are specific to this disease as opposed to other stem cells. CML LSCs vary from ALM LSC in that CML LSCs are defined as CD34+/CD38- fraction whereas AML LSCs are composed of heterogenous populations and aside from CD34+/CD38- they also are in CD34+/CD38+ and CD34-. The treatment method for these cancers is to disconnect the cancer from the growth promoting bone marrow environment thus making them more sensitive to conventional therapy. There are several compounds being studied as possible ways to disconnect the AML or CML from the bone marrow such as: BL-8040, CAR-LMC, Ruxolitinib, AMD3100, TH-302, Aflibercept, AS101, AMG 386, SRF231, TTI-621, CC90002 Hu5F9-G4, LY3039478. These compounds all have different target cites but are all designed to disconnect the cancer from the growth advances provided by the bone marrow. IL-1RAP has also been shown as a good marker to target CML LSCs in a more selective manor because of its specific expression in CML LSCs. Inducing apoptosis is a common approach to AML treatment (Houshmand, et al., 2019).

Antibodies targeting IL-1RAP were thought as a possible way to treat CML LSCs without harming normal stem cells. The antibodies mAb81.2 and mAb3F8 were used and generated by the hybridoma technique. The cell cultures were bone marrow and peripheral blood from healthy volunteers and CML patients. The CML cells were stimulated with IL-1B, IL-33, IL-36 or SCF. IL-1B and SCF resulted in a slight expansion of CD34- CML progenitor cells. The stimulation as a result IL-1B on CD34+/CD38- cells resulted in a 30-fold increase in cell numbers as opposed to normal cells that responded weakly. In vivo samples were studied to show the therapeutic effects of IL-1RAP antibodies. Mice who expressed IL-1RAP were treated with mAb81.2 which resulted in prolonged survival. The treatment proved to be very effective to the point where the treatment was stopped after 45 days, yet the mice survived an additional 12 days with 2 mice living until day 101. Several of these mice displayed significantly lower bone marrow leukemic cell levels compared with

control mice. These antibodies are effective because they block the signals of IL-1 as well as initiating effector cells to kill tumor cells. This treatment does not affect normal human stem cells because normal stem cells do not express IL1RAP (Agerstam, et al., 2016). This study shows tremendous promise of utilizing the stem cell theory's principles as a route of treatment.

Conclusion

While there is a lot of data supporting each understanding of cancer development, there seems to be one correct approach to cancer; it appears that the correct answer is not one of these three theories rather a combination of the immunological theory and the somatic mutation theory. The immunological theory seems almost entirely correct based on the utilization of CAR-T and CAR-NK, DC-CIK as successful methods of treatment as well as the case-report of a tetanus shot proving to increase cancer prognosis. The issue with solely using the immunological theory is that it better explains the treatment of cancer and what should happen to prevent cancers from developing (NK cells) but it does not address why a cancer cell forms. Rather it only states once a cancer forms the immune system should prevent it from developing. The somatic mutation theory explains why a cancer starts to develop but the immunological theory explains how to prevent it from continual growth. These theories, in tandem, are the best explanation of cancer development.

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The Biocompatibility of Various Dental Materials

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Abstract

Dentistry is a continuously evolving field with new materials and technologies constantly innovated. The oral cavity presents a harsh environment in which restorative and implant materials must be able to withstand. Aside for meeting the appropriate physical and chemical standards, it is important that dental materials be biocompatible. Biocompatibility relates to the material's ability to function in the body without causing harm to living tissue. It is necessary to analyze the materials being used and determine whether they interact with the body in a detrimental manner. This will enable dental professionals to choose the most beneficial material to utilize for patient safety and overall health.

Introduction

Tooth decay is a prevalent health issue faced by the general population. In fact, according to the National Institute of Dental and Craniofacial Research, 92% of adults between the ages of 20 to 64 have had dental caries in their permanent teeth. Our teeth are comprised of four tissue layers. Enamel, dentin, and cementum, are the three outer layers of hard tissue, which surround the soft pulp tissue layer containing nerves, blood vessels, and connective tissue. Cavities form when bacteria and food debris combine with saliva to create a layer of plaque which sticks to the teeth. Plaque contains acid which begins to dissolve the enamel of our teeth and cause dental caries when the plaque layer is not removed. Tooth decay is an active process that will continue to progress through the multiple layers of tooth structure if left untreated.

There are various restorative treatment options that exist to treat damaged teeth. Choosing the right material is important considering that our teeth are exposed to constant wear and tear. At times, teeth can regress beyond the ability to be repaired. Fortunately, implant replacement has become a viable option, with the use of various metals and synthetic materials. Materials must be durable as well as viable in an aqueous environment.

Amalgam and composite resin fillings are the two most common materials used to fill cavities. Amalgam is composed of liquid mercury combined with an alloy made of silver, tin, and copper solid particles. Dental amalgam is inexpensive and easy to work with, making it a convenient option for dental restorations. Composite resin material is made of a ceramic and plastic compound which makes it a good tooth colored alternative. At present, titanium is the gold standard in implant dentistry. On the other hand, there are other materials being explored in cases where titanium may present issues of biocompatibility.

However, when introducing any foreign substances into the human body there is always the risk of detrimental side effects. Some of the components in these filling materials can be cause for concern. For example, the use of dental amalgam gives rise to mercury vapor exposure. In contrast, composite resin fillings may release bisphenol A (BPA) and other monomers into the blood stream. Both of these restorative materials contain components that can have toxic effects on the human organ systems and may be hazardous to our health.

This review will explore the biocompatibility of these restorative and replacement materials and analyze the possible long-term side effects that they may have on one's overall health.

Material and Methods

Research was done by studying original research articles and scientific papers found on the Touro College online library. Specific scientific databases such as ProQuest and EBSCO were utilized, and additional information was obtained by analyzing articles found.

Discussion: Amalgam

Amalgam fillings, also known as silver fillings because of their appearance, are one of the oldest used materials in restoring decayed teeth. The low cost, ease of placement, and durability make amalgam a great option for dental fillings. The material is composed of a powdered alloy of silver, tin, copper, zinc, and elemental mercury. Directly prior to placement, the components are mixed together to activate the filling, which makes it soft and pliable for easy placement. It is not necessary for the area receiving the amalgam to be completely dry, which makes it a good choice in locations that are difficult to isolate from saliva.

With a composition of about 50% elemental mercury by weight, there is a concern regarding the toxicity of this material. Interestingly, although it has been in use for over one hundred years, dental amalgam has not undergone the regulatory proof of safety testing required of materials intended to be implanted into the human body. Therefore, the question remains as to whether the components of this material interact with the body in a harmful way.

Inhaled mercury vapor can cause damage to the brain, kidneys, and other major organs. After being inhaled into the lungs, it is transported through the blood to the brain. Liquid mercury is dangerous because it vaporizes at room temperature and can easily be inhaled. Although amalgam fillings harden rapidly and remain in the solid metallic form once placed in the tooth, studies have shown elevated levels of mercury in the blood and urine

following restorative procedures utilizing amalgam as the material of choice (Nicolae, et. al., 2013). This indicates that there is some inhalation of mercury vapor prior to the material hardening.

Aside for the inhalation upon initial placement of amalgam fillings, low levels of elemental mercury vapor are released when amalgam filled teeth are subject to stress due to chewing, brushing, bruxism, and ingesting hot foods. Measuring urinary mercury concentration is a good method of analyzing the long-term influence due to the presence of such fillings. A comprehensive study utilizing this method of analysis was conducted within the Canadian population (Nicolae et. al., 2013). Factors considered included age, gender, and the number of filled surfaces. Of all groups, 95.42 – 98.23 % of participants had mean urinary mercury levels below 5 ug Hg/L. When compared to a normal range of up to 20 ug Hg/L, these concentrations do not seem to be cause for concern. Overall, less than 5% of participants had mean urinary mercury levels that qualified for possible reevaluation.

Although amalgam seems safe to use in the mouth, there is a potential concern for dental professionals who experience daily exposure (Jamil et. al., 2016). A recent study demonstrated that people working in the dental environment who handle amalgam do have increased levels of mercury concentrations in their blood. Mean concentrations in both dentists and dental assistants were above the normal range of 20 ug/ L, with dentists at a mean concentration if 29.835 ug/L and assistants at 22.798 ug/L. Also, the study demonstrated a correlation between dentists who had more years of experience with greater increases in mercury levels in the blood. This seems to indicate that there is somewhat of a cumulative effect. In addition, working longer shifts was associated with higher concentration of mercury in the blood (Jamil et. al., 2016).

With the phase down of amalgam fillings, this should be less of a concern. Although dental professionals are still utilizing amalgam fillings sporadically, other fillings have become more popular. This would suggest that the occasional use of amalgam for a filling would not contribute significantly to an increase in mercury concentration in the blood. A possible method of preventing increased mercury concentration in the blood of dentists would be to implement appropriate regulatory safety measures, such as wearing protective masks and gloves.

The New Zealand children's amalgam trial (CAT) was a study that explored the impact of exposure to mercury from amalgam fillings on neuropsychological and renal function, focusing specifically on children as the population (Bellinger et. al., 2007). The randomized sample size was 534 participants, and at baseline there were no amalgam restorations present. Over a five year period, there was an average of 15 tooth surfaces restored per patient. When measured, the group with amalgam as the dental material of choice for restorations had significantly higher mercury levels in the blood. However, this did not translate into impaired neuropsychological and renal function.

A possible explanation can be that this was a study conducted with children as the participants. Increased mercury levels may have a cumulative effect. Perhaps adverse health effects would not appear until later in life. For this reason, it would be appropriate to take precautions with pregnant women and children who are still developing. In fact, studies have shown that mercury easily crosses the placenta as well as the blood brain barrier (Magos, Clarkson, 2006).

Furthermore, with the increase of exposure to electromagnetic fields due to common sources such as Wi Fi routers, laptops, mobile devices, and MRI, there is a new aspect to consider regarding the safety of amalgam fillings. Although fillings have been shown to release mercury vapor even after being placed, countless studies have demonstrated that the release is generally at extremely low dosages which is not a cause for concern. However, the presence of electromagnetic fields increases the release of toxic mercury from amalgam fillings (Mortazavi et. al., 2015). This creates an extra concern specifically for pregnant women who have amalgam fillings. Mercury circulating in the blood easily crosses the blood brain barrier, as well as the placenta (Magos, Clarkson, 2006).

An in vitro study was conducted to examine this new concern regarding amalgam toxicity. The goal was to test the effect of electromagnetic fields on the release of mercury from amalgam fillings. The study included twenty healthy premolar teeth that had been removed for orthodontic treatment. The teeth were all prepared to be filled in an identical way, by the same dentist. They were then restored using amalgam and placed into artificial saliva. The control group was stored in an environment away from any exposure to electromagnetic fields. The experimental group was placed in the vicinity of a Wi-Fi router that was actively exchanging data with a nearby laptop.

Following electromagnetic exposure, the mean concentration of mercury in the artificial saliva was tested. The concentration in the saliva containing teeth that had been exposed was .056 mg/L, in contrast to the unexposed control group that had a mean concentration of .026 mg/L. The results clearly indicate that the radiofrequency radiation given off via Wi-Fi devices can increase mercury release from amalgam fillings (Paknahad et. al., 2016). Being that it is one of the first studies exploring

this concern, it would be necessary to conduct further studies before stating that it is a concern.

There are people who remain concerned with the presence of amalgam fillings, and even desire to have such fillings removed. However, removing such filling for no other dental related reason may cause more harm than benefit. This is because drilling old fillings would cause the release of additional mercury vapor and would cause the patient to unnecessarily inhale additional mercury vapor.

A study was conducted to determine whether the removal of amalgam fillings alleviated symptoms of health issues attributed to the presence of mercury. The presence of these symptoms had no other medical or psychological explanation. The study consisted of 90 patients between the ages of 20 -50 who reported 10 symptoms of health problems that were suspected to be due to amalgam. There were three groups; a group whose fillings were removed, another group that underwent biological detoxification therapy in addition to filling removal, and a final group that did not remove fillings but participated in a lifestyle improvement regimen.

To begin, baseline mercury levels were taken to be compared to the end results. In addition, all participants were given a 50 item symptom list to complete, ranking each item 0-3, and combining the scores for a weighted sum score. The amalgam fillings were removed and replaced by other materials over a period of time, in the two groups chosen to have fillings removed. The fillings were removed one quadrant at a time with a wait period of one week between treatments. The group undergoing biological detoxification therapy, took vitamin supplements in addition to filling removal.

Mercury levels measured in the blood and urine were significantly lower in the two groups to have their fillings removed as compared to the group that did not have any fillings removed. The 50 item symptom list was completed by all participants at 6, 12, and 18 months following initial treatment. All groups showed a decrease in the main complaint sum score, with a slightly larger decrease for the two groups to have the amalgam removed (Melchart et. al., 2008).

It is difficult to demonstrate a correlation between the amalgam fillings and attributed symptoms of health issues. The results of this study indicate that although the presence of amalgam fillings increase levels of mercury in the blood, they are not the source of any known adverse health effects. Therefore, it would be unwise to have such fillings removed if not deemed necessary for improved oral health.

Amalgam continues to be used as a great option in restorative dentistry. Research thus far has demonstrated that amalgam remains a biocompatible material that does not cause harm to the human body. Although it does increase mercury levels, the concentration is too slight to be considered significant. Specifically in the case of posterior teeth where aesthetics are not as much of a concern, it may actually be a preferable option. This is because it can withstand stronger chewing forces.

On the other hand, being that it is not the only source of environmental exposure to mercury, there is a movement to utilize other available materials in cases where it will not compromise treatment outcomes. In addition, appropriate protective safety measures should be taken to protect dental personnel who are more frequently exposed. Thus far the use of amalgam has not been linked with adverse health effects. Further studies are necessary to determine the association between electromagnetic fields and the release of mercury from amalgam fillings.

Resin-Based Composite

Resin-based composites (RBC) are a more recent innovation in restorative dentistry. These fillings are made up of a mixture of ceramics and plastics. RBC are often preferred, as they match the color of teeth and have a nicer esthetic appearance. However, these fillings may contain Bisphenol A (BPA), and other components that are toxic when released as monomers. BPA is used to synthesize various monomers that make up RBC, such as BPA–glycidyl methacrylate (BisGMA). Often, residues of BPA remain in the process of synthesizing RBC material (Luo, et. al., 2016).

BPA is in the class of xenoestrogens. Xenoestrogens are chemicals that are known to be endocrine disrupters, and they inhibit normal hormone function (Zimmerman-Downs, et. al., 2010). BPA is thought to be related to various diseases including diabetes, heart disease, obesity, as well as immune and reproductive disorders. The European Food Safety Authority has established a tolerable daily intake of no more than 4 ug/ kg of body weight per day.

Most of the BPA that humans are exposed to comes from people's diet. Upon entering the body and passing through the digestive tract, BPA passes through the liver and is detoxified. The liver converts most of the BPA from its free unconjugated form to a non-estrogenic conjugated form. This prevents it from interacting with the body in a harmful way. However, research has shown that even after fasting, the concentration of unconjugated BPA is higher than predicted (Stalhut, et. al., 2009). This demonstrates that it is not metabolized immediately, but remains in the body for some time.

In addition, BPA entering the body via the skin, as well

as oral or respiratory mucosa, bypasses initial liver metabolism and circulates in the blood for a longer period of time in its unconjugated form. This presents a concern specifically when dealing with dental treatment. Materials that contain BPA or precursor molecules may leech these harmful chemicals into saliva and enter the blood stream. Various studies have been conducted both in vitro and clinically to determine if the use of these materials in dental fillings negatively impacts overall health.

A study was done to test whether the presence of composite fillings was related to increased levels of BPA in saliva. The study consisted of 40 volunteer participants between the ages of 20 -35 who were patients of dental clinics in Bergen, Norway. All of the participants underwent a comprehensive dental evaluation and were given a score between 1 and 3 based on the number of existing composite restorations. The experimental group consisted of twenty individuals with at least 6 tooth surfaces restored with RBC. The control group was made up of twenty people without any composite fillings. Five ml. of saliva was collected from all participants and stored in BPA free test tubes.

The collected saliva samples were then tested using a liquid chromatography technique. Both the unconjugated, as well as total BPA concentrations were measured. In the experimental group, eight out of the twenty were found to have saliva BPA concentrations above the detectable limit of .1 ng/mL. Within the control group there were 3 participants with concentrations above the detectable limit. However, in both cases the concentration was still very low (Berge et.al. 2017). This study seems to indicate that the presence of RBC alone is not the cause of BPA related health concerns. However, there are factors that limit this study (i.e. sample size), and further studies are necessary to explore this issue.

An in vitro study was done to test cytotoxicity of resin based composite fillings. The harmful effects of these materials are decreased once placed in dentin, but not completely eliminated. The release of toxic materials from composite materials was previously thought to be a concern only within the first 24 hours following placement. Therefore, many studies were designed focusing on the potential harm caused only following initial placement. This study aimed to test whether or not there is a long-term release of toxic materials into our body systems.

Various common composite materials used were prepared in lab dishes and light cured. The specimens were then split into three groups; a control group stored as is, a second group aged in lab simulated artificial saliva for seven days, and a final group also in saliva for 14 days. High- pressure liquid chromatography was used to test the components of the artificial saliva for presence of harmful substances. The results indicated that although the release of these substances decreased after 24 hours, a cytotoxic effect on cell activity remained even after a two week wait period. This study suggests that further clinical testing should be designed with a focus over more than a 24 hour release period (Al-Hiyasat, et. al., 2005).

Composite fillings require a bonding agent, which assists the material in adhering to a prepared tooth structure. 2-hydroxyethyl methacrylate (HEMA) and BPA are components contained in bonding agents that can be cytotoxic to human gingival fibroblast (HGF). In a recent study, cell cultures were grown using healthy human gingival tissue to test various bonding agents. It demonstrated that both HEMA and BPA negatively impact cell viability of HGF although they exhibit different patterns of cytotoxicity. Although the cytotoxic effect was reduced after 24 hours, this is a matter of concern that requires further study being that all of the bonding agents displayed some level of cytotoxic activity on HGF (Reddy, 2017). The study was important as it displayed that different bonding systems had varying effects, with some being more harmful to HGF cells than others. Until an improved method is developed it is necessary to evaluate and choose a low risk bonding agent.

When using RBC, the technique for placing them varies. Composite fillings are soft and pliable for placement, and then cured with a blue light to harden the material into a strong durable filling. These fillings are available in flowable, paste like, and bulk consistencies. Interestingly, studies have demonstrated that the method used for placement is related to the degree of release of toxic monomers and BPA into circulation (Pongprueksa et. al, 2015). They are generally placed in 2 mm increments to ensure adequate polymerization and reduce polymerization shrinkage stress. For deep fillings, a bulk fill composite has been developed that can be cured in 4 mm increments. Fillings that are not cured completely can result in monomers leeching into the oral cavity and reaching pulp tissue.

The efficiency of polymerization is important, as composites with low degree of polymerization release more monomers. The degree of conversion (DC) can be measured by means of spectroscopy. A study was conducted with the objective of determining how the DC varied based on the consistency of RBC used, and how it related to monomer elution. It also analyzed the change in release of monomers over time. Cylindrical samples were prepared using three types of RBC. They were filled in two 2 mm thick layers or one 4 mm bulk layer, and light cured to polymerize.

For all of the composites, there was a mean DC between 60 and 70%. There was a significant difference in total monomer release depending on the composite type. The paste-like composite showed the lowest monomer elution. This can be attributed to the fact that it has a lower resin content to begin with. In addition, it is administered in layers allowing for better curing. Four mm bulk placement resulted in a lower DC and higher monomer release. Evidently, curing through a thicker layer of composite material is not as effective in complete polymerization of the material. This results in greater release of toxic monomers. In addition, fillings that are not fully cured can lead to gaps forming between the filling and the tooth structure. This can lead to secondary caries.

The study demonstrated that monomer release is dependent on both the composite type and the method of application. Therefore, the usage of paste-like composite and a layered placement technique seem to be the preferable method of treatment. This would limit the unnecessary release of monomers and BPA. In addition, it was found that rinsing and scrubbing fillings following placement further reduces the escape of harmful monomers. This extra step should be implemented for a safer procedure.

During the placement of RBC, composite dust particles are released into the surrounding environment. These nano sized particles become airborne and upon inhalation can travel deep into the lungs. This may present a concern for patients, and for dental personnel who perform multiple such procedures daily. RBC are composed of methacrylate monomers, which have been shown to provoke allergic reactions. It is important to investigate whether the composite dust that escapes into the atmosphere releases methacrylate monomers.

A recent study analyzed the dust particles released from four common composites used in dentistry. Samples of composite were light cured and polished in an enclosed chamber using a diamond bur. The dust was collected by means of a cyclone vacuum for analysis. The particles collected underwent various examinations by means of microscopy and spectroscopy. They were shown to release unpolymerized methacrylate monomers (Cokic, et. al., 2017). This poses a respiratory risk to those in the surrounding environment. Interestingly, dental workers are prone to developing asthma and other respiratory issues, although the cause has not been verified. Composite dust particles released in the air may be the culprit of this observed phenomenon. Further measures should be taken to reduce the inhalation of composite dust. Perhaps better safety masks should be implemented to reduce the inhalation of unhealthy composite dust.

Amalgam vs. Composite: Which to Choose?

The biocompatibility of materials used in restorative dentistry relates to how well it can interact with living tissue. According to the above data both amalgam and composite fillings contain components that can create issues of biocompatibility. However, although studies indicate the presence of toxic materials circulating in the body following dental procedures, the concentrations generally remain below levels that are harmful. Therefore, the question remains as to which material is preferred for treatment.

With the increased focus on esthetics, many patients opt for composite fillings. Particularly when dealing with anterior teeth, it definitely seems to be a better option. However, when considering which material to use for posterior teeth, amalgam does have some advantages. This is because the back molars bear a lot of stress from chewing forces. Amalgam has been shown to withstand more strain and is less prone to cracking or needing to be replaced. In addition, composite fillings are sensitive to temperature, and shrink upon exposure to heat, which can cause increased tooth sensitivity. Furthermore, shrinkage of composite material creates a gap between the tooth and the filling. This is known as leakage, and is a big cause of secondary caries formation. However, with proper oral hygiene much of this can be avoided.

Implants

Prosthetic implant devices are an evolving field in dentistry, and choosing the right materials are key to a successful procedure. Implants provide an excellent replacement option for teeth that are no longer restorable and need to be removed. Titanium is often the material of choice, as its osteophilic nature makes it highly biocompatible. Titanium is very reactive, and will rapidly form a layer of titanium oxide when exposed to an aqueous environment (such as the mouth) or air. This forms a boundary at the interface between the implant material and biological environment, which protects the material from corrosion. Therefore, the metal ion release into the body is limited and generally unreactive (Kumar et. al, 2016).

Although less prevalent than other metal allergies, titanium allergies do exist (Lahori et. al., 2015). An allergic reaction can create a rift in this oxide layer formation and may impair the biocompatible function. Allergic reaction to titanium is caused by the release of ions interacting with native proteins in the nearby environment. This may cause a hypersensitive reaction and rejection of the implant. Determining the presence of titanium allergies and working with alternative materials is an area of continued research. Titanium allergies may be the culprit to otherwise unexplainable implant failures.

Another potential issue of this otherwise successful material is its elasticity. Young's modulus is a mechanical property which measures the stiffness of a solid material. It is a measurement determined by the slope of the stress-strain curve of a material. At 113.8 GPa, the elastic modulus of titanium is significantly higher than bone (18.6 GPa). This makes it less flexible than bone and can result in strain upon implant insertion and subsequent implant failure (El Hajje et. al., 2014). The implant material will act to shield the bone from stress, which eventually can lead to it loosening and losing contact with the surrounding tissue.

Zirconium has been introduced as a possible alternative implant material. Clinically, it has demonstrated success similar to traditional titanium implants. Studies have shown little difference in osseo-integration between the two materials. In addition, zirconium implants actually display reduced microbial growth as compared to titanium. This decreases plaque formation on the implant and surrounding tissue, which is important for the long-term success of an implant.

Peri-implantitis is the inflammation of the gum and bone structure in the area of a dental implant. It is caused by the buildup of trapped bacteria and can lead to bone loss. An in-vitro study was conducted to explore the adhesion of oral bacteria to different implant materials. It included both titanium and zirconium, as well as a combination of both. The results displayed that oral bacteria have less of an affinity for zirconia (Al- Radha et. al., 2012). This suggests that zirconia implants can lead to better implant results and more successfully prevent the development of peri-implantitis.

Over time, recession of soft tissue surrounding implants can occur. This can lead to exposure of implant parts and is a particular concern when dealing with anterior teeth. Also, titanium implants can result in discoloration of gingival tissue. In patients with a thin gingival biotype or high smile line, such discoloration is readily apparent. Zirconium implants may provide a solution to this issue as they result in a more esthetic appearance (Bhasin, et. al., 2015).

Polyetheretherketone, or PEEK, is a new material being explored as a possible alternative implant material. This may be an effective way to circumvent the titanium allergy, as well as provide a more esthetic option. In addition, with a low elastic modulus it can prevent issues of stress-strain distribution. This is in contrast to both titanium and zirconium, which possess higher elastic modulus (El Hajje et. al., 2014). However, being that its elastic modulus is actually lower than that of bone it is necessary to reinforce the material in order to use it successfully (Schwitalla et. al., 2015.

A finite element analysis technique, which is commonly used to test dental materials in vitro, was used to study the stress distribution on the jaw following implant placement. It focused on titanium vs PEEK as the materials to be analyzed. A 3D model of the left mandibular jawbone was produced and an implant screw and abutment was inserted. Three different implant materials were used in the study to be compared, including titanium, and two different forms of commercial PEEK.

Circular contact areas were used for the purpose of testing the degree of stress caused to the different implant materials. Force was applied to the occlusion areas of the simulated implant via a specialized device, and the stress, deformations, and contact pressure were measured. The results of the study indicated that PEEK reinforced with 60% vertical carbon fibers was similar in stress distribution as titanium. Pure PEEK displayed higher stress damage. Perhaps this can be attributed to the fact that the elastic modulus of pure peek is lower than that of cortical bone. Reinforcing it with vertical carbon fibers makes it strong enough to withstand more stress, yet still maintain a lower elastic modulus than titanium (Schwitalla et. al., 2015).

The characteristics of PEEK suggests that it possesses the potential to be a good alternative to titanium. However, long term studies regarding clinical success are lacking. Further research is necessary before implementing the use of this synthetic material as a final abutment for implants.

For the most part, titanium implants have a track record of success and continue to be used as an excellent replacement option. It is a highly biocompatible material that generally integrates into the bone well. In the case of allergies or hypersensitivity, there are other options being explored. Zirconia is a good alternative as it has shown similar clinical success and may even have higher antimicrobial properties. In addition, it seems to be a preferred option in the case of anterior teeth and thin gingival biotypes.

Conclusion

The above studies indicate that the field of biomaterial compatibility requires further attention. It is necessary take a closer look, even though they are already being utilized in the dental field. Amalgam as an older material is less of a concern. However, in regard to composites, future studies focusing on the long term and cumulative effect should be designed. This information is important in being able to further improve the biocompatibility of dental materials. On the other hand, with both of these materials, the release of toxic components is shown to be below baseline levels of what is harmful to the body.

Therefore, in terms of clinical applications the decision of which material to utilize can be made by practicing dentists. The choice can be made based on the individual needs of each patient and situation.

When dealing with implants, titanium remains an excellent option. Although it is a small percentage, there are some people who experience hypersensitivity or allergies to this material. There are other materials being explored with similar success rates. Some of these materials have shown good potential experimentally, yet clinical case studies are lacking. Further studies are necessary before implementing the widespread use of such materials.

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What are the Most Appropriate Treatments, Preventions and Family Planning Options for Patients with Factor V Leiden?

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Abstract

The management of Venous Thromboembolism in terms of treatments and prevention has been well researched for patients who have been discovered as positive for Factor V Leiden genetic clotting disorder, in either the homozygous or the heterozygous genotype. In fact, the research and development of new agents called Direct oral anticoagulants aim to treat and prevent clotting events while minimizing the risks of bleeding. Although several risk factors have been identified in relation to VTE, Factor V Leiden mutation presents a seemingly higher risk due to its unpredictable incidences of clotting events, even in patients with optimal health characteristics. Yet, we haven't transitioned to routine testing of the common population for the Factor V Leiden as a preventative measure, rather the focus has been on those at risk due to family history of VTE or if clinical decisions might depend on it, such as the proper choice of contraception for female patients. Additionally, the increased risk of thrombophilia during pregnancy raises concern for proper medical management that would benefit the mother, without harming the baby, at any point of the pregnancy.

Introduction

For many people, the thought of having a blood clot is terrifying and rightfully so because it is potentially fatal and essentially invisible. A blood clot can appear in the leg, in the lungs or even in the brain. Also called a thrombus, a clot represents an accumulation of blood in a solid state, essentially caused by coagulation processes. Although coagulation is an important and vital function of homeostasis, the resultant clot formation may obstruct blood vessels enough to limit perfusion of their field of supply. Medicine has shown that many risk factors for developing thrombi are acquired and depend on a patient's physical or medical conditions such as obesity, hypertension, atherosclerosis, or even pregnancy. However, different genetic mutations appear to be linked to increased risk of thrombosis, such as Factor V Leiden, named after the city of Leiden in the Netherlands, where it was first identified while researching links between oral contraceptives and increased cases of thrombosis (Vandenbroucke et al., 1994). Factor V Leiden is the most common genetic mutation causing increased risk of thrombophilia, or abnormal coagulation, therefore treatment options should be carefully considered for patients with clotting history, as well as preventative measures for those who have not presented with thrombi. In view of the association of blood clots with family planning measures, this review will focus on women with Factor V Leiden, considering female individuals with or without a history of thrombophilia.

Background:

Pathophysiology of Factor V Leiden

The factor V Leiden thrombophilia, also called Activated Protein C resistance, stems from a genetic mutation that affects parts of the clotting cascade, a physiological process necessary for proper regulation of coagulation (Van Cott et. al, 2016). More specifically, Activated Protein C (APC), along with a cofactor Protein S, normally inactivates some coagulation factors, hence acting as an anticoagulant system. The mutation results in a decline in that inactivation, which has the potential to increase the coagulation activity. As for all genetic mutations, they can be present as heterozygous with one abnormal copy of the gene, or homozygous, for which both copies of the gene are mutated, the latter increasing the risk for thrombophilia by 80-fold. Regarding the population distribution of the Factor V Leiden mutation, it appears to be more prevalent in Europe, followed by Africa, and less prevalent in America (Rees, Cox, 1995).

Methods

To complete this review, multiple scientific scholar databases and peer-reviewed articles mainly found on the Touro College online library were used. The Proquest, Browzine, Google scholar, and Dynamed databases were utilized with specific associations of key words in order to optimize the research and the results necessary to complete this study.

Discussion

When exploring the Factor V Leiden mutation and its relation to increased risk of venous thrombosis, it is first essential to make the distinction between individuals who have had a previous clotting event such as a deep vein thrombosis or a pulmonary embolism and those who have not. In fact, treatment options, preventative measures and family planning possibilities may be affected by a positive history of thrombophilia, regardless of the presence of any physiological risk factors, not to be dismissed, but to be utilized in conjunction with factor Factor V Leiden mutation at the time of medical management (Anderson, 2003). Those risk factors should be touched upon for reference and comparison throughout this review.

Non-genetic Risk Factors of Venous Thrombosis

The risk factors for VTE (venous thromboembolism) mainly consist of obesity, long periods of immobility, surgery, smoking, age, pregnancy and use of oral contraceptive pills, according to the NYU VTEC center and the

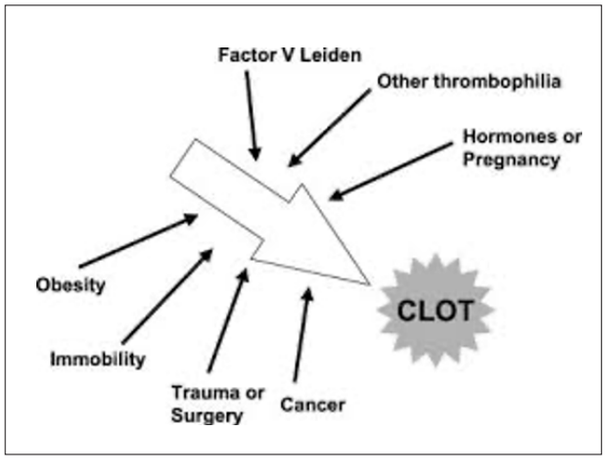

Figure 1: Most common risk factors of Venous Thromboembolism

Cornell center for blood disorders, both specializing in venous thrombosis. Although common knowledge supports their statement, others might argue about the true character of "risk factor" for some of the elements above mentioned. For instance, obesity appears as a weak link to increased VTE, based on multiple studies, therefore it should be carefully analyzed when considering treatment or even prophylaxis (Anderson, 2003). Similarly, although age seems to count as a risk factor for many diseases or medical complications, its direct connection to increased VTE is questionable, especially due to the variety of ethnicities throughout the world and their life standards, as described by an epidemiology review with respect to China presenting an impressively low rate of DVT per year (Cushman, 2007). Therefore, we should denote the importance of evaluating an association of physiological and potentially genetic risk factors when treating or preventing venous thrombosis.

Treatments and Preventions of Venous Thromboembolism

It is essential to distinguish between patients who have had a VTE event and those who haven't, as it may affect their treatment approach, even if they are positive for factor V Leiden. Additionally, this section will analyze whether treatments and preventions will be influenced by the homozygous vs heterozygous factor V Leiden status.

When discussing treatment options, we refer to patients who have actually had a DVT or a pulmonary embolism and require treatment with anticoagulant. Although the review focuses on patients with Factor V Leiden, it is important to make the comparison with patients who do not have any genetic mutation that would favor VTE, in order to better understand the rationale for treatment and prophylaxis.

Duration of Treatment

A minimum of 3-6 months of anticoagulation therapy is recommended for all patients who suffered a VTE, regardless of physiological or genetic risk factors (Kearon, Akl, 2014). However, if a VTE is unprovoked, meaning without obvious risk factor, it is highly recommended to pursue anticoagulation for at least 12 months, or perhaps indefinitely to avoid recurrence, as highlighted by multiple studies compiled in one of the articles on UPTODATE, an online medical reference, that strongly emphasized the reduction in rates of VTE recurrences in patients who had been on anticoagulation long term (Lip, Russell, 2019).

Moreover, it is debatable whether a genetic mutation causes unprovoked VTE or if it is provoked by the mutation. Even if provoked, opinions about the duration of treatment vary widely, especially if the patient's genotype is heterozygous for factor V Leiden; in fact, many providers will not treat indefinitely for a VTE provoked by a genetic factor, (Lip, Russell, 2019). More surprisingly, the actual difference in VTE incidence between both Factor V Leiden genotypes seems questionable. Indeed, when analyzing the number of actual cases and the percentage of recurrence after a first event, only a slim difference between homozygous and heterozygous percentages in VTE events (Perez Botero, Ormsby, 2016). Therefore, we can argue that the Factor V Leiden genotype shouldn't be used alone to distinguish between lengths of treatment.

On a separate note, it is essential to take into consideration the bleeding risk associated with taking anticoagulant agents, commonly named "blood thinners". As a matter of fact, all anticoagulants increase the bleeding risk to some extent and require vital risk versus benefit assessment when initiating treatment for VTE and determining duration of treatment. Bleeding associated with anticoagulation can range from a simple hematoma or easy bruising, to life threatening gastrointestinal or intracranial hemorrhage (Garcia, Crowther, 2019). Evidently, the longer a patient is on anticoagulation treatment, the higher chances he/she has of developing internal bleeding events, and those shouldn't be taken lightly, therefore must be investigated even with minimal symptoms, such as coughing up a little blood. In fact, the Mayo clinic recommends precaution when prescribing blood thinners especially to older patients (>75 years old) who present higher chances of GI bleed (Harringa, 2019). On a similar note, when treating a patient with factor V Leiden with anticoagulation on a long-term basis, one ought to carefully monitor the bleeding effect of the prescribed agent. Indeed, we can also include female patients with factor V Leiden, for which continuous anticoagulation therapy will result in heavier menstruations. Although, we previously

argued that the factor V Leiden genotype alone doesn't determine the length of treatment, those patients will need long term treatment since the factor V Leiden is a genetic component, and by definition cannot just disappear, as opposed to obesity. Therefore, if it is a constant risk factor, it will most likely represent a constant threat. The next section will present various anticoagulants that have been discovered and manufactured.

Different Anticoagulants on the Market

Table 2: Pharmacotherapy Options

Medication	Drug Class	Mechanism of Action	Daily Dosage	Renal Dosage Adjustments	Reversal Agent
Warfarin (Coumadin, Jantoven)	VKA	Inhibits formation of vitamin K dependent clotting factors II, VII, IX, X and proteins C and S	Dependent on INR	No	Yes; phytonadione (Mephyton)
Dabigatran (Pradaxa)	DOAC—direct thrombin inhibitor	Directly inhibits thrombin	150 mg twice daily	No	Yes; idarucizumab (Praxbind)
Apixaban (Eliquis)	DOAC—factor Xa inhibitor	Directly inhibits factor Xa	10 mg twice daily for 7 days, followed by 5 mg twice daily	No	None
Betrixaban (Bevyxxa)	DOAC—factor Xa inhibitor	Directly inhibits factor Xa	Single dose of 160 mg, followed by 80 mg daily for 35-42 days	CrCl >30 mL/min: no dose adjustment CrCl 15-29 mL/min: 80 mg single dose, followed by 40 mg daily CrCl <15 mL/min: use not recommended	None
Edoxaban (Savaysa)	DOAC—factor Xa inhibitor	Directly inhibits factor Xa	60 mg daily	CrCl ≥50 mL/min: no dose adjustment CrCl 15-50mL/min: 30 mg daily CrCl <15 mL/min: use not recommended	None
Rivaroxaban (Xarelto)	DOAC—factor Xa inhibitor	Directly inhibits factor Xa	Treatment: 15 mg twice daily for 21 days, followed by 20 mg daily Recurrence risk reduction: 10 mg daily	CrCl ≥30 mL/min: no dose adjustment CrCl <30 mL/min: avoid use	None

CrCl: creatinine clearance. DOAC: direct oral anticoagulants; INR: international normalized ratio; VKA: vitamin K antagonists.
Source: Reference 19.

Table 1: Oral Anticoagulant comparative table

Based on the table above, multiple factors are to be considered when initiating a patient on oral therapy following an acute VTE event, especially when the patient is positive for factor V Leiden, and will most likely require long term anticoagulation therapy The drug class can be taken into account . In this case, both warfarin and the rest of the agents called Direct Oral anticoagulants (DOACs) are acceptable for treatment of VTE (Milling, Frontera, 2017). Furthermore, the daily dosage varies among all AC agents, from once a day with Warfarin or Xarelto, to twice a day with Eliquis or Pradaxa. Therefore, it raises an obvious question of medication compliance. Surely patients would be more likely to comply with their medications if only they needed to be taken once a day instead of twice a day. In that respect, Warfarin or Xarelto seem to be optimal options. However, as indicated on the table above, the daily dose of warfarin is dependent on a standardized international blood test called INR. In fact, patients on warfarin need to check their INR blood test quite often as their dose of warfarin will be adjusted based upon the results. Therefore, the frequent change of coumadin dosage undoubtedly will result in patients confused with dosage, that is without considering frequent venipunctures to test the INR. In addition, warfarin is a vitamin K antagonist, which means all foods high in vitamin K will counteract the effect of coumadin; hence, those foods should be avoided or eaten in small portions all throughout the duration of the treatment with warfarin; a factor to consider greatly since a lot of greens or even garlic can interact with warfarin (Mayo Clinic, 2018).

The next component of the table includes the creatinine clearance, representing a potential risk of certain DOAC agents such as Xarelto, on the renal function. Although it doesn't directly concern a DVT or a PE, certain patients who present with blood clots events, may also suffer from other medical conditions such as renal disease, which in this case can render the choice of AC more challenging.

The last component of the table indicates the possibility to reverse AC agents due to overuse or excess bleeding for example as a result of hypovolemic shock or major trauma. Warfarin's reversal agent is Vitamin K. Moreover, a reversal agent became approved by the FDA for both Eliquis and Xarelto this year, by the name of Andexanet Alfa (Cuker et. al., 2019).

Besides for the factors indicated on the table above, it is essential to also mention the potential risks of bleeding and consequent serious medical conditions such as GI bleed and intracranial hemorrhage. According to multiple trials, it appears that DOACs have a lower risk of intracranial hemorrhage or even GI hemorrhage than warfarin and therefore should be used as a first choice, if all contraindications have been carefully considered (Garcia, Crowther 2019).

Additionally, considerations must be made for female patients who are pregnant, because all the above-mentioned agents, whether Warfarin or DOACs are not indicated during any of the stages of pregnancy. Alternatives

will need to be used as needed for treatment of a VTE during pregnancy or even as a prophylactic agent; those will be contemplated in the family planning section.

The duration of anticoagulation treatment varies with the unprovoked versus provoked status of a VTE, in patients with either Factor V Leiden genotype, heterozygous or homozygous. A blood thinner treatment is designed to treat the current thrombosis, and even more importantly to prevent recurrence. The nature of the AC agent will depend on the availability of a reversible agent, the patient's current renal status and the level of compliance a provider attempts to achieve, apparently more in favor of DOACs due to decreased risk of internal bleeding, compared to warfarin. Recent tendency has it to get tested for genetic conditions such as Factor V Leiden, generally due to family history of clotting. Therefore, if a patient has a Factor V Leiden mutation, there may be some preventative measures to consider in certain cases, even if that individual has never experienced a VTE.

Prevention of Venous Thromboembolism for Patients with Factor V Leiden

There are several non-genetic risk factors to venous thromboembolism (VTE), such as obesity, age, recent surgery or long immobilization states. If we also consider the positive Factor V Leiden mutation genotypes, we can understand that certain patients are at serious risk for developing thrombi, whether from a physiological or genetic standpoint, or both. Moreover, patients who have had a VTE can certainly develop another one, hence the necessity for treatment, and preventative measures. Let's explore some risk factors and analyze the preventative measures that would best benefit those at risk.

First of all, we can raise the question regarding testing for genetic mutations such a factor V Leiden for patients who have had a VTE or are family members of Factor V Leiden. As a matter of fact, knowing that condition exists can be part the prevention process. It seems that newborn or infant screening for Factor V Leiden or testing of the population is not recommended, however the recommendations seem strong for female patients of child bearing age with personal or family history of VTE, as well as those under 50 years of age with a personal history of Thromboembolism (Grody, et. al, 2001). While testing family members of Factor V Leiden genotypes carriers is important in certain cases, it appears it doesn't necessarily prevent the occurrence of VTE, unless those patients are being educated on the mutation. It is especially important to educate about VTE risks and implications in different aspects of healthcare such as after a surgery, while on oral contraceptive or even during pregnancy (Segal et. al, 2009).

Regarding the prophylactic interventions using medications, patients undergoing surgery or long-term immobilization following a surgery such as a knee or hip replacement, will be offered anticoagulation prevention via a low molecular weight heparin (LMWH). The most common of those agents is called enoxaparin, with brand name "Lovenox", available as an injectable product, that can be self-administered by the patient, but requires several calculations to determine proper dosage based on weight and monitoring with a blood test called the "Anti Xa activity" that calculates its efficacy (Busti, 2015). More precisely, the LMWH acts as an inhibitor of a factor Xa, one of several proteins involved in the coagulation cascade, which results in clotting formation, either in a normal ratio or abnormal, with consequences such as VTE. .

Another commonly used agent for prophylaxis, especially following a surgery, is warfarin, previously mentioned. In fact, it may seem undesirable for a lot of patients to go through daily injections. Although the use of prophylaxis Warfarin has been associated with more than 50 % reduction of VTE post orthopedic surgery, LMWH was concluded as a superior alternative for protection against thromboembolism post-surgery (Paj, Douketis, 2019). Moreover, the use of low dose Aspirin has been contemplated as well for prevention of VTE but is most commonly used for cardiovascular prevention such as protection from strokes or myocardial infarctions.

Although the goal of anticoagulant agents is for preventing VTE, the bleeding consequences are not to be ignored. Therefore, hospital settings also offer non-pharmacological options such as an IPC device, intermittent pneumatic compression device, that improves venous circulations in upper and lower extremities to prevent edema, as well as venous thromboembolism. However, since data hasn't shown the IPC device as a complete alternative to medicated options, according to "uptodate" latest research(Paj, Douketis, 2019), it is becoming common practice to start off with IPC devices for patients with a high risk of bleeding, and then transition to a blood thinner medication agent, as presented by the AHRQ, a government agency in charge of research and quality in healthcare systems (Maynard, 2016).

Testing for Factor V Leiden is not commonly done with the general population but focuses on family members of those with the genetic mutation. In regard to prophylaxis agents for long term immobility, such as post-surgery, different agents are available and will depend on the patient's abilities to self-administer daily injections, as well as the risk of bleeding, yet it is always important to measure the levels of risk, especially for those patients with a genetic component such as factor V Leiden mutation. The

factor V Leiden genetic mutation was discovered in relation with increased risks of VTE for female patients on oral contraception. Indeed, it appears that an important amount of the research regarding Factor V Leiden thrombophilia is associated with female individuals and attend to pregnancy or contraception related matters.

Female Patients with Factor V Leiden-Family Planning

Female patients of child-bearing age who are positive for factor V Leiden need to be aware that their genotype can affect their choices when dealing with contraception and family planning.

Contraception

It is believed that oral contraceptives, especially estrogen containing agents have the potential to increase the risk of VTE; indeed, the estrogen will increase the plasma concentration of certain clotting factors and fibrinogen, all of which are favorable to clotting formation (Busti, 2015).

Although several sources have pushed for testing patients for Factor V Leiden prior to prescribing oral contraceptives, it represents a large population of female patients being tested and consequent precautions to be taken for life with constant awareness of the genotype, when there is no guarantee one of those women will ever experience a thrombosis event. Rather, a review suggests recording a comprehensive personal and familial medical history or VTE when prescribing oral contraceptives (OCP) (Rosendaal, Koster, et. al, 1995), which seems more appropriate and targeted.

It is important to understand that the association of factor V Leiden and use of oral contraceptives cause great thrombosis risk, and has been long studied and concluded as such (Choe, Suh, 2019), therefore a female patient should always mention it to her doctor before being prescribed any oral contraceptives. Luckily, several contraceptive options have become available on the market to satisfy all preferences and medical conditions, which becomes handy for those with a history of VTE or known Factor V Leiden. For example, the oral contraceptives containing only progesterone have been categorized as little to non-affecting coagulation. Therefore, these can become an ideal option for those women at higher risk of developing VTE, such as those with factor V Leiden genotypes. Similarly, Intrauterine devices (IUD) containing only progestin, such as the Mirena, are an excellent alternative to oral contraceptives for those who can't seem to comply to the daily regimen of birth control, especially when indicated to be taken at the same time every day. It is however essential to bring to light the fact that fewer studies were conducted on progesterone-only agents, therefore more data may be necessary to fully draw the conclusion of its higher grade of safety in terms of VTE prevention (Tchaikovski, Rosing, 2010). In addition, The Journal of Community Hospital Internal Medicine published an article this year warning about the progestin-only IUD, when a female patient in her early 30's with Factor V Leiden suffered a pulmonary embolism while on that intrauterine device (Jean, 2019). Therefore, although it appeared at first that only estrogen containing oral or intrauterine agents were associated with increased risk of VTE incidences, we find it questionable whether the progestin only agents are truely safe alternative for those patients at risk such as the Factor V Leiden genotypes, or even those without the genotype but with a history of clotting. Luckily for those individuals, there is now a non-hormonal intrauterine device available such as the Paragard IUD, releasing copper as a contraception method, therefore seemingly the ideal option for those of at VTE risk. In its mechanism, the copper acts as a toxic environment for sperm, which significantly reduces the chance for fertilization of the egg (Higginbotham, 2018).

Though it seems like the ideal option, it is interesting to present the reported increase of menstrual bleeding associated with the Copper Intrauterine device, as well as cramping (Galan, 2016). Furthermore, the idea of having an internal device can be disturbing for a lot of patients and has shown serious medical consequences such as ectopic pregnancies or infections, although only shown in low percentages.

To sum it up, it appears that although ample options are available on the market for contraception, the choices become limited for patients with Factor V Leiden or patients with personal or even a family history of VTE. Although intrauterine devices optimize compliance with the regimen, they aren't always the preferred options for contraception due to their potential serious consequences. Ultimately, patients at risk of developing VTE should resort to using the copper IUD, because it doesn't contain any hormones, despite its potential increase of bleeding risk, which can be even more exacerbated for those patients who already take daily anticoagulant.

Factor V Leiden plays an important role in a patient's choice for contraception, but also during pregnancy or when planning to become pregnant.

Pregnancy

Pregnancy represents a risk factor on its own for Venous Thromboembolism. During pregnancy, a patient will be considered in a hypercoagulable state, or increased tendency to coagulate, due to higher levels of fibrinogen and certain clotting factors, as well as a vascular damage or

reshaping that the increase in weight and fluid can cause (Gibson, Powrie, 2009). Following that thought process, a risk factor should be avoided, or if cannot be, should be carefully managed; in this case management of pregnancy for patients at risk of VTE.

Patients with Factor V Leiden and/or those with a history of VTE should carefully monitor their cycles as to know when to get pregnant because of the high probability those individuals will need to modify the types of AC they take. Multiple studies have examined the optimal options for prophylaxis AC in patients who are at high risk of developing VTE or those who have a history of VTE. It appears that the current recommendations suggest the use of heparin injections throughout pregnancy and up to 6 weeks after delivery, because heparin does not cross the placenta and is not transmitted via breast milk. Therefore, it is considered a relatively safe option for both the mother and baby (Gibson, Powrie, 2009). However, while Heparin is safe for pregnancy, it is important to distinguish between unfractionated heparin, which has been linked to osteoporosis and thrombocytopenia when used long term, and low molecular weight heparin (LMWH), with less incidence of the above mentioned (De Santis, et., al, 2006). An example of LMWH is Lovenox, with the generic name enoxaparin, and has demonstrated success as a prophylactic agent against VTE in several instances world-wide, one of which was reported regarding a young pregnant patient in Nigeria with factor V Leiden homozygous who was treated with LMWH during her pregnancy due to a DVT (Dogara, et, al, 2018). Since pregnancy might occur while on an anticoagulant, it is essential for those individuals to be aware of their cycles in order to start the appropriate AC agent as soon as soon they become pregnant. The use of direct oral anticoagulants (DOACs) are still contraindicated during pregnancy. In fact, it is due to the lack of data proving them safe, the few results demonstrating a high rate of miscarriage, as well as relatively significant amount of fetal abnormalities for patients on DOACs, concluded to be directly related to the DOACs, but poorly investigated (Lameijer, et, al, 2018).

Female patients who are aware of a risk factor for VTE, usually genetic such as factor V Leiden mutation, can evidently be more prepared and educated on the precautions to take regarding anticoagulation management during pregnancy. However, many patients in fact find out about their Factor V Leiden mutation during pregnancy, as they experience a DVT or pulmonary embolism, requiring emergency treatment with LMWH, or after suffering multiple spontaneous miscarriages. Interestingly, some studies have compared the rate of first trimester miscarriages in patients with known factor V Leiden and those with known regular genotype, and the incidence of early pregnancy miscarriage doesn't seem to have a close relationship with Factor V Leiden. Rather it does when comparing later term miscarriage, possibly caused by clotting within the uterine vasculature (Jivraj, et., al, 2009). Furthermore, while Factor V Leiden had been thought more risky, it doesn't appear to affect the management of pregnant patients for VTE prevention, since several studies have yet to discover significant differences in clinical pictures between homozygous and heterozygous, (Gat, et, al, 2014).

Other forms of prevention for VTE during pregnancy include compression stockings however these have received a low grade of compliance among those women due to discomfort. Moreover, it usually can improve blood circulation in lower extremities but are limited to lower extremities.

As with all patients, pregnant patients with factor V Leiden should in addition keep a healthy lifestyle, combining a balanced diet and regular exercise, to keep proper perfusion, for theirs and their babies' sake.

Conclusion

The high incidences of VTE has stimulated much research to find optimal forms of treatment and preventative agents in the forms of Anticoagulants, whether oral or injectable, for patients at higher risk of developing Venous thromboembolism, such as those individuals with the Factor V Leiden genetic mutation. Although the newer DOACs have become more popular due to decreased necessity for close monitoring, decreased bleeding risk, and statistical efficiency in the prevention of thrombosis, it appears they still cause issues for pregnant patients, and require more extensive research to prove their safety. Fortunately, low molecular weight heparin injections have been used for management of pregnant patients since they don't cross the placenta, and they have been utilized often as a post-surgery prophylaxis on a short-term basis. Regarding the management of patients who wish to utilize contraception measures, careful attention is to be applied due to the elevated clinical relationship between the use of estrogen and episodes of VTE, but unfortunately most of the contraceptive options do not seem adequate for those high-risk female individuals, therefore requiring further research and development. Although Factor V Leiden appears to be the most popular genotype associated with VTE, multiple other genetic mutations have close relationships with increased risk of thrombosis. Since the pregnancy represents a high risk factor on its own, perhaps the thought of prenatal

testing with a thrombophilia panel doesn't seem inadequate, although the question remains about a reasonable response to the screenings in terms of prophylaxis, that would be beneficial to patients' physical health without being detrimental to their mental health, once aware that an important genetic mutation is present, especially at sensitive times such as during pregnancy.

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Treating Anemia of Chronic Disease

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Abstract

Anemia of chronic disease (ACD) is the result of altered iron metabolism, blunted erythropoiesis, and shortened red blood cell survival caused by inflammatory cytokines. Hepcidin plays a key role in anemia of chronic disease by inducing endocytosis of the iron exporter ferroportin. Iron is trapped in enterocytes, hepatocytes, and macrophages, and is unavailable for hemoglobin synthesis. When treating the underlying disease is not an option, or anemia is severe, ACD should be treated. While oral iron supplementation is not suitable for ACD, intravenous iron may be effective. Erythropoiesis stimulating agents are a common approach to treating ACD, but a large percentage of ACD patients are refractory to ESAs. Additionally, there are many safety issues associated with ESA therapy. Concomitant IV iron supplementation increases patient response to ESAs and may reduce safety concerns as well. Several new, more targeted approaches to ACD treatment are being studied. Isocitrate supplements have been shown to improve ACD by minimizing the iron restriction response to increase erythropoiesis. Antibodies such as infliximab and tocilizumab, which neutralize the inflammatory cytokines TNF-α and IL-6, respectively, decrease hepcidin expression and improve hemoglobin levels. Finally, antibodies and speigelmers that target hepcidin directly are being developed. These new therapies, alone or in combination with IV iron or ESAs, show promise in alleviating anemia of chronic disease.

Introduction

Iron is an essential element for life. From the smallest microbes to the most complex of organisms, all require this vital nutrient to survive. As such, one of our bodies' many host defense mechanisms is to limit the amount of iron available to pathogens. Inflammatory cytokines cause an increase in the expression of hepcidin, a liver protein that is a key regulator of iron metabolism. Hepcidin binds to ferroportin, the only known exporter of cellular iron, and causes it to be internalized and degraded (Nameth et al., 2004a). Thus, iron is trapped inside the cells, out of reach of bacteria, cancer cells, or other pathogens.

When inflammation becomes chronic, however, this innate defense mechanism becomes counterproductive. Too much of the iron from senescent red blood cells is sequestered within macrophages, rather than recycled for erythropoiesis. Patients with chronic inflammation often develop a unique form of anemia, known as the anemia of chronic disease (ACD). ACD is the second most common type of anemia, caused by some cancers, chronic infections such as HIV, autoinflammatory diseases such as rheumatoid arthritis, and other diseases that feature chronic inflammation such as inflammatory bowel disease or heart disease. While treatment for these patients must focus primarily on curing the underlying disease, treating the anemia may greatly improve the patients' quality of life. Because of the unique mechanism by which the anemia develops, treatment of ACD can be complicated. Treatments often used to treat other forms of anemia, such as iron deficiency anemia, may be ineffective, or even detrimental. This paper examines several possible approaches for treating anemia of chronic disease, given its unique pathogenesis.

Methods

The information in this article was compiled by review and analysis of articles located using PubMed, ProQuest, and Google Scholar. Access was provided by the Touro College Library system. Emphasis was placed on original studies, although review articles were also used. References cited in the articles provided further reading. Articles discussing anemia of chronic kidney disease (ACKD), a related but different disorder were not included in this review.

Discussion:

Pathogenesis of Anemia of Chronic Disease

Hepcidin is a small peptide containing multiple disulfide bonds that is secreted by the liver. It was first isolated from human urine (Park et al., 2001). Initially recognized for its anti-microbial properties, it soon became clear that hepcidin is a key regulator of iron metabolism. Iron-overloaded mice overexpress mRNA coding for a peptide closely homologous to human hepcidin. Additionally, iron depletion results in a decrease in hepcidin expression (Pigeon et al., 2001). Genetically modified mice overexpressing hepcidin were born with pale skin and died soon after birth. Those that survived had severe microcytic iron deficiency anemia (Nicolas et al., 2002a).

A 2004 study by Nameth et al. provided a breakthrough toward understanding the role of hepcidin in iron homeostasis. Using in vitro cells expressing ferroportin (Fpn) labeled with green fluorescent protein (Fpn-GFP), they demonstrated the effect of hepcidin on ferroportin. Hepcidin binds directly to Fpn, inducing its endocytosis. Upon addition of hepcidin, Fpn-GFP disappears from the cell surface, and is localized in intracellular vesicles. (Nameth et al., 2004a). Hepcidin binds directly to ferroportin and induces rapid ubiquitination of several lysine residues of ferroportin. This tags ferroportin for internalization (Qiao et al., 2012). Ferroportin is subsequently degraded by lysozymes.

Ferroportin (also called MTP-1) is an iron transporter located at the surface of cells involved in iron metabolism,

including duodenal enterocytes, hepatocytes, macrophages, and placental cells. It is structurally similar to the DMT1 class of metal transporters (Abboud and Haile, 2000). Ferroportin acts as an iron exporter, releasing iron into the bloodstream. Fpn null/null mice are not embryonically viable. Inactivation of ferroportin at birth results in severely anemic mice with accumulation of non-heme bound iron in enterocytes, macrophages, and hepatocytes (Donovan 2005). In vitro, cells expressing ferroportin only in the presence of an inducer, accumulate ferritin when ferroportin is inactive. Induction of ferroportin results in a clearance of ferritin from the cell (Nameth et al., 2004a).

There are no known cellular iron exporters other than ferroportin. The removal of ferroportin from the cell surface inhibits transport of iron to the serum. Iron is trapped within the cell and stored as ferritin. Hepcidin levels, and thus ferroportin levels, are modulated by serum iron levels. Iron overload activates a BMP (Bone Morphogenetic Protein)/ SMAD signaling pathway which causes increased hepcidin expression. Hemojuvelin, a protein that is mutated in juvenile hemochromatosis patients, plays an important role in this pathway as a coreceptor of BMP (Babbit et al., 2006). Hepcidin expression is downregulated in response to anemia and hypoxia, allowing the iron necessary for erythropoiesis to be released into the plasma (Nicolas et al., 2002b).

Hepcidin also increases as part of the acute phase response to inflammation. The inflammatory cytokine Interleukin-6 (IL6) is the primary inducer of inflammatory upregulation of hepcidin mRNA expression. In vitro, the increase of hepcidin stimulated by inflammation is inhibited by the addition of anti-IL6 antibodies. IL-6 infusion in humans causes an increase in urinary hepcidin, along with a decrease in serum iron and transferrin saturation (Nameth et al., 2004b). IL-6 activates a JAK/STAT signal transduction pathway. The immediate -165 base pairs of the hepcidin promoter are necessary for hepcidin induction by IL-6. Deletion of the STAT binding motif within this promoter fragment inhibits IL-6 activation. In particular, the transcription factor STAT3 is required (Falzacappa et al, 2007). Chronic inflammation results in increased hepcidin and anemia in WT mice, but not in STAT3 knockout mice (Sakamori et al., 2010).

In patients with chronically elevated IL-6, hepcidin is persistently elevated. The resulting lack of ferroportin causes iron to be retained within the cells. Dietary iron is not absorbed into the plasma. Iron stored in hepatocytes of the liver is not released. Most significantly, iron from senescent erythrocytes is sequestered in macrophages in the spleen. Rats with ACD had higher levels of ferritin in the spleen than healthy controls, with less iron being released from macrophages (Theurl et al., 2010). The resulting hypoferremia limits the amount of iron available for erythropoiesis.

In addition to the functional iron deficiency induced by hepcidin-mediated iron sequestering, inflammatory cytokines have been shown to have other effects which contribute to anemia of chronic disease. Specifically, Interferon-γ (IFN-γ) has been shown to suppress erythropoiesis. Inflammation inhibits the wave of reticulocytosis normally seen in cells treated with erythropoietin (Epo) in vitro. Neutralization of IFN-γ reverses this effect. Treatment of mice with anti-INF-γ antibodies prevents inflammatory suppression of erythropoiesis (Thawani et al., 2006). This blunting of erythropoiesis may explain why ACD is often normocytic and normochromic. Other anemias caused by inappropriately high hepcidin levels, such as iron refractory iron deficiency anemia, are severely microcytic and hypochromic. IFN-γ suppression of erythropoiesis seems to compensate for the lack of bioavailable iron by producing fewer normal RBCs, rather than many abnormal ones (Nameth & Ganz, 2014).

Further contributing to anemia, erythrocytes have a moderately shortened life span in inflammatory conditions. Biotin labeling of erythrocytes in mice showed a complete RBC turnover by day 15 in mice with inflammation, compared to day 36 in control mice (Thawani et al., 2006). In humans, erythrocyte life span in patients with rheumatoid arthritis (RA), often used as a model for ACD, is significantly shorter than in healthy controls. Red blood cells from these patients transfused into healthy individuals showed normal survival. This indicates that the shortened survival was not caused by defects in the red blood cells. Rather, inflammation causes a decrease in erythrocyte life span by some extrinsic mechanism (Freireich et al., 1957). The premature destruction of erythrocytes is likely the result of increased macrophage activity in the spleen in response to various cytokines (Nameth & Ganz, 2014).

Tumor Necrosis Factor (TNF-α) and Interleukin-1 (IL-1) were among the earliest inflammatory cytokines to be implicated in anemia of chronic disease. However, the mechanisms involved are still unclear. Both TNF-α and IL-1 reduce hepcidin expression in vitro (Song et al., 2013). It seems that their role in ACD is indirect, via regulation of other cytokines. Cytokine-cytokine interactions are complex, and researchers still have a lot to learn before the pathogenesis of ACD can be fully understood.

Treating Anemia of Chronic Disease

Anemia of chronic disease is a secondary effect of an underlying illness. ACD is usually mild. As such, the major focus of treatment protocols for ACD patients must be on

curing the primary disease. As the inflammation caused by the disease subsides, inflammatory cytokine levels return to baseline. Consequentially, the anemia will be resolved without the need for anemia-related treatment. However, often the underlying disease is not curable. Anemia may also have a profound negative impact on the patient's quality of life. The most common complaint of patients with ACD is fatigue, which can be debilitating. In such cases, direct treatment of the anemia of chronic disease is recommended. As with any medical intervention, the benefits of treatment must be weighed carefully against any possible risks. It is important to note that anemia in chronically ill patients is often multifactorial. Anemia of chronic disease may occur in conjunction with true iron deficiency anemia, or anemia of another cause. It is necessary to correctly diagnose the cause of anemia before deciding on a treatment option. Finally, certain treatments may not be suitable for certain patients, depending on which underlying disease is causing the inflammation.

Oral iron supplementation, the obvious solution for iron deficiency anemia, is not appropriate for anemia of chronic disease. The removal of ferroportin from the basolateral surface of duodenal enterocytes prevents oral iron from being adequately absorbed into the plasma. Much of the minimal iron that is absorbed will be diverted into hepatocytes (Weiss & Goodnough, 2005). Intravenous iron therapy may be effective in treating anemia of chronic disease, because parenteral administration allows it to bypass the "mucosal block" in the duodenum. Indeed, several trials have shown that IV iron therapy is beneficial for anemia of chronic disease in cancer patients. A pilot study showed a significant increase in hemoglobin levels in cancer patients with non-iron deficiency anemia in response to weekly doses of ferric hydroxide sucrose (Abdel-Rezeq et al, 2013). A single dose of ferric carboxymaltose improved hemoglobin levels in chemotherapy patients with ACD. This improvement was maintained for a minimum of 8 weeks. (Hedenus et al., 2014). A large study comparing the efficacy of IV iron and oral iron supplementation in cancer patients found that both treatment options induce a similar increase in hemoglobin levels, although the response time was faster for IV iron. IV iron also caused less side effects than oral iron. The efficacy of oral iron seen in this study is surprising, as it conflicts with many other studies. However, this study included patients with iron deficiency anemia (Birgegard et al, 2016). Trials that were specific to patients with functional iron deficiency showed IV iron to be superior.

Several concerns have been raised regarding the safety of iron supplementation. Iron overload is associated with oxidative stress and cardiovascular events. Additionally, iron sequestering is an innate host defense mechanism. Iron supplementation may increase the risk of infections and tumor growth. (Weiss & Goodnough, 2005). Although the trials reported here have indicated that intravenous iron is safe for cancer patients, these trials only followed patients for short periods of time. Long-term studies are necessary to establish whether IV iron is truly safe for patients with anemia of chronic disease (Rodgers & Gilreath, 2019). Most of the research regarding efficacy of IV iron monotherapy for anemia of chronic disease has been in chemotherapy patients. Further experimentation is necessary to assess whether parenteral iron is useful in cases of ACD caused by illnesses other than cancer.

Among the earliest treatment options available for anemia of chronic disease are Erythropoiesis Stimulating Agents (ESAs). This category includes recombinant human erythropoietin (rHuEpo) and its derivatives, Epoetin-α and Darbepoetin-α. ESAs target the blunted erythropoiesis characteristic of ACD and should minimize the iron-sequestering effect of hepcidin as well. Pharmacological levels of erythropoietin cause a dose-dependent decrease in hepcidin expression in vitro (Pinto et al., 2008). In another study, pretreatment with erythropoietin partially prevented the increase in hepcidin normally induced by inflammation in mice. This effect was dose dependent. Pharmacological doses of Epo were effective, but the endogenous increase in erythropoietin in response to hypoxia was insufficient to reduce hepcidin expression. IL-6 levels were similar in Epo-treated and control mice. However, STAT3 phosphorylation decreased significantly. Suppression of STAT3 is likely the mechanism by which erythropoietin decreases IL-6 induced hepcidin expression (Huang et al., 2009). Erythropoiesis stimulating agents have been proven effective for ameliorating anemia of chronic disease in humans. In a placebo controlled double blind trial, normal hemoglobin levels were achieved in 94% of rheumatoid arthritis patients with ACD treated with rHuEpo. Interestingly, rHuEpo also reduced disease activity in RA patients (Peeters et al., 1996). ESAs are likewise used to treat anemia of chronic disease in cancer patients.

Unfortunately, there are serious safety concerns regarding ESAs. Although few side effects were reported in early trials of ESAs for rheumatoid arthritis patients (Peeters et al., 1996, Nordström et al., 1997), ESA treatment is associated with numerous adverse effects, especially in cancer patients. In a meta-analysis of 53 trials including close to 4,000 patients, mortality increased by 17% in cancer patients with anemia treated with ESAs compared to controls (Bohlius et al., 2009). In 2007, the FDA issued a black box warning stating that "ESAs

increase the risk of death, myocardial infarction, stroke, venous thromboembolism, thrombosis of vascular access, and tumor progression or recurrence [in cancer patients]". Venous thromboembolism is one of the most prevalent adverse effects of ESAs. This may be because ESAs induce iron-deficient erythropoiesis (IDE), by increasing erythropoiesis despite the lack of bio-available iron caused by ACD. This results in elevated platelet counts, which increases the risk for thrombolytic events (Henry et al., 2011).

Aside from increased safety concerns, the functional iron deficiency of ACD limits the efficacy of ESAs. Many patients do not respond to ESA treatment. Response rates vary from trial to trial. One study, in RA patients, reported that only 14.6% of patients achieved target hemoglobin levels. Decreased iron levels and more severe inflammation were predictive of a poor response. In an extension to the study, four out of five initial non-responders who were now given iron supplementation in addition rHuEpo treatment did show significant improvement in hemoglobin levels (Nordström et al., 1997). Hemoglobin does not increase significantly in response to ESAs in mice overexpressing hepcidin (Sasu et al., 2010). In another study, rats with higher pre-treatment levels of hepcidin, which correlates to lower iron availability, were shown to respond poorly to ESA treatment. Although ESAs decrease hepcidin expression somewhat, and thus decrease splenic iron retention, this effect is inadequate. Serum iron remains low, and ESA treatment does not effect an increase in hemoglobin in rats with very high hepcidin levels (Theurl et al., 2014).

The limitations of iron-deficient erythropoiesis to ESA treatment has led researchers to investigate whether concomitant iron supplementation could improve the safety and efficacy of ESAs. Indeed, many studies have shown positive results when erythropoiesis stimulating agents are combined with intravenous iron. Parenteral iron administration is preferable to oral supplements, as oral iron is not well absorbed by patients with ACD. IV iron could correct ESA-induced iron-deficient erythropoiesis and improve response to rHuEpo in rheumatoid arthritis patients with anemia of chronic disease (Arndt et al., 2005). Encouraging results were seen in cancer patients with ACD as well. Patients receiving supplemental iron intravenously achieved higher hemoglobin levels in response to epoetin-α than patients receiving epoetin-α with oral iron or epoetin-α alone. Response rates were significantly improved in the IV iron group as well. In the IV iron group, 73% of patients responded to the ESA treatment, compared to only 45% and 41% in the oral and no iron groups, respectively (Henry et al., 2007). Auerbach et al. similarly reported that cancer patients who received combination of darbepoetin-α and IV iron were more likely to reach target hemoglobin levels, with more rapid and significant improvement than patients who did not receive IV iron. Interestingly, the incidence of adverse effects, including thrombolytic events, was similar in both groups (Auerbach et al., 2010). By contrast, post-hoc analysis of data from the first study showed that IV iron decreased the likelihood of elevated platelet counts and venous thromboembolism (Henry et al., 2011). One trial failed to show a significant difference between chemotherapy patients receiving darbepoetin and intravenous ferric gluconate versus oral iron or oral iron placebos. Additionally, many adverse events were reported among those patients receiving parenteral iron (Steensma et al., 2010). However, this study had several limitations. The overall iron dose was lower than in other studies, potentially limiting the benefit of IV iron. By contrast, the individual doses of iron administered were 50% above the recommended dosage, which may explain the excessive number of adverse events. Finally, the study was terminated early, with few patients completing the study (Steensma et al., 2010 & Aapro et al., 2011). Nevertheless, the high incidence of adverse events in this study highlights the need for additional studies as to the safety of parenteral iron.

Experimental Treatment Options

Another option for targeting the blunted erythropoiesis of ACD which is currently being studied is isocitrate supplements. Aconitase is a Krebs's cycle enzyme responsible for converting citrate into isocitrate. Insufficient iron levels result in decreased aconitase activity, to produce less isocitrate. Aconitase activity is used by the body as an "iron sensor". Decreased isocitrate results in downstream inhibition of erythropoiesis by sensitizing erythroid progenitor cells to the inhibitory effects of IFN-γ (Richardson, et al., 2013). This is known as the iron restriction response. Supplementation of exogenous isocitrate inhibits the iron restriction response via a PKC mechanism (Bullock et al., 2010). Isocitrate does not change serum or stored iron levels, nor does it alter erythropoietin levels (Kim et al., 2016). Rather, it seems to "fool the system" into increasing erythropoiesis despite hypoferremia.

Low doses of isocitrate supplementation reversed anemia of inflammation in a model of rat arthritis by preventing the iron restriction response (Richardson et al., 2013). However, later studies found that much higher doses were necessary to achieve even transient results in mice. Administration of high doses of isocitrate caused increased inflammation in these mice (Kim et al., 2016).

The necessity of such high doses to improve anemia of chronic disease may limit the efficacy of isocitrate as a treatment for ACD in humans. Recent research has shown that treatment with a combination of isocitrate and fumarate is effective in curing anemia of chronic disease in a murine model, even at low doses (Goldfarb et al., 2019). Further research, including human trials, is necessary to determine whether isocitrate may be a clinically appropriate treatment for ACD. It seems reasonable to assume, however, that isocitrate treatment may have limitations similar to those seen with ESAs, as bypassing the iron restriction response would lead to iron deficient erythropoiesis. As with ESAs, supplementation with IV iron may be beneficial.

ACD is caused by the result of the interaction of various cytokines. Recent research has focused on inhibition of these inflammatory signals using humanized antibodies as a target for treating anemia of chronic disease. Inhibition of Tumor Necrosis Factor-α (TNF-α) is a common treatment option for the auto-inflammatory disease Rheumatoid Arthritis. Treatment with infliximab, an anti-TNF-α antibody, reduces hepcidin levels and improves blood parameters in RA patients. TNF-α alone actually decreases hepcidin mRNA expression in vitro. Paradoxically, TNF-α also upregulates IL-6 production, resulting in the downstream upregulation of hepcidin. Inhibition of TNF-α reduces IL-6, thereby indirectly reducing serum hepcidin levels, albeit not as effectively as direct inhibition of IL-6 (Song et al., 2013).

Tocilizumab, an IL-6 inhibitor used to treat multicentric Castleman's disease (MCD) and rheumatoid arthritis, effectively reduces anemia of chronic disease in these patients. Tocilizumab is a humanized anti-IL6 receptor antibody. It binds to the IL-6 receptor and prevents IL-6 binding (Mihara et al., 2011). Tocilizumab completely inhibits IL-6 induction of hepcidin expression in vitro. Short-term treatment of MCD patients with tocilizumab results in decreased hepcidin and amelioration of anemia. These results continue when Tocilizumab was given over a longer period of time. Hepcidin expression keeps decreasing until normal serum hepcidin levels are reached and hemoglobin increases. Other iron-related parameters improve as well (Song et al., 2010). Tocilizumab decreases serum hepcidin and improves iron related blood parameters including hemoglobin, serum iron, ferritin, and MCV in rheumatoid arthritis patients as well (Song et al., 2013). In monkeys with anemia of chronic disease, IL-6 blockade with tocilizumab caused a rapid decrease of serum hepcidin. Hemoglobin levels and RBC improvement was drastic at first, then slowed and gradually continued until they returned to pre-inflammation levels (Hashizume 2010). The amelioration of anemia by tocilizumab seems to be due to inhibition of IL-6 induction of hepcidin. However, as IL-6 plays a role in the pathogenesis of MCD and RA, and tocilizumab reduces disease symptoms, it may be that the improvement of the underlying disease plays a role in decreasing anemia as well.

A major concern with tocilizumab treatment is its immunosuppressive effect and increased risk of infection. Additionally, since tocilizumab suppresses biomarkers of infection, it is necessary to observe patients closely for symptoms that may indicate infection (Mihara et al., 2011). The STREAM study, which followed RA patients receiving tocilizumab monotherapy over a period of five years showed the drug to have a good safety profile. The majority of the adverse events reported were mild, and the benefits of the drug on patient quality of life outweighed these events (Nishimoto et al., 2009). It remains to be seen whether tocilizumab and other anti-cytokine antibodies are safe and effective for treating ACD caused by other diseases.

Perhaps the most targeted approach to treating ACD is direct inhibition of hepcidin activity. Monoclonal antibodies (mAbs) and speigelmers which bind to hepcidin and prevent its interaction with ferroportin are promising developments. A Phase-1 study of the anti-hepcidin mAb LY2787106 in humans showed that the antibody was safe and well-tolerated in cancer patients with few serious adverse effects reported. However, the drug elicited only a transient increase in serum iron, which did not translate into improved hepcidin, serum ferritin, and TSAT levels (Vadhan-Raj et al., 2017). While ineffective alone, this antibody may be useful in treating ACD in combination with other drugs.

As an alternative to antibodies, Noxxon Pharma of Germany has developed an anti-hepcidin speigelmer called Lexaptepid Pegol (Nox-H94). Speigelmers are oligoribonucleotides with L-stereochemistry which bind to the target protein and inactivate it in a manner similar to antibodies. The advantage of a speigelmer over antibodies is that it does not elicit an immunological response in patients. Its L-stereochemistry prevents the development of anti-drug antibodies (ADAs) and also makes it nuclease resistant (Schwoebel et al., 2013). Indeed, patients receiving lexaptepid did not form antibodies specific to the drug (Boyce et al., 2016), while some patients receiving the mAb LY2787106 did develop ADAs (Vadhan-Raj et al., 2017). In vitro, lexaptepid inhibits the hepcidin-induced decrease in ferroportin expression. In monkeys with ACD caused by IL-6 injection, lexaptepid pegol partially improved reticulocyte hemoglobin levels, hematocrit, and erythrocyte counts (Schwoebel et al., 2013). A

placebo controlled double blind study of lexaptepid pegol in healthy humans showed a dose-dependent increase in serum iron, transferrin saturation, and serum ferritin. Reticulocyte hemoglobin did not increase in healthy volunteers but would be expected to increase in patients with ACD (Boyce et al., 2016). Lexaptepid temporarily prevents the inflammation-induced hypoferremia caused by endotoxin injection in heathy individuals (van Eijk et al., 2014). While the results have been promising in trials involving healthy individuals, further trials in ACD patients are necessary in order to know whether Lexaptepid can be clinically useful.

One aspect which may limit the effectiveness of both anti-hepcidin antibodies and speigelmers is the relatively high production rate of hepcidin. High doses of these hepcidin-binding ligands are necessary to keep up with production of new hepcidin (Fung & Nameth, 2013). Further experimentation, particularly in patients with anemia of chronic disease, will show whether this indeed limits the clinical value of such treatments. Even if these drugs are ineffective alone, they may be useful in combination with other treatments. Low dose antibody neutralization of hepcidin was shown to increase responsiveness to ESAs in mice with ACD. The combination of the antibody and darbepoetin-α completely corrected anemia in these mice (Sasu et al., 2010). If this is shown to be the case in humans as well, these drugs could take the place of parenteral iron, thereby eliminating the potential safety hazards associated with IV iron.

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Is Rapamycin an Effective Anti-aging Drug?

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Abstract

Scientific and pharmaceutical advancements have revolutionized medicine. Many once- debilitating or fatal diseases can now be managed with medication. However, the search for a cure for the inevitable aging diseased state remained futile until recently. Rapamycin has been studied for its possible longevity effects, providing promising results for the development of anti-aging therapies. This paper evaluates the benefits and risks of rapamycin use. While rapamycin cannot be supported as a safe anti-aging drug, rapamycin studies have elucidated parts of the aging pathway, providing a breakthrough for anti-aging research.

Abbreviations

FKBP - FK506 Binding Protein
AKT – protein kinase B

Introduction

Hutchinson-Gilford Progeria Syndrome (progeria) is a rare genetic disease caused by a mutation on the LMNA gene which encodes for the Lamin-A protein. This causes a modified form of Lamin-A, known as progerin, which has an internal deletion of fifty amino acids (Graziotto et al. 2012). Wild-type Lamin A is the scaffolding of a cell, which holds the nucleus together. Researchers believe the variant Lamin-A leads to an unstable nucleus that causes the symptoms of progeria (The Progeria Research Foundation).

Children with progeria show early signs of aging, which include loss of body fat and hair, growth failure, stiffness of joints, aged looking skin, hip dislocation, cardiovascular disease, stroke, and generalized atherosclerosis. The lifespan of children with HGPS is fourteen years, with the common cause of death atherosclerosis (The Progeria Research Foundation).

The Progeria Research Foundation is currently conducting a clinical research trial to test a possible cure for Progeria. The trial is testing the combined use of Lonafarnib and Everolimus on HGPS patients. Lonafarnib is a farnesyltransferase inhibitor. By inhibiting the attachment of a farsenyl group to progerin, the progerin is unable to inhibit the cell function (The Progeria Research Foundation). Everolimus, a derivative of rapamycin, promotes the autophagy of the progerin (Graziotto et al. 2012).

Increased levels of progerin have been found in the skin and arteries of normal, aged individuals (McClintock et al. 2007, Olive et al. 2010). The correlation between increased progerin and the physiology of aging is seen in the rapid aging of HGPS patients with high levels of progerin and the decrease in age-associated symptoms upon autophagic degeneration of progerin. Thus, the correlation between HGPS and normal aging suggest rapamycin as a possible drug to promote longevity in the general population (Graziotto et al. 2012).

In 2009, a study showing that rapamycin extends the lifespan of mice was published. This was the first study to realize the anti-aging properties of rapamycin on mammals (Harrison et al. 2009). Since then, rapamycin has been explored as a possible anti-aging drug for humans.

Method

Articles sourced in this paper were obtained by searching Touro's database and Google Scholar. Some articles were referenced in review articles and the original articles were retrieved. Additionally, data was obtained from the Progeria Research Foundation and the lecture of Michael Hall who discovered and studies TOR.

Discussion

The Mechanism of Rapamycin

Rapamycin is a macrocyclic lactone secreted by the bacterium streptomyces hygroscopicus which was discovered on the Easter Island (Tee 2018). Is was originally intended for use as an antifungal. However, its immunosuppressive and anti- proliferative properties rendered it unsuitable as a safe antifungal treatment. Instead, these properties of rapamycin led to its development for other purposes, and rapamycin along with its derivatives are FDA approved for use as immunosuppressants and an anticancer agent (Lamming 2016).

Rapamycin combines with FKBP to inhibit the activity of the mechanistic target of rapamycin (mTOR), which is part of the phosphatidylinositol 3-kinase-related kinases family (Hall 2016). The mTOR protein forms two protein kinase complexes - mTORC1 and mTORC2, both balancing cell growth and metabolism with degradation. The mTOR pathways responds to favorable growth conditions, such as adequate nutrition, resources, and growth factors by stimulating anabolic processes (Kennedy, Lamming 2016, Laplante, Sabatini 2009). Conversely, stressful conditions in the cell inhibit the mTOR pathway, thereby inducing catabolic processes (Laplante, Sabatini 2009).

The two mTOR complexes have some shared components. They both contain the mTOR core, the regulatory protein Deptor, and mLST8/GβL to ensure complex assembly and stability (Kennedy, Lamming 2016).

In mTORC1, the mTOR protein kinase interacts with the scaffold protein Raptor, and the AKT substrate PRAS40 (Kennedy, Lamming 2016). As shown in in Figure 1, MTORC1 controls many processes, including protein synthesis, lipid synthesis, autophagy, mitochondrial metabolism, and biogenesis (Laplante, Sabatini 2009). Many of the functions of mTORC1 are inhibited by acute exposure to rapamycin which causes a decrease in biosynthesis and an increase in biodegradation (Tee 2018, Hall 2017).

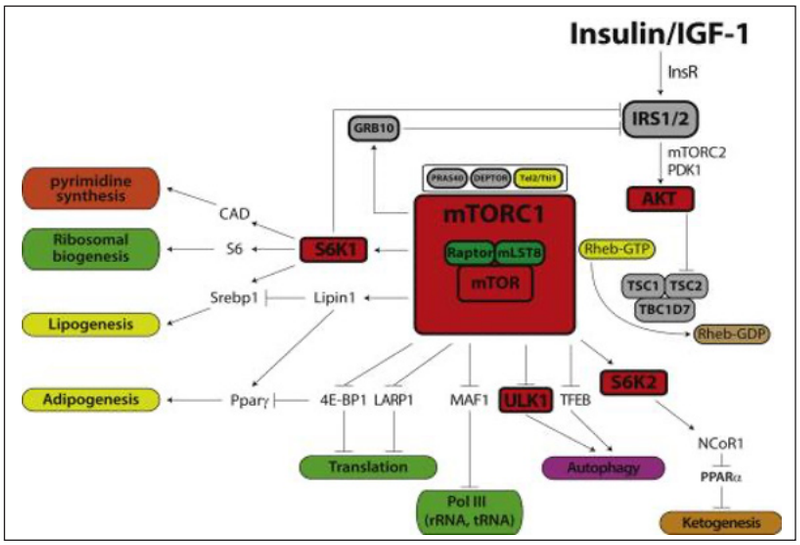

Figure 1. Diagram of the pathways involving mTORC1 (Kennedy, Lamming 2016)

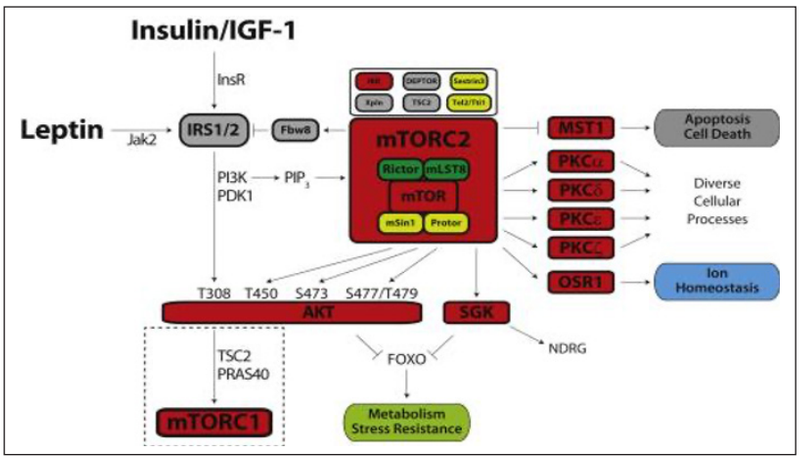

Figure 2. Diagram of the pathways involving mTORC2 (Kennedy, Lamming 2016)

MTORC2 contains the scaffold protein Rictor, the protein subunits mSIN1 and Protor-1/2 (Laplante 2009). Prolonged rapamycin use inhibits mTORC2 by binding to free mTOR and preventing it from forming the mTORC2 complex (Johnson et al. 2013, Sarbassov et al. 2006, Tee 2018). MTORC2 is stimulated by IGF-1, insulin, and leptin (Kennedy, Lamming 2016). MTORC2 regulates mTORC1 via the phosphorylation of AKT. AKT phosphorylates TSC2, which is part of the TSC complex. TSC2 promotes the conversion of Rheb-GTP, which activates mTORC1 to Rheb- GDP. Therefore, the phosphorylation of TSC2 inhibits this conversion process, allowing the Rheb-GTP complex to remain in its active form and activate mTORC1 (Manning, Toker 2017). AKT also phosphorylates PRAS40It, which is an inhibitor of mTORC1 in its unphosphorylated form (Sancak et al., 2007). The other regulatory functions of mTORC2 include regulating cell survival, metabolism, proliferation, and cytoskeleton organization (Laplante, Sabatini 2009). The pathways involving mTORC2 are outlined in Figure 2.

What Anti-Aging Effects does Rapamycin have on the Body?

MTORC1 controls many processes linked to aging. Therefore, the inhibition of these processes by rapamycin contribute to the drug's anti-aging effects (Johnson et al. 2013). The suggested mechanisms of reduced aging by mTORC1 inhibition are discussed in this section.

MTORC1 inhibition promotes autophagy, which is the recycling of amino acids and degradation of damaged macromolecules and organelles. Autophagy in the cells declines with age, and it is proposed that the accumulation of damaged particles contribute to cellular dysfunction of aged individuals (Cuervo 2008). MTROC1 inhibition promotes autophagic mediated longevity, as supported by studies on yeast and invertebrate (Johnson et al 2013).

Regulation of mRNA translation is another process that may contribute to the anti-aging benefits of rapamycin. Under favorable growth conditions, mTROC1 promotes mRNA translation and protein synthesis. Inhibition of mTORC1 reduces translation, and studies on yeast, nematodes, fruit flies, and mice provide evidence that regulation of mRNA translation increases lifespan (Kaeberlein, Kennedy 2011). A main reason for the increased lifespan accompanying global mRNA translation reduction is the differential protein translation of specific mRNA. Molecular evidence is seen in yeast; when mTORC1 is inhibited Gcn4 is preferentially translated and increased translation of Gcn4 increases lifespan. Similar mechanisms are seen in studies of nematodes and fruit flies (Johnson et al 2013).

Another function possibly contributing to the anti-aging benefit of rapamycin is reduced inflammation through mTORC1 inhibition. Hyperactivation of mTORC1 causes inflammation which is present in many age-related diseases such as kidney disease, vascular inflammation after angioplasty, atherosclerotic plaques, and lung infection. Therefore, rapamycin use reduces inflammation associated with these diseases. (Johnson et al 2013).

Additionally, studies on yeast link mTORC1 inhibition to

the activation of a stress response pathway. The increased stress resistance promotes lifespan extension. Also, regulation of mitochondrial function and enhanced stem cell function seem to contribute to the longevity effects of rapamycin (Johnson et al 2013).

Rapamycin Studies on Mammals

The 2009 study conducted by Harrison et al. on 1,901 mice provides evidence for the longevity effects of rapamycin on mammals. In comparison to the control group, rapamycin-fed female mice had a 13% lifespan extension, and male mice had a 9% lifespan extension. The average age of the mice was 600 days, which is the equivalence of 60 years in humans. Thus, the study demonstrates rapamycin's effectiveness when started late in the lifespan.

In 2019 Sills et al. studied the safety of rapamycin on 23 middle-aged marmosets who were fed rapamycin for nine months. The marmoset showed no significant side effects and only minor effects on hematological markers. Since marmosets are biologically similar to humans, the study suggest similar outcomes would occur in humans.

Rapamycin Side Effects in Humans

Although rapamycin has been found to increase the lifespan of flies, mice, and marmosets, few studies have tested its anti-aging effect on humans (Graziotto et al. 2012). This section will review negative side effects of rapamycin treatment and the evidence to support the drug as an anti-aging therapy for humans.

Many studies on rapamycin have been conducted on immunocompromised patients. Side effects of renal and heart transplant patients taking rapamycin for its immunosuppressive property include delayed wound healing, interstitial pneumonitis, anemia, high cholesterol and triglyceride levels, infection, edema, and gastrointestinal symptoms. (Baur et al. 2011, Ekberg et al. 2010, Graziotto et al. 2012). Long term side effects of rapamycin include glucose intolerance, decreased insulin sensitivity, and increased risk of new onset diabetes (Johnston et al. 2008, Lamming 2016). While these reported side effects may seem to outweigh the potential benefits of rapamycin, it is important to consider that the adverse effects may be influenced by the patients' underlying condition or drug interactions (Kraig et al. 2018).

A small-scale study found that short term everolimus treatment boosted the immune response of normal, elderly individuals to the flu vaccine. This suggests that rapamycin improves immunosenescence, one characteristic of aging. Since rapamycin is also known for its immunosuppressive properties, its effects on the immune system seem to be dependent on disease, age, and/or antigen (Mannick et al. 2014).

Another small-scale study of a similar population found rapamycin use safe for elderly individuals with stabilized medical conditions. This population did not show a significant change in plasma glucose, insulin, and insulin sensitivity, which contrasts with the increased risk of diabetes seen with transplant patients. Additionally, there was no significant elevation in triglyceride levels, which was also a concerning side effect seen in previous studies. The side-effects reported in the study group were limited to a facial rash and GI symptoms. However, the study did not reveal any change in cognitive functioning, physical performance, or immune parameters. While these results do not support the anti-aging benefits of rapamycin, the limited side-effects on normal individuals support the safety of rapamycin use for further clinical testing (Kraig et al. 2018).

Despite the promising results of life extension seen in mammals, only a limited number of clinical trials with small sample sizes were conducted on humans. Therefore, rapamycin must undergo further studies to determine its efficacy.

Conclusion/Further Research

The substantial side-effects associated with rapamycin use in transplant patients makes it difficult for it to be approved as a preventative aging treatment. However, the discovery of life extension by inhibiting mTOR provides a basis for the search of other anti-aging treatments that work by a similar mechanism. One suggestion is MTORC1 specific- inhibition, which is sufficient to extend lifespan and may have reduced side effects. Studies have demonstrated the importance of mTORC2 in human physiology and metabolism (Lamming et al. 2016). For example, mTORC2 plays an important role in B-Cells, T-cell and macrophages (Byles et al. 2013, Festuccia et al. 2014). Additionally, prolonged ex-vivo exposure to rapamycin inhibits lipogenesis in rat hepatocyte which can be attributed to the disruption of mTORC2 (Brown et al. 2007, Kennedy, Lamming 2016).

Kennedy and Lamming found that while chronic exposure to FKBP12-rapamycin complex inhibits mTORC2, chronic exposure to the rapamycin-FKBP51 complex does not inhibit mTORC2. The binding affinity of rapamycin with FBPK51 has an inverse relationship with the inhibition of mTROC2. Therefore, a rapamycin analog with a strong affinity for FKPB51 should be studied as a mTORC1 specific inhibitor (2016).

Another possibility, which is not well-studied, is to harness the benefits of rapamycin by eating a low-protein diet. Amino acids are one of the stimuli for mTORC1, but not mTROC2 (Lamming 2016). Studies show leucine consumption affects mTORC1 activity in humans, and a short-term protein-free diet reduces mTORC1 signaling

(Moberg et al. 2014, Harputlugil et al. 2014). The branch-chain amino acids - leucin, isoleucine and valine – enhance the mTORC1 activity in skeletal muscle, liver, pancreas and adipose tissue in rodents. Therefore, a diet with limited proteins or specific amino acids may provide a more natural approach to reduce aging (Lamming 2016).

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Is PNC-27 and PNC-28 the Best way to cure Cancer?

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Abstract

Immunotherapeutic agents have been researched for many decades as an alternative treatment for cancer. Current research demonstrates that immunotherapy is safer than radiotherapy or chemotherapy. This is attributed to immunotherapy's mechanism of utilizing the body's own defense system, as opposed to absorbing harmful chemicals. Two forms of immunotherapy that have been effective in curing cancer without the added danger of chemical toxicity are PNC-27 and PNC-28. These peptides were created by a supercomputer at SUNY Downstate Medical Center in New York in 2000. PNC-27 and PNC-28 work with the MDM2 -P53 tumor suppressor complex. It acts as a competitive inhibitor for binding, increasing the half-life of P53 in the cell and assisting with the elimination of cancer cells (Sarafraz-Yazdi E, Bowne WB, et al 2010). These immunotherapy agents also have the ability to bind to the cell membrane and lyse the cell. The clinical trials for PNC-27 and 28 were successful, and the drug is currently in use outside of the United States. Although this form of immunotherapy does come with some side effects, research illustrates that this form of immunotherapy can be a successful strategy in eliminating the cancer and ensuring that a relapse does not occur.

Abbreviations

MDM2- Mouse Double Minute 2 Homolog P53- Tumor protein, Tp53
HDM2- Human Double Minute Homolog 2

Introduction

Cancer is one of the three leading causes of death across the globe. Although researchers have been discovering cures for many diseases, the cure for cancer still eludes them. There are over 240 different forms of tumors that currently exist, and each type comes with a unique mechanism that can help it evade treatment. Tumors are caused by the ongoing replication of a cell that does not adhere to cell regulations for replication (Vassilev A, DePamphilis, 2017). Due to the fact that tumors arise from our own cells, in order to cure cancer, researchers have to come up with a treatment that exclusively destroys malignant cells, keeping the healthy cells intact. While there are many cures being evaluated in current research that target all cells, PNC-27 and PNC-28 have the ability to target cancer cells only.

There are millions of cells in the human body. In order for cells to thrive, the cells need to be replicated to ensure continuity. New cells are constantly being created as old cells are dying. Cells are converted to tumor cells when the checkpoints that are responsible for monitoring the division do not proceed as expected. (Chao et al., 2017) When the cell metastasizes and travels to another region within the body, the tumor is transformed into a potentially lethal cancer.

When a cell goes through the division process, it replicates all of its chromosomes and sends an identical copy to the new version of the cell that was formed. The process by which this is done is called mitosis. The cell has three phases that ensure that cell replication is proceeding correctly. These three checkpoints that ensure the legitimacy of DNA are known as the M Phase (Mitosis), G1 Phase (Gap 1), and the G2 Phase (Gap 2). At the G1 phase, the accuracy of the DNA is evaluated before proceeding. At G2, the chromosome duplication is assessed. (Veron, 2017) After these checkpoints, the cell goes on to produce identical daughter cells. In a normal, healthy cell, if a cell does not pass these checkpoints successfully, the cell is destroyed. These checkpoints are the main regulators of replication and ensure a low rate of errors in the DNA.

The process of mitosis guarantees perpetuation of life, but it can prove lethal if it proceeds unchecked. Genetic mutation (regardless of whether it was caused by carcinogens or through random errors during DNA replication) takes place in cells throughout the lifetime of an organism (Ahluwalia, 2009). When one of these mutations results in the breakdown of cell cycle regulation, it gives rise to an "unregulated proliferating descendant clone of that particular cell" (Cooper, 2000). This clone of cells can form a tumor. Tumors arise with significant frequency, but most tumors pose slight risk to their host cell due to tumor cell localization. These tumors are known to be benign tumors. It is generally evident when a tumor is benign since the cells bear a close resemblance and role comparable to normal cells. (Mims, et al. 2015).

One of the most significant stages in the diagnosis of cancer patients is the series of events leading to the development of tumor cell invasion and metastasis. Cancer metastasis is the dispersion of cancer cells to tissues and organs outside the original site of the tumor. Once a tumor has left its original source, it travels and forms additional tumors in other organs. This process occurs in three main developments: invasion, intravasation, and extravasation.

Metastasis occurs due to a loss of cell to cell adhesion. Initially, the malignant tumor cells isolate from the main mass at the original source. Subsequently, the cells proceed to invade the surrounding stroma. This development causes the secretion of substances to degrade the basement membrane and extracellular matrix of the organ it has invaded (Shields JA, Shields CL, 2016). The secretion of substances helps express the proteins that mediate the control of the tumor's motility. In addition to the tumor's ability to migrate to other organs, there is a process called angiogenesis. The process of angiogenesis is necessary for the tumor to exist and keep itself satiated. Angiogenesis is the development of new blood vessels that aid with local diffusion. This helps the cell sustain

itself with nutrients, oxygen and transporting metabolic wastes (Nowak-Sliwinska P, 2018).

After invading other organs, the tumor then goes through the introversion stage. Introversion allows for the tumor to make pathways throughout the blood so it can travel through the body and settle in another location. The tumor networks with the endothelial cells through biochemical interactions. This process is regulated by a carbohydrate-carbohydrate reaction.

Adhesion to the endothelial cells creates more solid bonds to penetrate the endothelium and the basement membrane (More SK, Vomhof-Dekrey EE, Basson MD., et al 2019). Through this process, the new tumor reproduces.

As mentioned previously, the common driving force behind cancer is a genetic mutation, but there are many distinctions in how cancer can evolve. There are different mutations that can alter the genes standard structure and function. It is generally not possible to determine what the underlying cause of the mutations. However, research has linked different risk factors to an increased probability of cancer. Carcinogens and viral or genetic factors have all been linked to cancer. For example, exposure to the environment plays a role in how genes will be transcribed. The atmosphere and our food contain many toxins that can cause mutations. UV radiation that is induced by exposure to the sun without ensuring proper protection causes thymine dimers, which researchers believe causes skin cancer. Smoking is also a significant cause of cancer.

Chemicals in tobacco smoke harm the cleansing system that the body employs to remove toxins. Thus, smokers are not as capable of handling toxic chemicals as those with healthy lungs and blood (O'Keeffe LM 2017). Moreover, smoking damages DNA, and generates potent carcinogens which not only affect the trachea, bronchial system and lungs, but also permeate the entire system.

Methods

Data was collected using ProQuest and PubMed databases, and the National Library of Medicine. The images, graphs, and diagrams are in the research articles referenced. Additional materials were found in the SUNY (State University of New York) Downstate research library.

Radiation Therapy Mechanism:

Treatment of cancer originally involved surgical removal techniques alone. Over the previous centuries, radiation therapy has been discovered as an alternate treatment. Radiation therapy treats cancer by focusing beams of intense energy on a specific part of the body to eliminate cancer cells. Some of these forms of energy typically includes X-rays, but can also use high-energy particles or waves, such as gamma rays, electron beams, or protons. The larger the amount of energy involved in the therapy, the more penetration is accomplished by the ionizing ray into the cancer tissue (Orth M, Lauber 2014). The ionizing ray kills the cells that are actively dividing. Although radiation is effective for preventing division of rapidly dividing cells, it can cause only minimal breaks in the DNA. Nevertheless, these breaks stop cancer cells from replicating and producing, activating their elimination. It can be used to shrink early stage cancer, or treat advanced symptoms of cancer.

Radiation therapy attempts to strike a balance by eliminating the harmful dividin cells and minimizing damage to the normal cells around the tumor site. Normal cells can often repair much of the damage caused by radiation. The majority of people with cancer receive radiation therapy as part of their treatment plan, along with a combination with other treatments.

Physicians prescribe radiation therapy to treat all forms of cancer, including benign tumors. Although radiation therapy is effective, research shows that radiation therapy has a large number of side effects. These include tooth decay, third degree skin burns, and difficulty breathing (Murphy, et al. 2019). Radiation therapy is rationed, and there is a limit to the amount of radiation people can safely receive. Depending on how much radiation an organ has received during the first round of treatments, the body may not have the ability to receive radiation a second time.

The major lethal side effect of radiation therapy is the relapse of the cancer. Because radiation therapy damages healthy cells, these damaged cells can metastasize to other locations and cause a recurrence of the cancer. Most types of leukemia cancer, including acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and acute lymphoblastic leukemia (ALL) are all forms of cancer that originate from radiation exposure. Myelodysplastic syndrome (MDS), a bone marrow cancer that may evolve into leukemia, has also been associated with previous radiation exposure" (Cidon,EU 2016). Not all cancers may return immediately after radiation therapy. Research shows that solid tumors take much longer to develop, and don't come back quickly. Most of these cancers are not spotted for years after the radiation therapy has caused the damage, and some are diagnosed more than a decade later. We may be inevitably perpetuating the disease itself by using radiation therapy as an option.

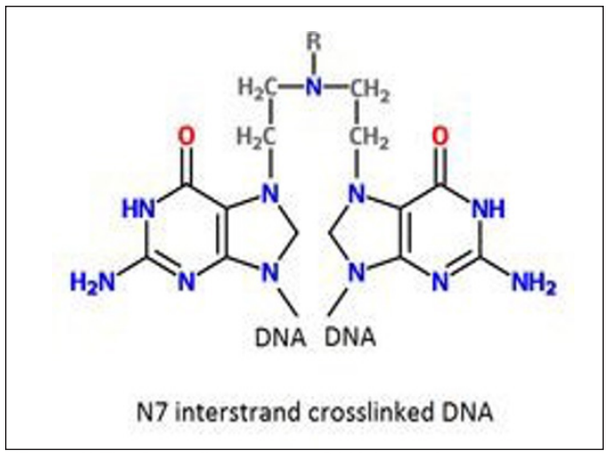
C Hemotherapy Mechanism:

A common form of treatment that is currently in use is chemotherapy. Chemotherapy is a systemic treatment,

and travels through the bloodstream. The treatment is often given to the patient for a specific time, ranging from 6 months to a year. In cases where there is no possible cure, chemotherapy may be given to extend the patient's life, or relieve painful symptoms. The drugs are usually given by IV or orally. Chemotherapy has a lower probability of damaging cells that are at rest, such as most normal cells. Physicians can prescribe the treatment after surgery was performed to remove the original tumor, in order to eliminate the individual cancer cells that may have metastasized before they are able to form a tumor.

The mechanism of chemotherapy is to eliminate cancer cells by targeting different cells at different phases of the cell cycle in order to halt the replication. The chemotherapy damages the genes inside the nucleus of cells, and attack cells that are at the point of splitting. Some damage the cells while they are replicating their gene before they split (Johnstone RW, 2016). Some chemotherapeutic agents attack the DNA of the cell by adding a methyl group, in order to prevent them from producing additional clones of the cancer cell. These are known as alkylating agents, and they attach to DNA, RNA, and proteins through covalent bonding.

Alkylating agents will attack at any point in the cell cycle. There are many types of alkylating groups, such as nitrogen mustards, nitrosoureas, and aziridines. The molecules bind to the DNA and change the DNAs conformation. DNA is composed of a double strand, and the

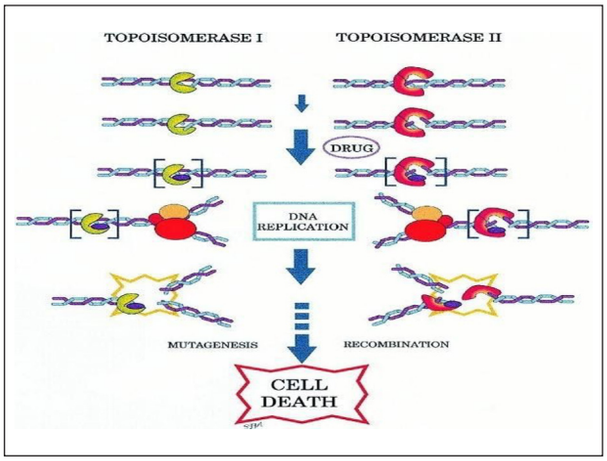


(Figure 1) The figure illustrates two DNA bases that are cross-linked by a nitrogen mustard (Siddik ZH 2005

molecules may either bind two times to one strand of DNA (known as a "intrastrand crosslink") or may bind once to both strands (called a "interstrand crosslink")" (Figure 1). If the cell attempts to replicate or fix cross-linked DNA during cell division, the DNA strands break (Cancer Chemotherapy and Biotherapy, 2005). This leads to a form of programmed cell death called apoptosis.

Another chemotherapy drug that interferes with transcription and translation is a topoisomerase inhibitor. When the DNA double-strand helix is unwound during DNA replication or transcription, the adjoining DNA (that has not been opened yet) is wound up extremely tightly. The topoisomerase inhibitor produces single- or double-strand breaks into DNA, reducing the tension in the DNA strand ahead of the replication point (Figure 2) This allows the normal unwinding of DNA to happen during replication or transcription. Inhibition of topoisomerase does not allow either of these processes to continue (Nitiss JL, 2007). Other chemotherapy drugs interfere with cell metabolism. This blocks off nutrients and oxygen supply, which causes growth arrest.

Research has shown that chemotherapy has many side effects. Some of these side effects include fatigue, hair loss, infection, fertility problems, weight loss, damage to



(Figure 2) Topoisomerase I and II Inhibitors (Huda W. 2016)

lung tissue, heart problems, kidney problems, and nerve damage (Uzun, 2019).) Studies show that one of the primary drawbacks is that chemotherapy does not achieve remission. One reason is that the original treatment is not successful in eliminating all the cancer cells. The remaining cancer cells that are left in the organ grew into a new tumor. Another reason that chemotherapy is not successful in achieving remission is that some cancer cells have metastasized to other parts of the body and started forming a new tumor in the new location. Cancer cells may also develop a mechanism that makes it become resistant to treatment when it relapses, thereby blocking a possible cure (Stadlr, WM, 2014).

Immunotherapy

Researchers have discovered that one of the best methods

for treating cancer is immunotherapy. Immunotherapy is a biological therapy that utilizes substances made from living organisms to treat cancer. Some immunotherapy treatments use genetic engineering to improve immune cells' cancer-fighting capabilities and are commonly referred to as gene therapies. Many immunotherapy treatments for preventing, managing, or treating different cancers can also be used in combination with surgery, chemotherapy, radiation, or targeted therapies to aid their overall effectiveness (Yang, Wang, Wang, 2019).

Immunotherapy boosts the immune system's ability to identify, target, and remove cancer cells. While many cells replicate naturally, this behavior is tightly regulated by an assortment of factors, including the genes within cells. When growth is not required at certain points, cells are instructed to halt growth. Cancer cells acquire defects that cause them to ignore these inhibitive signals, and their replication proceeds in an unregulated and rapid manner. Because cancer cells grow and behave through abnormal methods, this can alert the immune system of a potential threat, which can recognize and eliminate cancer cells through a process called immunosurveillance.

Immunosurveillance is an extremely effective method for eliminating pathogens from the body, but the process isn't always successful when it pertains to cancer. Even though the immune system can prevent or slow cancer growth, cancer cells have ways to evade destruction by the immune system. For instance, cancer cells may have genetic changes that make them appear less discernible to the immune system, have proteins on their surface that turn off immune cells, or change the normal cells around the tumor so they interfere with how the immune system responds to the cancer cells (Pardoll D. 2015).

Cancer cells develop ways to evade and escape the immune system, which allows them to continue to thrive and metastasize to other organs (Prendergast GC, Jaffee EM, 2007).

Therefore, immunotherapies are designed to enhance the cancer-fighting capabilities of immune cells and utilize the body's own protective measures to fight the tumors.

NC-27 and PNC-28 Peptides

PNC-27 and PNC-28 are synthetic peptides designed to specifically target and destroy cancer cells. Therapeutic peptides have the ability to treat an extensive range of diseases and cancers and contain a large number of advantages over proteins or antibodies. They are easily synthesized in a lab and are not toxic to the human body. They are manufactured to have a very specific affinity for the targeted cell. PNC-27 and PNC-28 were created using a super computer by Dr. Matthew Pincus and Dr. Joseph Michl of SUNY Downstate Medical Center. The peptides were created with a specific fold that only interacts with a particular structure present on cancer cells. It can be administered by nebulizer, vaginal or rectal suppository or intravenously at the tumor site. Many patients travel to foreign countries to receive this treatment. PNC-27 and PNC-28 have been used with exceptional results, and over 500 patients have had a high success rate with the drug since 2007. PNC-27 and PNC-28 peptides have been shown to be highly effective in specifically targeting a large variety of different cancers. These peptides have had high success rates with breast cancer, leukemia, melanoma, and pancreatic cancer lines. It has proven to be the most effective when taken simultaneously with immune system boosters, proper hydration, and a diet that avoids excess sugars and red meat.

P 53 Mechanism for PNC-27 AND PNC 28

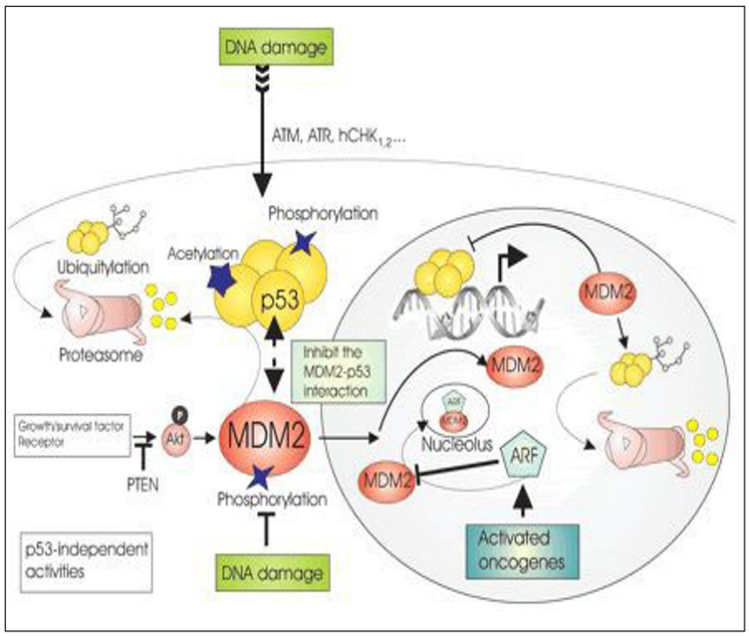
TP53 or "tumor protein 53" is a gene that is located on the seventeenth chromosome of humans. It codes for a protein that regulates the cell cycle and hence functions as a tumor suppressor. P53 has been described as "the guardian of the genome", referring to its role in keeping the cell stable by preventing genome mutation (Uehara 2018). TP53 has 4 domains that are responsible for activating transcription factors, tetramerization of proteins, recognizing DNA sequences, and checking damaged DNA (Sabapathy et al 2019).

The p53 tumor suppressor has a very significant role in growth arrest, DNA repair, and apoptosis, and it causes a response to different attacks on the cell. Rapid induction of high p53 protein levels by various stress types prevents inappropriate propagation of cells carrying potentially mutagenic, damaged DNA (Zhang et al., 2014). In normal cells, p53 is an extremely unstable protein that has a half-life ranging from 5-30 minutes. The p53 protein is present at very low levels in the cell, due to continuous degradation. This continuous degradation is primarily regulated by MDM2. Research over the past decade has assigned MDM2 as the central controller of p53 by regulating the p53 tumor suppressor function (Carotenuto, 2019).

A mutation of the p53-MDM2 complex during the induction of p53 can lead to the accumulation of active p53 in the cell (Figure 3). Consequently, p53 half-life extends from minutes to hours. This can aid p53 in stopping cancer. Both PNC-27 and PNC-28 were tested as agents that would competitively block p53 from interacting with HDM-2. By acting as a competitive inhibitor, the peptides would prolong the half-life of p53 in cancer cells. This eventually leads to more efficiency in inducing apoptosis of cancer cells.

Mechanism through Membrane and Affinity to HDM2

The PNC-27 and PNC-28 cancer peptides are substances that causes the death of cancer cells only. PNC-27 and PNC-28 accomplish this due to their affinity for binding to a protein called HDM-2. Cancer cells have high levels of HDM-2 present in their cell membranes. (Ehsan Sarafraz-Yazdi, Wilbur B. Bowne, 2010). Dr. Ehsan Sarafraz-Yazdi (Sarafraz-Yazdi E et al 2015) explains in detail how the PNC-27 and PNC- 28 cancer peptide manifests themselves, and what their mechanisms could mean for progress in the field of cancer research. The study points out that the peptide's mechanism hinges on the fact that there are formations of oligomeric pores in the plasma membrane of tumor cells. (American Association for Cancer, 2010) The oligomeric pores are present exclusively in cancer cells. Additionally, the observations illustrated that PNC-27 and PNC-28 were capable of distinguishing between cancerous and non-cancerous cells.



(Figure 3) A chart of the disruption of mdm2 and the p53 complex (Hashimoto N, Nagano H, 2010)

In a 2009 study, researchers discovered that the " three-dimensional structure of PNC-27's and PNC-28's p53 residues of its amino acids may be superimposable onto the structure for the same residues bound to HDM-2." This discovery alerted researchers to the fact that that PNC-27 and PNC-28's could target HDM-2 in cancer cells' membranes (Pincus, et al (2011)).

Upon further research, it was discovered that by "inserting untransformed cells that are not prone to PNC-27 and PNC-28 with HDM-2 containing a localized membrane signal, these cells then became inclined towards PNC-27 and PNC-28". This research established that the PNC-27 peptide was able to exclusively mark HDM-2 in the membranes of cancer cells and destroy them through membranolysis, while at the same time leaving the healthy cells alive (Sookraj, et al (2010). Additional research established that PNC-27 and PNC-28 uses the entire peptide when destroying cancer cells, as opposed to fragments. (Cancer chemotherapy and pharmacology, 2010).

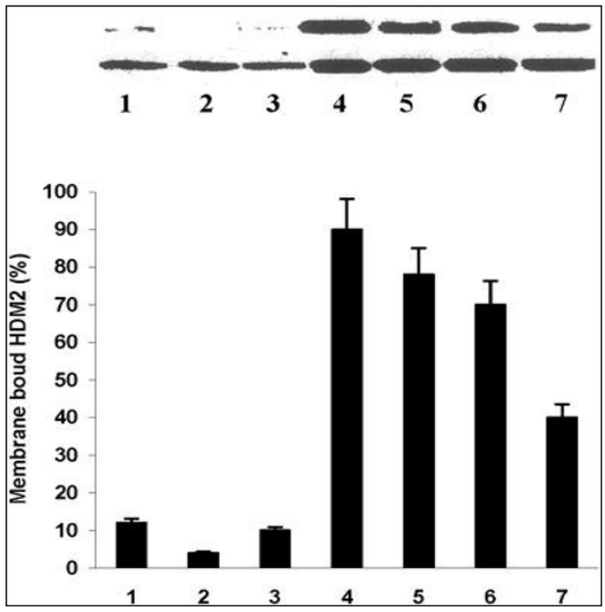
"The PNC-27 peptide has an HDM-2 binding domain that has the same residues 12-26 of p53 and a transmembrane-penetrating domain (Sarafraz-Yazdi, et al, 2010). PNC-28 is a p53 peptide from its HDM2-2-binding domain (residues 17-26), which contains the penetratin sequence enabling cell penetration on its carboxyl terminal end. This domain is connected to a transmembrane-penetrating sequence, also referred to as the membrane residency peptide (MRP). When p53 binds to the HDM-2 protein through the 12–26 amino acid sequence, HDM-2 causes ubiquitination of p53 that targets it for proteolysis in the proteasome. Both PNC-27 and PNC-28 peptides , which contains a sequence that is identical to that of PNC-27 but lacks the first six amino acid residues of PNC-27 (i.e., p53 residues 17-26), are highly toxic to a wide variety of cancer cells with IC50 values that range from around 75 ug/ml (18.6 uM) to 200 ug/ml (50 uM)." "While PNC-27 and PNC-28 are toxic to cancerous cell, neither peptide affects normal cells that are in culture, even at the highest doses (around 500 ug/ml) tested". This discovery illustrates that both peptides would not be toxic to the human body, in contrast to the effects of many chemotherapeutic agents (Davitt K, Babcock BD, 2014).

In a newer study that was analyzed in 2014 and published in Annals of Clinical & Laboratory Science, researchers concluded that "the anti-cancer peptide, PNC-27 and PNC-28, induces tumor cell necrosis of a poorly differentiated non-solid tissue mammalian leukemia cell line that depends on expression of HDM-2 in the plasma membrane of these cells." (Davitt, et al (2014). Studies based on information discovered in earlier experiments illustrated that the PNC-27 and PNC-28 peptides were able to destroy solid tissue tumor cells through the mechanism of binding to HDM-2 proteins in their cell membranes, an effect which was an effect that was different from the p53 activity in those cells.

The scientists wanted to determine whether PNC-27

peptide would also be effective against non-solid tissue tumor cells (Davitt et al., 2014). Their study determined whether the non-solid tissue tumor cells expressed HDM-2 in their membranes in the first place (solid tissue cells were found to do so), and find if PNC-27 could cause these cell's death by the HDM-2 binding. The researchers observed that these non-solid tumor cells do express HDM-2 in cell membranes.

They also noted that the PNC-27 peptide could cause cell destruction through membranolysis through the same HDM-2 binding mechanism as it did in the solid tissue tumor cells, independent of the p53 pathway. The research established that the peptides are successful for both solid and non-solid tumors, making it a valuable drug that could treat a variety of cancers.



(Figure 4) "Blots of whole cell lysates (Lower) and membrane fractions (Upper) for H (M) DM-2 in different cell lines as follows: Lane 1, MCF-10-2A; lane 2, BMRPA1; lane 3, AG13145 fibroblasts; lane 4, TUC-3; lane 5, MIA-PaCa-2; lane 6, MCF-7; lane 7, A-2058. The first three cell lines are untransformed; the remainders are different cancer cell lines. Each bar graph shows the percentage of HDM-2 in whole cell lysate that is present in the membrane of each cell line listed above. The numbers on the X axis of the bar graphs correspond to the lane numbers shown in the blots." (Safaraz-Yazdi E, Bownwe WB, 2010)

Studies and Trials on PNC-27 and PNC-28

Electron microscopy was utilized to observe the levels of HDM2 in the cell membrane (Wang, et al 2010). Parts of the membrane were isolated, and whole cell lysates from several different cancer and untransformed cell lines are shown in the figure below. The cells were blotted for HDM-2 (Figure 4). "In the lower section in the table, it can be seen that all whole cell lysates had positive results for the blotting procedure for HDM-2. On the upper section for the blots shown in the figure, the membrane fraction of each cancer cell line (lanes 4–7) is seen to contain significant levels of HDM-2. In contrast, the three untransformed cell lines (lanes 1–3) were discovered to contain low levels of HDM-2 in the region22 of the membrane. The percentage of whole cell lysate of HDM-2 present in the membrane fractions of the cell lines is shown in the lower section of the bar graph." (Safaraz-Yazdi E, Bownwe WB, 2010) The graph illustrated that the fractions of HDM2 present in the membranes of the cancer cell lines have an increased level of four-fold to nine-fold.

When the PNC-27 peptide was tested, researchers noted pain levels drop in the average time of a week. Three weeks following the administration of the peptide, the subjects sometimes developed flu-like symptoms. This is an indicator that the immune system had the ability to recognize and react to the death of cancer cells (Sookraj, Ka, Bowne WB 2010). Additionally, when the cancer killing peptide PNC-27 was administered after tumor growth had occurred at a different site from the original tumor, the tumor decreased in size, followed by a gradual increase in tumor growth that was "significantly slower than growth in the presence of control peptide." If the cancer killing peptide is immediately senttouro to the tumor, researchers concluded that the peptide would be successful in treating cancer. (Prendergast, Jaffee, 2007). At six weeks, the researchers noted an increase in lactate dehydrogenase and bilirubin levels.

At ten weeks, a large amount of tumor breakdown is noticeable. At the same time, the tumors become softer and pliable. In addition, some increase in the size of the tumor itself occurred simultaneously. However, this can often be attributed to inflammation because of immune system response. At about three months, researchers saw that the subjects of the study exhibited better energy levels and less cancer-related symptoms.

A study published in the International Journal of Cancer (2006) writes that researchers discovered that PNC-28 was able to reduce the rate of cancer cell growth in an organism at a quicker pace. Research tested PNC-28 to analyze its function to halt the replication of cancer cells. When PNC-28 was given over a two-week timeframe, the PNC-28 caused complete destruction of these tumors. (Science Daily, 2006) When administered simultaneously with tumor implantation, PNC-28 blocked the tumor growth entirely in the duration of the two-week period of administration, as well as two weeks after treatment, followed by weak tumor growth that leveled off at

low tumor size (Bowne WB, Sookraj KA, 2015). The trials on both drugs supported the theory that immunotherapy is able to eliminate cancer cells without causing damage to the human system.

All methods and studies in this paper have been completed, and the drug is currently being sold out of the United States. It is in trial stages to be sold in the United States as well. PNC-27 and PNC-28 are novel drugs that bring much promise to the field of cancer research, and promises an alternative to the other harmful treatments being used today. However, larger studies should be conducted to reduce the side effects that are experienced by patients taking the drug. There were no attempts in proving that PNC-27 is harmful for ingestion, unless contaminated by an outside source. There are multiple factors involved in creating immunotherapeutic peptides as treatment to cancer. Other approaches to improving the drug would be to further elucidate the mechanism by studying various models of the disease. By examining different models, this will hopefully give a deeper perspective and aid the efforts for a cure.

Conclusion

Cancer is a disease that has caused an upward trend in mortality rates. Although some progress has been made in cancer research for a cure, there are many drawbacks to the current treatments available. The current treatments are chemotherapy and radiation. While both of these treatments can successfully eradicate tumors and cancer cells, they also tend to damage healthy cells. There is also potential for a relapse because not all cancer cells are targeted during treatment, as some cancer cells may drift to other organs and rebuild there. As a result, many cancer patients succumb to the toxic effects of cancer treatments rather than the cancer itself. With wealth of evidence in support of immunotherapy, this alternative treatment shows great promise because it does not adversely impact healthy cells. This is because the underlying concept of immunotherapy is to invest the body's healthy cells with resources to be able to naturally combat the cancer cells. This is done by PNC-27 and PNC-28 peptides. Through research and study on animal subjects, the PNC-27 and PNC-28 peptides have been shown to be highly effective in selectively targeting a wide variety of specific forms of cancer, including pancreatic cancer, breast cancer, leukemia, melanoma, and additional cancer lines. Since PNC-27 is non-toxic, patients can get rid of the cancer in a healthy manner, while aiming to follow a lifestyle based on holistic health and wellness. When properly administered, PNC-27 and PNC-28 puts cancer on the defensive, resulting in outcomes that include significant pain reduction and, in many cases, lengthening of life. This research supports the evidence that PNC-27 and PNC-28 are one of the most effective ways to treat cancer.

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Intra-Ocular Vascular Endothelial Growth Factor Inhibiting Agents: Indications, Efficacy, and Alternatives

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Abstract

One of the most revealing parts of the human body is the eye. In fact, systemic conditions and diseases are commonly manifested in the eye and display pathology that reflect disorders. An example of a chorioretinal vascular condition that can present itself in the eye is diabetes mellitus, which if left uncontrolled can damage the eyes and a person's vision. Age related macular degeneration is specific to the eye, potentially leading to irreversible loss of central vision. Intravitreal injections containing vascular endothelial growth factor inhibiting agents appear to be the leading lines of defense against diabetic retinopathy and macular degeneration progression, helping to reduce both the pathological and visual manifestations of these chorioretinal vascular diseases.

Background

Diabetes is the fourth leading cause of health complications, making many people susceptible to diabetic retinopathy. In fact, the prevalence of diabetes is expected to increase by 7.7% by 2030, leaving many people at risk (Abbas et al., 2017). Age related macular degeneration is another neovascular condition of the eye which can reduce visual acuity to 20/800 in a patient's better eye by 60%, resembling a patient who "is bedridden with a catastrophic stroke" (Folk & Stone, 2012). These diseases are capable of impairing the daily functioning of a patient if they are not treated and monitored. Most ocular diseases can be identified with a dilated eye exam. Treatments with delivery of vascular endothelial growth factor inhibitors to the retina via intravitreal injection has gradually become the first line of treatment for ocular conditions such as diabetic retinopathy and macular degeneration. Pan-retinal photocoagulation (PRP) is a laser treatment that can also be used in controlling these conditions and preventing the progression of vision loss. Research is currently being done on both of these methods in an effort to determine their use in ocular disease.

Hypothesis

In most circumstances, vascular endothelial growth factor inhibiting medications can be implemented in a treatment plan to alleviate both the visual complaints and the pathological findings associated with chorioretinal vascular conditions. In some cases, however, this treatment option may not be appropriate due to potential risks and another treatment may be indicated.

Methods

Research for this paper was conducted with the use of ProQuest, EBSCO, and Pub-Med databases. Figures used throughout include corresponding sources from research articles.

Diagnostic Methods

Conditions such as age-related macular degeneration and diabetic retinopathy can be diagnosed with a proper exam. Ophthalmic tropicamide, a mydriatic, is used in order to give the clinician a proper view of the fundus. Imaging methods such as optical coherence tomography (OCT), fluorescein angiography, and wide field fundus imaging may also be used for future comparison during treatment. Diagnostic guidelines have been established to aid in classifying the type and severity of ocular disease. Findings, diagnostic methods, visual complaints, and prognosis of these conditions will be discussed in the following sections. Popular treatment options will also be outlined.

Diabetic Retinopathy

Diabetic retinopathy (DMR) is a complication resulting from diabetes mellitus Type 1 or 2. It appears to be one of the leading causes of blindness in patients aged 20-65 (Stewart, 2012). Currently, 336 million people suffer from diabetes and are therefore at risk of developing diabetic retinopathy, otherwise known as DMR (Abbas et al., 2017). Diabetic damage to the eye is a result of macular edema, which in more advanced cases, can lead to intraocular bleeding, detachments of the retina, macular ischemia, and retinal neovascularization. Possible risk factors for DMR include hyperglycemia, elevated blood pressure, and elevated serum levels (Buffolino & Park, 2019). Patients can usually control risk factors with lifestyle modifications including diet and exercise, yet it is important to note that specific patient demographics can predispose people to diabetic retinopathy.

Firstly, individuals diagnosed with diabetes at an earlier age tend to be more vulnerable to developing the condition. DMR becomes more prevalent as the age of diabetes onset is younger. "What is hitherto unknown is whether the increased prevalence of complications associated with early-onset disease is simply a consequence of the longer duration of disease, a consequence of a more severe metabolic phenotype, or in fact something specific to the diabetic milieu in younger patients that makes tissues more inherently susceptible to hyperglycemic damage" (Wong et al., 2008). Long-term exposure to elevated blood glucose levels seems to lead to oxidative stress on the eyes (Stewart, 2012). By the same token, retinopathy is not common in the first five years of a Type 1 diabetes onset, and diabetic retinopathy tends to be present after 20 years of having Type 2 diabetes (Cook et al., 2008).

Secondly, in addition to patient age, ethnicity is also associated with DMR. African Americans, Hispanics, and South Asians are especially prone to diabetic retinopathy. African Americans seemingly have reduced glycemic control, which can lead to retinal complications due to higher A1C values. The internationally recognized mark differentiating safe and unsafe A1C values is 7% (Long et al., 2017). A study showed that over a six-year period, 72% of African Americans with Type 1 diabetes mellitus developed retinopathy (Buffolino & Park, 2019). Furthermore, the combination of male sex, African American ethnicity, duration of standing diagnosis, and value of hemoglobin A1C, were "positively associated with retinopathy severity" (Long et al., 2017).

Before any signs of diabetic damage appear on the retina, choroidal vessels may constrict due to hypoxia and vascular changes can develop. Furthermore, it is important to note that in the early stages of damage to the retina, the patient may not be able to discern changes in visual acuity; the condition is usually diagnosed in a later stage of diabetic retinopathy (Wang & Tao, 2019). About 50% of patients with diabetes do not come for yearly screening visits (Buffolino & Park, 2019). The first markers of diabetic damage in the eye are the results of swelling and leakage of blood vessels because of poorly controlled blood sugar levels. The natural synthesis of vascular endothelial growth factors (VEGF) results in the disruption of the blood-retina barrier that leads to the spread of serum proteins leading to edema (Stewart, 2012). Bleeding near the macula can noticeably blur the patient's vision. According to Mayo Clinic's webpage on diabetic retinopathy (n.d.), the patient may also complain of fluctuating vision. This can occur because of uncontrolled blood sugar levels that cause the lens to swell and alter the refraction of light in the eye. Two other common visual complaints are impaired color vision and floaters (Vien, n.d.). Over time, the body may begin to generate new blood vessels in order to properly supply the eye with oxygen, which severely impacts the patient's vision and everyday functioning. Other lesions associated with diabetic retinopathy such as microaneurysms, hemorrhages, cotton wool spots, and hard exudates can be seen.

Diagnosis and Prognosis of Diabetic Retinopathy

Diabetic retinopathy is classified into either proliferative or nonproliferative diabetic retinopathy (PDR/NPDR) and then further broken down into mild, moderate, and severe. This classification considers the degree of damage present on the retina. The job of the clinician is not an easy one to classify the stage of diabetic retinopathy. With the help of wide field fundus imaging, the ophthalmologist is able to document and compare any changes to the appearance of the retina. The wide field images below can demonstrate the classifications of diabetic damage to the retina based on any associated findings.

Additionally, optical coherence tomography (OCT) is used to provide cross-sectional scans of the retina which can also help in determining the presence and degree of edema or cotton wool spots. Layers of the retina such as the RPE and choroid may also be altered.

Age-Related Macular Degeneration

Age related macular degeneration (AMD) is the most common cause of blindness in patients older than age 65. In industrialized countries, blindness due to AMD had a reported 1.47% prevalence and affected 1.75 million people in the United States alone (Eng, et al., 2019). The deposition of yellow, extracellular material underneath the retinal pigment epithelium that form drusen, indicative of photoreceptor cells that are degenerating is a key component of AMD (Virgili et al., 2015). The RPE must reuse materials in order to help revitalize photoreceptors. A defect in the cycle suggests the poor quality of enzymes that are responsible for breaking down those materials (Sarks et al., 1994), leading to degeneration of the RPE as drusen evolve into geographic atrophy. Extracellular deposits or atrophy of the RPE near the retina can lead to significant loss of central vision. As the drusen degenerate, they give a white appearance to the retina with "irregular margins and foci of calcification before disappearing to leave a focal patch of atrophy" (Sarks et al., 1994). Most patients complain of blurred or distorted vision or a scotoma (Cook et al., 2008). Risk factors for AMD include smoking, hyperlipidemia, and hypertension. Age-related macular degeneration is also strongly tied to a genetic predisposition to the point that children and siblings of patients with AMD are automatically considered suspects for the condition. Many sources rank genetics as an important contributor to the likelihood of developing age-related macular degeneration. Various studies show that people of African descent have a reduced risk for AMD, than does the Caucasian population (Delcourt, n.d.). Smoking increases the risk of AMD as well.

Although much about AMD and its pathogenesis is still unknown, studies show "that the largest single genetic factor contributing to AMD is a variant of codon 402 in the gene encoding complement…[that] strongly supports the long-held belief that the immune system is an important contributor to AMD" (Folk & Stone, 2012). In some observed cases of AMD, inflammatory mediators and other molecules have been found at the macula, indicating that there is indeed a connection between the condition

and inflammation. Furthermore, the release of excess activated macrophages in chronic inflammatory diseases is linked to cellular damage at the macula. Electron microscopy and immunohistochemistry methods were used to detect the presence of inflammatory biomarkers at sites of atrophy and neovascularization in patients with AMD (Cachulo & Costa, 2017).

Prognosis and Classification of AMD

Age-related macular degeneration is diagnosed with a dilated retinal examination. Evaluating a case of AMD requires paying attention to changes at or near the macula. Age-related macular degeneration begins with the dry form, characterized by the presentation of drusen. Number, size, and location of drusen are helpful in determining the progression of the condition. Additionally, drusen with indistinct borders seem to be indicative of the advancement of AMD (Cachulo & Costa, 2017).

As AMD progresses, the retinal pigment epithelium layer begins to show signs of atrophy. A case of AMD is characterized as wet or neovascular once new blood vessels begin to grow over or underneath the RPE (Folk & Stone, 2012). The generalized term "late AMD" refers to two specific presentations on the macula with exudates which can either be geographic atrophy or vascular AMD. Progression from dry to wet AMD occurs for 15% of patients complaining of severe loss of central vision and presenting with geographic atrophy that develops (Eng et al., 2019). Patients may notice visual distortion of straight lines. Ten percent of patients with AMD suffer from this more advanced form. More than 200,000 patients in the United States alone are diagnosed per year, accounting for 90% of all severe vision loss (Stewart, 2012). In a large study done, "the prevalence of large drusen increased from about 1.5% in Caucasians aged 40-49 years, to more than 25% in those aged 80 years or more" (Delcourt, n.d.). This statistic is important to consider because it indicates that Caucasians appear to be more at risk for developing AMD than people of other demographics such as Hispanic American, African American, and Chinese (Delcourt, n.d.). Of course, as the name of the condition suggests, the likelihood of developing AMD increases exponentially with age.

VEGF in Ophthalmology

Angiogenesis is a process that can be both lifesaving and necessary, yet in some cases can be detrimental. Throughout the body, the natural process for generating new blood vessels is crucial for maintenance related to growth (embryonic or otherwise) and repair. Yet in circumstances of hypoxia and inflammation, it allows the growth of tumors and is linked to the destruction of tissue (Stewart, 2012). Angiogenesis is also important when neovascularization is required such as after myocardial infarction. However, in ophthalmic cases, the generation of new blood vessels can lead to hyperpermeability that can harm vision by leading to retinal detachments and edema between the retinal layers. The primary molecule that is associated with angiogenesis is vascular endothelial growth factor. "Within the posterior segment of the eye, VEGF is produced by retinal pigment epithelial cells, neurons, glial cells, endothelial cells, ganglion cells, Muller cells, and smooth muscle cells" (Stewart, 2012). The primary cells of interest leading to the harms from AMD and DMR however, are vascular endothelial cells. Generally, the synthesis of VEGF is triggered by tissue hypoxia that can either be caused by "primary vascular occlusive disease or anaerobic tumor metabolism" (Stewart, 2012). The mechanism leading to the proliferation of vessels is complex but it results in mitosis and swelling of the endothelial cells, as well as vasodilation, helping new blood vessels develop. Specifically, VEGF works to encourage growth along the endothelial cells and dissolve the spaces between them, leading to the breakdown of the blood-retinal barrier that makes capillaries leak and new, potentially detrimental, blood vessels form (Stewart, 2012). VEGF-A, a subfactor of this molecule, is linked to growth and permeability of newly-developing blood vessels. VEGF inhibitors block the receptors that natural VEGF would bind to, preventing angiogenesis. "Long-term blockade of VEGF-A [receptor] causes shrinkage and maturing of the vessels so that they no longer leak. The accumulation of fluid within and beneath the retina dissolves, and the photoreceptors reattach to the underlying retinal pigment epithelium" (Folk & Stone, 2012). By using a medication that reduces the permeability of the retinal vessels and stopping their growth, leakage is also reduced, preventing swelling in order to bring the patient's visual acuity back to baseline (Folk & Stone, 2012). It is interesting to note that these drugs are capable of resolving findings associated with two very different conditions in that one is systemic and can target multiple parts of the body, while the other is localized in the eye.

VEGF Inhibitors: A Comparison

Although all VEGF inhibitors prevent neovascularization, there are several subclasses of this drug. Their generic names aflibercept, bevacizumab, and ranibizumab will be used throughout this paper (Folk & Stone, 2012). The efficacy of the medications as they compare to each other is still being studied.

Bevacizumab received approval from the Food and

Drug Administration for the treatment of colon cancer. Bevacizumab also binds to VEGF-A, which led clinicians to think that the drug may also be effective in treating neovascularization and exudates on the retina. Although there is no FDA approval for its intravitreal use, bevacizumab is proving itself effective (Folk & Stone, 2012). When compared to other medications in its class, bevacizumab has a longer half-life. One dose of the drug can reduce levels of natural VEGF in the blood by 117 times in just one day and when measured after one month, still maintained a four-fold reduction (Stewart, 2012). Furthermore, many clinicians recommend this drug because of its vast difference in cost compared to others. Being prepared in larger doses than is necessary for the eye means that the medication can be divided for multiple doses (1.25 mg. on average), reducing the cost even further. Whereas a dose of ranibizumab costs $2000, bevacizumab, although regarded as off label therapy, costs $75 per treatment (Folk & Stone, 2012).

Ranibizumab was developed specifically for use in the eye by fragmenting the bevacizumab molecule for higher affinity by five to twenty times more than bevacizumab (Stewart, 2012). A study looking at regular monthly treatment with ranibizumab shows that the first five years of treatment produce the best results in terms of visual acuity, but then results seem to approach baseline levels over time thereafter (Nishikawa et al., 2019). For treatment with ranibizumab, the MARINA study attempted to determine effective dosage of the medication. At random, 716 patients with neovascular AMD were treated with monthly 0.3mg or 0.5mg injections of the drug, or sham treatment for 24 months. Results showed that 92% of patients that were treated with 0.3mg of ranibizumab and 90% treated with 0.5mg lost less than 15 letters when being tested for changes in visual acuity. Furthermore, 26.1% of patients that were treated with 0.3mg and 33.3% with 0.5mg treatment had a 15-letter improvement or greater.

When aflibercept is compared to the other drugs, its increased binding affinity to VEGF-A receptors combined with its larger molecular size allows for a higher efficacy and hence, less frequent treatment. When used on a two-month basis after three monthly consecutive loading doses, results compared to monthly treatment with ranibizumab (Stewart, 2012). Patients with neovascular AMD who were treated with aflibercept intraocular injections were able to sustain improved vision for four years. Even though after one year some regression was present, 94.5% of patients still had improved vision. "Thus, visual gain [of aflibercept] in the first year is generally favorable, but long-term outcome is not as promising" (Nishikawa et al., 2019).

Conclusively, visual acuity was shown to improve with all three classes of treatment, but specific data was shown by a study conducted by DRCR.net in which 600 Americans with diabetic edema participated. The average age was 61 and most people had diabetes for over 15 years. Visual acuity before the study was 20/32 or worse and patients were administered either of the three drugs at random and monitored on a monthly basis in the first year of the study. When comparing the visual acuity of patients after treatment, those on aflibercept for two years were able to read 3.5 more lines compared to ranibizumab patients who read 3, and bevacizumab patients read 2.5 (Wells, 2016). Aflibercept showed better efficacy after the one- and two-year mark. "By two years, 41 percent of participants in the aflibercept group received laser treatment to treat their macular edema, compared with 64 percent of participants in the bevacizumab group and 52 percent in the ranibizumab group." Ultimately, with treatment involving any of the three drugs, visual acuity was improved to 20/32 and 20/40. There were even studies showing the efficacy of bevacizumab in improving patients' visual acuity from 20/235 to 20/172. The results of the study suggest that there is a slight difference between the frequencies at which the drug must be readministered depending on the specific type of medication chosen. According to the DRCR study done, there was no significance to using one medication over another for treatment of mild macular edema when visual acuity is 20/40 or better. However, in cases of 20/50 or worse vision it has been shown that Eylea (aflibercept) performed better than ranibizumab and bevacizumab (Wells, 2016).

Risks

Although this modality of treatment is usually the first line of defense against further damage to the retina, existing risks should be considered. Firstly, for all the subclasses of intravitreal injections used for treating chorioretinal disorders, the risk for eye infection and inflammation is the same. These are consequences of the administration and not the drug itself (Wells, 2016). Injection into the vitreous chamber also raises the risk for endophthalmitis, retinal tear or detachment at the area of injection, and vitreous hemorrhage because of penetrative trauma (Pershing et al., 2013). These risks must be kept in mind when developing a treatment plan for a patient suffering with other kinds of ocular disease as well. Furthermore, because additional fluid is added to the vitreous cavity when the medication is given, an elevation in intraocular pressure from increased volume in the eye can result.

A study was done spanning academic centers Stanford University Hospital & Clinics and Mayo Clinic. Patients who received anti-VEGF injections between January 1,

2006 and December 31, 2010 participated in order to determine the relationship between administration of the drug and intraocular pressure elevation taking place within 60 days of the injection. Intraocular pressure asymmetry between the eyes by a value of more than 3mm Hg was also considered. Those with a history of glaucoma or elevated IOP in the past did not qualify to participate. "A total of 11,828 bevacizumab injections and 10,354 ranibizumab injections were administered during the study period at the two clinical sites. In this series, 21 eyes of 18 patients developed elevated IOP of 24mm Hg of higher within 60 days of previous injection" (Pershing et al., 2013). Most patients were Caucasian females who received treatment for wet AMD and presented other pathology related to elevated IOP, such as narrow anterior chamber angles on gonioscopy. Most of the 21 eyes needed treatment for elevated pressure, 76% required prolonged treatment, and 19% resolved with no treatment. It was found that a risk of increase in IOP persisted even later in treatment after multiple injections to the eye. This risk was reported regardless of the subclass of anti-VEGF used, however a higher chance of IOP elevation in ranibizumab treatment was reported (Pershing et al., 2013). This risk is especially important to consider if the patient in question has glaucoma or is a glaucoma suspect, as a subtle rise in intraocular pressure may be detrimental. In such a case, the drug should be administered and the patient carefully monitored for changes in IOP that may lead to an acute attack of glaucoma.

Additionally, receiving anti-VEGF drugs alongside having coexisting cardiovascular conditions may lead to adverse effects. The SUSTAIN trial showed that although there is no link to myocardial infarction, a 10% incidence of stroke was found in patients with previous cerebrovascular disease that were also being treated with VEGF inhibiting medications (Stewart, 2012). In patients receiving regular treatment with ranibizumab, there was a higher incidence of heart attack, stroke, or death from cardiovascular or unknown conditions (Wells, 2016).

Finally, the nature of this treatment option for AMD/DMR is that frequent, monthly follow up visits are required to monitor and readminister the drug, rendering compliance of treatment more difficult to upkeep. Inherent drawbacks to the use of VEGF inhibiting agents are its cost and frequency of treatment as it relates to burden on the patient. Perhaps lowering the overall cost of the treatment (medication and visit) while creating a medication with a longer half-life or efficacy will allow more patients to undergo long term treatment, preventing blindness (Baek et al., 2019). In some cases, the use of a laser to clear drusen and prevent the furthering of neovascularization may be more appropriate, as will now be discussed.

Use of Pan Retinal Photocoagulation

Although traditional cases of diabetic retinopathy and macular degeneration now indicate treatment with intravitreal injections, this has not been the case for long. In the past, the first line of defense was treatment with blue-green Argon laser in order to create lesions around the area of retinal neovascularization and promote the regression of abnormal blood vessels and drusen. This option to help DMR and AMD is called pan retinal photocoagulation. In this technique, a beam of light from the laser is focused onto the retina using a special lens. The strength of the laser now used is usually weaker, with studies trying to determine the efficacy of subthreshold laser treatment to avoid applying more energy than necessary. Up to 300 lesion spots may be delivered, with each one between 100µm and 200µm in size. "Subthreshold photocoagulation delivers light energy with very short impulses that are absorbed by the RPE only, aiming to spare the neurosensory retina". Adjusting the strength, pulse duration, and frequency of the laser makes the treatment effective yet more gentle on the choroid in an attempt to reduce potential photoreceptor loss. Although the mechanism of treatment is not fully known, some ideas explain its efficiency.

Firstly, the laser may help in clearing remnants left by phagocytic cells and macrophages along the choroid. It is hypothesized that the accumulation of these cells leads to the development of drusen. Another train of thought is that the laser may precipitate the RPE layer to release cytokines and growth factors that are able to "modify the biochemical processes underlying the clinical manifestations of the retinal disorder, rather than simply destroying drusen, and may also act on the drusen remote from the site of laser energy application" (Virgili et al., 2015). Also, the laser energy is capable of causing the temperature of the retina to rise slightly, leading to the release of heat shock proteins. They "act as chaperones for refolding misfolded proteins in aging cells, thereby rejuvenating retinal pigment epithelium (RPE) cells and restoring their cellular function" (Eng et al., 2019).

A study included patients with extrafoveal neovascular presentation with drusen. Patients were 50 years of age or older and visual acuity either was 20/100 or worse (Silva, 2011). The laser used was a conventional blue-green Argon laser. Energy was set to be powerful enough to create the necessary lesions or controlled burns on neovascular areas. In this study, attention was not paid to maintaining sub-threshold energy. By using pan retinal

photocoagulation, the likelihood to stabilize the visual acuity of a patient doubled for those eyes, showing a 58% reduction in the risk to have severe vision loss.

Highlights from the Macular Photocoagulation Study Group (Silva, 2011) are shown here:

A. Five years after treatment, average visual acuity was 20/125 in the group treated compared to 20/200 in patients not treated with PRP.
B. Within the five-year span of treatment, 54% of patients had signs of recurring severe vision loss, with the majority of cases taking place within two years of treatment.
C. Smoking after PRP treatment was linked to accelerated recurrence of severe vision loss, as 85% of patients smoked more than 10 cigarettes per day compared to 51% in the non-smoking population.

An additional analysis of 12 studies of 2,481 eyes that were treated with subthreshold pan retinal photocoagulation showed similar results. This study included treatments done with 514nm and 810nm lasers. Conclusions of the review showed that pan retinal photocoagulation treatment is effective in reducing drusen count and thereby improving visual acuity in patients with dry AMD with the 810nm laser being consistently more effective. However, the study mentions that in order to obtain a significant improvement in visual acuity, the treatment must clear a substantial amount of drusen. Additionally, the study found that among the eyes that were treated, there was "no significant change in the risk of developing choroidal neovascularization or geographic atrophy" (Eng et al., 2019) and that more research would need to be done to conclusively confirm the efficacy of the 810nm infrared diode laser compared to the 514nm laser.

Comparing the treated patients with the observed patients in the study, the following data was obtained in regard to the relationship of diminishing drusen and treatment with pan retinal photocoagulation (Eng et al., 2019):

1. In five out of the 12 studies in which visual acuity was measured, it was determined that there was no difference in visual acuity in the groups who had and had not received treatment. Yet, the remaining studies showed a correlation between receiving treatment and improved visual acuity. This indicates that although pan retinal photocoagulation may be effective in clearing drusen from the retina, perhaps more research is necessary to conclude the effectiveness of the laser in improving the visual outcome for the patient.
2. In regard to the development of choroidal neovascularization (CNV), three of nine studies that followed up on this mentioned that there was no development of CNV. An additional three however noted that eyes treated with subthreshold pan retinal photocoagulation were slightly more likely to develop CNV.
3. Geographic atrophy was also monitored in this series of studies. It was reported that two studies showed GA in treated eyes, while another study showed that although the incidence of developing geographic atrophy in treated eyes was 3.9%, the likelihood was higher in eyes that were not treated, at 14.0%.

After five years, following up on patients showed 64% of eyes that were not treated with the laser developed more severe vision loss compared to 46% of patients that had treatment with PRP. Yet, the recurrence rate of vision loss was 54%, with 75% of those recurrences taking place in the first year (Cook et al., 2008).

Conclusion

Currently, there are a number of available treatment options to reduce visual complaints and pathological findings in patients with chorioretinal vascular conditions. Interventions such as subthreshold pan retinal photocoagulation and vascular endothelial growth factor inhibiting agents are still undergoing various studies, yet they do appear to be effective and promising options for patients battling age related macular degeneration and diabetic retinopathy. There are reasons why a clinician may choose one over the other in hopes of achieving the maximum benefit for the patient, depending on cost, compliance, and coexisting conditions. Although vascular endothelial growth factor inhibiting agents have become the first line of defense against neovascularization and other such complications of ocular disease, there are still reasons why they may not be the appropriate treatment option for a given patient as there are associated risks. Furthermore, some clinicians believe that using alternatives such as PRP may be a better option altogether. On the other hand, an advantage of VEGF inhibiting agents over pan retinal photocoagulation is "prompt regression of neovascularization and the preservation of peripheral and night vision" (Buffolino & Park, 2019). Undergoing treatment with VEGF inhibiting medications for patients with severe vision loss may not be recommended as the drug may not be able to restore vision (Baek et al., 2019), however in most circumstances, VEGF inhibiting drugs can and perhaps should be implemented to alleviate both the visual complaints and the pathological dangers associated with chorioretinal vascular conditions. Potential risks previously discussed such as IOP elevation, inflammation, visual and pathological damage, and coexisting conditions

may need to be considered on a case by case basis, necessitating another modality of treatment. Nevertheless, it should be stressed that without a proper dilated exam, very little can be observed and treated; imaging methods can also be used to monitor the stability or progression of these diseases.

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Causes and Mechanisms of Crohn's Disease

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Abstract

Crohn's disease and ulcerative colitis are the two most prevalent inflammatory bowel diseases (IBDs) in Jewish and Caucasian populations, affecting as many as one in 250 individuals. Nevertheless, the underlying causes of both disorders are not yet fully understood and remain unknown. However, current evidence suggests that the exaggerated inflammatory response, more commonly referred to as IBD, is believed to arise from dysregulation of the gastrointestinal (GI) immune system in genetically predisposed individuals who are exposed to environmental triggers. Recent advances have identified multiple IBD susceptibility genes; however, only a few environmental determinants of IBD have been consistently identified. The difficulty in understanding the etiology of IBD is in part due to the complex interactions between genes and the environment. Additionally, autoimmune mechanisms are believed to play a role in the development of IBDs, but the target antigens and the underlying pathways have not been sufficiently characterized and identified. IBD is commonly referred to as an "idiopathic disease;" a disease with an unknown cause (Health &Medicine, 2016; Sartor, 2006). This paper examines the possible causes of IBD, specifically highlighting Crohn's disease.

Key Phrase

GI- Gastrointestinal Tract
IBD- Irritable Bowel Disease
CD- Crohn's Disease
UC- Ulcerative Colitis

Introduction

Crohn's Disease (CD) is a chronic autoimmune disease mainly affecting the gastrointestinal (GI) tract extending from the mouth to the anus; however, it's most commonly found in the ilium, leading to the alternate name ileitis (Newman & Siminovitch, 2003). It's characterized by thickened areas of the GI wall with inflammation extending through all four layers including the mucosa, submucosa, muscularis externa, and serosa (Politics & Government week, 2017; Ruthruff, 2007). The inflammation is somewhat similar to that of ulcerative colitis, and commonly leads to abdominal pain, diarrhea, vomiting, weight loss, anemia and fever (Torres et al. 2017). However, some patients may experience mouth and skin sores along with joint stiffness and swelling (Creek, 2017). Unfortunately, patients suffering from CD are at a higher risk of developing other autoimmune disorders such as osteoporosis, thyroid disease, as well as colorectal cancer (Baumgart & Sandborn, 2012; O'Sullivan, 2009; Bae et al. 2014). Symptoms range from mild to severe and treatment varies depending on the severity of the disease. Typically, a doctor will perform a series of tests before confirming CD. These tests may include a physical exam, lab tests, stool samples, CT scans, MRI, endoscopy and a colonoscopy (Baumgart & Sandborn, 2012), in which a small flexible camera is inserted through the rectum to distinguish abnormalities. Together, these tests detect abnormalities which may exist throughout the intestinal track thus indicating IBD.

While there is currently no known cure for CD, treatment options are available (Akobeng, 2008; Michail et al. 2013). It is estimated that roughly ten percent of patients who undergo surgery will have prolonged clinical remission (Baumgart, 2012; D'Incà & Caccaro, 2014). The goal of treatment is to reduce inflammation and to improve the prognosis by limiting complications (Newman & Siminovitch, 2003). Noninvasive treatment options include anti-inflammatory medication, antibiotics, and immunosuppressant drugs. Anti-inflammatory medication controls and suppresses inflammation, thereby decreasing the frequency of flare ups. With proper treatment over time, periods of remission can be extended, and flare ups can be reduced. Alternatively, immunosuppressant medications, such as corticosteroids, aid by suppressing the immune system, thus allowing the intestinal tissue to heal.

However, in some instances, more invasive treatment plans are required. Despite significant progress in treatments for CD, it's estimated that as many as seventy-five to ninety percent of patients suffering from the disease will undergo surgery to relieve symptoms at some point during the patient's life. (Politics & Government Week, 2017; Liu et al. 2017). At times, CD creates complications that are deemed a medical emergency, such as a bowel abscess, a fistula (which may result from an unaddressed abscess), uncontrolled bleeding, or intestinal blockage, in which case surgery may be required. As an aside, patients may also require surgery as a result of previous medications having caused severe side effects, or simply because the medications have stopped working as effectively as the body adapts and immunizes itself against the medication (Torres et al. 2017).

There are several types of surgeries that can be performed, the most common one being a bowel resection. This involves the removal of all parts of the damaged intestine and is performed when parts of the small bowel are diseased or blocked (Simon et al. 2003). A partial colectomy is another type of bowel resection in which the surgeon removes the damaged parts of the colon. It can be performed openly or laparoscopically. When performed openly, the surgeon manually cuts an extensive incision; alternatively, the surgeon may prefer to work laparoscopically, making several small incisions, thereby allowing him to work outside the body. In some instances, a total colectomy is required in which the surgeon

removes the entire colon. This is generally required only in severe cases in which the colon is highly inflamed, and medication is ineffective (Travis, et al. 2011). A colectomy often requires further procedures to reattach the disconnected portions of the digestive system, thus permitting waste to leave the body. For example, in some instances, an ostomy is performed. This surgery is categorized as a lifesaving procedure as it is performed when stool is unable to be eliminated from the body through the rectum (Travis et al. 2011). In this procedure, the colon is removed, and a stoma is created on the abdomen. Wastes are then allowed to pass through the surgically created stoma into an ostomy bag, a prosthetic bag which holds stool and can be emptied regularly.

Common risk factors of these surgeries include bleeding, bowel obstruction, infection and pulmonary embolism.

Methods

Data was collected using ProQuest and PubMed databases through Touro College's online library. Among the key-phrases used were "Crohn's Disease", "irritable bowel disorder", "epigenetics", as well as "hygiene hypothesis."

Genetic Factors

There is a significant amount of evidence suggesting that genetic factors play a key role in the triggers of IBD. Genetic mutations lead to the body's defective defense against microbes in the digestive system, allowing the immune system to attack the lining of the digestive tract, thereby causing inflammation indicating IBD. The genetic analysis of complex diseases, such as IBD, is difficult to identify for several reasons. First, the disease isn't categorized into a single entity, but rather into groups of heterogeneous disorders, where environmental and genetic factors play a significant role in disease expression. Additionally, ethnic differences that may exist between patients suffering with IBD are associated with different mutations (Bae et al. 2014). Other difficulties faced include the relatively low frequency of IBD, about .1-.4 percent, amongst populations (Michail et al. 2013); statistics showing that only about ten percent, first-degree relatives are affected with the same disorder (Sartor, 2006; Pena, 1998); as well as the presence of genes with minor genetic effects, are some of the difficulties faced with when identifying disease susceptibility. Nevertheless, there is an emerging understanding of the inherited aspects that predispose an individual to IBD (Newman & Siminovitch, 2003).

The evidence for genetic involvement in IBD comes from ethnic e.g., Ashkenazic Jews and family-based studies on blood relatives affected with CD, associations of IBD with known genetic syndromes, diseases with known genetic predisposition such as autoimmune diseases, and more recently through genetic marker studies (Barber GE, Yajnik V, Khalili H, et al. 2016). Various genetic markers have shown different levels of correlation with IBD. Some have failed to show a consistent association with IBD, including blood groups, secretor status, a 1-antitrypsin, and immunoglobulin marker genes. Studies on these markers can be divided into those in which a genetic element is proven and those in which a genetic predisposition may be present (Pena, 1998). Several polymorphisms in the genes encoding different cytokines and their receptors have been described and are potential candidates to be studied scientifically.

There are currently multiple studies being carried out to help identify the genetic mutations, causes, and susceptibility in developing IBD. In addition, technological progress in genetic testing and DNA sequencing permitted many genome-wide association studies (GWAS), which helped identify new single nucleotide polymorphisms (Loddo & Romano, 2015). Correspondingly, over the past few decades, there have been significant advances in the understanding of genetic causes linked to IBD. (Newman & Siminovitch, 2003). The studies described below are population-based studies in areas that extend over a period of time, making them most likely to give fair, unbiased results. These studies have confirmed the increased prevalence of IBD in family members as compared to those in the general population. These include studies based on twins, families, and the Jewish Ashkenazic ethnic group.

The data derived from studies performed on twins powerfully supports the view that IBD is linked to a genetic component. There is a significant increase in the concordance of IBD in monozygotic twins compared to dizygotic twins, particularly with Crohn's disease. Twin studies for CD have shown fifty percent concordance in monozygotic twins compared to less than ten percent in dizygotic twins (Loddo & Romano, 2015). Environment plays only a negligible role in predisposition for CD. Indirect evidence supporting the necessity of predisposing genes for the development of the disease in the presence of a common environment is the low prevalence in spouses of patients with IBD. Spouses who both suffer from CD, is deemed as a highly unusual occurrence, as frequent as can be expected by chance alone (Dudley, 1995)

Family-based studies consist of studying linkage analysis in sibling pairs and parental transmission in genome-wide screening using microsatellite markers. Microsatellite markers, otherwise known as simple sequence repeats (SSRs), which can be defined as segments of DNA where the nucleotide sequence repeats. Familial aggregation of IBD has been well documented in North American and European studies. It is well established that first degree relatives, particularly siblings, are at much greater risk in

developing IBD as compared to those in the general population. For example, if one parent has CD, the child has a ten percent increased risk in developing CD; and even if both parents suffer from CD, that likelihood increases to thirty-five percent (Pena, 1998). It has been shown that siblings of patients suffering from CD experience a seventeen to thirty-five times greater likelihood of developing CD (Akobeng, 2008).

Another significant study performed on ethnic populations proved that genetic variants are indeed associated with CD. People of Ashkenazi Jewish heritage have an increased risk and a higher prevalence in developing CD as compared to non-Jews (Baumgart & Sandborn, 2012; Newman & Siminovitch, 2003). Several studies have proven that CD is two-to-four times more prevalent among individuals of Jewish decent compared to non-Jewish Europeans living in the same area (NewsRX Health & Science, 2012). Currently, there are seventy-one genetic variants that have been identified in Ashkenazi Jewish ancestry that increase an individual's risk of developing CD. (Baumgart & Sandborn, 2012). In a study performed examining over six thousand individuals whose Jewish ancestry was confirmed by a large number of genetic markers, several variants were detected that are associated with the increased risk of CD. The involvement of twelve recognized CD risk variants in Ashkenazi Jews was confirmed and identified in novel genetic regions, as opposed to in non-Jewish European populations (Kenny et al. 2012). Further studies of these regions may aid in the discovery of biological pathways affecting susceptibility to CD and lead to the development of innovative and more effective treatments. This study not only proves the genetic origin of CD, but also demonstrates the value of genetic studies in secluded ethnicities, such as the Ashkenazim. Jewish heritage is an independent risk factor for developing CD. Genetic anticipation is the earlier onset of a disease in the offspring of parents with the disorder; a phenomenon featured in other genetic diseases, it has now been identified in Crohn's (Polito et al. 1996).

While genetic studies have been highly successful at identifying the genetic risk factors for CD, these studies have proven virtually nothing about why one person will exhibit only mild symptoms of CD while another patient may require surgery to treat their condition. However, a familial link is often noticed in the symptoms of related patients. Researchers recognize that family members with CD often tend to have similar progress through treatments (NewRX, 2017). Furthermore, researchers looked at the genome of two thousand seven hundred patients who shared similar symptoms and severity of CD. By comparing these patient's DNA, the researchers discovered four genetic variants including FOX10, IGFBP1, MHC, and XACT that influence the severity of a patient's condition. Strikingly, none of these genes have been shown to affect the risk of developing CD. This fact suggests that it is likely that genetics not only plays a role in the diagnosis of CD, but in the patient's prognosis as well (Obesity, Fitness & Wellness Week, 2017).

Environmental Factors

Although the precise cause for CD is unknown, it's believed that environmental factors also play a role in the development of the disease. Several environmental risk factors, such poor diet, as well as the side effects of breastfeeding, smoking, stress, and drugs, are believed to trigger inflammation. Additionally, childhood exposure towards an overly hygienic environment, seem to allow CD to manifest in predisposed patients.

Food is believed to promote the maintenance of gut integrity. Although the direct cause and effect relationship between the gut microbiota and IBD is unclear, targeting the intestinal microbiota allows for prevention of disease, as well as potential research concerning new treatments. Diet plays a significant role in a patient suffering from CD, as the body is unable to digest and absorb food appropriately, thereby causing malnourishment in patients with CD (O'Sullivan, 2009). Furthermore, many patients with CD are anemic, as well as deficient in vitamin D, folic acid and vitamin B12 (Torres et al. 2017; Creek, 2017). Patients are recommended to avoid certain foods such as raw fruits and vegetables, dairy, fatty, greasy, spicy, and high fiber foods, as these foods are more difficult for the body to digest and absorb, and commonly irritate the bowel (Livie et al. 2006). While it has not been proven that a poor diet is a direct cause of CD, research has shown a common thread, that a proper diet is helpful in several ways by reducing one's predisposition to developing CD. Moreover, diet has also been used as a treatment option for CD (Akobeng, 2012). Similar research has proven that maintaining a healthy diet is equally as effective in putting a patient in remission as corticosteroid treatments, particularly in the case of children (BMJ, 1997). Nutritional repletion is effective both by affecting the gut mucosa as well as by decreasing intestinal permeability. There is some evidence that the enteral diet has a direct effect on the gut mucosa by reducing cytokine production and the accompanying inflammation, thus leading to decreased intestinal permeability (Hanaway, 2006).

Breastfeeding promotes colonization with microbiota such as Bifidobacteria by providing them with the HMOs (human milk oligosaccharides) they require to thrive. According to one study, when specific HMOs, GOSs

(short-chain galactooligosaccharides) and FOSs (long-chain fructooligosaccharides), were fed to infants in the first six months of life, they later had fewer incidences of autoimmune reactions linked to gut microbiota, including recurrent wheezing, and allergic urticaria. Accordingly, Crohn's which is an autoimmune disease, may be affected similarly by breastfeeding (Torres, et al. 2017). Maternal secretory IgA, a component of breast milk has been proven to affect the composition of intestinal microbiota and the expression of genes associated with intestinal inflammation. Additionally, a negative correlation has been found between infants' exposure to breast milk and the development of early onset IBD, suggesting breastfeeding has a preventative effect on IBD development (Stiemsma et al. 2015).

While cigarette smoking is notorious for initiating health risks such as lung cancer and heart disease, it is believed to play a role in the development of IBD as well. Although the pathogenesis for IBD is unclear, cigarette smoking is associated with IBD outcomes as it exacerbates the condition. Analysis of multiple studies has confirmed that active smokers are at increased risk for acquiring CD (Newman & Siminovitch, 2003). Furthermore, smoking has repeatedly been proven to worsen the prognosis of CD as it promotes a fistulizing phenotype, aggravates disease course, and produces a decreased response to medical treatment. (Ole et al. 2009). Interestingly, while smoking has negative ramifications for Crohn's sufferers, it may have a protective effect on those with a genetic disposition towards UC. Active smokers were proven to be at a lower risk for developing UC in comparison to those who never smoked and those who quit smoking. This phenomenon may be linked to nicotinic acetylcholine receptors, which are present in mucosal epithelial cells of the bowel, as well as on T cells. However, clinical trials of nicotine replacement in IBD have only yielded a modest benefit at best; thus, nicotine alone may not be the driving factor (Ole et al. 2009).

Despite the significant consequences of smoking associated with patients suffering from IBD pathogenesis, the highest incidence of CD occurs in countries with a low prevalence of smoking such as United States and Canada (Rook, 2012). Furthermore, the majority of patients with CD are not current smokers and the majority of smokers do not develop CD, proving that smoking or lack thereof likely plays a role in the pathogenesis of only a subset of IBD patients (Torres et al. 2017). These studies display little correlation between smoking and IBD. Nonetheless, all IBD patients should be encouraged to quit smoking due to the deleterious negative health effects associated with smoking.

The role of psychological distress and personality as predisposing factors for the development of CD remains controversial. The debate concerning this matter has been ongoing for over eighty years without reaching a common ground, as attempts to investigate the role of psychological factors have unveiled conflicting results. However, over the past several years, it has become evident that psychological stress is at least associated with CD. However, that stress may be the result rather than the cause of chronic and longstanding CD. Unpredictable flare-ups and changes in body image can also lead to additional stress. This stress may precipitate an exacerbation of the disorder, thus continuing a vicious cycle of flare ups.

Multiple theories have been presented suggesting explanations on the unknown environmental exposures that may interact with the immune system and result in abnormal inflammatory intestinal response. However, the most predominant theory is the hygiene hypothesis, which explains the link between exposure to specific microbial allergies and the prevention of certain diseases. This hypothesis theorizes that when one's childhood has had excess hygiene and a lack of exposure to enteric pathogens; the child may not develop sufficient immunity towards pollution and environmental contamination. Thus, it will inevitably impair microbial competence of the GI immune system and its ability to recognize new antigens, thereby predisposing children to immunologic disorders further on in life (Sabe et al. 2017). While the effect of lack of exposure is believed to be most profound during early childhood, the ideal timing and degree of exposure required remains unclear. The hygiene hypothesis also theorizes that multiple childhood infections and poor hygiene protects and prevents an individual from developing Crohn's disease by allowing the host to develop tolerance or immunity to agents that may trigger Crohn's disease later on in life (Stiemsma et sl. 2015). Additionally, it is entirely probable that the impact of various exposures are not mutually exclusive; such that disease development depends on the dose-response interactions between exposures, and this reflects how strongly one's exposure may protect or increase the risk conferred by CD susceptibility mutations) (Sabe et al. 2017).

Interestingly, the first Crohn's disease gene identified, CARD 15(caspase-activation recruitment) also referred to as the NOD2 (nucleotide oligomerization domain gene), found within IBD1 locus is involved with the innate immune system and the response of monocytes upon perceiving a bacterial encounter. The abnormal and unconstrained inflammatory response that may occur in individuals with NOD2/CARD15 mutations is likely to be involved with the pathogenesis of Crohn's disease. Polymorphisms in the CARD15/NOD2/IBD1 locus have been associated with the highest risk for CD

development. Recent evidence shows that polymorphisms cause reduced functionality of the immune system and exacerbated phenotype (Newman & Siminovitch, 2003). Counterintuitively, countries with poor sanitary conditions such as Africa and the Middle East reportedly have a relatively low frequency of Crohn's disease (Torres et al. 2017), while more developed countries such as Canada and the United States have the highest rates of CD. It is believed that endemic parasitic infections may favorably affect the immune system as to prevent disease by stimulating T-helper type2 (Th-2) cells, which in turn downregulate Th-1 cells and prevent the exaggerated Th-1 response associated with Crohn's disease. Building on this theory, the ova of Trichuris suis (eggs of a pig whipworm), have been successfully used to treat Crohn's disease patients, as the intake of these parasites elicit Th-2 cells thereby suppressing Th-1 cells from activating (Guarneret et al. 2006; Summers, et al. 2005).

Many physicians treating patients with IBD do not believe that stress or diet are direct causes of IBD. Yet, due to the lack of understanding of the etiology of IBD, no known direct cause and cure exist for the disease. The Crohn's and Colitis Foundation and medical researchers suggest that foreign substances found in the environment or external agents such as bacteria may interact with a weak immune system, thereby producing an immune response of inflammation which the body is unable to stop (McGovern, Gardet, Törkvist, et al. 2007).

Epigenetics

It has become apparent that epigenetics plays a significant role contributing to the development of CD. Epigenetics can be defined as mitotically heritable changes in gene expression without shifting the DNA. Gene expression can be altered by changes to the structure and function of chromatin. Different cells in the body are characterized by different functions and different levels of gene expression despite each sharing the same genetic code. This variation in gene activity from cell to cell is achieved by mechanisms and processes that are collectively termed epigenetics. The main epigenetic mechanisms include DNA methylation, histone modification, RNA interference, and the positioning of nucleosomes. These epigenetic mechanisms, in particular DNA, allow the onset and reactivation of disease that's triggered by environmental factors that rapidly break the mucosal barrier, thus stimulating an immune response or altering the balance between beneficial and pathogenic enteric bacteria. Different genetic abnormalities can lead to similar disease phenotypes; these genetic changes can be broadly characterized as causing defects in mucosal barrier function, immunoregulation or bacterial clearance. These new insights will help develop better diagnostic approaches that identify clinically important subsets of patients for whom the natural history of disease and response to treatment are predictable. Methylation appears to be of great importance in the interaction between genome and the environment. Variation in DNA methylation is a well-recognized cause of human disease and is likely to play a pivotal role in the cause of complex disorders such as CD.

Discussion and Conclusion

Inflammatory bowel disease is sectioned into two divisions, CD and UC. Both are complex multifactorial disorders characterized by chronic relapsing intestinal inflammation. However, the etiology of both diseases remains largely unknown as it is characterized as an idiopathic disease (Sartor, 2006). Understanding the causes and molecular mechanisms of CD and UC is a leading challenge in gastroenterology research, due in part to the relatively low frequency of these disorders. Although significant effort has been made to help identify genetic and environmental factors that may increase the risk of IBD, little is known about IBD-specific factors. Recent research has suggested that IBD is caused by a complex interplay between genetic predispositions of various genes, combined with an abnormal interaction with environmental factors as a result of a perceived threat. Epidemiological evidence for a genetic contribution is made apparent by twin and family studies of CD patients. Genetic variants that stimulate IBD have a substantial effect on gene function. These variants are so rare in allele frequency, that the genetic signals aren't detected in genome-wide association studies of patients with IBD. With recent advances in sequencing techniques, roughly fifty genetic disorders have been identified and associated with IBD such as osteoporosis as well as thyroid disease. Monogenic defects have been found to alter intestinal immune homeostasis through many mechanisms. Candidate gene resequencing should be carried out in early-onset patients in clinical practice.

The evidence that genetic factors are the prime contributors to disease pathogenesis confirms the insignificant role of microbial and environmental factors. Epigenetic factors can mediate interactions between environment and genome. Epigenetic mechanisms could affect development and progression of IBD. Epigenomics is an emerging field, and future studies could provide further insight into the pathogenesis of IBD.

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Is Hyperbaric Oxygen Therapy Effective For Treating Autism?

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Abstract

Autism spectrum disorder (ASD) is characterized as a developmental disability caused by abnormalities in brain function. Studies link ASD with various physiological abnormalities, such as cerebral hypoperfusion, oxidative stress, inflammation, and mitochondrial dysfunction. Studies show that diminished blood flow to the brain demonstrates a connection between several core autistic behaviors, and the hypoperfusion generally worsen with age, growing more prevalent in older children. Although hyperbaric oxygen therapy ("HBOT") is not yet approved by the United States Food and Drug Administration) ("FDA"), several studies performed internationally have proven its efficacy in treating people with autism. Even so, the FDA does approve of its use for other medical conditions such as decompression sickness (the bends) experienced by divers, treatment of air or gas embolism, carbon monoxide poisoning , and thermal burns. Hyperbaric oxygen therapy chambers expose the patient to 100% oxygen (unlike the 21% oxygen found in the air we breathe) by increasing the air pressure in the chamber up to three times higher than normal air pressure of 1 atm. This allows the lungs to take in more than the expected amount of oxygen and thus be distributed throughout the body to the cells that are deprived of oxygen. This paper examines the effectiveness of HBOT in children with ASD, by reviewing the available research.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by "persistent deficits in social communication and social interaction across multiple contexts and restricted repetitive patterns of behavior, interests, or activities," (APA, 2013). ASD includes autistic disorder, pervasive developmental disorder, and Asperger syndrome. Approximately 1 out of 110 people in the United States are currently affected by ASD (Rice, 2006). The cause of ASD is unclear. Although several genetic syndromes, such as Fragile X and Rett syndromes, have been linked with ASD, the majority of ASD cases are not due to a simple single gene or chromosomal disorder; studies have estimated that genetic syndromes only account for 6-15% of ASD cases (Schaefer & Mendelsohn, 2008). Recently, research and clinical studies in ASD have linked physiological abnormalities, such as cerebral hypoperfusion, inflammation, oxidative stress, mitochondrial dysfunction and immune dysregulation (Rossignol & Frye, 2012). With this perspective, ASD may consist of physiological irregularities rather than merely a CNS disorder (Herbert, 2005). Treating ASD is not a simple matter; there are a limited number of treatments. The behavioral aspects of ASD are manageable with the help of behavioral therapies. Applied behavioral analysis (ABA), specifically, has been proven to lead to advances in certain children with ASD. In two years, ABA brought about remarkable improvements in the IQ and behavioral issues of several children with ASD (Lovaas, 1987). After four years, ABA researchers at the Wisconsin Early Autism project found analogous results of improved IQ and behavior (Sallows & Graupner, 2005). Conversely, researchers in Norway found that eclectic therapy (a form of psychotherapy) was successful in the treatment of children with ASD who were observed for only one year. The results proved to supersede the effective outcomes of ABA (Eikeseth, et al., 2002). When it comes to cognitive and behavioral transformations in children with ASD, behavioral therapies are not an ideal approach, as they are quite time demanding. Additional treatments for ASD that aim to treat physiological irregularities have been conveyed, yet, a majority of these forms of treatments have not been critically assessed. However, many studies were performed to test the effects of HBOT to treat the physiological abnormalities possessed by people with ASD, many of which have shown promising results.

Methods

The research incorporated in this paper was retrieved from original research papers and scholarly articles obtained through Touro College's library of databases, such as ProQuest and EBSCO. Google scholar, and PubMed were also used to acquire data. The articles chosen were critically assessed, compared, and analyzed to evaluate if hyperbaric oxygen therapy is effective for treating autism

History of Hyperbaric Chambers

Hyperbaric air or "air under pressure" is a medical treatment that dates back over 150 years. In 1662, a British physician created a sealed chamber in which the air was compressed and decompressed using valves and oxygen. This was extraordinary;it preceded the discovery of oxygen and was utilized as a treatment for respiratory diseases (Clarke, 2008). The idea of employing pressurized environments developed further in 1879, when the French surgeon, Fontaine, built a pressurized mobile operating room. He observed that inhaled nitrous oxide had greater efficacy when used under pressure. Patients treated in an increased pressure environment were not as cyanotic as those treated in standard conditions. Additionally, his patients experienced improved oxygenation (Fontaine, 1879). In the early 1900's, Dr. Orville Cunningham, a professor of anesthesia, found a greater improvement in heart disease victims when they resided

closer to sea level than when they were living at higher altitudes. Later, hyperbaric chambers were utilized by the military, in the 1940's, to treat deep-sea divers who experienced decompression sickness. In the 1950's, physicians first used HBOT during lung and heart surgery, which steered to its use for carbon monoxide poisoning in the 1960's. Since then, over 10,000 clinical trials and case studies have been achieved for several other health-related purposes, with the great majority reporting successful outcomes (Ohnishi, et al. 2000).

Hyperbaric oxygen therapy (HBOT)

Hyperbaric oxygen therapy (HBOT) is the medical use of oxygen in a pressurized environment, and involves breathing 100% oxygen while inside a hyperbaric chamber that exhibits pressures greater than sea level (1 atmosphere). The increased pressure permits oxygen to dissolve and saturate the blood plasma (autonomous of hemoglobin), which yields an extensive array of valuable physiological and cellular outcomes. This noninvasive therapy is the most reliable way to provide ample oxygen to all organs of the body. The standard treatment lasts for 60-90 minutes, during which the patient lies down and breathes regularly (Feldmeier, 2003). The use of hyperbaric oxygen treatment (HBOT) was implemented as a treatment method for numerous clinical disorders. It has been proven in several clinical studies to enhance the body's inherent ability to regenerate and repair. It is used as a therapy to facilitate the healing process in both chronic and acute disorders. Decompression sickness, stroke victims, arterial gas embolism, healing of wounds, severe carbon monoxide poisoning and smoke inhalation are only some of the common uses of HBOT. However, in 2005, researchers began to consider using HBOT to improve behavioral and physiological abnormalities found in individuals with ASD (Leach, et al. 1998). This paper will evaluate the current data on treating ASD with HBOT. First, the effects of HBOT on physiological irregularities in children with ASD will be evaluated. Then, the effects on autistic behaviors will be examined. Finally, the apparent contrary effects of HBOT in ASD and restrictions of studies will be assessed.

Cerebral Hypoperfusion in ASD and the Effects of HBOT

Cerebral hypoperfusion is characterized as insufficient blood flow in the brain and is one of the common physiological abnormalities possessed by individuals with ASD. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) were used in studies to discern hypoperfusion exhibited in the brains of people with ASD versus normal healthy brains (Bjørklund, et al. 2018). Both PET and SPECT scans are types of nuclear imaging tests that utilize radioactive tracers to create three-dimensional images. However, they differ in the radiotracers used. Studies have proven these scans to be a reliable measure of cerebral blood flow in observing what areas of the brain are receiving more blood flow. (Amen, et al. 2011; NIH CC, 2002). Common autistic behaviors such as need for consistency (Ohnishi, et al. 2000), habitual behaviors (Starkstein, et al. 2000), disability in processing emotions and facial expressions (Critchley, et al. 2000), and delayed language development (Wilcox, et al. 2002), are linked to hypoperfusion. Moreover, hypoperfusion is related with increasing age in children with ASD (Wilcox, et al. 2002). It is possible that HBOT could improve cerebral hypoperfusion in ASD. Numerous studies were completed that evaluated the effects of HBOT to treat various neurological disorders, such as chronic brain injury and traumatic brain injury. The results of the SPECT scans were evaluated before and after the delivery of HBOT at low pressures (1.3 to 1.5 atm). These studies showed significant results (Harch, et al. 2012), (Golden, et al. 2002). Histograms correlated to the pre and post SPECT scans were analyzed with a widely used medical images software called Osirix DICOM. Results from both scans showed significant pattern differences beneath the cerebral cortex in the central area of white matter, known as the centrum ovale. The pre-HBOT images displayed a larger array of counts in the histograms than in the post-HBOT. The narrower range of counts in the post-HBOT corresponded to the increase of perfusion to the lacking areas. The effectiveness was studied through many areas in addition to SPECT: physical exam, symptoms, and psychological measurement (Harch, et al. 2012). Moreover, numerous case studies have reported enhancements in cerebral perfusion after HBOT treatment. One study involved a child with ASD who received one hour of HBOT at 1.3 atm and 24% oxygen per day for ten days (Heuser, et al. 2002). The SPECT scans revealed increased perfusion. However, the effects of ten treatments only lasts several months. For longer lasting effects (6-18 months) more treatments are necessary (20-60 treatments). Behavioral improvements were detected in this child including cognitive and memory functions. The child became sympathetic, started to point and articulate, and interact with others. Another child with ASD, who received 40 treatments of HBOT at 1.3 atm and 24% oxygen, also presented behavioral improvements, such as, improvements in speech and communication and fine motor skills (Rossignol, 2008).

Another study involved 108 children with ASD who

experienced decreased perfusion in their temporal lobes as measured by SPECT scans (Kinaci, et al. 1970). They received HBOT at 1.5 atm with 100% oxygen for an hour a day for 50 days. After their treatments, SPECT scans revealed that 82.40% showed improvements in their temporal lobes, 85.26% in their frontal lobes, and 75.75% in other areas. Furthermore, clinicians did an Autism Treatment Evaluation Checklist (ATEC) on 54 of the individuals and significant improvements were noted: 85.5% in Sociability, 79% in Speech/Language/Communication, 75.2% in Health/Physical/Behavior, and 87% in Sensory/Cognitive Awareness. This study revealed major improvements in the physical, behavioral and cerebral perfusion of children with ASD after HBOT (Kinaci, et al. 1970). This study is reliable due to the large sample size, unbiased SPECT imaging, and therapist assessments.

Oxidative Stress in ASD and the Effects of HBOT

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Some children with ASD experience oxidative stress. Some researchers believe that HBOT indirectly causes an increase in oxidative stress due to the amplified production of reactive oxygen species ("ROS") from the elevated oxygen levels (Alleva, et al. 2005). Therefore, they are reluctant to use HBOT on children with ASD, since some of them already have elevated oxidative stress levels (Rossignol, et al. 2007). A contributing factor to their increased oxidative stress levels might be due to the excessive amounts of oxygen supply to their cell's mitochondria, which can escalate the production of ROS. In contrast, HBOT has proven to upregulate antioxidant enzyme production that can aid with detoxification issues found in children with ASD such as glutathione peroxidase, superoxide dismutase (Gulec, et al. 2004), and catalase (Nie, et al. 2006). The antioxidant enzyme level increase can protect against harm initiated by ROS (Rossignol, 2007). Additionally, minor elevations in ROS created by HBOT can be advantageous, as they increase mitochondrial biogenesis (Gutsaeva, et al. 2006).

Two studies have observed the influences of HBOT on oxidative stress in children with autism (Audhya, 2007; Rossignol, et al. 2007). The first study included administering HBOT at 1.3 atm daily to 48 children with autism. Catalase, glutathione peroxidase and superoxide dismutase (SOD) levels were screened before beginning HBOT and again after day 1 and day 32 of HBOT (Audhya, 2007). Results demonstrated a 1.9-fold increase of the original catalase levels after the first day and a 90% improvement after 32 days. Glutathione peroxidase showed a 1.4-fold increase after 1 day and a 1.2-fold increase after 32 days from the initial HBOT. Finally, a 4.5-fold increase of SOD was observed after 1 day and a 4.7-fold increase after 32 days of starting HBOT. The results of HBOT on antioxidant enzymes can be considered conditioning.

The second study involved 18 autistic children from ages 3-16. This open label, prospective study included administering 40 treatments of HBOT, with 24% oxygen, at 1.3 atm, to 12 children, and 100% oxygen at 1.5 atm to 6 children. Blood markers were taken before and after 40 treatments (Rossignol, et al. 2007). Researchers noticed dramatic improvements in speech, socialization, GI function, and strength. The effects persisted months after the study was completed. Furthermore, insignificant changes were seen in plasma-oxidized glutathione levels. This proves that oxidative stress markers did not worsen because oxidized glutathione is transported from within the cells when intracellular levels surpass the redox capacity (Dickinson and Forman, 2002). However, the intracellular oxidative stress levels in the children with ASD did not diminish with the lower HBOT pressures (Rossignol, et al. 2007). The open-label nature of this study and the two diverse treatment groups (one group who received 24% oxygen at 1.3 atm versus the other group who received 100% oxygen at 1.5 arm), as well as the absence of a control group, are some of the limitations of this study. Nonetheless, the prospective nature of this study strengthens it.

Inflammation in ASD and the Effects of HBOT

Some individuals with ASD experience inflammation, such as neuroinflammation, gastrointestinal inflammation and immune dysregulation as supported by recent studies. Numerous studies have conveyed atypical inflammatory indicators in children with ASD. For instance, increases in Tumor Necrosis Factor-alpha (TNF-alpha) (Chez, et al. 2007), and neopterin (cellular immune system activation marker) (Messahel, et al. 1998), were related in studies of children with ASD. Additionally, studies showed that children with ASD, who reported having gastrointestinal inflammation, have analogous symptoms to one suffering from inflammatory bowel disease (IBD) (Ashwood, et al. 2003). Anti-inflammatory results were reported after treatment with HBOT. A decrease in pro-inflammatory cytokines (including TNF-alpha, IL-1 and IL-6) (Weisz, et al. 1997), and an increase in counter-inflammatory IL-10 levels were reported after HBOT (Buras, et al. 2006). Moreover, improvements in IBD were reported in a systematic review that used HBOT (Rossignol 2012). A study reported a decrease in production of interferon-gamma by lymphocytes with HBOT at 2.0 atm/10.5% oxygen, but an increase in interferon-gamma with 100%

oxygen administered at 1.0 atm (Granowitz, et al. 2002). Therefore, it is concluded that the result of HBOT on reducing inflammation is facilitated through the pressure-related component and not particularly on the delivery of oxygen.

Unfortunately, a series of recurring events can cause amplified inflammation which can lead to escalated cerebral hypoperfusion. This may cause hypoxia, which leads to production of HIF-1a (hypoxia-inducible factor-1alpha) which induces inflammation (Nathan, 2003). HIF-1a is fundamental in the innate immune system, as it triggers inflammation by myeloid cells (Cramer, et al. 2003).

The effects of HBOT on biomarkers of inflammation in children with ASD were inspected in two prospective studies. The first study included 12 children who were given 40 treatments of HBOT at 1.3 atm/24% oxygen and 6 children who were given HBOT at 1.5 atm/100% oxygen. Biomarkers were measured before and after the treatments by testing the participants' blood specimens taken before and after the treatments (Rossignol, et al. 2007). Improvements were seen in C-reactive proteins (non-specific inflammation markers), as their levels decreased in the total study population ($p=0.021$). The most significant decrease was seen in children with the highest levels of C-reactive proteins prior to the study. These children also showed behavioral improvements. SRS (Social Responsiveness Scale), ABC-C (Aberrant Behavior Checklist – Community), and ATEC (Autism Treatment Evaluation Checklist) were filled out by the parents and were used to calculate the scores for each child before and after their treatments.

The second study measured plasma cytokine levels, including some related to inflammation prior to and after HBOT. Ten children with ASD participated in this 20 week study with 80 sessions of HBOT given to them at 1.5 atm/100% oxygen (Bent, et al. 2012). Although the study reported behavioral improvements, negligible modifications were observed in cytokines. Yet, the authors mentioned that none of the children actually had irregular cytokine levels prior to the study, so notable variations could not be seen.

In another study, a child with ASD who suffered from eczema and bowel inflammation, with a distended abdomen, displayed significant improvements after 40 treatments of HBOT at 1.5 atm and 100% oxygen over the course of a month (Rossignol 2008).

Mitochondrial Dysfunction in ASD and the Effects of HBOT

Mitochondrial dysfunction is observed in some people with ASD (Rossignol & Frye, 2011; Frye & Rossignol, 2011). HBOT is considered a possible treatment for mitochondrial dysfunction, although treatments for this condition are minimal (Rossignol & Frye, 2011). The outcomes of HBOT on mitochondrial dysfunction were measured in various studies.

In a controlled study HBOT at 1.5 atm and 100% oxygen was administered to 69 patients who obtained severe traumatic brain injury (TBI), within 24 hours of injury. Significant increases in brain oxygen concentrations were seen, as well as increases in cerebral blood flow and reduced CSF lactate levels (elevated CSF lactate is an indicator of mitochondrial dysfunction). Results also showed improvements in mitochondrial function and brain metabolism after contrasting both room air treatment of 21% oxygen and 100% oxygen given at normal pressure (1 atm) (Rockswold, et al. 2010).

HBOT can make more electron transport chains in mitochondria. In 2008, a study was conducted on healthy rat tissue (Kurt, et al. 2008). HBOT was done on the rat and the tissues were looked at before and after the treatment. Results showed that the mitochondria were working more efficiently. Mitochondria work more efficiently through physical exercise. During exercise, the cells are stressed and mitochondria are making more electron transport chains. Similarly, with HBOT, the cells are slightly stressed and they make more electron transport chains thereby working more efficiently and experiencing less oxidative stress.

Furthermore, a study was conducted in 2006, which showed it is possible to increase the number of mitochondria in the brain cells of rats. This phenomenon is called mitochondrial biogenesis (Gutsaeva et al. 2006). This was not demonstrated in humans, because it would require risky brain biopsies to be performed on patients. This can be one possible mechanism as to why HBOT is working. Although these results seem promising, no clinical studies were performed to test the effects of HBOT on mitochondrial dysfunction in children with ASD.

Behavioral Measurements in ASD and the Effects of HBOT

Most of the studies performed using HBOT for children with ASD have inquired behavioral rather than physiological factors. In a current systematic review, two double-blind, arbitrary, controlled trials were reviewed regarding the use of HBOT in children with ASD (Ghanizadeh, 2012). The first study examined the outcome of giving 33 children with ASD, 40 treatments of HBOT with 24% oxygen at 1.3 atm, which included 2 treatments each day for 5 days a week, over the course of 4 weeks. This study was performed in comparison to 29 control

children with ASD who were given room air that was slightly pressurized (1.03 atm/21% oxygen) (Rossignol, et al. 2009). Noteworthy improvements were detected in the treated children on the ATEC scales (questionnaire that measures changes in severity of ASD in response to treatment) by the caregiver, as well as on the CGI scales (Clinical Global Impression rating scales: measures the severity of symptoms, treatment response and the effectiveness of treatments) by the clinicians and parents. The results showed improvements in language, eye contact, cognitive attentiveness and overall functioning. Strengths of this study comprise of the involvement of 6 medical centers (which limited possible biases correlated to a specific site study), assessments by blinded parents and clinicians and the use of a control group.

The second study examined the outcome of giving 18 children with ASD, 80 treatments of HBOT with 24% oxygen at 1.3 atm, within a 15 week duration. These results were compared to 16 children who were treated with a placebo, involving air flowing through a chamber at normal pressure. Subsequently, ABA therapy was given to both groups, yet insignificant changes were observed using various behavioral scales (Granpeesheh, et al. 2010). These undesirable results can be attributed to the fact that rigorous ABA therapy was given during the administration of HBOT which did not seem successful (Ghanizadeh, 2012). On the other hand, this study is reliable because of the blinded evaluators as well as the control group. The contrasting results can be ascribed to the discrepancies among the two controlled trials (Ghanizadeh, 2012). For example, the age and quantity differences of the participants, as well as the magnitude of treatments. One study delivered 10 hours of HBOT per week (Rossignol, et al. 2009). While the other study provided 5 hours per week (Granpeesheh, et al. 2010). There were also probable distinctions in ASD severity.

Antagonistic Properties of HBOT in ASD

Among the many studies performed, minimal unfavorable outcomes were reported of HBOT usage for people with autism. One study stated that "HBOT was safely administered to autistic children and all participants were able to finish 40 HBOT sessions without any major adverse events" (Rossignol, et al. 2007). A different study informed minor antagonistic effects, including 4 kids with earaches, 2 kids with otitis media and other individuals who were autonomously affected with insomnia, seizures, lethargy, amplified vocal sensitivity and hyperactivity (Bent, et al. 2012). In a controlled study, one of the participants experienced frequent urination and a skin rash which the doctor attributed to yeast as the causing factor. Other participants withdrew from the study, specifically one child who experienced regressing symptoms of asthma after several HBOT treatments. The physicians did not think the two were related, but removed the child as a safeguard. Another child withdrew from the study before completing it due to anxiety. One participant in the control group experienced diarrhea and abdominal swelling, yet still completed the experiment. In addition, another control group participant experienced worsening of pre-existing eczema. Seizures or barotrauma, however, were not detected in neither the treatment nor control groups (Rossignol, et al. 2007). In a study performed on the effects of HBOT in Thai autistic children, it was reported in the data analysis that "there was no serious adverse effect in any case and tinnitus was a mild side effect in one case and it went away in one week" (Chungpaibulpatana, et al. 2008).

According to the FDA, "patients receiving HBOT are at risk of suffering an injury that can be mild (such as sinus pain, ear pressure, painful joints) or serious (such as paralysis, or air embolism). Since hyperbaric chambers are oxygen rich environments, there is also a risk of fire" (FDA commissioner, 2013) .

Restrictions of the Studies Performed

Most of the studies performed contain restraints that contribute to the erratic results among them, including the retrospective approaches, the absence of control participants and the minimal amount of participants. Moreover, the open trials increase bias, as both the participants and the researchers are aware which treatment is being given. However, two of the controlled studies did not contain these restraints. Furthermore, other studies utilized observational methods which are not efficient when analyzing modifications in memory and attentiveness (Jepson, et al. 2011). Unfortunately, the long-term effects of HBOT are unknown because once the study was complete researchers did not follow up on the long-lasting effects. Additionally, a majority of the studies focused on behavioral modifications rather than physiological modifications. Earlier studies proved that ABA therapy in individuals with autism is only effective when done over a substantial period of time. For example, ABA therapy studies displayed significant behavioral changes over a one to four year duration (Lovaas, 1987; Sallows & Graupner, 2005).

It is common for the physiological modifications seen in autistic children treated with HBOT to be observed before intellectual and developmental enhancements due to the intricacies of brain maturation. Studies have found significant physiological improvements after a short time

in participants who were treated with HBOT, specifically modifications with cerebral hypoperfusion. While most of the studies noted behavioral enhancements in several children with autism, the studies only persisted for a few months. This short duration of time is not enough to determine the influence HBOT has on development. Further studies are necessary to investigate the long lasting results of HBOT in people with autism.

Many studies noted behavioral and physiological advances when treating autistic children using HBOT. Yet, noteworthy developments were not found when researchers from a common group performed two studies (Granpeesheh, et al. 2010; Jepson, et al. 2011), and insignificant results were noticed in a different minor study (Lerman, et al. 2009). The inconsistent outcomes among the studies may be attributed to the autistic children's opposing responses to HBOT (Jepson, et al. 2011). To illustrate, children who possess certain physiological abnormalities such as mitochondrial dysfunction, cerebral hypoperfusion and inflammation might be more prone to display improvements. Nevertheless, most of the studies performed to test behavioral aspects did not analyze the chemical aspects such as levels of oxidative stress or inflammatory markers. However, one behavioral study did analyze alterations in the levels of cytokine, but insignificant modifications in cytokines were seen because all the participants already had regular cytokine levels prior to their HBOT treatment (Bent, et al. 2011).

Further investigation is required including participants with ASD who possess specific physiological abnormalities and which analyze variations in these physiological issues such as cerebral hypoperfusion and inflammation. Noteworthy improvements were related in studies which administered HBOT sessions more frequently. This also seemed true in studies that used HBOT to treat patients with traumatic brain injuries, (Harch, et al. 2012). More studies, however, are needed to analyze several HBOT factors, such as the ideal oxygen and pressure limits required to treat patients with ASD.

HBOT in Thailand

In 2008, the first study in Thailand was performed to determine the effects of HBOT to treat autistic children and whether it is safe or not (Chungpaibulpatana, et al. 2008). The study included 7 Thai autistic children who received 10 sessions of HBOT once a week at 1.3 atm. Before and after the treatments, five areas were evaluated: "1. Social development 2. Fine motor and Eye-hand coordination 3. Language development 4. Gross motor development 5. Self - help skills" (Chungpaibulpatana, et al. 2008). Results showed advances within these five domains with 75% of children who displayed progress while 25% showed no reaction to the treatment.

FDA Approval

Although HBOT is currently being used as a treatment method to treat various disorders in Thailand such as autism, it has not been clinically verified or cleared by the FDA to cure or be successful in the treatment of autism, cancer, or diabetes (FDA, 2013). However, the FDA did approve the use of HBOT for several other conditions. These include "treatment of air or gas embolism (dangerous 'bubbles' in the bloodstream that obstruct circulation), carbon monoxide poisoning, decompression sickness ('the bends'), and thermal burns (caused by heat or fire)," (FDA, 2013).

Disadvantages

Dr. Paul Claus, medical director of Mayo Clinic's Hyperbaric and Altitude Medicine Program, relates risk factors of HBOT. The increased oxygen of the chamber supports combustion which poses a risk for fires in the facility. Therefore, necessary precautions are taken. The patients are required to remove their street clothes since polyester is fuel for fire. The patients are provided with cotton blend clothing instead. Additionally, the treatments are quite expensive; they are approximately $1,500 an hour in most hospital settings (Claus, 2017). However, if more studies are done documenting improvements of HBOT, then hopefully the cost will be covered by the healthcare system or insurance companies to further benefit those with autism. .

Conclusion

"There is no one-answer for many questions but there are many answers for one question. Multiple factors are the possible causes of autism. The curative factors may be from multidisciplinary approaches," (Chungpaibulpatana, et al. 2008). Numerous studies have demonstrated that HBOT is an effective treatment for children with autism. The pressures utilized during treatment with HBOT (1.5 atm/100% oxygen maximum) are proven to improve the common physiological abnormalities in individuals with ASD; such as, cerebral hypoperfusion, oxidative stress, inflammation and mitochondrial dysfunction. Furthermore, studies that targeted the behavioral measurements in ASD also showed positive results even though most of those studies did not use control groups. The two studies that did utilize control groups however related opposing outcomes. However, multiple crucial variations between the trials were noted. Taken together, the studies imply that the use of HBOT in children with autism correlates

with marginal antagonistic effects and is handled well. In conclusion, HBOT is a harmless and possibly beneficial treatment for kids with autism. However, additional studies are necessary before it can become an internationally accepted treatment method. Future studies should be more specific and directed towards the specific physiological abnormalities within the various ASD subdivisions and should use control groups as well to promote efficiency and determine which people can gain from HBOT treatment. Furthermore, why don't the hospitals in Thailand publish anything regarding their routine HBOT usage? They value intellectual property not academic publications. If it were not more clinically effective than the use of HBOT would not be used regularly and its ineffectiveness would spread like wildfire. Based on the studies performed, overall, the use of hyperbaric oxygen therapy appears to be a promising treatment for children with ASD.

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Effects of High Fat Diets

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Abstract

With obesity on the rise, and subsequently numerous diseases, many people are looking for newer ways to counter the effects of the influx of fatty, high sugar and unhealthy foods they are consuming. The most common and widely used method is a diet plan. The key to both a healthy and effective diet is knowing what the diet does to the body. There are short term diets which have adverse effects if kept for too long and long-term diets which are aimed for a healthy lifestyle. Every diet is based on the increased and decreased consumption of different macronutrients and therefore, each affects the body differently. The Ketogenic Diet is a high fat consumption diet that is gaining credence from its success rate. However, due to it being a relatively new diet fad, there are not too many who understand the long-term adverse effects of a high fat diet.

Introduction

We live in a world of instant gratification. Smart phones, smart cars and smart homes are the new social norm. Another idea of the now world we live in is takeout food. Instead of preparing meals, one can be picked up or even delivered to your home at minimal charge. This would seem to be useful for person who is too busy to cook but is leading to a major problem in western culture – obesity. With obesity on the rise, many people are looking for newer ways to counter the effects of the influx of fatty, high sugar and unhealthy foods they are consuming. The most common and widely used method is a diet plan.

The word diet is simply the amount of food or drink a person consumes. When people speak about a diet though, they are referring to a special course of controlled or restricted intake of food and or drink for a particular purpose. Magazines, websites, and health moguls are promoting new diets and fads almost daily. It has reached a point where weight loss has become a societal pressure leading people to come up with numerous 'quick fix' diets to lose the 'excess' weight so as not to deviate from society.

Aside from body image concerns, there are many chronic illnesses that are related to obesity. To name a few, hypertension, diabetes, joint pain, and cardiovascular diseases, such as an acute myocardial infarction, are all largely products of unhealthy chosen lifestyles and poor dietary habits. While there are certain diets that are great in preventing, or at least not promoting, various diseases they are mistakenly used by healthy individuals who are possibly harming themselves as they are unaware of the side effects.

The aesthetics of being leaner and thinner have become the idol of, and completely overtaken, the views of almost every individual today. It has become such an obsession that new diet plans are arising daily. Some plans are more beneficial than others and some are just fads, but they are all developed on the same basis of energy expenditure.

To start with, the two biggest fat loss myths must be disproven. First, calorie counting does not work. For those who want nothing more than to hear that they can get lean and fit by only paying attention to what they eat and not how much, this idea is what they thrive on. This is total fools play since how much you eat is significantly more important than what you eat. For example, John Cisna, a science teacher, lost 60 pounds in six months eating food bought solely from McDonalds every day. He wanted to burst the lie that people believe about food intake. While his diet plan may not have been too nutritious, it proved that you can lose weight by eating whatever junk food you would like as long as you are minimizing your calorie intake (Peterson, 2015). This kind of plan will give the wanted weight loss; however, such an eating regimen will slowly affect his visceral health if it is kept up. These types of fast foods are high in fat without many vitamins which can cause, amongst many other issues, high cholesterol levels and high blood pressure due to the buildup of plaque and therefore narrowing of the arteries.

The proper way to diet is to have a plan that is both balanced as well as nutritious. A balanced plan is consuming the proper percentage of both macro and micronutrients. Nutritious foods are ones that are rich in those nutrients that are needed daily for an organism to uphold its proper functions. Food is the material we take into our bodies to nourish it with the (hopefully) proper nutrients and other substances it needs for energy, health, and growth. Macronutrients are the substances our bodies require in large amounts. Namely protein, carbohydrates, and fats. There are also some minerals needed in relatively large quantities, but this paper will focus on fats, carbohydrates and protein as most diets pertain to altering the consumption of one of such food groups.

Secondly, it is important to understand the one most important thing about weight loss. It comes down to energy balance which is the correlation between energy intake and energy expenditure (Lean, Astrup, & Roberts, 2018). Put in more than you take out and you will have an excess amount of energy meaning you will gain weight. Eat about as much as you burn daily, and your weight will stay the same. Take in less energy than you use and there will be a deficit and you will lose weight. This is not some wild un-based theory either. According to the first law of thermodynamics, energy cannot be destroyed nor created, but can only change its form within a system. Our bodies absorb the food we eat and transform the stored energy into energy we can use. Muscles convert

the stored energy for mechanical energy, digestive system for body fat (chemical energy) and our organs convert energy into thermal energy. This is evidence how energy is never lost, but rather converted to the form each part of our body needs based on demand.

The process of our body transforming the food we intake to usable energy is one of the purposes of energy expenditure. Energy expenditure (EE) is the amount of energy that a body needs to carry out its physical and physiological functions and is measured in calories. It is the combination of a basal metabolic rate (BMR) or standard metabolic rate (SMR) and their activity level. Basal metabolic rate represents the minimum energy expenditure for a resting and fasting animal at thermoneutrality (Nilsson & Nilsson, 2016). This means BMR is the lowest sustainable energetic (caloric) cost required to keep one's body functioning at rest. Activity level is movement that requires additional energy aside from one's BMR. Activity level is not only pumping iron five days a week at the gym, it is also taking the stairs rather than the elevator during the day, mowing the lawn, changing a tire, or pushing a shopping cart. The more physical activity a person is involved in, also adds to a greater amount of energy a person expends and therefore the more s/he needs to consume.

Aside from physical activity there are other factors that play a role in determining a person's BMR - gender, body mass index, age and yes, even genetics. Due to these various factors, nutritional requirements differ from person to person and are therefore evaluated based on kilogram of body weight and every person will alter the amount based on their energy exertion during both health and disease.

It should come as no surprise then that consuming less energy than your body requires for a couple weeks or months would account for less energy in the body and therefore a leaner and leaner body type. Since energy balance has been proven to be the basic regulation for weight loss and weight gain it is found at the baseline of every proper diet plan.

In addition to dieting, it is both scientifically proven as well as seen in people's success that exercise in synchrony with a healthy diet plan is the most advantageous. Individuals who exercise along with being careful with what they eat will lose weight. They expend more calories than they take in daily and the deficit results in a loss of body weight. The Cleveland Clinic agrees and explains why exercising without a diet plan is not too effective for weight loss (Team, W., 2018). They state that in order to lose weight one of two options must be done; either one must burn more calories by working out than s/he consumes in one day or take in fewer calories alone than one's body uses daily. To summarize, putting one's body into an energy deficit will cause weight loss and this is the basis for the majority of diet plans.

The two main types of diets are long-term plans and short-term plans. Long term diets aim to benefit a person's healthy well-being for the long haul. The results may not be instantaneous, yet the programs are easier to follow. These diets do not restrict a certain food group completely which prevents the process from becoming too arduous for someone who thinks that diets are restraining. The overall objective is a healthy lifestyle.

Alternatively, short term diets are the quick fixes. These diet plans usually see results within a week of following them. Diets that fall under this category are ones where quick weight loss is desired for aesthetic looks or necessary in preventing larger health issues, like cardiovascular disease or diabetes. These usually entail many restrictions which aside from making the dieter unwilling to stick with the plan, it makes them unhappy to comply with the rules.

One severe version of a short-term diet is known as crash dieting. These diets are severely low total calorie plans and are sustainable for a couple weeks at most. They are mostly used by bodybuilders and other professions before a performance where by eating very minimally, they can accentuate the appearance of their muscle hypertrophy. The problem begins the week after the diet is over. Many people find motivation to continue such a restricted diet in the promise of the prize. The logic behind knowing the situation is temporary gives way to individuals being able to have greater self-control which is a staple in crash diets. Additionally, in such a case not having an end goal also makes such diets extremely difficult and not very long lasting in general.

While these diets may help people lose much more weight than they would on a gradual diet plan, they are worse for the person in the long run. As an individual loses weight rapidly, their metabolism slows down. The metabolism lowers because the body tries to preserve its energy stores. This helps the short-term diet process by decreasing the 'hunger' a person would feel due to the body's adaptation to the caloric decrease. However, after such a severe low-calorie diet is over and an individual loses thirty or more pounds, their metabolism does not rise back up right away. At this point, the body requires fewer calories due to the decrease of energy metabolized. Adding much more calories to a metabolism that is designed to burn fewer amounts per day will result in increased weight and will likely leave the person worse off than where s/he started.

A large example is shown in the weight loss program

The Biggest Loser, which is a 30-week weight loss television program for bariatric adults. In 2016 a study was done on six men and eight women who participated in the show to see if the weight they lost while on the show stayed. Part of the 'Biggest Loser Study' found that the majority of the show's contestants regained a significant amount of the weight they had lost within six years after exiting the show. A few even ended up heavier than they had been before they started the competition (Fothergill et al. 2016). It was as if their bodies were intensifying their effort to pull the contestants back to their original weight (Oshin, 2020).

This phenomenon can happen to anyone, not just those on low-calories diets. For every kilogram of weight loss a person experiences, their metabolism slows down around twenty to thirty calories. But while it is burning less, the body also wants to eat more – the appetite of someone losing weight increases, making the person want to consume about one hundred calories more than they were having before. The process, known as adaptive thermogenesis, serves the purpose of regulating an organism's energy balance after changes in diet.

The body has a certain amount of fat – a "set point" – that it is comfortable with from a survival standpoint and wants to maintain. When a person begins vigorous training combined with a restrictive diet, the body responds by thinking it is under threat. The training may increase the body's muscle mass, but when the diet becomes too restrictive – which varies from person to person – leptin, the satiety hormone, begins to drop, somewhat like a starvation alarm. Leptin manifests itself as hunger and you either don't get as full from your meals, or you can't go as long between meals without getting hungry.

Nowadays, there are various lauded fad diets. One of which is called the Ketogenic diet, more commonly known as the keto diet. This diet is a short term diet based on two main goals. The primary goal is to greatly reduce the number of carbohydrates consumed. Without the intake of carbohydrates, the body cannot synthesize glucose, it's main energy source, and therefore, will need to resort to an alternate fuel. Secondly, fat consumption needs to be increased in order to become the new primary source of energy.

To reduce the intake of glucose one must consume fewer carbohydrates. Carbohydrates are the primary source of energy production in the body. When the body is deprived of carbohydrates due to reducing intake (to about less than 50g per day), insulin secretion is significantly reduced and the body enters a catabolic state. Glycogen storage depletes which forces the body to go through certain metabolic changes. Two metabolic processes that come into action when there is low carbohydrate availability in body tissues are gluconeogenesis and ketogenesis.

Gluconeogenesis is when the body produces its own glucose. This is primarily done in the liver using lactic acid, glycerol, and certain amino acids (alanine and glutamine). When glucose availability drops further, gluconeogenesis is not enough to keep up with the needs of the body and ketogenesis begins and provides the body with ketone bodies as an alternate source of energy in the form of ketone bodies. Ketone bodies synthesized in the body can be easily utilized for energy production by heart, muscle tissue, and the kidneys. Even though the brain normally uses a large percent of the carbohydrate intake to sustain its function, it can learn to use ketone bodies as they are able to pass the blood brain barrier as well.

The ketogenic diet consists of seventy percent fat, twenty five percent protein and five percent carbohydrates. The mechanism by which the Ketogenic diet induces weight loss is by minimizing carbohydrate intake, which reduces insulin production, and thereby decreases the amount of glucose stored as fat in cells. Digested fat does not convert to glucose and thus does not activate insulin secretion. The Keto diet emphasizes high fat consumption based on the physiological metabolism of fat, where it is lysed to energy instead of being stored in adipocytes. Fat calories consumed are not stored. For the body to replace the energy it lacks from glucose, due to low carbohydrate intake, the body turns to its current reservoir of fat storage, in addition to the fatty foods, which in turn facilitates weight loss.

The basic ketone bodies that accumulate in the body during ketogenic diet, namely beta-hydroxybutyrate and acetone, are formed from the oxidation of fatty acids into acetoacetate. This metabolic state is referred to as "nutritional ketosis" and as long as the body is deprived of glucose, this ketotic state will remain.

Since ketone bodies are released only in small concentrations and don't alter the blood's pH, nutritional ketosis is quite safe. The issue arises when ketones build up in the body mainly due to diabetes, especially if combined with a disease or severe starvation. Accumulation of ketone bodies dramatically lowers the pH (increase acidity) of the blood which leads to ketoacidosis and if left untreated can eventually cause severe dehydration, coma or death.

Why are ketone bodies used as an efficient energy source in the body? Ketone bodies produce more adenosine triphosphate (ATP) in comparison to glucose, and are therefore used as an efficient energy source to the body. One hundred grams of acetoacetate generates

9400 grams of ATP, and 100 g of beta-hydroxybutyrate yields 10,500 grams of ATP; whereas, 100 grams of glucose produces only 8,700 grams of ATP. This allows the body to maintain efficient fuel production even during a caloric deficit (Masood, et. al, 2020).

An increased fat presence in the gastrointestinal tract increases satiety by stimulating the secretion of orexigenic hormones, an appetite stimulant, like GLP-1 and CCK. These hormones delay gastric emptying and decrease motility which allows food to pass through the system at a slower rate. This causes an individual to feel satiated for a longer period. This may also be the reason why many people on the ketogenic diet lose weight. Individuals on such a diet consume large amounts of fat which keeps them full in between meals. Therefore, it prevents them from snacking throughout the day and eating excessive amounts of unnecessary calories.

Over the past decade, nutrition science has produced robust evidence that high-carbohydrate diets contribute to obesity and chronic disease, and that very low-carbohydrate, ketogenic diets provide a healthy alternative. Since then, another study concluded that low-carbohydrate diets should be avoided just as much as high carb diets because they are both associated with higher overall mortality and shorter average lifespans (Bellemare & Finaret, 2018).

Both high and low percentages of carbohydrate diets were associated with increased mortality, with minimal risk observed at fifty to fifty five percent carbohydrate intake. Low carbohydrate dietary patterns favoring animal-derived protein and fat sources, from sources such as lamb, beef, pork, and chicken, were associated with higher mortality, whereas those that favored plant-derived protein and fat intake, from sources such as vegetables, nuts, peanut butter, and whole-grain breads, were associated with lower mortality, suggesting that the source of food notably modifies the association between carbohydrate intake and mortality.

The ketogenic diet was originally developed to help children with epilepsy. According to Greene,et al.(2003) "…ketones, unlike glucose, may be unable to deliver the immediate and large amount of energy necessary to initiate or sustain seizure activity". In this way, seizures can be decreased as the glucose necessary to sustain such high brain activity is not available. Furthermore, seizures would subside or become less severe in times of fasting. Fasting prevented seizure spikes. Researchers further thought of what would happen if they were to find a diet with similar effects as if one fasted - ketone bodies. Epileptics would be able to control their seizures somewhat possibly and thereby some part of their life for longer periods of time. This led to the high fat, low carb ketogenic diet and it worked because like fasting, ketone bodies were produced and not taking in excess sugar which also peaks the strength of seizures. For certain people with epilepsy, the ketogenic diet should be followed, according to the guidelines of a certified professional, as the benefits may outweigh the disadvantages for individuals. The diet regimen may have a higher mortality rate but for them, they would rather be in better control of their lives.

In addition to preventing seizures, the ketogenic diet is believed to be related to other long-term effects as well. Since it is a relatively new concept, there hasn't been that much long-term time for research but an excess of fat in the body has many effects. Although this is the case the diet seems to be effective. A study on eighty three obese patients, with a body mass index greater than thirty five, who took part in a twenty four week ketogenic diet; reported a significant decrease in the body weight, increase in high density lipoproteins, and decease of low density lipoproteins (Dashti et al.(2004)

People have become very interested in this diet due to its ability to reduce weight. However, people are not aware of the long-term effects this may have on their overall health. An evidence-based case study reported that the ketogenic diet can increase the risk for cardiovascular disease by twenty six percent each year. This statement is backed up by the evidence they found about this diet in particular. The ketogenic diet increases LDL, low density lipoprotein, levels because of the increased intake of saturated fat (Dashti, et al. 2004).

LDL is a lipoprotein mainly made from cholesterol, which transports lipids from the liver to peripheral tissue for it to be stored or utilized as energy. Cells in the body have receptors that bind to LDL and engulf it via endocytosis. The cholesterol esters in the LDL can be stored or hydrolyzed into cholesterol and fatty acids to be used for hormones, bile, or the cell membrane. LDL is known as the unwanted cholesterol in the blood because of its function. It is undesirable to have stored cholesterol (a form of fat) in the organs, tissues, and cells throughout the body.

The more fat you eat - and a ketogenic diet requires a lot of fat - the more low density lipoproteins are produced, but when receiving cells are saturated by cholesterol (lipids) the residual low density lipoproteins remain in the bloodstream. This is dangerous as it can cause atherosclerosis - plaque buildup in the arteries which can lead to various cardiovascular emergencies.

Another negative side effect of high fat diets is Biliary Disease. Biliary disease is a condition that affects the bile duct whose purpose is to pass bile to the small intestine.

For example, Cholecystitis, the production of gallstones in the gallbladder, which can occur from an overconsumption of fat or increased blood cholesterol. Gallstones can block the bile duct thereby prohibiting the passage of bile. This will prevent fat from being broken down, digested, and absorbed. In theory this may sound great because if fat is not absorbed it will pass through our digestive tract without increasing the calorie intake. This is true although it will cause fatty stool and anal leakage.

An additional overwhelming issue that will be caused by Cholelithiasis (gallstones) is pancreatitis (inflammation of the pancreas). The reason why these two issues are connected is because the bile duct from the gallbladder and the pancreatic duct merge before entering the intestine. Bile from the liver and enzymes from the pancreas both pass through this common duct. When gallstones are lodged in this area, the pancreas cannot release enzymes such as proteases, lipases and amylase into the intestine. When this happens, a process known as auto digestion begins to occur where proteases like trypsin and chymotrypsin begin to digest pancreatic tissue instead of being released to break down proteins taken in by the body.

Other negative side effects of the keto diet include edema, swelling catalyzed by fluid buildup, fat necrosis, a lump of dead or damaged tissue and pancreatic hemorrhage, bleeding in the pancreas. Another issue that can arise from the keto diet is fatty liver, an increased build-up of fat in the liver. Non-alcoholic fatty liver disease (NAFLD) is asymptomatic and therefore, an individual will be unaware of this disease until the condition worsens. People on the keto diet consume such large quantities of fat that their livers overload. This is especially dangerous if sustained, because their energy stores stem from fat consumed being lysed to ketones. If the liver minimizes its bile secretion, less fat will be utilized for ketone bodies and thereby, drain the body's energy stores. This leads to fatigue, dizziness, lack of energy and motivation which in turn leads to the people being lax in their health and exercise - countering the reason they started the diet in the first place. Only once a fatty liver further manifests into hepatitis will these symptoms become prominent will the dieter seek medical attention.

A positive side effect of the ketogenic diet also primes us to burn the excess fat that so many of us are carrying around our waists. Our glucose levels return to normal, we become less insulin resistant, we shed fat, and significantly reduce our risk of developing chronic disease. Another significant benefit is the production of ketone bodies, now the subject of intense scientific investigation due to their many health-promoting properties including protection of the brain and nervous system, reduction of systemic inflammation, the root cause of cardiovascular disease, and anti-cancer effects.

Low carbohydrate diets, which restrict carbohydrate in favor of increased protein or fat intake, or both, are a popular weight-loss strategy. However, the long-term effect of carbohydrate restriction on mortality is controversial and could depend on whether dietary carbohydrate is replaced by plant-based or animal-based fat and protein. One widely followed long-term diet that has been proven to be very beneficial and effective is the Whole Food and Plant Based diet more commonly known as the vegan diet.

In another study 15,428 adults aged forty five to sixty four were evaluated periodically through interviews where they filled out semi quantitative food frequency questionnaires. Typical portion sizes were provided as reference so that they could estimate their intake of each food item consumed. The results were adjusted for age, sex, race, total energy consumption, diabetes, cigarette smoking, physical activity, income level, and education.

In conclusion, the low carbohydrate diets that substituted the carbohydrates for animal derived fat and protein sources, such as from lamb, pork, beef, and chicken were accompanied by higher mortality. In comparison, the low carbohydrate diets that replaced carbohydrates with fat and protein from a plant base such as from vegetables, nuts and whole grain were associated with mortality. This finding suggests that the source of protein and fat in one's diet can change mortality showing that these two things are related. The optimal quantity of carbohydrates consumed in a diet should be between fifty to fifty five percent of total intake. For this reason, less than fifty percent or more than fifty percent of carbohydrates consumed seems to be ineffective (Seidelmann et al 2018).

Long-term effects of a low carbohydrate diet with typically low plant and increased animal protein and fat consumption have been hypothesized to stimulate inflammatory pathways, biological ageing, and oxidative stress. On the other end of the spectrum, high carbohydrate diets, which are common in Asian and less economically advantaged nations, tend to be high in refined carbohydrates, such as white rice. These types of diets might reflect poor food quality and confer a chronically high glycemic load that can lead to negative metabolic consequences.

One diet plan based on this philosophy is the vegan diet. A study was aimed clarifying the association between vegetarian, vegan diets, risk factors for chronic diseases, risk of all-cause mortality, incidence, and mortality from cardio-cerebrovascular diseases, total cancer and specific type of cancer (colorectal, breast, prostate and lung), through meta-analysis. This comprehensive meta-analysis reported a significant protective effect of

a vegetarian diet versus the incidence and/or mortality from ischemic heart disease (-25%) and incidence from total cancer (-8%). A vegan diet conferred a significant reduced risk (-15%) of incidence from total cancer (Dinu, et. al., 2017).

In conclusion, finding a diet that suits an individual is not so simple. According to the International Society of Sports Nutrition (ISSN), there are two main positions in regard to diets. The nature of the diet and the way it influences the body composition. Each diet type has its own various sub classifications that fall under the main eating style. Therefore, while diets are a good way to lose weight, there is a point where they can do more harm than good. The keto diet is a great plan to stick with for a short while; however, consuming mostly fat every day, takes a toll on the body. Although the ketogenic diet is popular there are many long term diets that are undoubtedly safer and healthier for the body.

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