The Science Journal

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

Tenth Anniversary Edition

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The cover illustration, created by Professor Antony O'Hara of the Digital Multimedia Design Program, is a composite of all of the previous Science Journal covers.

Letter from Dr. Robert S. Bressler

Dear Readers,

I have always avoided inserting a message from the editors or myself in this journal. However, I just felt that this occasion called for some sort of statement. One of the several tasks suggested to me when I first accepted my position at Touro was to get a Science Journal re-established. It was probably more than coincidence that Rivka Borger and Michelle Gordon (now Grunin) approached me with the same request. In my previous 44 years of teaching I had never been in involved in a similar activity. The three of us plowed ahead with the overriding goal of keeping this a student publication from start to finish. Given the multiple facets of my new job, it was difficult to maintain the priority for this one. I am not sure that starting this journal could have been accomplished without the strong motivation and commitment of Rivka and Michelle, and of course all of the student editors and contributors. We have enjoyed support and expertise from two individuals in particular, Dean Robert Goldschmidt and Mrs. Esther Greenfield. Dean Goldschmidt has championed our efforts and provided the funding. Mrs. Greenfield has always been there to find even the most miniscule errors in my "printer ready" versions. She has unknowingly challenged me to continuously move even closer to perfection. Thank you both!

The Science Journal is available on line at http://las.touro.edu/departments/biology/. At that site, you can open each paper and see the full content. You will also be able to see the mastheads. That will document the student nature of this publication. Submitted articles are sent to the Editorial Board members for peer-review (I review them as well). The reviewer's comments are then forwarded to the authors for their rebuttal or acceptance. After all of that they are formatted, assembled, and sent to the printer. A few years ago, Professor Antony O'Hara of the Digital Multimedia Imaging Department learned about the formatting and layout difficulties I was having. He graciously volunteered to involve his students in the effort and has performed those tasks for us when students were not available. Additional thanks are extended to Professor Atara Grenadir, Chair of the Art Department, who has arranged for Art students to assist in designing and producing the more recent covers. Please enjoy the variety of articles we put forth for you.

Sincerely yours,

Robert S. Bressler, Ph.D.

Letter from The Charter Chief Editors

Dear Readers,

The Science Journal of the Lander College of Arts and Sciences has been in existence for ten years. Ten years is a major accomplishment, not only in how far we have come, but in how much wonderful and amazing scientific research has been written and published throughout ten years of excellence.

When we were students in the Biology track of LCAS, we were interested in working on our own research, and looking for opportunities to advance, write, and publish work. Dr. Bressler, head of the Biology department, encouraged our idea, and decided to revive the Touro Science Journal. The Touro Science Journal had been in existence previously, but with Dr. Bressler's guidance, we revived the idea, revamped the Journal, its peer editorial committee drawn from interested students, and managed to produce something unique: a science journal filled with excellent research, done and edited by the Touro students themselves.

It gave students who had a desire to read and learn, to publish and to review, a place to submit their work, where they could undergo for the first time in their college years, peer review and processing, and to see their final work in print. It showcased original topics and ideas, and both the students and professors gained in the creation. Editors changed as the student body graduated and shifted, but the idea and concept remained the same, allowing it to become the gold standard for a Touro student to show their research.

We feel privileged to have started it on that path, and we hope that it will continue in the future to allow students a taste of what taking a research concept from start to the finished publication, can be. We owe a tremendous gratitude towards Dr. Bressler, for encouraging us in our notion, helping us give it its start, and continuing to produce and maintain it every year since, and for being the amazing science teacher and professor that he is. He gives of his time, his devotion, and thinks big- and that's exactly what this Journal has become: from a thought to an amazing 10 year anniversary. We also owe gratitude towards the Dean of Touro, and all the excellent scientific faculty, for funding, support, producing the research, their time, effort and help every step of the way.

We both graduated from Touro College and pursued careers in the medical and biological sciences: Michelle, defending her PhD thesis this year as a research geneticist, and Rivka, who has been working as a Physician Assistant for over 6 years. We are sure that Touro College and the Lander School of Arts and Sciences gave us the background and start we needed to pursue our goals.

We are honored to present to you the tenth edition of the Science Journal, and we hope that it will continue for many, many more years.

Sincerely,

Rivka and Michelle

Charter Chief Editors

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Most Efficient Methods to Treat Breast Cancer

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Abstract
Breast cancer is rampant in today's world. Because there are many different cases and so many different ways to classify breast cancer, a multidisciplinary approach must be taken. Many patients undergo breast conserving surgery which creates a need for the eradication of any remaining tumor residue through radiation. Fifty Gy of radiation should be applied to the breast with an additional 16 Gy as a boost. If a mastectomy is performed to remove a large tumor and 4 or more positive lymph nodes were present, radiation should be applied as well. If the tumor expresses HER2 protein, Trastuzumab should be given to "turn off" the protein. If a patient's cancer is hormone receptor positive, Tamoxifen should be given. If, however, the patient is premenopausal, the Tamoxifen must be given together with ovarian suppressors.

Introduction
Breast cancer can almost be considered an epidemic in today's world. It is the most frequently occurring cancer in women worldwide. (Li, 2016) It has become evident that breast cancer diagnoses have increased because screening methods have become more efficient and because overall life expectancy has been lengthened in recent years. In most cases, breast cancer is treated by removal of the tumor and surrounding areas. Sometimes neo-adjuvant (prior to surgery) therapy is given. Alternatively, treatment only begins after surgery. Afterwards radiation is applied to the tumor site and surrounding areas. In addition, if the tumor is hormone receptor (estrogen or progesterone or both) positive, patients will undergo hormone therapy as well as chemotherapy. This paper will discuss radiation therapy and hormone therapy as a systemic treatment but will not cover chemotherapy.

Methods
This article was written through the analysis of various original research and peer reviewed journal articles. Access was obtained through PubMed and the Touro College Library.

Results and Discussion
Breast Conserving Treatment and Mastectomy
Breast cancer treatment has shifted from total mastectomy toward breast conserving therapy. From the 1970's, studies have shown that breast conserving surgery with radiation have the same effects as the Halsted (radical) Mastectomy for tumors up to 5 cm. (Veronesi, et.al 2013) When a patient with breast cancer undergoes breast conserving therapy, radiation of the breast and some surrounding areas is usually applied (Aebi, et.al 2011). Even after mastectomy, radiation is required if there are four or more lymph nodes found that ae positive for cancer.. There was no observed benefit from radiation in women with node negative disease. (EBCTCG, McGale, et.al. 2014) Post-mastectomy radiation is recommended if the tumor is 5 cm or larger, there are at least 4 positive nodes, a positive margin of resection, or skin involvement. However, because of the psychological effects of mastectomy, breast conserving surgery is now considered an acceptable form of treatment even when the tumor is large, as long as the breast can be reshaped. Now, about 25% of women diagnosed with breast cancer undergo mastectomy, usually because of large tumor size, location of the tumor, or local recurrence following a previous breast conserving surgery. (Orecchia, 2015)

In order to reduce the psychological effects and cosmetic drawbacks of mastectomy, the skin sparing method has been introduced. One recent development in the skin sparing method is nipple-sparing mastectomy which allows for the preservation of the nipple areola complex. However, there has been increased recurrence behind the areola following nipple sparing mastectomy. Therefore, radiation is performed on patients that are at greater risk of recurrence. There is no standard practice in patients who have an intermediate or low risk and radiation's role in this case is unclear. (Orecchia, 2015) Although further research is needed, this new method can open up new opportunities for better methods of breast reconstruction.

Comparison of Mastectomy, Lumpectomy, and Lumpectomy plus Irradiation
A study was performed to see whether mastectomy, lumpectomy, and lumpectomy plus irradiation had different effects on survival. Follow up after 20 years showed no significant difference in survival between the group that underwent mastectomy as opposed to the groups that underwent lumpectomy with or without radiation. This study did not show any risk of radiation causing cancer in the contralateral breast which is a common concern. The findings at 20 years of follow up did indicate that lumpectomy with irradiation significantly decreases recurrence when compared to lumpectomy without irradiation. However, perhaps, if wider margins were removed at the time of breast conserving surgery, the need for radiation afterwards would be eliminated. (Fisher, et.al. 2002)

Effects of Radiation following Breast Conserving Surgery
Breast conserving surgery can be performed on women with a less advanced stage of breast cancer by removing any microscopic residual of the cancer. However, macroscopic bits of the tumor can remain in the breast or nearby area; therefore radiation is commonly used to prevent local recurrence and distant metastases. In one study, 10,801 women were included to see how radiation can reduce the risk of recurrence and death

including different variables, such as age, tumor size, and nodal status. This study differs from previous studies in three ways. Firstly, it assumes that all recurrence whether it is locoregional or distant is related to the tumor that preceded it. Therefore all recurrence is considered as first recurrence. Secondly, previous studies obtained follow up results for 10 years, and this one goes up to 15 years. Lastly, previous studies considered death of unknown causes within the first 10 years to be attributed to breast cancer, but this study attributes it to other causes. (EBCTCG, Darby, et.al. 2011).

When radiation was applied after breast conserving surgery, the 10-year risk of recurrence dropped to 19.3% as opposed to 35.0% in women that did not undergo radiotherapy. The risk reduction in this case is 15.7% for women who had radiotherapy following breast conserving surgery. Three quarters of the recurrence in those that had no radiation therapy was of a local nature while fewer than half of those in the radiation group had a locoregional recurrence. In addition, radiation reduced the risk of cancer related mortality to 3.8% in 15 years, which suggests that for every 4 recurrences avoided due to radiation, one death was prevented.

Radiation halved the risk of annual first recurrence and it reduced cancer related death by a sixth. However, mortality without recurrence (death from other causes) was slightly higher during the first 10 years in the radiation following breast conserving surgery group than those who did not have radiation. The higher death rate in the radiation group can be attributed to the toxicity of the radiation. However, this is not considered significant because it is such a minuscule percentage. On average, including all the women of all the different groups (node status, age, endocrine receptor responsiveness, etc.), during a 15-year period, one death was avoided for every four recurrences avoided by year 10.

This study considers any instance of recurrence, no matter where it occurs, as a first event rather than only considering locoregional recurrence as a first event as other studies have done. This gives more accurate results than previous studies. Different kinds of recurrences cannot be ignored because radiation is assumed to reduce all recurrences. Because this study gathered data from patients with a wide range of risks and overall, radiation halved the risk of recurrence, it can be assumed that it will reduce the risk in future patients as well by a half. (EBCTCG, Darby, et.al. 2011).

Boost

After 50 Gy radiation on the breast, a 16 Gy boost is recommended except for in patients with a low risk for local recurrence. (Aebi, et.al 2011) As stated previously, radiation prevented one death in a 15-year follow up for every four instances of local recurrence prevented in a 10-year follow up. However, because of the intensity of the radiation, fibrosis frequently occurred which caused a poor cosmetic result. (Bartelink, et.al. 2007) In one trial, the boost dose was lowered from the previously accepted 25 Gy to 16 Gy because of the high percentage of patient fibrosis which resulted from the intensity of the boost. There were 5,569 breast cancer patients who were included in the boost vs. no boost study. The patients were no different from each other with respect to surgery or whole breast irradiation. Local recurrence in the no boost group after 10 years was 4% higher than in the boost group. Incidence of fibrosis at 10 years was 4.4% in the boost group as opposed to 1.6% in the no boost group. Although a boost of 16 Gy was shown to decrease the rate of local recurrence in this study, no improvement has been discovered in disease-specific survival or overall survival. However, this could be because of the success of salvage mastectomy which was performed on the no boost group when they began to exhibit signs of local failure. The risk of fibrosis for those in the boost group is not of a significant level. (Bartelink, et.al 2015).

The above study was done over a period of 10 years. A different study was performed which included 20-year follow up for all patients. This trial was performed to see if a boost dosage of 16 Gy decreased the rate of recurrence. A 5-year follow up study showed that it does decrease recurrence. A 10-year follow up showed that when a boost is delivered, the rate of salvage mastectomies decreases. In the 20-year follow up, local recurrence was 13% in the no boost group as opposed to 9% in the boost group. Incidence of fibrosis was a little bit higher (about 4%) in the boost group. The cumulative percentage for salvage mastectomies performed on the boost group was 6.4 vs. 10.3 for the no boost group. The fact that there was no improvement in overall survival in the boost group seems to show that the boost is not needed. However, this must be the result of the high rate of successful salvage mastectomies performed. The boost the number of salvage mastectomies by a third. (Bartelink, et.al 2015)

Effects of Radiation following Mastectomy

Post mastectomy radiation is used to treat women with four or more positive lymph nodes to ensure complete removal of any residual tumor foci. However, the role of radiation in treating women post mastectomy with one to three positive lymph nodes still remains uncertain. As of now, post mastectomy radiation is not given to women who have node-negative disease. The following study included 8,135 women who had 1 to 3 positive lymph nodes. About 20% of the women had node-negative disease, 72% had node positive disease, and the nodal status for the remaining ones was unknown.

Radiation therapy was shown to significantly decrease the rate of recurrence and breast cancer death in the group that had 1 to 3 positive lymph nodes. Radiation was not seen to have any added positive benefit in women who had node negative disease. Radiation reduced the 10 year risk of recurrence in women with 1 to 3 positive lymph nodes from 45.7% to 34.2% which is an overall absolute benefit of 11.5%. Today, radiation treatments cover more of the high risk areas, such as the chest wall. In addition, the doses are lower than they used to be so the risks of radiation have decreased. Therefore, the proportional gains of radiation today are probably greater than the results of this study. However, within the 20 years in which this study has been performed, methods of detection and treatment have improved; therefore the absolute benefits of radiation are lower than those reported in this study. (EBCTCG, McGale, et.al 2014).

Intraoperative Radiotherapy

There is new research in the field of intraoperative radiotherapy to treat many different kinds of cancers including breast cancer. Instead of a few weeks or months of radiation post breast conserving surgery, patients can be treated with a dose of radiation at the surgery itself. Although the average time of radiation has decreased over the last couple of years, it is still a strain and inconvenience on the women who must travel to the hospital every day for about 30 days to receive treatment. Another advantage of intraoperative therapy over mastectomy is that if recurrence occurs after breast conserving surgery and a mastectomy is needed, it is easier to perform it with the now popular method of skin and or nipple sparing procedures if no radiation was applied to the skin because radiation can cause necrosis. (Veronesi, et.al. 2013) In addition, intraoperative radiation therapy can target the cancerous tissue while protecting the normal tissue around the tumor. (Najafipour, et.al, 2015).

In one trial, the effects of intraoperative radiation on a number of different cancers was tested. Intraoperative radiation was found to increase survival in patients with pancreatic cancer. When used on patients with early breast cancer, it was found to decrease life expectancy by less than a day. Although it may increase operating room load, it is generally a cost effective and considered a safe method to treat breast cancer. (Najafipour, et.al, 2015) However, more research must be done to see if this new method has any real advantage.

In another study, 1305 patients were randomized, 654 belonging to the external radiotherapy group and 651 to the intraoperative group. Only 11% had more than 4 positive nodes. In this study, ipsilateral breast tumor recurrence was defined as "the sum of local recurrence plus 2nd ipsilateral tumors." Local recurrence was considered the recurrence of the carcinoma at the previous location. Within five years of follow up, there was a significantly greater instance of recurrence in the intraoperative radiation therapy group than in the external radiotherapy group. Overall survival, however, did not differ significantly between the two groups. Predictions are that in the future, better methods will be used to differentiate between patients who are at higher risk of recurrence and therefore need external whole breast irradiation or intraoperative radiation plus external radiation. (Veronesi, et.al 2013) It must be noted that this study only measured 5 years which considered short term follow up in a study of this sort.

Testing for Endocrine and HER-2 Receptivity

Trastuzumab

Overexpression of HER2 protein occurs in 20-25% of breast cancers, causing high-grade tumors, increased growth rates, early systemic metastasis, and reduced rates of overall and disease free survival. (Slamon, et.al 2011) Trastuzumab (Herceptin) is given many times in conjunction with chemotherapy, but it can also be given alone. Trastuzumab does not cause the negative side effects that other chemotherapy medications are known for, such as alopecia, myelosuppression, and severe nausea. However, Trastuzumab has been known to cause cardiotoxicity in 1.4% of those to whom it was administered to. (Piccart-Gebhart, et.al 2005) Various studies were performed to find out how much Trastuzumab actually improved disease free survival and whether the increase in cardiotoxicity was a cause for concern.

One study included 4,482 women. Both groups received Trastuzumab after undergoing primary treatment (surgery, chemotherapy, radiation, ect.). One group received it for one year, the other for two. Patients with a history of cardiac trouble were excluded from this study. The study shows that Trastuzumab given after primary treatment reduces the rate of recurrence by 50%. It does not seem to make a difference what type of chemotherapy is received. The risk of cardiotoxicity is low; however, it is possible that this is because there is a short follow up in this study. In addition, there is a concern that longer term follow up will show that Trastuzumab does not reduce disease recurrence in the central nervous system. The results of this study indicate that one year of Trastuzumab should be used following primary treatment of breast cancer. (Piccart-Gebhart, et.al 2005).

A study was performed to see whether an anthracycline-based regimen increased Trastuzumab's toxicity. There were 3,222 women who were divided into three groups. One group underwent an anthracycline regimen. In the second group, the patients received anthracycline with Trastuzumab. In the third group, the patients only received Trastuzumab. All three groups

underwent an intense cardiac monitoring schedule. A significant benefit was seen in patients who received Trastuzumab as opposed to patients who did not. There were no apparent differences between the two Trastuzumab goups; however the trial was not set up to discern a difference between them. Congestive heart failure occurred more often in the group that received anthracycline in addition to Trastuzumab. In addition, treatment time is decreased when anthracycline is not given. (Slamon, et.al. 2007).

Hormones and their effect on Breast Cancer and breast cancer treatment

In the Million Women Study conducted in the UK, over 1 million women between the ages of 50-64 were recruited to have a breast screening every three years. The women were given a survey to complete shortly before their screening. (The questionnaire can be viewed at http://www.millionwomenstudy.org). The purpose of the study was to see if and how hormone replacement therapy affects breast cancer. In total, 50% of the population had used hormone replacement therapy at some time. The results of the study proved that recent or current users of hormone replacement therapy are at increased risk of breast cancer. Those using estrogen-progestogen combinations have a four times greater risk than those using estrogen-only preparations. However, past users of hormone replacement therapy have almost no increased risk. In all, hormone replacement therapy results in 5 to 6 more cancers per 1,000 women with 5 years use and 15-19 extra cancers per 1,000 women with 10 years use. (Million Women Study Collaborators, 2003).

The Million Women Study, because of its large number of participants and span of time, has been the source of much controversy. It has been pointed out the women who participated in the study are not a good sampling of the general population because women that use hormone replacement therapy are more likely to be concerned about their risk of cancer and go for a mammogram. (Eden, 2010) However, the Million Women Study represented 53% of the population, so although this may be 53% of the population that is more likely to be affected by hormone replacement therapy, over half a population is a good indication of the rest of the population.

Hormone Therapy

Soon after a patient is diagnosed with breast cancer, she is tested to see whether her tumor is hormone receptor positive of negative, defined by estrogen and/or progesterone receptivity. Accurate assessment is critical in deciding whether to treat with hormone therapy; however, accurate results are difficult to obtain.

In the past, tumors that showed less than 10% positivity were not given hormone therapy because they seemed similar to patients who tested negative for hormone receptivity. Recently, however, The ASCO/CAP guidelines were revised to treat women in the 1%-9% positivity group with hormone therapy. Now 3% more women are being treated with hormone therapy. The problem is that although for a small minority of cases, hormone therapy has a good positive result on those in the 1-9% group, physicians must consider the benefits of endocrine therapy versus its cost and side effects. (Yi M, et.al 2014) The goal of this study, as noted above, was to show that perhaps the benefits of treating the 1-9% group with endocrine therapy do not outweigh the costs. However, this study has limitations. They retroactively collected the data and because of that, the Estrogen receptor status is not known for certain because the patients obtained their diagnosis outside the facility where this study was performed. In addition, the study's limited sample size makes it very difficult to analyze the data based on results from adjuvant chemotherapy and endocrine therapy.

A later study shows that even with a very small percentage of Estrogen receptor positivity, adjuvant hormone therapy cut breast cancer mortality by a third for fifteen years. Those who had hormone receptor treatment were found, initially, to have lower rates of recurrence than those without; however, they do risk relapse for up to fifteen years despite usage of Tamoxifen for five years. (EBCTCG, Davies, et.al. 2011).

The suppression of hormones and the target of HER2 receptors is a not a new development in medicine. Two cohorts, comprised of 7,178 patients, were studied to see whether current hormone treatment is more effective than that of the past. One cohort was diagnosed recently, and the other was diagnosed from 1986-1992. Relapse event was any disease recurrence. In all, 1,700 patients had relapsed in 9 years of follow up. The result showed that the current treatments are more effective because they are used for longer and they are more efficient. However, patients with ER+ tumors do seem to have a late relapse and ER- tumors have an early relapse in patients that were recently diagnosed. (Cossetti, et.al 2014).

Another study was performed in 1996 to see whether there is a difference and how significant of a difference 2 years of Tamoxifen makes compared to 5 years of treatment . The results showed that 5 years was more effective. They did not test past 5 years; therefore, they do not know if more than 5 years would be more effective. (Swedish Breast Cancer Cooperative Group, 1996).

Premenopausal hormone receptor positive breast cancer is treated by ovarian ablation or suppression. The ovaries still

function in premenopausal women and they are the main site for hormone production. The aromatization of androgens in the adrenal glands produces hormones in postmenopausal hormone-receptor positive breast cancer. Aromatase Inhibitors (AI's) inhibit the production of those hormones. AI's fall into two categories: non-steroidal and steroidal AI's. Across the board, AIs are superior to Tamoxifen. The ovaries are only temporarily suppressed when women are premenopausal for preservation of fertility. (Li, et.al. 2016).

The Premenopause/Post-menopause treatment methods

Tamoxifen is the standard drug to treat hormone receptor positive breast cancer. Premenopausal patients, however, are often resistant to Tamoxifen. Therefore ovarian function suppression medications are combined with Tamoxifen in order to reduce disease recurrence in pre-menopausal women. Ovarian function suppression medications increase toxicity, complicating overall treatment regimens. (Kim et. al, 2016).

Two trials were performed, the Tamoxifen and Exemestane Trial (TEXT) and the Suppression of Ovarian Function Trial (SOFT), to determine whether the aromatase inhibitor Exemestane improved survival in premenopausal women when used in conjunction with Tamoxifen as opposed to women treated with ovarian suppressors with Tamoxifen. The combined data from the two studies shows that Exemestane plus ovarian suppression as opposed to Tamoxifen plus ovarian suppression significantly improved survival and decreased risk of recurrence. (Pagani et. al, 2014) However, in the SOFT study, the women who remained premenopausal and had an increased risk of recurrence and therefore needed adjuvant chemotherapy, the addition of ovarian suppression treatments improved survival rates (Kim et. al, 2016) Because these women received additional chemotherapy, it is not clear whether it is the chemotherapy or the ovarian suppression that increased survival.

A Korean Study was performed to compare ovarian function suppression with Tamoxifen versus just Tamoxifen in young, premenopausal women who had undergone chemotherapy. The main purpose of this study was to compare the 5-year disease-free survival rates between the two groups. The Korean Breast Cancer Society focused on this age range because although South Korea has an overall smaller number of breast cancer diagnoses compared to the world at large, 48.7% of breast cancer diagnoses are in premenopausal women under the age of 50 which is a much higher percentage of young women than in other countries.

The advantage of this study over other studies is that there is repeated evaluation of ovarian function, which allows for better selection of patients that should be receiving ovarian function suppression treatments, avoiding unnecessary side effects. Ovarian suppression causes menopausal symptoms which significantly alter the the quality of life. These symptoms can lead to a lack of patient compliance and can also destroy physician-patient relationship (Kim et. al, 2016). This study shows the benefit of Tamoxifen plus ovarian suppression therapy in premenopausal patients with Estrogen receptor positive breast cancer treated with chemotherapy. This trial helps determine the optimal endocrine therapy needed based on ovarian function status of premenopausal patients.

Conclusion

The patient must undergo a mastectomy if reconstruction of the breast post breast conserving surgery is impossible or not feasible. However, mastectomy is not as radical as it once was. Moreover, now there are new skin sparing methods, such as the nipple areolar sparing method which allows for a more natural reconstruction after surgery. Post mastectomy radiation is given in women with 4 or more positive lymph nodes, but in women with 1-3, the question still remains. Studies show that in women with 1-3 positive lymph nodes, recurrence is reduced by over 10%.

In about 75% of cases, patients undergo breast conserving surgery. Except in cases of very low risk of recurrence, patients must have radiation afterwards. However, perhaps better tumor residue removal will be employed which can eliminate the need for radiation therapy. After breast conserving surgery, radiation reduces risk of recurrence within 10 years from 35% to 19%, a significant difference. Radiation prevents one death for every four recurrences prevented (a 3.8% decrease). Radiation does increase toxicity; however, the percentage is so low, that they give radiation anyways. However they do try to keep dosages low. A radiation boost after the radiation treatment regimen decreases recurrence by 4% over a period of 10 years and after 20 years. There is no difference in overall survival between the group which received a boost and the one that didn't because of salvage mastectomies and other retroactive treatments performed. The risk of fibrosis does increase by the same percentage as survival in the boost group.

Trastuzumab is given to those that test positive for HER2 expression. Although it does increase cardiotoxicity in 1.4% of cases, it has less side effect than other chemotherapy medications. However, the risk of toxicity may be low in this study because of a short (5 year) follow up. A longer follow up may show a larger percentage of toxicity.

Hormone replacement therapy increases one's risk of getting breast cancer. Those who take an estrogen-progesterone

combinations have a four times greater risk of developing cancer than those who take estrogen only. Each woman together with her doctor must decide whether the benefits outweigh the risks in taking hormone replacement treatment. This treatment increases the quality of life for post-menopausal women; however if someone has an increased risk of developing breast cancer, they should not take this treatment. The Million Women Study does represent most of the population. However, sometimes large studies cannot take certain factors into because although they do divide their sample by different characteristics, there are too many people to be very exact and specific.

New guidelines have been set up, so now women with 1-9% hormone positive lymph nodes get hormone therapy. However the study that proved that this change is necessary (from the previous about 10%) has limitiations. Firstly, they collected the data retroactively, so they could not be certain that all of it was correct, and they had a limited sample size. Those that are hormone receptor positive and take hormone therapy for five years do initially have decreased recurrence, but they do risk relapse for up to 15 years.

In the study where a group received hormone therapy from 1986-1992 was compared to a more recent group, it was proven that hormone therapy is more effective now because the therapies are more efficient and they are used for longer than they once were. Although those with hormone receptor positivity have a better prognosis, they are at risk for a relapse albeit much later than those with hormone receptor negative cancer. Hopefully new research and discoveries will lead to a more permanent and better prognosis.

Premenopausal women are resistant to Tamoxifen, so it is usually applied together with ovarian suppressors. These suppressors can increase toxicity, but they do improve. As the Korean study noted, repeated evaluation of ovarian function can allow for better selection of patients which can avoid unnecessary side effects which lower quality of life.

All the above research shows that there is no one best method to treat breast cancer. Treatment must have a multidisciplinary approach. Each patient must be treated as an individual with all symptoms and disease specifics taken into account.

There are various limitations that studies have. One limitation is that studies, by their very nature, must be performed under certain assumptions, like assuming that death of an unknown cause within 10 years is disease mortality which may or may not be the case. In addition, a large sample size can cause a "one size fits all" attitude. Trials involving breast cancer patients have an advantage because there are many people who have breast cancer. However because of the large study size, at times there is not enough specification of treatment. One woman may seem to have the same diagnoses as another, but some important differences may not be accounted for. For example, a woman who tests positive for HER2 receptor and has 8.5% hormone receptor positivity will be given Trastuzumab and hormone therapy, but perhaps she has a risk of heart disease in her family and the increased cardiotoxicity will be more harmful for her than the average patient. It is not enough for doctors to be up to date on different treatment advances, they must create a treatment plan that takes each individual patient's needs, background, and overall health into account.

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Effects of Moderate Intake of Alcohol on Coronary Heart Disease

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Abstract

The french paradox is caused by an inconsistency with scientific experimentation: The French have a very high intake of saturated fat and cholesterol, which is associated with coronary heart disease, yet they have un-commensurately low levels of deaths due to this disease. It has been proposed that alcohol, as a part of the French diet, is the factor that helps decrease the risk of heart disease in the French population by 20-30%. Research has been gathered from many medical journals to create a larger and more accurate perspective to determine if alcohol has any effect on coronary heart disease. Experiments indicate that alcohol does indeed have protective effects on the cardiovascular system through various mechanisms including: High-density lipoprotein (HDL) increases, Apolipoprotein A1-A2 increase, Hemostatic factors, and insulin sensitivity increase.

Introduction

The French Paradox, a term coined by Serge Renaud in 1986, has stymied scientists for years. The paradox is presented by the fact that France is one of the world's highest consumers of saturated fat and cholesterol-containing foods. Yet, as a whole, the French people have lower rates of coronary heart disease than the rest of the Western world (cited in Ferrieres, 2004; cited in Agarwal, 2002). This presents a paradox since it has been firmly established that large amounts of cholesterol and saturated fat increase the risk for Coronary heart disease (Shestov, et al, 1993). How could a nation that consumes more cholesterol and saturated fats than the United States of America still have a significantly lower CHD mortality rate?

One possible explanation for this conundrum was proposed in 1986: the French have a larger intake of alcohol consumption than Americans. It was, therefore, hypothesized that alcohol, through various biological mechanisms, can lower the risk for Coronary heart disease (Rimm, 1999). The detrimental effects of alcohol have been tested and proven. However, most of these terrible effects stem from long term alcohol abuse and binging. Perhaps viewing alcohol from a moderate intake perspective will present different data. The purpose of this paper is to research and assess this hypothesis to determine if a moderate intake of alcohol has a deterrent effect on coronary heart disease.

Method

This is a meta-analysis of more than twenty papers including many original experiments. The information gathered in this paper has been collected from numerous sources including databases such as PROquest and JSTOR. Additionally, a great deal of information was gathered from medical journals publicly available on the internet or in libraries. All of this information was read, analyzed, and compared to determine each piece's respective authority and veracity. Each article was also assessed in accordance with its respective levels of diversity and sample size significance.

Results

In this review, the words "moderate intake of alcohol" will be used. This term obviously excludes excessive consumption of alcohol as well as total abstinence from alcohol. For laypeople, the term "moderate intake of alcohol" has a wide and subjective purview that will always fall somewhere between abstinence and excessive alcohol consumption. For the purposes of the experiments referenced herein, moderate intake of alcohol refers to 15-30 milligrams of ethanol (the alcohol found in what are commonly considered alcoholic beverages, such as beer, wine and spirits) per day. This is the amount typically contained in one to two alcoholic beverages. Any alcoholic consumption below or above 15-30 milligrams may have similar effects. However, this level of consumption it is not within the purview of this research paper.

When focusing on the intake of a moderate amount of alcohol (15-30 mg of ethanol), several experiments have found positive effects that alcohol exerts, directly or indirectly, on coronary heart disease. Graphically, it is represented as a J or U shaped curve. Below is a summary of the effects of alcohol on relative risk of death (Tabl1 1). Some experiments show that moderate levels of alcohol intake will decrease the risk of coronary heart disease by as much as 20 to 30 percent (Rimm et al, 1999b; Emberson & Bennett, 2006; Pearson, 1996). There are numerous mechanisms proposed for this decreased risk. Some have greater scientific support than others. This paper will present those mechanisms that have sufficient scientific ground upon which to stand. The various mechanisms that will be discussed are:

High-density lipoprotein increase; Apolipoprotein A-1 and A-2 increase; Change in lipoprotein size; Increase in fibrinolytic activity; Decrease in fibrinogen; Decreases in platelet aggregation; and Increasing insulin sensitivity.

Apolipoprotein A-1 A-2 Increase

In a process similar to High density lipoprotein increase, moderate intake of alcohol is hypothesized to increase the transport rates and concentration of Apolipoproteins A-1 and A-2. These proteins are produced in the liver and are part of the large complex of HDL that helps start the process of removal of "bad cholesterol" from the blood stream by HDL (De Oliveira e Silva et al, 2000; van der Gaag et al, 2016; Frank & Marcel, 2000).

There are two primary ways that Apolipoproteins assist in the removal of bad cholesterol:

First, in particular, Apolipoprotein A-1 is a cofactor for the enzyme lecithin cholesterol acyltransferase (LCAT) which converts cholesterol into cholesterol esters (http://www.ncbi.nlm.nih.gov/gene/335; Frank & Marcel, 2000). The cholesterol esters are more hydrophobic and help form the sphere of HDL that gets transferred in one of two ways: directly, through specific HDL receptors to the liver; or indirectly through very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) conversion. Therefore, an increase in the concentration and transport rates of Apolipoproteins A-1 and A-2 will speed up the process by which the HDL will esterify and remove cholesterol from the blood.

Second, in addition to its role in speeding up the rate of cholesterol removal by HDL, Apolipoprotein A-1 has also been associated with prostacyclin stabilizing factor, which is involved in anticlotting. By stabilizing prostacyclin, better known as Prostaglandin I2 (PGI-2), platelet aggregation is inhibited which helps decrease the chance of clots forming. The positive effects of inhibiting platelet aggregation will be discussed in more detail later in this paper (Yui et al, 1988).

Change in Lipoprotein Size

Experiments have also shown that ethanol can increase particle size of HDL and LDL. Small, dense LDL's have been associated with a risk of Coronary heart disease of three times that of an individual with large "fluffy" LDL. Additionally, an increase in the size of these lipoproteins has been associated with cardioprotection (Mukamal et al, 2007).

All of the above mechanisms hypothesize how alcohol creates a potential downgrade of risk for heart disease based upon changes in lipid profile. More particularly, these hypotheses are based upon increases in HDL-Cholesterol and Apolipoprotein concentrations as well as lipoprotein size.

The Hemostasis Hypothesis

The next set of mechanisms that hypothesize how moderate alcohol consumption has a possible downgrade on heart disease are based on hemostasis. Put simply, these hypotheses suggest that alcohol affects the way the blood clots and breaks down clots, thereby reducing the risk for coronary heart disease.

One possible hemostatic mechanism proposed is that moderate intake of alcohol can decrease fibrinogen concentration (Mennen et al, 1999 Stec et al, 2000). Experiments have shown that the moderate intake of alcohol decrease the levels of circulating fibrinogen by 18-20 percent (Wang, Barker, & Fuller, 1999). This decrease can cause a significant reduction of the risk for coronary heart disease. Fibrinogen is a blood soluble plasma protein that is activated by the enzyme thrombin and produces the protein fibrin. Elevated levels of fibrinogen in the blood can lead to potentially devastating effects on the heart. First, it can cause an increase in platelet aggregation. Second, it will increase fibrin levels which promote coagulation, which leads to an increase in blood clotting. Third, elevated levels of fibrinogen increase plasma viscosity, which is associated with an increased severity of coronary heart disease (Junker et al, 1998; Stec et al, 2000), In some scenarios, these factors can cause acute clot formation and block arteries, including coronary arteries, leading to myocardial infarctions. Finally, elevated fibrinogen levels play a role in atherosclerotic plaque build up. A moderate intake of alcohol will decrease fibrinogen levels thereby decreasing the above factors.

On the antonymic aspect, alcohol has been proven to increase the levels of fibrinolytic activity (Pikaar et al, 1987; Aikens et al, 1998; Sierksma et al, 2001). In addition to alcohol decreasing fibrin building, it is also able to increase fibrin catalysis. Experiments have shown that moderate levels of alcohol can increase levels of plasminogen activator, which converts plasminogen to plasmin, one of the enzymes that catalyzes fibrin clots. As a result, the hemostatic process of fibrinolysis is increased. By increasing the fibrinolytic activity in the body, clots are less likely to form thereby reducing strokes and ischemic incidents. In fact, many medications that treat heart diseases work through similar mechanisms by inhibiting Plasminogen activator inhibitor-1, which acts exactly as its name indicates -- by inhibiting the inhibitor, the activator is increased (Vaughan, 2011).

Another hemostatic aspect of moderate alcohol intake is its effect on platelet aggregation. Experiments have shown that alcohol can decrease platelet aggregation, subsequently decreasing the risk of cardiac incidents (Zhang et al, 2000; Pikaar et al, 1987; Renaud & Ruf, 1996, Parson, 1996). Platelet aggregation is a pivotal player in controlling and carrying out hemostasis. Moreover, it is a significant factor when it comes to atherslerotic heart disease. Atherosclerosis starts with the oxidation of lipoproteins which are responsible for creating lesions on the arterial wall. These lesions expose the endothelium thereby allowing platelet receptors to open to accepting signals that allow platelets to begin adhering to one another. Platelets are also responsible for amplifying the signal that attracts leukocytes such as monocytes and lymphocytes. These cells have been previously proven to advance atherosclerosis through many mechanisms including the uptake of cholesterol up to the point of rupturing. Additionally, platelets contain chemokines within them that are released when platelets are activated. These chemokines are also factors that play a role in the propagation of ahterslerosis (Kaplan & Jackson, 2011; Weber, 2005).

Insulin Sensitivity Increase

Alcohol has been shown to increase insulin sensitivity in insulin resistant individuals. (Sierksma et al, 2003; Joosten et al, 2008; Fachinni, Chen, & Reaven, 1994). There is a correlation between these individuals and higher risk for coronary heart disease. The precise mechanisms for this correlation are unknown but hypothesized as follows (Sparks, Sparks, & Adelhi, 2012). In one hypothesis, muscle and fat tissue resistance to insulin causes an increase in plasma insulin and free fatty acid concentrations. Subsequently, this deadly combination will lead to Very low density lipoprotein (VLDL)-triglyceride secretion , which leads to higher triglyceride plasma levels. Another possible hypothesis suggested is that in insulin resistant individuals, there is a defect that does not allow insulin to properly regulate VLDL-triglyceride secretion thereby leading to hyperglyceridemia. In both of these mechanisms, the end result is an increase in triglycerides found in the plasma which, through an additional mechanism, is associated with coronary heart disease (Hokanson, 2002).

Discussion

Many scientists disagree with these hypotheses set forth above. Some attack the conclusions themselves while others question the experiments based on external biases and statistical errors. One proposed argument is that the control group of abstainers from alcohol in the experiments had been tainted by ex-drinkers who became abstainers due to health risks. These individuals either realized themselves or were directed by medical providers that they had to stop drinking for their overall health and/or their potential for a cardiac event. If this were true, then using these individuals as the control for the experiments would completely ruin the results as these individuals are much more prone to having cardiac problems due to their previous consumption. However, this contention has been accounted for in many experiments. The experiments have made sure that their control groups consisted of individuals who have never been excessive drinkers as well as others that have been lifelong abstainers from alcohol.

Another potential problem that has been raised concerning the experiments is the determination of what type of people make up the moderate alcohol intake group. The concern is that perhaps individuals who are moderate drinkers of alcohol come from healthier homes or have healthier lifestyles. These concerns extend to the possibility that they have potentially better exercise routines, higher education, and better health insurance.

With regard to the claims of cardioprotection by HDL increases, there is some question as to whether increasing HDL will even have any impact in cardiac incidents. According to Dr. Daniel Keene, pharmacologically increasing HDL and its effects did not improve the individual's risk for cardiac incidents (Keene et al, 2014). Now, this is strongly contended by many other experiments and it would seem likely that there is some other explanation for why Dr, Keene did not find any improvement in his experiments. However, this is a curious experiment that should be taken into account.

All in all, there is sound evidence and scientific proof to say with confidence that alcohol exerts some level of cardioprotection by increasing HDL concentrations, in particular the HDL2 and HDL3 subfractions. The mechanism behind this is not fully understood. However, the hypotheses reported above may shed some light on this area. The experiments supporting the cardioprotective effects of moderate alcohol use have been performed with significant numbers of subjects representing all people of all races, sexes and socioeconomic backgrounds. There have been over 40 experiments proving this point. Granted that some of these experiments do have some faults and biases with patients, but taken as a whole, the experiments clearly indicate a significant cardioprotective trait of alcohol. However, this only accounts for roughly 50 percent of alcohol's cardioprotection.

Regarding the other 50 percent of cardioprotection, there is also sound basis to say that alcohol will decrease circulating fibrinogen, increase fibrinolytic activity, and decrease platelet aggregation. All these factors have been proven by proper scientific experimentation. Although some may contest these results because a few of the experiments were conducted in vitro and not on people, the results were sound and the newer experiments have verified this to be true.

With regard to increasing insulin sensitivity there is a lot unknown about the mechanisms by which insulin resistance harms the cardiovascular system and how alcohol increases sensitivity. However, from all the experiments it would seem clear that there is an increase in the sensitivity even if we are not clear about how it happens.

Conclusion

Based on all the evidence and experimentation is seems evident that there are benefits to moderate alcohol intake. To go so far as to recommend alcohol as a remedy or even a supplement to those in risk of coronary heart disease does not fall under the purview of this paper. It may be true that alcohol will reduce the risks for heart disease up to 30 percent. However, we need to be cognizant of the potential risk and damage that alcohol may cause on other parts of the body. To summarize, medical professionals should not be so quick to dismiss alcohol as a deadly drug, rather they should research and weigh the cost versus benefit and advise their patients accordingly.

It would seem clear that since alcohol has cardioprotective attributes, the French paradox is not very paradoxical. The French diet includes a consistent moderate intake of alcohol, which would explain the lower coronary heart diseases rate in France. Although this may not be the only explanation for why the French cardiac mortality rate is much lower, alcohol intake is proven to be a valid hypothesis.

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From Squirrels to Cognitive Behavioral Therapy (CBT): The Modulation of the Hippocampus

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Abstract

The legitimacy of psychotherapy can often be thrown into doubt as its mechanisms of action are generally considered hazy and unquantifiable. One way to support the effectiveness of therapy would be to demonstrate the physical effects that this treatment option can have on the brain, just like psychotropic medications that physically alter the brain's construction leaving no doubt as to the potency of their effects. Beginning with the understanding of therapy as a behavior, this paper first questions the possibility of behavior effecting measurable change on the brain. Examining diverse samples of both animals and humans repeatedly shows that the excessive exercise of spatial memory and mapping activities, which rely on the hippocampus, correlates with targeted hippocampal growth and modulation. The hippocampus reliably enlarges when over exercised. With this correlation demonstrated, this paper returns to therapy to find that Cognitive Behavioral Therapy (CBT), of all psychotherapies, modulates the brain in the very same pattern effected by targeted spatial and mapping behavior. These twin correlations lend credence to each other and their surprising similarity is best explained by the hippocampus's chief role in declarative memory. Both spatial memory and CBT rely on skills and behaviors regulated by declarative memory, lunder the jurisdiction of the hippocampus. This paper aligns the strong evidence of the spatial memory- hippocampal growth correlation with the CBT- hippocampal growth observation to show that CBT does indeed leave observable effects on the brain and real impressions on the patient.

Introduction:

Objective

This paper will explore how targeted use of the hippocampus leads to its morphological modulation and growth, with the overall goal of demonstrating how targeted behavior can demonstrably alter the brain. Specifically, the hippocampal growth correlated to its cognitive-map character will be explored. The correlation between the practice of spatial mapping activities and hippocampal growth will be examined in animals and humans. With hippocampus growth in response to these specific activities firmly established, this paper will attempt to correlate this observation to an area where it can have real-world application: therapy. The therapy that will receive particular focus is Cognitive Behavioral Therapy (CBT). This focus is due to the observation that CBT, out of all psychotherapies, specifically effects brain modulation along the very same lines as spatial memory and practice of mapping skills.

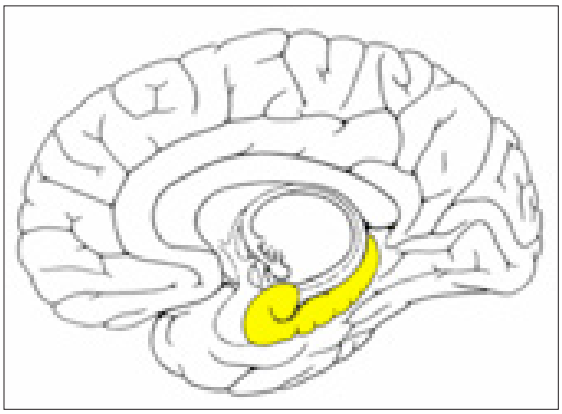

Figure 1: The human hippocampus, right lateral view (http://neurowiki.dk/images/8/83/Hippocampus0.png

What Is the Hippocampus?

The hippocampus is a seahorse shaped structure located in the medial temporal lobe of the brain. It has two lobes that each process a different type of memory. The right lobe is mainly responsible for viseo-spatial memory and the left lobe primarily works with verbal or narrative memory (Burgess, et. al. 2002). However, this lateralization is general; current research suggests that the storage and retrieval of both types of memories, especially the spatial subset, are more universally distributed across the whole hippocampus. This is evidenced by "cells coding for the same location [in reference to viseo-spacial memory] being distributed over the entire hippocampus." (Moser, Moser, 1998).

The hippocampus is considered the center of the declarative memory system. Declarative memory, also known as explicit memory, is the type of memory that can be consciously recalled and put into words, such as for facts and verbal knowledge (Ullman, 2004). It includes "episodic memories" which are autobiographical and personal, and "semantic memories," defined as general knowledge about the world (Burgess et. al. 2002, Schachter et. al. 2009). As part of its role in declarative memory "the hippocampus is central to the rapid acquisition of declarative knowledge about the environment, generating a so-called cognitive map." (Voermans et. al. 2004).

The cognitive map theory is the most current explanation for how organisms create and store memories of their environment. This theory "proposes that the hippocampus of rats and other animals represents their environments, locations within those environments, and their contents, thus providing the basis for spatial memory and flexible navigation." Essentially, the hippocampus builds a personal map of an organism's environment

as the organism navigates its way through it. Interaction with the frontal lobes of the brain "timestamps" each addition to the cognitive map, creating context and adding episodic character to the map (Burgess et. al. 2002).

The hippocampus has been observed to work in close conjunction with the caudate nucleus of the basal ganglia, a part of the brain responsible for forming associations between place and stimulus, thus leading to habitual behaviors. Both systems can work together, but non-competitively, in route recognition. When an organism returns to a previously visited location, the hippocampus uses its cognitive map to help recognize the location while the caudate contributes by recalling the personal memories of what occurred in this place the last time the organism was there. The hippocampus and caudate traverse different paths to come to the same conclusion: place or route recognition. Each are capable of recognizing a location on their own, via their alternative methods, but the possibility of enhanced recognition resulting from interaction between the two has been studied (Voermans et. al. 2004).

This is an example of the current trend in scientific research, in which many scientists study the interrelations and delocalization of brain function, based on the premise that "because the cognitive systems of the brain work in an integrated fashion, presumably the different memory systems do not work in isolation." (Voermans et. al. 2004). As such, much research has been done on the interactions and communication between the hippocampus and various other parts of the brain whereas, historically, focus had been placed on defining each brain area and its specific function. This makes focus on the morphology, function, and effects of one specific brain structure difficult but this paper will attempt to focus on the hippocampus specifically.

Methods

Information for this paper was obtained through various databases made available through the Touro College library. Relevant Internet searches, via Google, were also used to help lead to resources. Keywords included hippocampus, declarative memory, cognitive behavioral therapy, and brain modulation. Textbooks on therapy were consulted as well.

Discussion: Animal Studies of the Hippocampus

The clear effects of an organism's mapping behavior affecting physical changes on its hippocampus were initially observed in black-capped chickadees in Ithaca, NY. Chickadees are small, non-migratory, food-storing birds in the same family as jays and nutcrackers. These birds "showed a peak in relative hippocampal size in October, at the same time of year that food storing was reported to be greatest in this population of chickadees." (Sherry, Hoshooley, 2010). This peak was specific to the hippocampus; two other brain areas measured for control did not undergo any change at all. Additionally, this change was assuredly due to their increased mapping activity and not the alteration in day length (photoperiod) that occurs in the fall, as manipulating the day length experienced by captive birds had no effect on hippocampus size (Sherry, Hoshooley, 2010). Taking this together with previous studies that lesioned the avian hippocampus and observed how this specifically "disrupted memory for the locations of caches, because caching performance, feeding, and other behavior were not affected" assures the hippocampus's central role in cache mapping/memory (Sherry, Vaccarino, 1989). Therefore, it is safe to conclude that the correlation between the chickadees' behavior and their hippocampus size is causal. The chickadees' food storing activity, which involves tracking down nuts and seeds, hiding these finds in multiple caches, and most importantly recalling the location of each cache, exercises and expands the hippocampus.

In fact, the research team in this study worried that their observations may not be readily replicable because "in captivity, it may not be possible for birds to engage in enough food storing and cache retrieval to produce the changes in hippocampal size and neurogenesis observed in the wild." (Sherry, Hoshooley, 2010). This concern insinuates that once a certain threshold of practice of this behavior is reached, the hippocampal change is inevitable. It also shows that certainly it is some excessive level of practice of these activities, food storing and cache retrieval, that directly enlarges the hippocampus.

In a remarkably parallel finding, the hippocampi of Northeastern red squirrels, creators and hoarders of multiple caches of nuts, in an activity self-descriptively named "scatterhoarding," are larger than the hippocampi of their close cousins, the gray squirrels of the West Coast. The non-hoarding gray squirrels have no winter to contend with and so have no need to utilize the spatial mapping and recall skills specific to the hippocampus to scatterhoard for the future when food will not be available (Johnson et. al. 2010).

Notably, red squirrel hippocampus size has even been shown to fluctuate along with their hibernation patterns. When the squirrels are up and about, busily finding, hoarding, and creating mental maps of their caches, their hippocampi are larger than when their body temperature decreases to the point of initiating torpor, or hibernation, and thus cessation of all such activities (Millesi et. al. 2001). Like the chickadees, the red squirrels are clearly modulating their own hippocampi through their behavior.

Homing pigeons, known specifically for their mapping and spatial skills, are a natural species to look for the hippocampal

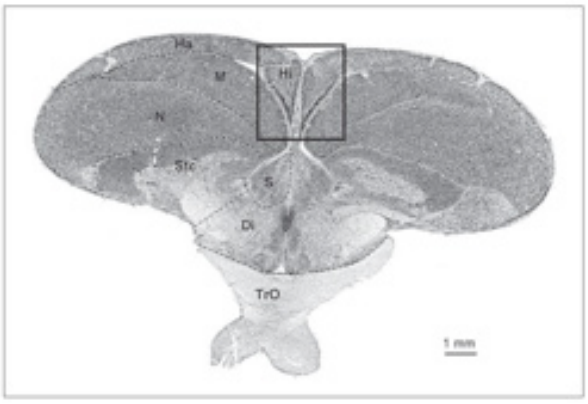

Figure 2: Coronal section through brain of homing pigeon with area of study, hippocampus, outlined. (Cnotka et. al. 2008)

growth observed in chickadees and squirrels. Citing studies similar to those discussed above, researcher Cnotka and her team hinge their study on the fact that "the hippocampus plays a critical role in processing spatial information both in birds and in mammals." (Cnotka et. al. 2008). And indeed, when these researchers examined the brains of the pigeonsthey found that homing pigeons possess disproportionately larger hippocampi for their body mass (Figure 2). A separate research team compounded this research by finding "morphological and histological differences in hippocampal tissue in homing and non-homing pigeons" (Shapiro, Wieraszko, 1996).

Additionally, comparing carrier pigeons that are allowed to fly, explore their barns, and map out new routes with pigeons that are confined to their cages, revealed the explorer pigeons to have measurably larger hippocampi (Cnotka et. al. 2008). The comparison between the explorer pigeons and the cage-confined pigeons directly parallels the above comparison between the red squirrels and gray squirrels. Both directly prove that when an animal stresses its hippocampus, the hippocampus will remodulate and enlarge.

Neurogenesis

Although it has been firmly established that animals who exercise their hippocampus directly affect their hippocampi's growth, the cause of this phenomenon has not been determined. The team of researchers who conducted the study on homing pigeons admit just that. They write, "in our study we have not determined what is responsible for this increase in volume, but it would be interesting to see why the hippocampus might be larger. Existing cells could increase their cell body size or build up larger dendritic arbors, new neurons or glia could be added, or there could be increased vascularization." (Cnotka et. al. 2008).

Meanwhile, the researchers who observed the hippocampus growth in New York chickadees were able to squarely implicate neurogenesis as the mechanism of growth. They did this by administering to wild birds "injections of tritiated thymidine, [3H] thymidine, which is incorporated into the nucleus of mitotic cells" (Sherry, Hoshooley, 2010). [3H]thymidine is a commonly used radioactive cell marker that newly forming cells incorporate into their DNA, thus differentiating the new cells from pre-existing cells (Toyohara et. al. 2002). The researchers then released the chickadees back into the wild. When they recaptured the birds, they found that "birds given [3H]thymidine in October had more labelled hippocampal cells when captured six weeks later than birds injected in August or February/March." (Sherry, Hoshooley, 2010). Evidently the birds generated more cells in their hippocampi in October, when their scatterhoarding activity levels were high, than in the months when they were not exercising their hippocampi excessively.

Neurogenesis would seem to be the most likely explanation for hippocampus growth but the possibility of new brain cells being created "on demand" should not be taken for granted. This is because "in most brain regions, the generation of neurons is generally confined to a discrete developmental period," and so growth would really not be possible at any time for most areas. Eriksson et. al., (1998), originally demonstrated the presence of progenitor cells, from which newborn neurons are generated and so the prerequisites for new cell growth, only in specific parts of the brain. Among these parts was the hippocampus. He showed this by injecting human cancer patients with "a thymidine analog, BrdU [that] is incorporated into the DNA of dividing cells" and then after the patients died, dissecting their brains to find labelled cells in the denate gyrus of the hippocampus (Eriksson et. al. 1998). These labelled cells indicate the presence of new neurons, and so progenitor cells, specifically in the hippocampus. This finding is crucial to correlating the animal studies to humans and offers the mechanistic explanation for how the hippocampus gets bigger. It shows that new neurons can indeed be grown in the hippocampus and that this growth can be initiated at any time – including whenever mapping skills are exercised. Eriksson's study essentially opened the door to neurogenesis and, as he ends off his report, "the potential to regulate human neurogenesis should prove to be an interesting area of investigation." (Eriksson et. al. 1998). The data in this paper enthusiastically supports this proposition.

Neuromodulation

In contrast, another research team focused on "the plastic processes that underlie long term potentiation" as the mechanism by which an organism can remodel its "mental map," or hippocampus. This team defines long-term potentiation as "a long-lasting, activity-dependent enhancement of synaptic strength that has been extensively studied in the hippocampus." (Kentros et. al. 1998). In their study, they observed how rats build their

mental maps by encoding each cell in their hippocampus with a different aspect of the place they are in. These programmable cells were appropriately named "place cells." It is then the "conjoint activity of place cells [that is] thought to be the basis of a map of the environment that the animal uses for solving spatial problems." (Kentros et. al. 1998). With this proposition for the construction of the mental map in place, the researchers then demonstrated that place cells, and the connections and interactions between them, can be reprogrammed based on the rat's activity. Essentially, the team demonstrated the ability of the hippocampus to remodulate, in addition to expand, in response to excessive use (Kentros et. al. 1998).

Taken together, notable hippocampal neurogenesis and neuromodulation correlates with the specific exercise of the hippocampus via practice of hippocampus-centric behaviors, namely those that rely on spacial mapping and recall activity.

Onward to the Humans

Having examined how the hippocampus specific behaviors of a wide range of animal species cause direct growth of the hippocampus, and accounting for this growth with evidence for neurogenesis and neuromodulation in the hippocampus, this paper turns to the likelihood of this causation occurring in humans as well. Are human brains, specifically the hippocampi, as manipulatable and malleable as those of rats, squirrels, chickadees, and pigeons?

Blithely referencing the sum of the extensive work that we have examined thus far, researcher Eleanor Maguire states, "the volume of the hippocampus in nonhumans is known to vary as a function of the demands placed on spatial memory." (Maguire et. al. 2006). With this firmly established, Maguire and her co-workers performed a landmark study comparing the brains of London taxi drivers with the brains of London bus drivers.

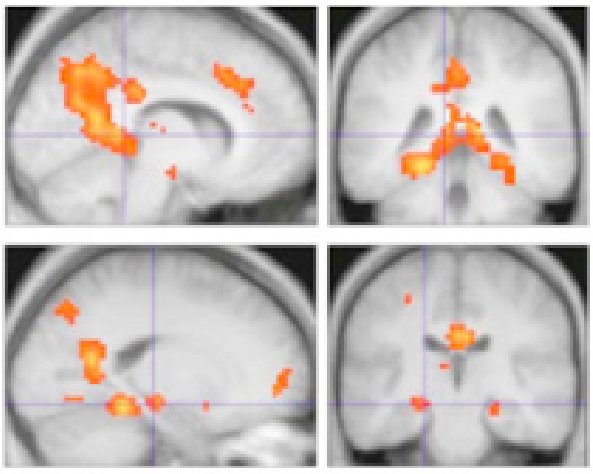

Figure 3: Hippocampal activation upon naviagtion through novel environments. (Maguire et. al. 2002

London is famous for its convoluted cityscape and so its taxi drivers are required to spend about two to four years studying its design and navigating its intricacies in order to be ready to pass the test required to get licensed as professional drivers. The taxi drivers' process to proficiency is rigorous and heavily reliant on the hippocampus. Just as it was observed with regard to the chickadees, squirrels, and pigeons, Maguire and her team report, "...years of navigation experience correlated with hippocampal gray matter volume only in taxi drivers [and not in London's bus drivers], with right posterior gray matter volume increasing and anterior gray matter volume decreasing with more navigation experience." (Maguire et. al. 2006). Since London bus drivers matched with London taxi drivers in "driving experience and levels of stress, but differed in that they follow a constrained set of routes," they provided the perfect comparison group to the taxi drivers who must learn and then navigate "25,000 streets and the locations of thousands of places of interest." (Maguire et. al. 2006, Woollett, Maguire, 2012) Therefore, comparing the taxi drivers, who must continuously re-navigate and calculate intricately mapped out routes, to bus drivers, who merely retrace a limited set of preset routes, shows that it is specifically the mapping activity that remodels and expands the hippocampus. Maguire confirms for animals and revolutionarily proves that for humans as well, that to modulate the hippocampus "the key factor seems to be utilizing a complex spatial representation over years of navigation." (Maguire et. al. 2006)

The clear growth of the hippocampi of London taxi drivers in response to their spatial mapping behaviors highlights that humans are indeed capable of modulating their own brains. People who spend years stressing their hippocampi will reliably enlarge them. The London taxi drivers study also shows that, surprisingly enough, changing one's own brain is really not that hard. This opens for consideration the reach of self-induced brain manipulation. To what extent can humans modulate their own brains, through what activities, and to what benefit?

The Relevance to Therapy

Answering these questions is of particular pertinence to psychologists, specifically those who practice therapy. This is because, "if psychotherapy is regarded as a form of learning, then the learning process that occurs in psychotherapy may produce alterations [in the brain]." (Gabbard, 2000). Researchers seek to highlight the physical, observable effects of therapy on the human brain to help prove the therapy's efficacy. And indeed, multiple fascinating observations have been made.

In 1992, a study contrasting different forms of treatment for depression found that "behavior therapy and fluoxetine [a common antidepressant] appear to produce similar decreases in cerebral metabolic rates in the head of the right caudate nucleus." (Gabbard, 2000). The caudate nucleus is part of the basal ganglia and responsible for "the acquisition of place-appropriate responses leading to habitual behavior," (Voermans et. al. 2004). While it does function in a memory system separate from the hippocampus, the two do have comparable enough roles that in the event of degeneration of one, the other can compensate (as detailed earlier in this paper). According to this study, "both memory systems support navigational memory, albeit based on the processing of different representations. It has been hypothesized that both systems work in parallel, receiving similar input information, but processing this information according to principles that emphasize different relationships among the elements of a given event or situation" (Voermans et. al. 2004). Importantly, the basal ganglia, like the hippocampus, does possess progenitor cells for growth by neurogenesis (Erikkson et. al. 1998).

The similarity between the roles of the basal ganglia and the hippocampus help the modulation of one stand in as evidence for the possibility of the modulation of the other. Similar functions means that similar activities will affect their size and shape. With the overwhelming evidence that this paper has examined for hippocampus-centric activities modulating the hippocampus, it follows that similar activities, only different in that they are reliant on the caudate, would modulate the caudate. If this is so then we can consider how the activities that the researchers found here to modulate the caudate, Behavior Therapy, might be similar to the activities that modulate the hippocampus (spatial memory and mapping behaviors).

Another research team comparing the effects of different mental health treatment forms showed that "cognitive behavioral therapy appears to cause biological changes in people with panic disorder." (Gabbard, 2000). These researchers first observed that individuals with panic disorders inappropriately release lactate in response to certain stimuli. This lactate then serves to trigger the panic attack. The researchers treated the individuals with CBT and tested the CBT's effectiveness by injecting them with the triggering lactate. The team saw that, after treatment, "the induction of panic by lactate... [was] effectively reversed through successful cognitive therapy. In other words, panic disorder sufferers in whom, before starting therapy, attacks were precipitated by injection of lactate no longer responded in that manner after therapy." (Shear et. al. 1991). While this study does not bring the physically observable effects of the therapy studied down to the level of the brain, it does provide another exhibit of therapy, an action, inducing physiologic, measurable effects.

A similar set of results, proving that performing the actions proscribed by therapy induces physiologic responses, comes from a research group who measured the variation of certain hormone levels of depressed patients in conjunction with CBT. They "observed that in a group of outpatients with mild major depression, responders to cognitive behavior therapy had significantly greater decreases in T4 levels and free T4 index than nonresponders" (Joffe et. al. 1996). The researchers propose that this is part of the whole "cascade of biological events that effect a therapeutic response in depression" (Joffe et. al. 1996). Here the physiological effects of therapy are found in hormone level variation, but it is assumed that digging a bit deeper would reveal modulation to the brain.

The Specific Effect of Cognitive Behavioral Therapy

Notably, these studies all tend to refer specifically, out of all the forms of therapy available, to Cognitive Behavioral Therapy (CBT). It seems that CBT is different from other forms of therapy and that it is especially effective in inducing measurable morphological brain change.

A practical guide for clinicians to employ CBT helps define this treatment method: "CBT is based on two central tenets: 1) our cognitions have a controlling influence on our emotions and behavior; and 2) how we act or behave can strongly affect our thought patterns and emotions" (Wright et. al. 2006). The cognitive portion of CBT works to resolve cognitive errors that contribute to an individual's disorder, such as overgeneralization and magnification, and the behavioral component consists of the patient learning and performing actual tasks such as breathing modification and journaling " (Wright et. al. 2006). CBT's in-practice structure consists of four basic steps that reflect these principles: 1. relabel unwanted thoughts as symptoms of a brain disorder, 2. reattribute these thoughts to the dysfunctional brain, 3. change behavioral responses even though the thoughts are still there, and 3. revalue the thoughts as less important (Beauregard, 2014). Based on the evidence thus far of actual behaviors modulating the brain center they rely on, it makes sense that the action centric, goal oriented CBT would be the type of therapy most likely to induce brain changes.

Dr. Aaron Beck, the founding father of CBT, stressed the importance of patient action and involvement in his or her own therapy. The structure of the patient - therapist relationship, termed "collaborative empiricism," expects the patient to work as an equal partner to the therapist in solving his or her problems. The patient is assigned behavioral tasks and homework assignments to personally, actively accomplish (Wright et. al. 2006). More than the therapy "being done" on the passive patient until the patient's problems are fixed, in the CBT model the patient is taught how to

remodel his or her own beliefs in conjunction with being told to perform self-driven actions. It is through fastidious participation and performance of these actions that the patient is "fixed."

Evidently, the actions proscribed by CBT rely on the hippocampus because, as this paper began to describe, multiple studies correlate the effectiveness of CBT with enlargement or modulation of the hippocampus. This relationship has been observed in patients treated with CBT for panic disorder (Beauregard, 2014), social phobia (Goldapple et. al. 2004), obsessive-compulsive disorder (Goldapple et. al. 2004), post-traumatic stress disorder (Levy-Gigi, Keri, 2014), spider phobia (Paquette et. al. 2003), and depression (DeRubeis et. al. 2008). And so, just as the London taxi drivers' spatial mapping behaviors modulated their hippocampi, the active CBT patient evidently relies on the hippocampus when he or she performs the CBT-assigned tasks and thereby enlarges his/her hippocampus.

CBT for Depression

An illustration of CBT's effect on the brain can be found in a clinical study comparing the effects of CBT and paroxetine (a standard antidepressant) on the depressed brain. Based on the premise established by multiple clinical trials "in patients with both mild and severe major depression consistently demonstrate similar rates of response to cognitive behavior therapy (CBT) and antidepressant pharmacotherapy," a team of researchers hypothesized that, while the observable effects of these two treatments are practically identical, their mechanisms of action are likely very different (Goldapple et. al. 2004). After administering a full course of CBT to a sample of depressed patients, the researchers imaged the patients' brains (Figure 5) and indeed found a pattern very distinct from that of paroxetine. They found that "areas of increased metabolism before to after treatment included the hippocampus and dorsal midcingulate" and that the changes effected by CBT were actually in the inverse direction of those caused by paroxetine (Goldapple et. al. 2004). CBT treatment effected "regional changes [that] involve sites similar, and in some cases identical, to those seen previously with paroxetine and other pharmacotherapies, but the changes were in the opposite direction." (Goldapple et. al. 2004). Where paroxetine decreased hippocampus size and connectivity, CBT enlarged it. Therefore, it is seen that modulating the hippocampus is a unique, effective method for treating depression that is distinctly accomplished by CBT. Notably, CBT was seen here to both expand and remodulate the hippocampus.

However, it seems that opposite conclusions were observed in a separate imaging study. Here, "MDD [Major Depressive Disorder] participants displayed a greater activation in the subgenual cingulate cortex, medial PFC, and left anterior hippocampus/amygdala before treatment, and a reduction in these brain regions after long-term psychodynamic therapy." (Beauregard, 2014). Essentially, the research team here saw therapeutic intervention shrink the hippocampus! A possible explanation for this opposite observation is the use of a different therapy, not CBT in particular, as intervention. Perhaps its mechanism is like that of paroxetine, which helps alleviate depression but via a pathway that modulates the brain into a pattern inverse to that of CBT (Goldapple et. al. 2004). Therefore, rather than confounding conclusions formed thus far, this contradictory observation can help reinforce the special effect CBT alone has on the hippocampus.

CBT for PTSD

A separate research team headed by Levy-Gigi examined the effects of CBT on the brains of patients suffering from PTSD. They found that "morphological changes associated with psychotherapy were confined to the hippocampal formation and cingulate cortex." (Levy-Gigi, Keri, 2014). PTSD is especially relevant as it has long been specifically correlated with smaller hippocampal size. Researchers in 1997 "showed that the left hippocampal volume in adults with post-traumatic stress disorder who had experienced childhood physical and sexual abuse was dramatically reduced when compared to that in matched controls." (Gabbard, 2000). Therefore a treatment that directly enlarges the hippocampus would likely be especially effective.

Indeed, Levi-Gigi's team reports that "the most noteworthy finding of this study was that clinical improvement during CBT in PTSD was associated with increased hippocampal size and elevated FKBP5 gene expression, a cellular regulator of the glucocorticoid receptor." (Levy-Gigi, Keri, 2014). First, confirmation of the way CBT enlarges the hippocampus is proffered. Second is the introduction of the gene FKBP5, a "regulator protein of the cortisol receptor and [since] abnormal cortisol secretion is linked to hippocampal atrophy, it is reasonable to hypothesize that the amelioration of FKBP5 gene expression had a causal role in the normalization of hippocampal volume." (Levy-Gigi, Keri, 2014). This hypothesis says that elevated levels of this particular gene, FKBP5, can be aligned with and seen as confirmation for hippocampal enlargement.

Levy-Gigi and her associates also returned to the previous discussion on what exactly is behind the hippocampal enlargement observed, considering how "possible mechanisms may be enhanced neurogenesis, increased neuronal size, and enrichment of dendritic arborization." (Levy-Gigi, Keri, 2014). According to the evidence examined previously, it is likely that the progenitor cells observed in the hippocampus, and so neurogenesis, is the mechanism behind the hippocampal growth observed here. Neuromodulation, or the reconnectivity and recharacterization of hippocampal cells, likely occurred as well.

CBT for Spider Phobia

Anxiety disorders, such as phobias, respond well to CBT intervention. This is because anxiety disorders typically include and/ or come from cognitive errors that CBT can specifically target and solve (Wright, 2006). One specific phobia studied in conjunction to CBT is spider phobia. The team behind the spider phobia study says that "although several psychological models have been proposed to explain the therapeutic effects of CBT, little is known regarding the neurobiological mechanisms underlying this form of psychotherapy." (Paquette et. al. 2003). To investigate this matter they administered CBT to a sample of spider phobic females, all of whom were deemed responders to this intervention. However, when the researchers examined the brains of their spider phobic subjects (via fMRI) before and after CBT, they found a decrease in hippocampus activity. Before intervention they found an overactivation of the hippocampus and after intervention they found this overactivity greatly decreased. This finding directly confounds the extensive research examined and discussed thus far. Therefore, the generalizability and validity of this study must be questioned.

The Connection Between Declarative Memory, the Hippocampus, and CBT

As laid out in the beginning of this paper, the hippocampus is considered to be the center of declarative memory, which is the collection of conscious memories people are capable of articulating. The foil to declarative memory is procedural memory, the contents of which are implicit, operating outside of conscious awareness (Gabbard, 2000). New research has begun to understand the effects of talk/ interpersonal therapy and psychoanalysis to be in the procedural, implicit realm. Sigmund Freud, father of psychoanalysis, himself alluded to implicit memory years before the concept was defined when he "stressed that what the patient does not remember will be repeated in the relationship between patient and analyst." This concept is defined as "transference," and in light of modern psychology can be seen as stemming from implicit memory (Gabbard, 2000). The patient in therapy will be implicitly affected by the therapist and his or her relationship to the therapist. Characteristic of this, he or she will not be able to explicitly articulate the effects of the psychoanalysis or psychotherapy even though he or she will exhibit behavioral change.

Illustrating this concept is the lament of psychiatrist Gabbard: "therapists are often disappointed when they see former patients and ask them what they feel was of most benefit to them during the years they were in psychotherapy. Much to the dismay of the therapist, patients often do not remember any of the psychodynamic formulations or interpretations that the therapist carefully constructed to provide insight. Instead, they remember a joke the therapist told, a belly laugh they shared, a moving moment of emotional connection, or a glance exchanged between therapist and patient when a special form of closeness was felt." (Gabbard, 2000).

Psychologist Lyons-Ruth interprets these moments in therapy as a form of "implicit relational knowing when something emotionally reparative transpires without involving the realm of insight or cognitive understanding." (Lyons-Ruth et. al. 1998). She and her colleagues believe such moments are a crucial part of the mode of therapeutic action. Alteration of implicit/ procedural memory and the manipulation of the manifestations of these types of non-declarative memories, such as transference, seems to be characteristic of all therapies besides for CBT.

CBT is unique in that it specifically targets explicit memory, rather than implicit memory. CBT does not attempt to grope its way through the murky depths of the subconscious, non-declarative, implicit realm. Rather, as illustrated above, CBT's two central focuses are on understanding and controlling cognition alongside altering behaviors in order to affect emotions and thoughts. Both these actions are necessarily declarative as they rely on the patient's explicit understanding, self-motivation, and complicity.

The hippocampus is the center of the declarative memory system and, stemming from this role, is its spatial mapping responsibilities. Therefore, the linkage between the identical effects of spatial memory/ mapping skills and CBT on the hippocampus is their reliance on declarative memory! Both make excessive use of the hippocampus and so enlarge it. The scatterhoarding squirrels, the homing pigeons exploring their barns, and the taxi drivers navigating London make use of the same exact memory modality and brain system as the traumatized, phobic, or depressed patient carrying out CBT-proscribed actions. Both groups over-rely on the hippocampus, in its declarative memory role, and so the same pattern of hippocampus enlargement emerges in both.

The diverse areas of research explored in this paper converge on the central point that exercising the hippocampus, via exaggerated practice of the skills and behaviors that it is responsible for, effects its measurable modulation and growth. The pointed use of the hippocampus's cognitive map function by the black-capped chickadees, the red-tailed squirrels, the homing pigeons, and the rats enlarged the hippocampus in each species. When the London taxi drivers exaggeratedly employed their parallel cognitive maps to navigate the city, they enlarged their hippocampi as well. Intensified use of the hippocampus in terms of its explicit memory responsibilities by CBT patients modulated the hippocampus along the very same lines. The search for an underlying feature to relate these actions leads to the very definition

of the hippocampus: the center of declarative memory. All of the parties discussed in this paper evidently over-employ declarative memory, and so the hippocampus.

Further Research and Conclusion

Based on the observations and correlations in this paper, further research could examine if or how other areas of the brain could be modulated. The declarative memory - hippocampal growth correlation is so clear and definitive that there must be similar trends in other areas of the brain. This paper has already observed that similar modulation is possible in the caudate nucleus. Perhaps identifying the specific skill that a different brain area is responsible for and then excessively practicing this skill would modulate or enlarge the corresponding brain area as well.

Further research could also examine whether the correlations in this paper could be looked at in reverse. Could invasively entering the brain and somehow forcibly enlarging the hippocampus effect an increase in spatial/mapping memory or explicit memory? And, assuming this enlargement were possible, could it be used as therapeutic intervention for the same patients that typically benefit from CBT?

Viewing the relationship in reverse has already been ventured towards by Maguire, the researcher who studied the hippocampal enlargement of London taxi drivers. She proposed "examination of the characteristics of those who succeed at taxi driver training, and [asking] whether innate pretraining cognitive factors and/or hippocampal volume are predictive of successful qualification." (Maguire et. al. 2006). In other words, do individuals with naturally larger or more malleable hippocampi, and so an inborn propensity for spatial memory, gravitate towards jobs that benefit from this characteristic, such as taxi driving?

The researchers who studied the effects of CBT on PTSD also mentioned viewing the correlation they observed in reverse. They proposed the "possibility that small hippocampal size is a premorbid vulnerability factor for PTSD" (Levy-Gigi, Keri 2014), looking at the hippocampus size first and the effects that follow after. If a smaller hippocampus could predispose an individual to disorders such as PTSD, then perhaps a larger than average hippocampus could protect them from this and other hippocampus-centric mental disorders. While still strictly conjecture, research on and validation of this theory might then lead to hippocampal enlargement becoming standard intervention for individuals deemed at risk for mental disorders correlated to the hippocampus. Having observed diverse correlations and multiple angles, this paper concludes with the vast potential for practical implications that may arise as researchers begin aligning and synthesizing some of these ideas.

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Jet Lag and the Biological Clock

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Abstract
Jet lag is a resulting disorder of flight travel across several time zones. Circadian rhythms, the approximately 24 hour system that our bodies follow, become distorted following such a trip, as the body's internal biological clock has not yet caught up to the current local time zone. Time cues and other external factors are necessary to adjust the clocks and reverse the negative effects of jet lag.

Introduction
Long flights across several time zones can result in jet lag, similar to what is known as travel fatigue. The effects of this jet lag are caused by the tight space of the aircraft, limit of food options, dehydration from the dry air, as well as climate and lighting changes. The consequences of such jet lag increases with every additional time zone crossed, as the internal body clock is unable to adjust properly. This can result in the inability to sleep at night and improperly timed daily functions. Circadian rhythms, the internal bodily processes that follow an approximately 24 hour biological clock, require an adjustment period to correct the negative effects of jet lag. Natural clocks follow a morning-night schedule which allows a person to function during daytime and reboot at night. Jet lag can cause a disorder in this natural system. Prolonged and repeated travel over time zones have been linked to health problems such as depression, irregular menstrual cycles, diabetes, fluctuating melatonin secretion, as well as other issues. Upon arrival to the new destination, the traveler should attempt to regulate his biological clock to the local time zone. Planning ahead will assist the traveler to maintain a healthy balance of the body's internal functions.. The goal of this paper is to understand what jet lag is, how it occurs, and how the internal biological clock can be adjusted to relieve or avoid the symptoms.

Methods
Research articles were obtained from the Touro Library database (tourolib.org), which lead the student to various sites including ProQuest, PubMed, among others. The articles were compiled from an assortment of science journals and experimental studies that discussed the definition of jet lag and its symptoms, the explanation of a body clock and its makeup, with testing and trials to prove the connection between the two. Each article was reviewed intensely, and the findings were subsequently arranged into a comprehensive paper in order to fully describe the term jet lag.

Discussion
Jet lag, also known as circadian dyschronism, is a temporary syndrome characterized by various physiological and psychological effects that occur following flights across several time zones, most likely resulting from circadian misalignment in the body. An assortment of symptoms have been linked to jet lag, including fatigue, coexisting with insomnia, irritability, disorientation, headaches, loss of concentration, gastrointestinal problems, such as: indigestion, loss of appetite, among others. Comparing the studies can be challenging due to the lack of jet lag symptoms. Yet, fatigue, difficulty concentrating, decreased alertness during the day, weakness, and lethargy have been found as consistent effects (Waterhouse et al, 1997).

The word 'circadian' originates in the Latin 'circa dia,' which means 'about a day,' because circadian rhythms do not equal exactly 24 hours, but vary in that range by each organism. These unalterable rhythms are caused by the constant ticking of the body's biological clock in the brain and other organs, and therefore keep many aspects of our body in sync - specifically behavior, metabolism, and physiology (Rosbash, 2003). Jet lag results from these circadian rhythms breaking out of their natural harmony when the body clock is unadjusted from traveling a great distance. Our sleep/wake cycles seem to be the most disturbed by the journey.

Crossing three or more time zones is what generally causes the desynchronization associated with jet lag. The more time zones crossed, the more severe the jet lag will be. Jet lag will not occur from traveling north or south. Interestingly, it will be worse from eastward travel than westward since traveling west extends the day, thereby allowing for easier correction. After a flight east, travelers are not tired during the local nighttime, which is daytime for their unadjusted body clock, but they get tired as the new day dawns, as it is nighttime for their body. After a flight to the west, people are more tired in the new evening at their destination and yet they awaken early. Although this also a challenging transition, the traveler will be able to go about his day and settle in (Waterhouse et al, 2007). In one study, subjects flew between Europe and the United States, over a 6 hour time difference, and those who traveled on a westward flight adjusted back to peak performance in about 2-4 days. Meanwhile, those who traveled eastward required 9 days to recover, because instead of shortening the day by 6 hours, most of the travelers' internal clocks were delayed by 18 hours (Coleman and Kim, 1998).

The body clock is located on the suprachiasmatic nucleus (SCN), which is connected directly to the retina and receives information about light from the eyes, as well as having receptors for melatonin type 2. Without external inputs and rhythmic time prompts, the body clock and its daily rhythms can continue, but with a period not exactly 24 hours, as circadian means

about 24 hours. Regardless of the exact amount of time, for the body clock to function properly it must be adjusted to the solar day via recurring signals in the environment, known as zeitgebers - time-givers.

A zeitgeber's effect on the body clock depends on the time at which it presents; a zeitgeber can produce a phase advance, phase delay, or no phase shift. A phase-curve response is the relationship between the time the zeitgeber is presented and the phase shift. The chief zeitgeber is the light-dark cycle and the secretion of melatonin when a person is asleep at night. The product of one's environment and lifestyle, the exogenous component, together with the body clock, the endogenous component, form a healthy rhythm when they are in sync. However, the span of days after a time zone transition causes the rhythm to falter, because while the exogenous component has changed, the endogenous component has not yet caught up to it. Theoretical statistics evaluate rhythms before and after a time zone transition, showing the loss of synchronization. The external light is being sent through the retina and is telling the body that it is daytime, but the internal body clock still feels as if it is meant to be nighttime, and therefore jet lag reigns. Table 1 advises when exposure or avoidance of bright light will assist in correcting the body clock upon arrival at the new time zone. It is based on the observation that light in the morning of body time will advance the clock, and in the evening of body time will delay it. These times may not coincide with actual day or night, and so wearing sunglasses or seeking a light box that mimics the sun may be necessary to keep the biological clock in its sync (Waterhouse et al, 1997).

Table 1. Use of bright light to adjust body clock on first day after time zone transition:

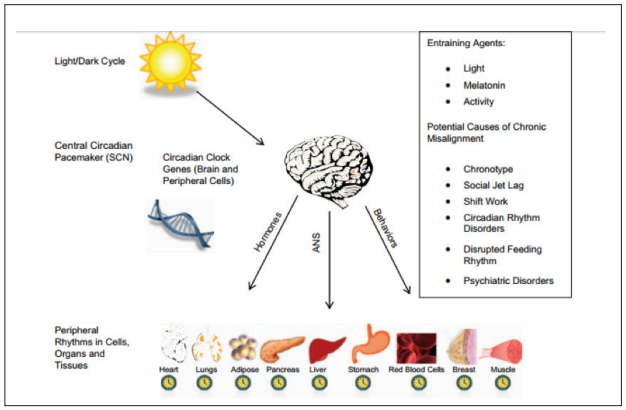
	Bad local times for exposure to bright light	Good local times for exposure to bright light
Time zones to the west		
4 h	0100-0700* (1:00 - 7:00 am)	1800-2400+ (6:00 pm - 12:00 am)
8 h	2100-0300* (9:00 pm - 3:00 am)	1400-2000+ (2:00 - 8:00 pm)
12 h	1700-2300* (5:00 - 11:00 pm)	1000-1600+ (10:00 am - 4:00 pm)
Time zones to the east		
4 h	0200-0800+ (2:00 - 8:00 am)	0900-1500* (9:00 am - 3:00 pm)
8 h	0600-1200+(6:00 am - 12:00 pm)	1300-1900* (1:00 - 7:00 pm)
12 h	@	@

**Will advance to body clock. +Will delay the body clock.*
@Treat as 12-14 h to the west, since the body clock adjusts to delays more easily than to advances. Source: Waterhouse et al, 1997

This concept of an endogenous clock following circadian rhythms has been around for centuries. A French astronomer named Jean Jacques d'Ortous de Mairan conducted a simple experiment on a mimosa plant, which he knew to be a heliotrope. He saw that it was also responsive to the sun by raising its leaves during the day and drooping upon the fall of darkness. De Mairan put a mimosa plant into a cupboard to observe its reactions when kept in the dark. He noticed that the leaves still continued to open and close rhythmically, seemingly by its own internal interpretation of night and day. His clear-cut finding from this was that the plant had an endogenous component with an internal biological clock (Foster and Kreitzman, 2014).

Baron and Reid describe circadian misalignment very clearly: "The term 'circadian misalignment' can describe a variety of circumstances both in the laboratory and natural environment. In the Oxford Dictionary (2010), 'misalignment' refers to 'the incorrect arrangement or position of something in relation to something else.' One of the most common types of misalignment studied is misalignment of the sleep/wake cycle in relation to the biological night. Other types of misalignment include misalignment of feeding rhythms to the sleep/wake or light/dark cycle, or internal misalignment of central and peripheral rhythms. Figure 1 presents a schematic depiction of the organization of the central and peripheral circadian rhythms. This diagram includes zeitgebers (light, melatonin and activity) as well as the potential causes of chronic circadian misalignment.

Figure 1



The timing of melatonin onset under dim light conditions (dim light melatonin onset or DLMO) and the core body temperature minimum are often used as markers of circadian timing. The timing of these circadian markers in relation to sleep/wake behaviors is commonly referred to as phase angle, and has also been used as a measure of circadian alignment. For example, the duration between circadian markers (DLMO or core body temperature minimum) and sleep timing (onset, midpoint or wake time) has been evaluated. Individuals with evening chronotype (preference for later timing of sleep and activity) have been shown in some studies to have a shorter phase angle between circadian markers and sleep, indicating that they sleep and wake earlier in their circadian phase. One of the most significant and immediate consequences of misalignment of the sleep/wake cycle to the biological night is sleep disturbance and/or daytime sleepiness. Insomnia, difficulty waking in the morning and

sleepiness caused by circadian misalignment are typical symptoms that lead patients to seek care in sleep clinics for circadian disorders. However, the physiological and psychological consequences are much broader than sleep/wake disturbance. These include changes in feeding patterns, metabolic function and risk for mood disorders. Other types of disruption include internal misalignment of rhythms, such as timing of central versus peripheral clocks. For example, research in animal models has demonstrated that altering availability of food timing shifts peripheral (e.g. hepatic) but not central rhythms." (Baron and Reid, 2014).

There are many bodily processes controlled by day-night rhythms, such as blood pressure control, feeding behaviors, and lipid and carbohydrate metabolism, among others. The circadian clock, a continuous internal system of transcriptional loops, is what controls this regulatory biological movement, and these looping pathways balance each other out. In example, transcription factors such as Clock and Bmal1 control the assembly of proteins like Per and Cry, which then regulate the assembly of Bmal1, resulting in the levels of Bmal1, Clock, Per and Cry fluctuating regularly. Various input signals, zeitgebers like light and hormones, can change these loops and 'reset the clock' so to speak (Staels, 2006). In another study of two groups of travelers, after a flight with a 6 hour difference one group remained inside a lab for a week while the other group spent time outside. The group that went out adapted to the new time zone in half the time that the indoor group needed. Local time cues, like light-dark cycles and meal times, among others, are what allowed the second group to synchronize quicker (Coleman and Kim, 1998).

The concept of "social jet lag" has been mentioned in current research as well. This is a form of persistent circadian disruption that corresponds to the time difference between routine sleep cycles during the work/school week and free time on weekends. It is a weekly discrepancy in the internal body clock, which can be comparable to journeying across three to four time zones twice a week for someone who wakes up at 6:00 AM during the week and sleeps until 9:00 or 10:00 AM on the weekend. There has been discovered an association in the affirmative between the extent of this "social jet lag" and body mass index, suggesting yet again that the interference of circadian cycles contributes to weight gain, among other possible issues (Summa and Turek, 2015).

It all seems to come down to the body clock and its adjustment. But what does that truly mean? What is this clock? It was first believed that there was a single master clock in the suprachiasmatic nucleus in the brain which regulated numerous biological processes within the body. It was later discovered that there are additional peripheral clock genes in the liver, kidneys, pancreas, heart, and other tissues. We now know that these clocks manage the activity of three to ten percent of genes in various tissues, and in some cases maybe even as much as 50%. If any of these clocks fall out of sync with the master clock, which is possible from sleeping at the wrong times, the frenzy within the body can lead to obesity, diabetes, depression, and many other complicated disorders. The clock gene in the heart sends signals in the early morning to prepare the heart for the harshness of awakening, an explanation as to why so many heart attacks occur at dawn hours. The peripheral clock in the liver functions strongly with the metabolism, contributing to the regulation of normal blood glucose levels over the course of the day to make certain there is a steady flow of energy for brain function and body processes. There is still a system required to combat excess blood glucose due to eating, with insulin the primary hormone in charge of that regulation by starting the removal of glucose from the bloodstream. Insulin is produced in the pancreas, where there is another biological clock that is vital to sustaining normal blood sugar levels. Any disruption of that clock can damage pancreatic function and result in diabetes, a metabolic disorder in which the body produces too little insulin and causes irregularities in blood glucose levels. An experiment was done by Marcheva and Bass in which pancreatic tissue from mice with mutations in circadian clock genes were isolated and examined. They observed that the amount of insulin secreted in response to a glucose stimulation decreased dramatically. They then bred mice with their pancreatic clocks deleted, and these offspring mice developed diabetes early in life and showed a drastic reduction in insulin secretion. Another experiment with mice allowed the scientists to observe a connection with Night-eating Syndrome, a disorder in which people would consume an excessive amount of food at night and develop obesity and/or metabolic syndrome. It was thought that this condition may be resultant of a deficiency in managing the circadian timing of hunger, causing patients a predisposition to weight gain and metabolic misalignment. These examples just provide further evidence for the fact that the multiple clocks throughout the body are required to maintain equilibrium by synchronizing their timing, all based on the leading master clock (Foster and Kreitzman(2014),. any disruption causes a multitude of health issues, including heart and stomach issues, neurological diseases and psychiatric illnesses among others (Summa and Turek, 2015). Due to the fact that circadian rhythms don't follow an exact 24 hour schedule, they have the ability to 'reset,' or shift, the phase in response to environmental time cues. The central clock in the SCN is mainly reset by light, owing to its direct pathway to the retina. The peripheral clocks, as mentioned above, are controlled by various signals from the SCN clock. For example, melatonin synthesis in the pineal gland, mentioned in depth below, is mainly affected by the light-reliant SCN clock, with nocturnal secretion (Wu et al, 2011).

The core body temperature (CBT) and melatonin secretion, as mentioned above, both play key roles in the body's natural ability to follow a healthy sleep/wake cycle. Professor Jim Waterhouse wrote in the British medical journal The Lancet that "the ease of getting to sleep and staying asleep depends not only on the previous wake time, but also on associations with the circadian rhythm of core temperature. Sleep is easiest to initiate when core temperature is falling rapidly or is at its lowest and most difficult when body temperature is rising rapidly or is high. Waking is the opposite of sleep initiation, because it happens when core temperature is rising or is high." (Waterhouse et al, 2007). In healthy people, core temperature will reach a low point between 3:00 and 5:00 AM, during which time most people are asleep, and steadily begins to increase at about 6:00 AM, reaching a climax at midday when most people are awake (Brody, 2007). CBT measurement is a standard physiological method for studying circadian rhythms, because its rhythm has been determined to be the most reliable endogenous circadian oscillator. Hamilos et al performed an experiment of CBT in patients with Chronic Fatigue Syndrome and documented that "the rhythm of CBT should be a valid benchmark for which to compare other circadian rhythms for desynchronizations" (Hamilos et al, 2001). There has also been a parallel seen between core body temperature and mental performance - performance rises with rising temperature throughout the early hours of the day, allowing for peak performance, and as it gets later, tasks that require central processing and short term memory decline due to the falling evening temperatures. There have also been changes in mood and performance observed under different groupings of the time of day and the amount of time awake, with more deterioration in mental performance with sleep loss (Waterhouse et al, 2007). Melatonin, produced mainly by the pineal gland and then secreted into the bloodstream, is a hormone that is secreted mostly at nighttime, when it is dark, seeming to stimulate the body to sleep. When there is trouble sleeping, oral ingestion of the chronobiotic drug melatonin can assist in inducing sleep. A phase response curve, which describes the relationship between a stimulus and a response, is the resulting reaction to melatonin ingestion, shows that taking it in the morning will cause a delay in circadian rhythms, and taking it at night will cause an advance in them. This phase response curve is about 12 hours out of phase, with the phase response curve of light, which would cause a phase advance in the morning and a phase delay at night (Brown et al, 2009). It seems that the circadian clock regulates the timing of melatonin secretion, although darkness is necessary as well because light will inhibit the secretion. After a long flight, the SCN-generated night-time cue will be set to the home nighttime before the clock adjusts, and thus if a traveler will enter a dimly lit room when it is nighttime at their home, melatonin secretion can occur and cause sleepiness (Sack, 2009). But taking melatonin in the evening and remaining in somewhat darkness can aid in falling asleep, which can additionally help acclimate the body to the local sleep/wake schedule. A word of warning for female travelers: interaction of melatonin and menstrual hormones can lead to a stop of the menstrual cycle. Female athletes can even be at a health risk from this, since many of them already have stalled cycles due to "overtraining" (Reilly et al, 1996).

In reference to athletes, there was a study done on elite athletes that recorded sleep loss and mood changes after long distance flights. Athletes that traveled more than five time zones to the west showed shifts in performance, including grip strength and back and leg endurance, which were in phase with their unadjusted body clocks. A similar result was seen in a group of Olympic athletes who journeyed over ten time zones to the east. It was even observed that for several days after an east or west flight over six to eight time zones, the athletes had distorted grip strength and weak performances in training sessions (Waterhouse et al, 2007).

Now that is it understood what jet lag is and the extent of its affects, counting the misalignment of the internal body clock, what can be done to alleviate the dyschronism symptoms? There are a number of ways to attempt to prevent jet lag, as well as suggestions for adjusting the body clock to combat the desynchronization once it emerges.

Trip planning can play a major part in prevention of jet lag. If possible and extra stress would not be caused, stopovers should be included in the flight plan. Breaking up the number of time zones being crossed by having a stopover can greatly diminish the likelihood of jet lag. Keeping hydrated is also necessary to counteract the dry air of the flight cabin (Waterhouse et al, 1997). Also, unless it is nighttime at the destination, sleep should be avoided during the flight, as it can anchor the rhythms to the home time zone. Staying on home time by remaining concurrent with one's circadian rhythms won't allow the internal clock to be disrupted; however it seems to make sense only for short trips. Supposedly, this approach was employed by President Lyndon B. Johnson when he met with Vietnamese leaders. This may be difficult, but if possible it can help avoid majority of jet lag symptoms. Conversely, coordinating with the local time of the planned travel location, from the get go can adjust the clock quicker and avert negative effects of the journey. This can be done by eating meals by the local schedule, going out in the daytime, going to bed at a reasonable time for the local environment, etc. A sleeping pill may be required the first night to assist in an easy transition, but hopefully after that the adjustment will continue smoothly (Coleman and Kim, 1998). Melatonin, which we have seen to be a somewhat 'darkness signal,' can be helpful in aiding clock acclimation, since it has the ability to induce sleep

when necessary. However, the dosage is unclear, and it would seem that the timing of administration is more relevant than the actual amount. In addition, a boost in caffeine consumption may combat daytime tiredness; however caution is required so as not to aggravate insomnia. Exercise has been proposed to alleviate jet lag symptoms, but it has not been studied in clinical trials (Sack, 2010).

Conclusion

From the research obtained, it is evident that jet lag has no exact, scientific definition, but that it is a lack of synchronization of the body's internal clocks and the external environment. It can take a few days following flight travel over several time zones for the body's circadian rhythms to realign. Repeated travel over an extended period of time can have the same long term consequences as those of shift workers - extended desynchrony with persisting symptoms that exacerbate with age. Resetting the clock requires zeitgebers, the most integral of which by far is the fluctuation of light and darkness. Adjusting is the primary goal to adequately offset jet lag. To do this, travelers should set up a specific itinerary of predetermined sleep and activity times, meal schedules and exposure to light versus remaining in dim lighting. The quicker the circadian system acclimates to the new time zone, the shorter the symptomatic period of jet lag will be. Assistance from the hormone melatonin may be necessary, a chronobiotic which adjusts the timing of the central body clock. Recently the drug called modafinil, meant to resist the tendency to fall asleep, has been registered for use in shift work sleep disorder, and although no records deal with its use in jet lag, the two have similarities and so perhaps this drug would aid in preserving daytime alertness (Arendt, 2009). Each individual must know his body and research the best methods for him to avoid or resolve jet lag.

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Is Deep Brain Stimulation a Viable Treatment for Parkinson's Disease?

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Abstract
Treatment options for those suffering from Parkinson's Disease are as diverse as its symptoms. With the advent of modern technology there are new and innovative treatments that are becoming available, such as Deep Brain Stimulation (DBS). Prior to exploring treatment options one must t understand the various causes of the disease. Treatment of the various motor and non-motor symptoms can include a combination of medication and surgical therapies. Among surgical interventions DBS is the treatment of choice. It has the fewest side effects and provides the greatest symptomatic relief.

Introduction
Parkinson's disease was first formally written about in 1817, by a prestigious English doctor, James Parkinson. He published a short pamphlet about the disease titled, "An Essay on Shaking Palsy". In the beginning of his paper he describes the symptoms of the disease as "involuntary tremulous motion, with lessened muscular power parts not in action and even when supported; with a propensity to bend, with trunk forwards and to pass from a walking to running pace; the senses and intellect being uninjured". (Elis, 2013) Parkinson was not the one who discovered the disease as there are sources referencing the disease dating back to the ancient Egypt and Mesopotamia. (Raudino, 2012) As time, technology, and science advanced, scientists and medical professionals were able to further understand the causes and symptoms of the disease. Treatment options for those suffering from Parkinson's are as diverse as its symptoms. With the advent of modern technology, there are new and innovative treatments that are becoming available. One of these new treatments is Deep Brain Stimulation (DBS). As with all new treatments, one has to assess its effectiveness, its side effects and how it compares with old methods of treatment. This paper will address these issues in relation to DBS and its treatment for Parkinson's disease.

Methods
This systematic review was composed after reviewing relevant journal articles about the subject matter. Articles used discussed causes of the disease, current treatment methods and alternative surgical interventions. Articles were obtained using search engines like Proquest and MedLine and articles from published journals.

Background
Prior to exploring treatment options, one must thoroughly understand the disease and its causes. Parkinson's has many different causes, some rooted in genetics, others in chemical imbalances. The genetic cause for this disease has been shown to include five different genes. (Pchelina, et. al. 2014) The first of these genetic proteins is α-synuclein, a neuronal protein which serves an unknown function. These proteins aggregate in the Lewy bodies, causing researchers to conclude that they play a major role in the protein composition of the Lewy bodies. Although mutations within α-synuclein are relatively rare; even patients with sporadic PD seem to exhibit protein aggregates in the Lewy bodies, leading researchers to believe that α-synuclein plays a major role in both genetic and sporadic forms of PD. Several independent family studies done on groups of people with PD show that α-synuclein mutations are a rare cause of PD. The largest analyses done on α-synuclein and PD indicated that allele length variability is associated with an increased risk for PD. (Pihlstrøm, Toft, 2011).

Mutations in the gene encoding parkin, a 465 amino acid chain protein is one of the causes of autosomal recessive PD. Parkin mutations in familial PD suggest that the ubiquitin-protease system has an important role in the disease. (Pickrel, Youle, 2015)

Mitochondrial abnormalities are also thought to cause the disease. These abnormalities lead to a failure of cellular energy production and increased free radicals. The newest gene discovered which confirmed these hypotheses is the PINK1 gene. (Pickrel, Youle, 2015).

Another gene associated with Parkinson's is the DJ-1 protein. Although its exact function is unknown, it is thought to help with proper protein folding. DJ-1 protein may be linked to abnormalities in the protein control system. Other studies show that DJ-1 mutations may lead to increased levels of oxidative stress. (Pchelina, et. al. 2014).

PD is not caused by genetic factors, although genetic factors increase one's risk for developing the disorder. Sporadic PD is when there are multiple genetic alterations which increase the risk of developing PD, but they do not cause PD. One such risk factor is prevalent among Ashkenazi Jews carrying the GBA gene mutation. Those who do have a GBA mutation are seven times more likely to develop the disease when compared to healthy control groups. (Feany, 2004).

The most common cause for PD is from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Motor symptoms appear when approximately 50%–60% of these neurons degenerate, causing a 70%–80% depletion of dopamine levels in the dorsal striatum. (Pickrel, Youle, 2015).

Discussion

Parkinson's disease is associated with a wide variety of symptoms, both motor and non-motor. The four cardinal motor symptoms associated with the disease are; Rest tremor, bradykinesia, rigidity and loss of postural reflexes. The rest tremors seen in PD are usually found in the distal extremities, but they can also be seen in the lips, chin and jaw. Bradykinesia can be described as slow movement and it is a hallmark of basal ganglia disorders. Although Parkinson's patients seem to have impaired motor programes, when provided an external stimulus many of them exhibit normal movement. This phenomenon is known as kinesia paradoxica. Rigidity is often one of the first symptoms of the disease, but it is often misdiagnosed as arthritis or bursitis. Neck and trunk rigidity leads to postural deformities. Additional postural issues include, extreme neck flexion, trunk flexion and/or scoliosis. (Jankovic, 2008).

Non-motor symptoms associated with the disease include; autonomic dysfunction, cognitive abnormalities and sleep issues, among others. Autonomic dysfunction features can include, orthostatic hypotension, sweating dysfunction, sphincter dysfunction and erectile dysfunction. Neurocognitive dysfunction was tested on 537 PD patients using the Neuropsychiatric Inventory. Eighty-nine per cent of patients exhibited symptoms for at least one of the Neuropsychiatric Inventory. The results of the Neuropsychiatric Inventory were as follows: Fifty-eight percent of patients showed symptoms of depression, 54% showed apathy symptoms, 49% exhibited anxiety symptoms and 44% showed signs of hallucinations. (Jankovic, 2008).

Sleep abnormalities often seen in PD were thought to be a side effect of the medications patients were given. More recently, some physicians are beginning to view it as a part of the disease. Researchers began looking at the hypothalamic hypocretin system, a system that regulates sleep/wake cycles, to see how this system differs in Parkinson's patients. They have found that patients with PD had almost 50% fewer hypocretin neurons than the healthy control group. (Fronczek, et. al. 2007).

There are no known preventions that will stop PD, however there are some substances which have an inverse relationship with PD. Caffeine has been found to reduce one's risk for PD. MPTP, one of the toxins associated with PD was injected onto the striatum of mice. This led to an 85% decrease in dopamine levels in the area. However when the mice were given moderate amounts of caffeine, there was only a 60% decrease in dopamine levels. This study leads to the conclusion that caffeine may help mitigate some of the symptoms of PD, however this relationship is not causal. (Holden, 2001).

Nicotine, found in cigarettes, decreases the risk for developing the disease. It also inhibits the MPTP pathway, ensuring that more dopamine receptors remain intact. Additionally, nicotine has been shown to reduce the activity of Monoamine Oxidase, which causes the oxidation of dopamine. Coffee drinkers have a 30% decreased risk of developing PD, while smokers have greater than a 30% reduction risk. (Martyn, Gale, 2003).

There is no known cure for Parkinson's Disease, however, there are a full array of medications and treatments to slow progression of the disease and to relieve some of its symptoms, both motor and non-motor. Drug therapy is the mainstay of treatment. Surgical intervention, such as deep brain stimulation is recommended in severe cases. Physiotherapy, speech therapy and occupational therapy have all been shown to help Parkinson's patients as well, particularly in advanced stages of the disease. (Rajput, Rajput, 2006).

The three main drugs being given to Parkinson's patients are, Levodopa, monoamine oxidase B (MAO-B) inhibitors and dopamine agonists (DAs). MAO-B inhibitors are given to patients with mild motor symptoms, usually in the early stages of the disease. When administered in conjunction with Rasagiline, patients dropped 3-4 points on the Unified Parkinson's Disease Rating Scale. When taken with other drugs, such as SSRIs, some patients began exhibiting serotonin syndrome symptoms. Dopamine Antagonists act directly on striatal dopamine receptors and have a greater effect on motor symptoms than MAO-B inhibitors. DAs are often prescribed as a first order treatment in patients who are young at age of onset. Side effects of DAs include; nausea, headaches, sleep attacks and Impulsive Control Disorder, among others. DAs are usually supplemented with levodopa in the later stages of PD. Levodopa, also known as the gold standard in PD treatment, is the first order treatment given to elderly Parkinson's patients. Levodopa is most successful at eliminating PD motor symptoms, however, it has many side effects. (Sprenger, Poewe, 2013).

Some side effects of levodopa include nausea and dyskinesia. Being on the drug long-term causes up to ⅓ of patients to develop dyskinesias. Between doses patients can experience painful muscle spasms and the reemergence of other PD symptoms. The National Health Service of England recommends keeping the dose of levodopa as low as possible to prevent motor complications. The Movement Disorder Society recommends taking levodopa with a DA to prevent long-term motor side effects. (LeWitt, 2008).

The most common surgical intervention for Parkinson's patients is Deep Brain Stimulation (DBS). Prior to the discovery of this treatment, surgical treatment for movement disorders involved

ablations. More specifically pallidotomy, thalamotomy, and more recently, subthalamatomies. Thalamotomy relieved many of the tremors and dopa-induced dyskinesia, however it often left patients with speech and cognitive deficiencies. Unilateral pallidotomy have been shown to significantly improve contralateral tremor, rigidity, bradykinesia, and dyskinesias. Side effect of pallidotomy include, weight gain, reduction in verbal fluency, and a higher incidence of recurrent depression in patients who have a prior history of the disease. Appropriate anatomical and careful physiological screening prior to placement of the lesions may decrease incidences of cognitive or neuropsychological damage. Subthalamatomies have been experimented with as a cheaper alternative to DBS. Preliminary findings indicate that there are fewer cognitive and speech side effects than in pallidotomy and thalamotomy. However postoperative chorea occurred in more than half of patients who underwent this procedure. This remains a concern with subthalamotomy. Additional research is necessary to ascertain whether this treatment is a viable alternative to DBS. (Walter, Vitek, 2004).

Deep brain stimulation is a highly effective surgical therapy for PD and other movement disorders (fig. 1). To qualify as a

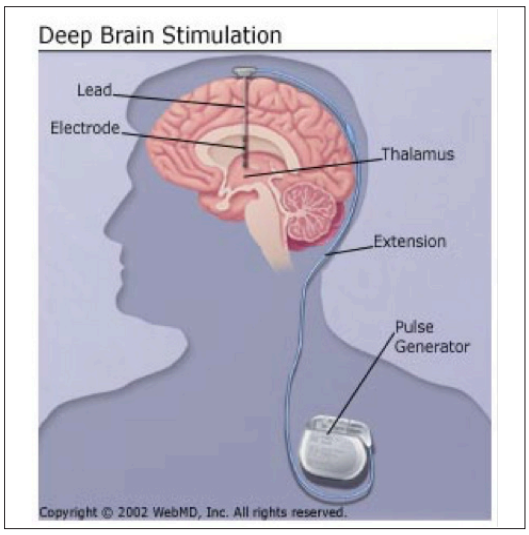

Figure 1. This drawing depicts the components of DBS

candidate for surgery, patients must be first undergo testing by a competent neuropsychologist and neurologist. A neurologist will review the patient's medical history and ensure that reasonable medical intervention has been applied. The patient will then meet with a neuropsychologist to discuss goals and expectations of the surgery. If a patient has unrealistic expectations, they are no longer an appropriate candidate for surgery. (Benabid, et. al. 2009).

Once a patient is found to be a viable candidate for surgery, they undergo basic preliminary testing. The main test involves a patient skipping a dose of levodopa at night. The patient then goes to the neurologist so he can assess the patient's symptoms without medication. Afterwards, the patient then takes his dose of levodopa, so the neurologist can see how it affects the patient's symptoms. Symptoms that do not improve with medication, usually will not improve with DBS either, hence the significance of this test (Farris, Giroux, 2011).

Once cleared for surgery the patient will undergo the procedure. The procedure involves the implantation of electrodes in the subthalamic nucleus, two lead coated wires and a neurostimulator, which is technologically similar to a pacemaker. (Farris, Giroux, 2011) Prior to the implantation of the electrodes, patients undergo stereotactic imaging to locate the subthalamic nucleus. Surgery for implantation of electrode and lead is usually done under local anesthesia, so the surgeon can determine the best location for the electrode within the subthalamic nucleus. Using microelectrodes, the surgeon will stimulate different areas to figure out where the patient will have the greatest symptomatic relief. Once the best tract is found the surgeon will replace the microelectrodes with the lead through various means. After this is done, the patient is closed up and the second part of the procedure takes place at a later date. (Benabid, et. al. 2009).

The second part of the procedure involves placing the neurostimulator in the subcutaneous pouch in the subclavicular area. This is done under general anesthesia. Once the neurostimulator is inserted and connected to the lead, the programming of the stimulator begins. Programming of the neurostimulator will usually begin a week after surgery. Voltage is increased gradually, while the patient is tested by the neurologist. Once an appropriate setting has been found for the patient, PD medication dosages are lowered to prevent dyskinesias. (Benabid, et. al. 2009).

Deep brain stimulation offers many advantages to ablations, although it is not a risk-free procedure. When performing ablations, the lesions placed on the brain are permanent and irreversible. With DBS, the neurostimulator can be turned off, or reprogrammed in case of complications. In cases of adverse effects, the neurostimulator can even be explanted. Some of the adverse effects of the implantation of the electrodes and leads in DBS include hemorrhaging, seizures and/or infection. Although these side effects are relatively rare (4%), it is important to know about them. With regard to the neurostimulator, battery depletion is an issue. Batteries have a median life of four years, but in patients with high voltage stimulation, they may last only one year. (Grill, 2005).

Studies done on the short and long term effects of the procedure on Parkinson symptoms show significant improvement in many of them. Following DBS of the subthalamic nucleus, levodopa dosages decreased on average 55.9%. Rigidity and bradykinesia symptoms decreased by 63% and 52% respectively after twelve months. Parkinsonian tremors decreased by 61% following subthalamic nucleus DBS. However, stimulation targeting the dorsal border of the subthalamic nucleus produced an 86% improvement in tremor symptoms. Gait and balance issues caused by PD are less likely to be helped by DBS. In a one year follow up study, patients were found to have the same gait and balance scores as their preoperative scores with drug treatment. (Fasano, et. Al. 2012).

One must also examine how DBS affects the non-motor symptoms of PD. As mentioned above, there are numerous non-motor symptoms which are part and parcel of the disease and DBS effects these symptoms as well in various different ways. Cognitively, DBS is safer than all other surgical interventions. The vast majority of studies indicate that patients have a decline in phonological and semantic verbal fluency tasks. This decline is noticeable shortly after surgery and may be the result of microlesions to the cortical-basal ganglia circuit, which is involved with word retrieval. An additional reason for this decline may be due to the withdrawal of dopaminergic drugs. Apathy seems to worsen following the procedure. This may be caused by the inactivation of dopaminergic receptors in the mesocortical and mesolimbic pathways after DBS. Many studies show a decrease in anxiety following DBS, this may be caused by the relief of motor symptoms. DBS seems to have little effect on autonomic symptoms of PD. Further research is necessary to better understand this. Sleep symptoms associated with PD, seemed to show improvement after DBS. This may be due to decreased bradykinesia and increased bladder capacity. (Fasano, et. Al. 2011).

Conclusion

Deep Brain Stimulation has been proven to be the most effective surgical intervention for those suffering from Parkinson's Disease. As with most medical issues, invasive surgery is not the first choice for treatment, but if necessary DBS is the gold standard in invasive treatments for PD. It provides relief to many of the motor symptoms associated with the disorder. Although success varies, it has developed into the most viable surgical treatment for PD.

List of Acronyms:

DBS	Deep brain stimulation
PD	Parkinson's Disease
MAO-B	Monoamine Oxidase B
DA	Dopamine Agonists

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Treatment Options for Skin and Soft Tissue Infections Caused by Methicillin-Resistant Staphylococcus Aureus

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Abstract

Staphylococcus aureus is a pathogen responsible for common skin infections, such as impetigo, cellulitis, folliculitis, and abscess and it is the most common cause for skin and soft tissue infections (SSTI). Humans are carriers of this microorganism and are responsible for facilitating its spread around the world. Over time it has developed resistance to multiple antibiotics, such as penicillin and methicillin, which has made S. aureus a persistent problem in the healthcare world today. Its methicillin resistance has given it the more commonly known name methicillin-resistant S. aureus (MRSA). MRSA was initially discovered solely in the healthcare environment and thus became known as healthcare-associated MRSA (HA-MRSA). With time, MRSA began to affect people with no previous exposure to a healthcare facility and was therefore called community-associated MRSA (CA-MRSA). Incision and drainage are often the first and best treatment option used against SSTI caused by MRSA. Antimicrobial therapy is also employed. Vancomycin is currently the leading drug, however other antibiotics such as linezolid, daptomycin, clindamycin, and trimethoprim-sulfamethoxazole are sometimes used due to a growing number of reports of vancomycin resistance. This paper examines the different options for the treatment of SSTI caused by MRSA by comparing different antibiotics, their mechanisms of action and resistance, dosages and administration, as well as adverse effects. No definite conclusion can be made as to the best or most effective treatment option for MRSA. Rather, each reporting of SSTI caused by MRSA needs to be evaluated on a case by case basis to determine the most appropriate choice of therapy. The various aspects of MRSA and therapy choices employed to combat it have been researched by the author using the Touro database, Google Scholar, and PubMed for various links to journals and articles that these databases provide.

Introduction

Staphylococcus aureus is a gram positive pathogen and the most common cause of skin and soft-tissue infection (SSTI) in the world. A study done in 2008-2009, spanning more than nineteen European countries and more than 3000 cases of complicated skin and soft tissue infections (cSSTI), found that about one third of these cases were due to S. aureus (Russo, et al., 2016). S. aureus is responsible for common skin infections such as impetigo, cellulitis, folliculitis, carbuncles, abscess, pyomyositis, and necrotizing fasciitis, as well as more deep-rooted infections that lead to blood stream infections, nosocomial pneumonia, and infection of wounds and surgical sites (Popovich, et al., 2008). S. aureus is so prevalent that about 25% of humans are consistent carriers of this microorganism, while 50% are observably intermittent carriers. Colonies of S. aureus can be found in the anterior nares and other areas of skin throughout the body.

Penicillin was the first antibiotic used to treat S. aureus infections; however, within a few years S. aureus developed resistance to this "miracle drug". A specific strain of S. aureus started to produce an enzyme capable of destroying penicillin, called penicillinase. The plasmid that produced the penicillinase quickly spread among the different strains of S. aureus. To combat this new development, methicillin, which is a semisynthetic β-lactamase-resistant penicillin, was created in 1959. After only two short years, the first case of methicillin-resistant staphylococcus aureus (MRSA) was reported. This case of bacterial resistance was the result of a more complex mechanism than found in the penicillin resistant strain of S. aureus. Methicillin works by blocking the protein penicillin binding protein (PBP), found in S. aureus that is associated with the construction and maintenance of the cell wall. Instead of using these PBP proteins, resistant strains of S. aureus acquired a new protein, PBP2a, which has the same function as PBP but is not susceptible to methicillin. PBP2a is encoded by the gene mecA, located on the chromosome, unlike the penicillinase gene which is found on the plasmid. This mecA gene is a distinguishing characteristic of MRSA and the presence of PBP2a means that S. aureus is resistant not only to methicillin, but to all β-lactam antibiotics, including synthetic penicillin, cephalosporin and carbapenem (Mastofsky, et al, 2011; Pantosti, Veniti, 2009; Popovich, Hota, 2008).

HA-MRSA

MRSA has become a worldwide problem and is common throughout hospitals as well as smaller healthcare facilities. Since its origins in the 1960s, the spread of healthcare-associated MRSA has become a public health concern (Mastofsky et al, 2011; Pantosti, Veniti, 2009). The common strains of MRSA found today originated from a few clones that developed independently of each other (Gardam, 2000). MRSA is spread by direct contact and most often in a healthcare setting where contamination can spread through the hands of healthcare providers (Okado et al, 2016). This spread of infection has led to the definition of HA-MRSA which is an annotation for healthcare-associated MRSA or for hospital-acquired MRSA. Patients with compromised immune system or those that have had extended hospital stays are more susceptible to the contraction of MRSA. In addition, use of antibiotics and undergoing surgery are factors that can contribute to MRSA infection. Once a patient has acquired HA-MRSA it is extremely difficult to eradicate and

the person can be a carrier for an extended period of time (Gardam 2000). A patient can be a carrier for HA-MRSA for longer than six months, long after his discharge from the hospital (Pantosti, Veniti, 2009).

In order to reduce the prevalence of HA-MRSA and to stop the spread of this nosocomial pathogen, hospitals take on two different approaches. The first recommendation is prevention based and includes barrier precautions, hand washing and environmental cleaning for MRSA patients. The second approach is an implementation of an antibiotic stewardship program. The purpose of the program is to control antibiotic prescribing in order to reduce the adverse effects of antibiotics, such as drug resistance and more specifically decreasing the spread of infection caused by multi-drug resistant bacteria. A 10-year program implemented in Saint-Joseph Hospital in Paris, France focused on applying a combination of these two aspects to decrease the prevalence of HA-MRSA found in their hospital. The program, implemented from 2000-2009, showed an 84% decrease in HA-MRSA colonization throughout the hospital. Researchers found that there was an increase in the use of alcohol based hand rubs (ABHR) from 6.8 L to 27.5 L per 1000 patient-days. Additionally, antibiotic use, measured with the Defined Daily Dose (DDD) per 1000 patient-days decreased by 31%. The implementation of this program and its results were significant in that it was conducted in a region known to be endemic for MRSA. From 2000-2009, France had MRSA rates greater than 25%, an alarmingly high rate (Chalfine et al, 2012).

CA-MRSA

In the 1990s a new strain of MRSA emerged. Known as community-associated methicillin resistant staphylococcus aureus (CA-MRSA) This new epidemiology has the capability of causing infections in otherwise healthy individuals who have had no previous exposure to a healthcare setting. These reported cases of CA-MRSA have statistically worse clinical outcomes than those of HA-MRSA (Mostofsky et al, 2011; Pantosti, Veniti, 2009). CA-MRSA is a more infectious form of S. aureus than HA-MRSA and can carry the genes that encode Panton-Valentine leucocidin (PVL) which is "associated with tissue necrosis and a greater severity of disease" (Russo et al, 2016). However, in most cases CA-MRSA causes skin and soft tissue infections (SSTI) such as furuncles, abscesses, impetigo, and cellulitis (Pantosi, Veniti, 2009). CA-MRSA is the most common cause for patients presenting with SSTI in emergency departments around the United States (Albrecht et al., 2015),

CA-MRSA has a number of distinguishing characteristics that differentiate it from HA-MRSA. Firstly, CA-MRSA, unlike HA-MRSA, is vulnerable to most non-β-lactam antibiotics and contains what is known as SCCmec element of type IV and type V. SCCmec stands for staphylococcal cassette chromosome mec and is the mobile genetic element for S. aureus that is responsible for its resistance to methicillin and other β-lactam antibiotics. This SCCmec element is encoded by the gene mecA and is divided into subtypes I-VIII (The Working Group etc., 2009). Conversely, HA-MRSA is multidrug-resistant to non-β-lactam antibiotics and contains SCCmec type I, II, III. A second difference, as mentioned above, is that CA-MRSA contains PVL, which is a strong virulence factor (Mostofsky et al, 2011; Pantosti, Veniti, 2009).

With the growing number of MRSA cases reported each year, the clearly defined lines between HA-MRSA and CA-MRSA are being blurred. Asymptomatic colonization of MRSA can persist for years and a HA-MRSA can be easily misconstrued for CA-MRSA. Cases have been reported of community-onset HA-MRSA, as well as nosocomial CA-MRSA infections (Mostofsky et al, 2011). The definitions abound as to how to clearly classify and differentiate between HA-MRSA and CA-MRSA. There are many uncertainties as to how to define prior hospitalization and length of time since hospitalization. This question is crucial as it determines if one is still a carrier of MRSA or not. The common definition of prior hospitalization is "hospitalization within six months to one year of current admission". Sometimes, MRSA acquired from long term care facilities and nursing homes is not considered healthcare associated, but rather CA-MRSA, skewing the statistics (Gardam,2009). One way to differentiate between the two strains it to test the isolate's susceptibility to non-β-lactam antibiotics, as this is one key difference between HA-MRSA and CA-MRSA (Popovich et al, 2008).

There are many different treatment options available to treat MRSA infections, including incision and drainage, oral antibiotics, parenteral antibiotics, and topical therapies. More than one mode of therapy can be used. Treatment options can be administered on both an inpatient and outpatient basis (Popovich et al, 2008).

The purpose of this paper is to review some of the current and leading parenteral and oral treatment options used to best treat SSTIs caused by both HA-MRSA and CA-MRSA. It will explore and compare different antibiotics, their uses, benefits and side effects in an effort to understand the most effective way to treat MRSA.

Methods

This study was performed through the analyzation of various original and peer reviewed articles which were accessed using databases such as the Touro Database, PubMed, and Google Scholar. The research collected in this study was used to understand MRSA, its effects and the best way to treat it when found as the cause of SSTIs.

Discussion

There are many different treatment options for MRSA that are currently in use or in development. Some are administered orally or parenterally, while others are administered topically.

Incision and Drainage

The most important part in the treatment of complicated SSTI (cSSTI) is the incision and drainage of the infection site. This procedure is common in the case of furuncle, soft tissue abscesses and other purulent SSTI. An estimated 80% of patients presenting in the emergency departments with acute, purulent SSTI require drainage. Most patients with infections caused by CA-MRSA are cured via incision and drainage alone and do not require any antibiotics. Thus, incision and drainage, where appropriate, is the first step to treating cSSTI caused by MRSA (Ruhe et al., 2007; Stryjewski et al., 2008).

However, there is still much to learn regarding the effectiveness of incision and drainage of infection sites. A study done on children with SSTI, with an infected site of greater than 5 cm, showed that pediatric patients are more likely to experience failure of incision and drainage alone and antibiotic therapy is usually necessary. Additionally, patients with other risk factors such as systemic illness, comorbidities, as well as multiple areas of SSTI are more likely to need antibiotic therapy in addition to or in place of incision and drainage (Popovich et al., 2008).

Vancomycin

Vancomycin is the standard drug used today to treat SSTI caused by MRSA (Stryjewski et al., 2008). Since its introduction in 1958, it has been used to combat gram positive bacteria with great success. Vancomycin is administered parenterally on an inpatient basis. Vancomycin, a glycopeptide, works against MRSA by inhibiting the bacteria's cell wall synthesis. It interacts with a peptide precursor of the peptidoglycan at an important site of attachment and thereby inhibits peptidoglycan polymerase and transpeptidation mechanisms. Penicillin is also bactericidal by hindering biosynthesis of cell wall; however, vancomycin inhibits an earlier stage of the peptidoglycan synthesis thereby eliminating cross-resistance (Wilhelm et al., 1999).

The clinical practice guidelines set by the Infectious Diseases Society of America states that the recommended dose for the average adult with normal renal function for intravenous vancomycin is 15-20mg/kg/dose every 8-12 hours. Dosage amount should not exceed 2000 mg regardless of a patient's weight. For more serious cases, in which a patient is systemically ill and has a suspected MRSA infection, the dosage level may be elevated to 25-30 mg/kg/dose. However, in such a case, one must be cautious for red man syndrome, a reaction associated with a high dosage of vancomycin that can cause anaphylaxis. As a precaution, infusion time can be lengthened to two hours and an antihistamine can be administered prior to the loading dose. Antibiotics can be administered on an empirical basis until culture results are obtained (Liu et al., 2011). For patients who have problems with renal function, careful monitoring should be ensured while administering vancomycin, as vancomycin can sometimes be associated with nephrotoxicity (Wilhelm et al., 1999).

As with the development of resistance to β-lactam antibiotics which led to the new subset of S. aureus, specifically MRSA, new reports have come out of vancomycin resistance. The levels of resistance range from intermediate susceptibility to full resistance of vancomycin (Pantosti et al., 2009). These new strains of S. aureus resistant to vancomycin are known as vancomycin-intermediate S. aureus (VISA) and as vancomycin-resistant S. aureus (VRSA) (Popovich et al., 2008). There is even a third category of vancomycin resistant bacteria known as hetero-VISA, that seems to be vancomycin susceptible when routinely tested, but contain a minority of cells that have intermediate vancomycin susceptibility. Upon exposure to vancomycin, these hetero-VISA bacteria can multiply in number and spread (Pantosti et al., 2009).

VRSA developed from a prolonged use of vancomycin in response to chronic MRSA infection. Over time, the bacteria changed from MRSA to VRSA by the plasmid exchange of vanA, the gene for vancomycin resistance, possibly from a co-infecting vancomycin-resistant enterococcus (Pantosti et al., 2009). A minimum inhibitory concentration (MIC) to vancomycin has been observed nationwide in MRSA isolates. This phenomenon, known as the MIC creep, has led to the lowering of the MIC breakpoint for vancomycin in 2006 to ≤ 2 μg/ml for susceptible, 4-8 μg/ml for intermediate, and ≥16 μg/ml for resistant. As a result, higher dosage amounts are being recommended for therapy, yet there is no substantial data to prove its efficacy and a higher dosage may cause greater toxicity, especially nephrotoxicity (Popovich et al., 2008).

S. aureus that displays a decrease in vancomycin susceptibility generally has phenotypic features that are different than the strains of both original S. aureus and MRSA. The main phenotypic change of vancomycin-susceptible S. aureus (VSSA) to cause resistance is a general thickening of the cell wall. Involved in this alteration of cell wall structure is an overexpression of PBP2 and PBP2a, increased level of abnormal muropeptide protein, increased amount of D-alanyl-D-alanine residue, and reduction of peptidoglycan cross-linkage (Sirichoat et al., 2016).

Linezolid

Many test results have shown Linezolid to be a comparable drug, in terms of results, to vancomycin. Linezolid is an oxazolidinone antibiotic that was first discovered in the 1990s and approved

for use in standard medicine in 2000 (Dumitrescu, Lina., 2014). Linezolid is the first antibiotic of this class to be brought to market. Oxazolidinone represents a new systemic antimicrobial class. Linezolid works by blocking protein function in the cell. It binds to the 50s subunit of the pathogenic cell which prevents it from complexing with the 30s subunit, mRNA, initiation factors, and formylmethionyl-tRNA. This inhibition prevents the formation of the protein initiation complex, preventing the translation step of protein synthesis. Other drugs that are classified as protein synthesis inhibitors, such as macrolides and tetracyclines, have different mechanisms, as each allows the process of mRNA translation to begin, while stopping peptide elongation. This mode of action of linezolid seems to work particularly well against staphylococcal infections. Additionally, its target site is unique and does not interfere with the mechanism for other protein synthesis inhibitors. Linezolid is bacteriostatic (Livermore, 2003).

Reported cases of linezolid resistance have been rare and a number of reasons for the low level of resistance have been proposed. First, linezolid is an entirely synthetic compound, so it is unlikely that naturally occurring mechanisms of resistance, such as those found in antibiotic producing organisms will be employed. Second, oxazolidinones inhibit ribosomal protein synthesis while other antibiotics of similar ribosomal protein synthesis mechanisms do not propose cross-resistance to linezolid. Additionally, linezolid acts by binding to the 23s rRNA of the 50s ribosomal subunit. There are multiple copies of the gene that code for 23s rRNA in each cell. For resistance to occur, mutations would be necessary in each of the copies of the gene. Because of the fear of resistance, caution should be employed when there is a long and repeated use of antibiotics (Meka, Gold, 2004).

Linezolid is administered either intravenously or orally. It has 100% oral bioavailability and therefore allows for rapid transition from parenteral form to an oral one, possibly resulting in early discharge from the hospital. This can result in decreased length of stay and a decrease in overall cost of treatment. MRSA can be treated with oral linezolid exclusively. Outcomes were the same for patients treated only with oral linezolid compared to intravenous vancomycin despite the difference in routes of administration and antibiotic (Dumitresu, Lima, 2014; Weiglet et al., 2005). Standard dosage of linezolid is 600 mg every 12 hours. For uncomplicated SSTI the recommended dose is 400 mg every 12 hours. Because of its 100% bioavailability, dosage amount is not dependent on the route it is administered (Moellering 2003).

Linezolid can cause some minor side effects, including gastrointestinal discomfort such as nausea, vomiting and diarrhea, and dermatological effects, like rash and itchiness. Most reported adverse effects ceased with the cessation of therapy. When therapy exceeded 14 days, 12.6% of patients studied, experienced hematologic effects such as decreases in platelet count, hemoglobin level, hematocrit and white blood cell count (Birmingham et al., 2003). Linezolid can also cause reversible myelosuppression when administration of antibiotics exceeds 14 days (Weigelt et al., 2005).

In clinical studies linezolid has performed comparably to vancomycin in treating cSSTI caused by MRSA, with possible advantage. A study showed that "linezolid [was] superior … to vancomycin in the MRSA subset. The difference between linezolid and vancomycin results was most dramatic in patients with abscesses and surgical-site infections caused by MRSA". Linezolid is more effective in treating SSTI caused by MRSA due to the enhanced penetration of linezolid into the skin and tissue (Weigelt et al.2005). Linezolid has also shown to be less nephrotoxic than vancomycin (Dumitrescu et al., 2014). In a study comparing the treatment of cSSTI caused by MRSA with linezolid and vancomycin in diabetic and non-diabetic patients, similar results indicated no significant difference in therapy of diabetic patients. However, for non-diabetic patients, results showed a greater success rate for patients treated with linezolid (Lipsky et al., 2011). Other studies have shown no significant benefit of using linezolid over vancomycin. Szczypinska et al. claims that by examining length of stay (LOS) as the determining factor of efficacy of an antibiotic, no significant difference was found between vancomycin and linezolid. As of now, linezolid is used as a secondary choice to vancomycin in cases of vancomycin resistance, despite the proven success and efficacy of linezolid in treating MRSA, in order to prevent the rise of linezolid resistance (2013).

Daptomycin

Daptomycin is another viable antibiotic for the treatment of SSTI caused by MRSA that was brought to market in 2003 (Popovich et al., 2008). Cell death is caused by rapidly depolarizing the bacterial membrane. The lipophilic tail of daptomycin, a cyclic lipopeptide compound, inserts itself into the bacterial cytoplasmic membrane, in this case MRSA, and causes a rapid depolarization of the membrane. This ultimately leads to a loss of DNA, RNA, and protein synthesis. Daptomycin is bactericidal at all of the bacterial growth stages, including the stationary phase (Aikawa et al., 2013; Gonzalez-Ruiz et al., 2016; Seaton, 2008).

Daptomycin resistance is rare but is a growing concern in the healthcare field (Hayden et al., 2005). The mechanism of resistance is unknown but it has been suggested that the mechanism of resistance may be due to irregular dltA transcription factor which "may result in a change of the bacterial cell membrane

fluidity and therefore lead to a reduced affinity of daptomycin to its target site" (Gonzalez-Ruiz et al., 2016). It has been hypothesized that the mechanism for daptomycin resistance is similar to that of vancomycin by VISA. Daptomycin is similar in size and weight to vancomycin and may not be able to penetrate the cell wall to reach the cell membrane, which daptomycin interacts with, if the cell wall thickens as it does in VISA. Daptomycin would never have the opportunity to reach the bacterial cytoplasmic membrane in the face of such an obstacle, resulting in daptomycin resistance (Cui et. al. 2006). Resistance to daptomycin without previous exposure has so far proved to be extremely rare, with an observation of only 0.04% of 10,000 cultures tested shown to have an MIC of 2 µg (Gonzalez-Ruiz et al., 2016).

The recommended dose of daptomycin to treat cSSTI is 4 mg/kg/day to be administered parenterally with a 30-minute IV infusion. Course of treatment for daptomycin is 7-14 days. Daptomycin use has not been approved for pediatric patients (Gonzalez-Ruiz et al., 2016; Seaton, 2008). Daptomycin is administered only once daily to reduce the risk of elevated creatine phosphokinase (CPK) levels and skeletal muscle toxicity. Elevated CPK levels were resolved upon discontinuation of daptomycin treatment. The most common adverse effects reported are constipation, nausea, and headache (Gonzalez-Ruiz et al., 2016).

In some cases, such as difficult-to-treat infections like recurrent MRSA due to vancomycin resistance, a higher dosage of daptomycin (≥6 mg/kg/day) may be recommended. A higher dosage of daptomycin would allow for rapid clearing of bacteria and would lower the risk for emerging resistance. Daptomycin has also been used in combination with other antimicrobial therapies to prevent the rise of resistance. Such combination therapies should be considered for patients who are at high risk of developing resistance to daptomycin alone. Daptomycin and linezolid combination therapy was synergistic in its effect and was bactericidal for MRSA. Daptomycin has also been used with rifampin, trimethoprim-sulfamethoxide, fosfomycin, and tigecycline (Gonzalez-Ruiz et al., 2016).

Many studies have been performed measuring the efficacy of daptomycin compared with that of vancomycin. Most show daptomycin and vancomycin to be comparable and a selection should be made based upon physician and patient preference, resistance factors and economic cost/ benefit analysis (Gonzalez-Ruiz et al., 2016; Kauf et al. 2015). Patients treated with daptomycin showed a greater probability of clinical success by day 2 than those receiving vancomycin therapy, however length of stay for patients from both antibiotic groups averaged to be the same- four days. Vancomycin is also significantly cheaper than daptomycin (Kauf et al. 2015).

Clindamycin

Clindamycin is especially useful in treating MRSA in an outpatient setting (Frei et al., 2010). Clindamycin is a lincosamide drug and belongs to the class of antibiotics known as macrolides, lincosamides and streptogramin B (MLS). Although similar in function to macrolides, lincosamides and streptogramin B are structurally different. Clindamycin works by binding to the large ribosomal subunit, which is the center responsible for catalyzing the formation of peptide bonds during protein elongation. Clindamycin blocks this ribosomal tunnel, allowing peptide elongation to continue until it reaches a point of steric hindrance caused by the clindamycin. This inhibition will lead to eventual dissociation of the peptidyl-tRNA from the ribosome. Once a peptide has reached a certain length on the ribosome, clindamycin loses its ability to inhibit protein synthesis (Tenson et al., 2003).

Resistance to clindamycin therapy is a cause for concern. The resistance mechanism involves modification of the drug binding site on the ribosome. The mechanism is the same for all MSL antibiotics, and is known as MSLB resistance. MSL drugs bind to the 23s rRNA-binding site. The erm gene is responsible for the methylation of the 23s rRNA-binding site. In the presence of the erm gene, resistance can be expressed constitutively as well as when induced into production. Because of the presence of the erm gene, resistance can occur during the course of clindamycin therapy (Lewis II et al., 2005; Popovich et al., 2008). In testing for clindamycin resistance, MRSA strains may appear susceptible, but can later develop resistance. To test for inducible resistance, a D-zone test is used. This a double-disk diffusion test in which two disks, a clindamycin disk and an erythromycin disk are placed on a plate growing S. aureus. The strain is inducible resistant if the zone of inhibition around the clindamycin disk facing the erythromycin disk is blunted, forming a D shape. Rates of inducible clindamycin resistance varies by region in the United States. Use of clindamycin should be determined by local rates (Popovich et al., 2008).

Clindamycin can be administered parenterally or orally for SSTI. When administered intravenously, the appropriate dosage is 600 mg every 8 hours. For oral administration, patients should be given 300-450 mg four times daily. Courses of treatment range from 10-14 days (Popovich et al., 2008). Clindamycin has a 90% oral bioavailability and can penetrate well into skin and skin structure. It is also less costly than some newer drugs used to treat SSTI caused by MRSA (Lewis II et al., 2005). Clindamycin may also inhibit the PVL gene that is common in CA-MRSA (Forcade et al. 2011).

Clindamycin is commonly associated with gastrointestinal side effects. Most common were reports of diarrhea and pseudomembranous colitis (PMC). In a study comparing gastrointestinal

effects caused by clindamycin and ampicillin, another medication known to have gastrointestinal side effects, it was found that 29.8% of patients developed diarrhea following clindamycin therapy. Patients were evaluated for side effects for six weeks following discontinuance of clindamycin therapy (Lusk et al. 1977).

The findings of a study involving a retrospective chart review to compare the efficacy of vancomycin to the efficacy of clindamycin indicated that vancomycin and clindamycin had similar treatment results (Frei et al. 2010). In some cases, clindamycin is used in conjunction with vancomycin as a combination antimicrobial therapy. However, it has been reported that clindamycin often "antagonizes the antistaphylococcol activity of vancomycin" (Deresinski, 2009).

Trimethoprim-Sulfamethoxazole

Trimethoprim-Sulfamethoxazole (TMP-SMX) is another antibiotic that is used for the treatment of MRSA. TMP-SMX is the most commonly prescribed oral antibiotic for outpatient treatment of CA-MRSA infections, in addition to clindamycin (Forcade et al., 2011; Johnson, Decker, 2008). TMP-SMX is a two part drug consisting of one part trimethoprim to five parts sulfamethoxazole. The interaction of these two drugs inhibits the bacterial synthesis of tetrahydrofolic acid, which is used in the production of bacterial nucleic acid. TMP-SMX inhibits two consecutive steps in the formation of folic acid. Sulfonamides inhibit dihydropteroate synthetase (DHPS), which is responsible for para-aminobenzoic acid to be catalyzed into dihydrofolate. TMP inhibits dihydrofolate reductase (DHFR) which causes dihydrofolate to be catalyzed into tetrahydrofolate (Huovinen, 2001; Michalek et al., 2015). TMP-SMX has also been shown to have anti-inflammatory and immunomodulatory effects, enhancing its antimicrobial capabilities (Michalek et al., 2015).

Resistance to TMP-SMX can be found against both drugs, trimethoprim and sulfonamides, with different mechanisms against each. Resistance of S. aureus to TMP is suspected of being caused by a single amino acid change in the dhfr gene, altering DHFR. A single amino acid mutation in the dhps gene is responsible for sulfonamide resistance of S. aureus (Huovinen, 2001). However, a study performed in three cities in the United States in 2005, showed that 97% of CA-MRSA isolates were susceptible to TMP-SMX (Johnson et al., 2008; Popovich et al, 2008).

TMP-SMX is most commonly administered orally with the standard dose of 160/800 mg twice daily for 7-15 days. TMP-SMX can also be administered intravenously with a dosage of trimethoprim (80 mg)/sulfamethoxazole (400 mg) per 5 ml to be given as 5 mg/kg every 6-12 hours (Michalek et al., 2015; Popovich et al., 2008). TMP-SMX has a high bioavailability, at around 85% for both complexes (Stein et al., 2016). Some recommend a higher dosage, 320/1600 mg twice daily for 7-15 days, of oral TMP-SMX to treat SSTI caused by MRSA. Yet Cadena et al. found that patients treated with the two different doses had similar clinical results. For the treatment of SSTI caused by MRSA, a higher dose may not be necessary (2011). A lower dose may also minimize gastrointestinal adverse effects (Michalek et al., 2015). In studies testing TMP-SMX patients were found to have side effects of diarrhea, nausea, vomiting, pruritus, and rash (Miller et al., 2015).

Studies comparing TMP-SMX to clindamycin, show TMP-SMX to be a comparable treatment option (Frei et al., 2010; Miller et al., 2015). TMP-SMX can be used in combination with clindamycin to treat pediatric patients (Stein et al., 2016). TMP-SMX bactericidal activity against MRSA was greater than that of linezolid, rifampicin, and clindamycin, other popular oral antibiotics used to treat MRSA. The use of TMP-SMX is economically beneficial as well. When compared to linezolid, at $1352 for a 10-day course of treatment, TMP-SMX is significantly cheaper, costing only $18 for a 10 day course. These numbers are average wholesale numbers in the United States (Kaka et al. 2006). According to Johnson et al. studies comparing the effectiveness of TMP-SMX to vancomycin, the leading drug, are lacking (2008). However, all VISA strains in the United States are susceptible to TMP-SMX, and TMP-SMX has been used, in combination with other drugs, in its treatment (Cosgrove et al., 2004).

Conclusion

Staphylococcus aureus is the leading cause for SSTI in the world. In an effort to combat this growing concern, many antimicrobial agents have been used. The method of defense was penicillin and then methicillin which lead to what is commonly known as MRSA. Initially found only in hospitals and other healthcare facilities, MRSA soon spread to the community at large. Patients with no previous healthcare exposure were now susceptible to MRSA. These infections are typically manifested as SSTI in the form of impetigo, cellulitis, folliculitis, and abscess.

With the rise of methicillin resistance, and subsequently resistance to all β-lactam antibiotics, new treatment options were needed. Vancomycin became the treatment option of choice. However, vancomycin resistance was soon reported as well, though not to the extent of methicillin resistance. Other antibiotics are also used to combat SSTI caused by MRSA. Daptomycin, a newer drug that is administered intravenously, shows great promise. Linezolid, can be administered both parenterally and orally, and therefore has potential to shorten hospital stay. Clindamycin and TMP-SMX are both popular oral drugs used in outpatient treatment of MRSA.

Regardless of the effectiveness and benefits of these non-β-lactam antibiotics, vancomycin remains the "gold standard" of treatment therapy for MRSA. Fear of developing resistance to alternative drugs limits the extent of their use and keeps vancomycin as the leading choice. Healthcare professionals and researchers need to remain alert to any signs of resistance of antimicrobial agents to prevent its spread and to help stop the increased presence of MRSA in hospitals and the community.

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Phage Therapy as a MRSA Treatment

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Abstract
This paper seeks to review how previous research on bacteriophage therapy can be utilized to improve the treatment of MRSA infections. Due to the growing phenomenon of antibiotic resistance, scientists are looking to utilize the natural antibacterial qualities of bacterial viruses called phages to fight MRSA infections. In order to improve the therapeutic methods of combatting MRSA, one must first understand the mechanics of how phages infect bacterial cells and lyse them with their proteins. The narrow host range of bacteriophages causes the infection of only the pathogenic bacteria and maintains the state of the normal flora. Research suggests that the combination of antibiotics and bacteriophage therapy is a more effective means of combatting MRSA than the utilization of each individual treatment on its own. The next step in developing this treatment option is isolating and classifying each specific bacteriophage and determining its host range. Matching the phages and their corresponding bacteria may seem like a daunting process; however, researchers discovered that phages are naturally located near the bacterial strain that they infect. An additional aspect of the ongoing research is the determination of what causes some phages to be more effective than others. Scientists note that not only does the phage have an inherent strength which is derived from its genetic makeup but, its strength in combating infections is also affected by the host's immunity. New findings lead researchers to believe that there may be a more direct method of treating MRSA infections. Although the phage in its entirety can be used to lyse bacterial cells, scientist have isolated a protein found within the phages that can lyse bacterial cells. Scientists hope to harness its power for a more sinuous treatment of infections.

Abbreviations
CDG- Chronic Granulomatous Disease
ICA- Inter-Cellular Adhesion
MRSA- Methicillin Resistant Staphylococcus Aureus
PAS- Phage-Antibiotic Synergy
SA- Staphylococcus Aureus
VRSA- Vancomycin Resistant Staphylococcus Aureus

Introduction
One of the growing challenges in healthcare is the rising occurrence of antibiotic resistance. Many pathogens have developed resistance to an array of drugs; the discovery of new antibiotics has not been keeping up with the rate of the evolving antibiotic resistant bacteria. Many are concerned that the medical world may return to the times when small infections will be lethal.

For example, Methicillin Resistant Staphylococcus Aureus (MRSA) is an example of a bacterial strain that is resistant to every antibiotic except for Vancomycin. This bug is responsible for most hospital acquired infections., and these bacteria can colonize anywhere from surgical sites, lungs, blood, heart, skin and even brains. (Jensen et al., 2015). Unfortunately, there has been a recent discovery of Vancomycin Resistant Staphylococcus Aureus (VRSA). The emergence of VRSA and its dangers have lead scientists and medical doctors to worry about the future of medicine.

Felix d'Herelle co-discovered bacteriophages in the early 1920s. Bacteriophage therapy utilizes the killing powers of nature's predators for bacteria; bacteriophages. Interest in phage therapy, and its associated research diminished once Flemings discovered the curative powers of penicillin. However, Russia and many Eastern European countries have continued studying this topic. The growing need for alternative treatments for bacterial infections gives rise to a resurgence of interest in this novel topic (Pincus et al., 2015).

A bacteriophage is a virus that binds to receptors that are only found in bacterial cells. Bacteriophages infect bacterial cells, reproduce and then lyse their host bacteria. Then the original phage's offspring then spreads and infects more bacterial cells. Bacteriophages have a specific receptor site and are thus antibacterial to a narrow range of bacterial cells. In addition, their specificity prevents them from infecting human cells. Bacteriophage therapy seeks to harness these powers and use them to treat human infections (Jensen et al., 2015).

Scientists are hopeful that bacteriophage therapy will successfully treat infections caused by many pathogens, specifically the hard to treat MRSA infections. Since research on this topic is fairly new, clinical trials need to be run prior to its institution in standard medicine. This paper will review some of the different experiments and their outcomes. The author wishes to determine how these experiments will impact the future of phage therapy.

Phage Infection Mechanism
Bacteriophages are viruses that infect bacterial cells. They are found all over the planet. The phages infect bacterial cells by binding the tips of their tails to receptor molecules on the bacterial cell. The viral DNA is then injected into the host cell. The virus now directs the creation of more phages, which can be created in less than thirty minutes. The phage bursts out of the bacterial cell, thereby killing the bacteria. The released phage's offspring continue to infect more bacterial cells (Pastagia et al., 2013).

Bacteriophages produce two proteins in order to lyse the cell: Holin, creates a pore in the bacterial cytoplasmic membrane, and Lysin interacts with the peptidoglycan in the cell wall. Lysin cleaves to the peptidoglycan bonds and ruptures the cell wall. Unable to withstand the osmotic pressure, the cell wall bursts (Wang et al., 2000). The released phages can then continue the cycle of infecting bacterial cells.

Narrow Host Range

As a bacterial virus, bacteriophages can be extremely host specific. Consequentially, they exhibit some major advantages in comparison to broad spectrum antibiotics. For instance, using phages to rid the body of infections does not pose a problem to the normal flora, whereas antibiotic treatments impact normal flora. The downside to the phage specificity is the rapid ability of the bacterial viruses to develop resistance, thus increasing the risk that the therapeutic effect is merely transient.

One way to circumvent this problem is to create a personalized phage cocktail specifically for each patient. This would entail culturing every infection, and then creating a specific mixture of bacteriophage from a phage library. This would ensure that the specific viruses could be combined in accordance with the different infectious pathogens present in the patient. In order for this to be a viable treatment option, the hospital would need to have a broad range of bacteriophage present at all times. One group of researchers studied how probable it is to have a wide resource of phages to treat infections.

The researchers purchased many strains of antibiotic resistant bacteria from both a lab and a hospital. In order to isolate the bacteriophages, they filtered sewage waste and then centrifuged the supernatant. Finally, the bacterial viruses were purified three times by plating and re-plating the plaques.

After multiple attempts, the researchers discovered that only one out of 117 strains of bacteriophages were effective against MRSA. After obtaining more samples of the bacterial viruses from other sources, the researchers still had difficulty isolating effective phage. The study resulted in the realization that it is not feasible to treat MRSA infections on demand; isolating effective strains presents significant challenges. In order to treat these infections, pre-made wide range cocktails would have to be available. It is important to note that on demand discovery of phages does seem possible for other bacterial pathogens such as Pseudomonas aeruginosa, Salmonella, and E. coli. Treating these infections with personalized cocktails is a viable option (Mattila et al., 2015).

Combination of Phage and Antibiotic Therapy

One way to use bacteriophage to combat infections is to combine phage therapy with antibiotic therapy. The combination of phages and antibiotics so far seems to be synergistic; the interactions between the two antibacterial agents provide a combined effect that is greater than the sum of the individual effects. One phenomenon that has been noted is called phage-antibiotic synergy (PAS). Research shows that administering sub-lethal doses of antibiotics brings about this effect, regardless of whether the pathogen is a multi-resistant or naïve bacteria or whether an old or new antibiotic is used.

Evidence suggests that a high dosage of antibiotics leads to a tremendous evolutionary advantage for antibiotic resistant pathogens. So, combining phage and antibiotics may be the solution to the medical dilemma. A lower dosage can be used to target naïve bacteria, and the bacteriophage can simultaneously inhibit the growth of the antibiotic resistant strains (Torres-Barceló et al., 2016).

Concerns in PAS Treatment

There are some concerns about the negative effects that can occur as a result of this two-fold treatment. Although there is no research that provides a basis for these fears, in order to ensure the complete safety of all patients, it is imperative to keep these concerns in mind. One major concern is regarding the possibility of breeding double resistant strains of a pathogen. This fear is an outgrowth of a similar phenomenon that has been noted for some antibiotic combination therapy (Torres-Barceló et al., 2016).

Researchers must also be mindful of specific antibiotic-phage combinations that inhibit the effectiveness of each individual component. For example, phages can block the bacteria's ability to absorb the antibiotic, or the antibiotic may affect the host in a way that limits the phage's ability to infect the bacteria or to produce sufficient offspring. Thorough screening of all the various combination before treatment can easily eliminate this concern (Torres-Barceló et al., 2016).

Finally, researchers caution that PAS may increase the virulence factors of certain infections. This phenomenon can be seen in the instance of quorum sensing, the communication between different bacteria cells, which can coordinate the production of some virulence factors. It has been observed that certain exposure to either phages or antibiotics has produced increased virulence. However, the bacterial viruses that target quorum sensing receptors can inhibit this response. Although there is little or no research indicating that these concerns are obstacles to the use of bacteriophage therapy, proper research must be done before combining antibiotics and phage therapy in treatment of infections (Torres-Barceló et al., 2016).

Isolation and Host-Range Determination of Bacteriophage

n order to harness the therapeutic powers of phages, the therapeutic index must be carefully studied and documented. One group of researchers bought bacterial cells from labs and public facilities. They isolated the methicillin resistant strains on agar plates. The next step required that they isolate the virulent phages. Upon obtaining samples from the environment, the researchers purified the samples by filtration and centrifugation three times (Jensen et al., 2015).

In the study they discovered 12 bacteriophages that exhibit lytic activity against SA and MRSA. In order to determine the host range, they took spectrophotometric assays of the cultures of phage-treated bacteria and performed spot testing. After further testing the cocktails on fabric and on glass, they found that the bacteriophage viruses were able to reduce the colony count on cultures of fabric and glass. Interestingly, the phage cocktails showed greater lytic activity against human MRSA then against non-human Staphylococcus Aureus (SA) and against methicillin-susceptible SA. In conclusion, they isolated 6 unique lytic phages that show strong lysis of MRSA (Jensen et al., 2015). The information gathered from this research now opens the door to further studying of these specific bacteriophages. In order to advance the applications of this study in medicine, the efficiency of these phages need to be clinically tested in humans.

Phage Treatment Viability

In order for the bacteriophage to be therapeutically useful, there must to be a way to store it in pharmacies. In order to examine phage viability, researchers stored phage in the freezer, refrigerator, and at room temperature. They concluded that the phage was most stable at room temperature, and was almost 100% viable after 60 days (Pincus et al., 2015). This is a positive discovery for the future of phage therapy, as the shelf life seems to be suitable for pharmaceutical storage and sale.

Isolation of Phages and Host Range Determination

Another area of life in which MRSA affects humans is its ability to infect livestock. Generally, animals are not harmed by these infections. However, they can be responsible for the transfer of infectious bacteria to humans, and this transmission has been documented. In particular, farmers and vegetarians who experience greater exposure to livestock are at a higher risk for this kind of infection. One group of researchers isolated three bacteriophages that were found in pig farms and tested the effectiveness of their antibacterial qualities (Kraushaar et al., 2013).

The methods used in the experiment are as follows. Researchers purchased bacterial strains, specifically MRSA CC398 strain 10-1355, from a laboratory. The phages were taken from a variety of sources including dust swabs, nasal swabs, and fecal swabs from four German pig farms. They then centrifuged, filtered, and tested the phages on MRSA lawn. The host range was tested on agar plates of 86 different MRSA strains CC398 strains and 34 other MRSA strains. The researchers also analyzed the DNA of the phages. None of the analyzed phages contained known virulence or resistant genes. Hence, the study could be conducted without concern that the phages would eventually cause more harm than good to the human host.

The MRSA strains belonging to complex 398 are commonly found on pig farms. These researchers aimed to combat livestock-associated MRSA infections by discovering the phages that naturally inhibit this pathogen. They screened 91 samples. Twenty percent of the samples exhibited lytic activity. An interesting phenomenon was noted in the course of this process. One of the farms was negative for both MRSA and anti-MRSA bacteriophages. This discovery is important, because it connotes that phages are found in proximity to the bacteria that they infect. Thus, researchers can assume that if they have a bacterial pathogen it is likely that phage can be isolated from that environment (Kraushaar et al., 2013). This can simplify the process for future research as the knowledge of where to find the right phages is now known.

Genetics' Effect on Phage Efficiency

The researchers studied three phages that are genetically related. The three PSa phages interestingly infect the same wide range of bacteria. Twenty- four MRSA strains were infected by these phages. These phages all have a receptor that researchers believe may be lipoteichoic acid. Of the three PSa types, type two and type three reduced the numbers of a specific MRSA strain more efficiently than type one. This aspect was observed with wider clearings on agar plates, but the plaques produced by type two and type three were ten times more reductive that the plaques produced by type one. The genotyping of the three phage's genes indicates that this property is a result of the differences in the lysis genes (Kraushaar et al., 2013). Further examination of the genes that favor greater bacterial lysis would be the next step in producing antibacterial cocktails for human medicine.

Another interesting result of this experiment was the discovery of mutant bacteria that were resistant to the phages used in the study. Even when the three different phages were combined in one cocktail, they still did not harm the mutant cells. This discovery is important as it highlights the necessity of combining more diverse phages in a cocktail to prevent resistance to similar phages (Kraushaar et al., 2013). This is another area of research that can be expounded upon based on the findings of this experiment.

Phage Therapy and Host Immuntiy

An important aspect of phage therapy research is the determination of specific bacteriophage's therapeutic index. Additionally, it is vital to explore if the bacterial virus is equally effective in all human hosts or if different factors contribute to the optimal productivity of the bacteriophage. One study explored the efficiency of phage SATA-8505 and its ability to prevent and treat infections caused by the MRSA strain USA300. The therapeutic index was examined in vitro with human cells and in vivo in mice. This specific strain was selected due to earlier research that proved its efficiency in combating MRSA USA300 (Pincus et al., 20).

All materials for the experiment, MRSA strain USA300, bacteriophage SATA-8505, and the phages were obtained from laboratories. Using agar plates, the researchers were able to confirm that phage SATA-8505 has the ability to kill MRSA USA300. This phenomenon was seen when they observed the colony morphology of culture plates two to four hours after phage inoculation.

Once it was clear that the phages were effective killing agents of MRSA, the researchers inoculated the mice with MRSA pathogens immediately after they injected bacteriophages into the skin. They then measured the diameters of the skin lesions for six consecutive days. The mice that were treated with an equal concentration of phages and bacteria displayed smaller skin lesions than the control group. The researchers also used Chronic Granulomatous Disease (CDG) immuno-deficient mice. CDG patients are more susceptible to MRSA infections due to their problematic neutrophil response. Interestingly, the CDG mice developed bigger skin lesions than the healthy mice; usually CDG patients develop MRSA infections on deep tissue, such as the liver. (Pincus et al, 2015). This could be a positive sign, for despite the fact that the skin lesions were big, the infections resembled those of healthy patients. One may think that due to the large lesion size, the CDG mice did not benefit from the phage therapy. Upon further examination, one may conclude that these mice improved relative to their own individual states of immunity and infection.

Unfortunately, the phage treatment does not seem to be a magical injection. Both groups of mice exhibited an increased lesional inflammatory cytokines. The fact that the actual lesion size was reduced while the bacterial count and cytokine response did not decrease led the researchers to hypothesize that although the administered dose of phage reduced the ability of the bacteria to inflict toxin-generated damage, it did not affect the viability of USA300 (Pincus et al., 2015). These researchers did a complete project. In order to completely understand this phenomenon, they repeated the experiment while changing the phage dosage and the original bacterial count. They discovered that an increase in phage administration brought about an increase in the inflammatory response in the CDG mice. This may indicate why bigger lesions that are produced when a higher dosage of phage is administered.

This discovery highlights the importance of phage dosage, especially in immunocompromised patients. The scientists noted that although the phage worked at certain concentrations, when the dosage was increased, negative side effects, such as an inflammation of the infectious site, were observed. Further research is required in order to configure the optimal dosage for treating patients while incurring minimal or no harm. The researchers make a point in warning clinicians that before phage therapy is used on any immuno-deficient individual, the dosage must be reconfigured. Extrapolating data from healthy individuals would not work for the CDG patients who were more affected by increases in dosage.

Once researchers noted that the phages induced an inflammatory response in mice, they investigated whether a similar response could be noted in human cells. When human blood cells were exposed to dosages of phage, the human cells did not produce inflammatory responses. The response of human keratinocytes was also investigated. When compared to the diluent treatment, phage induced a small, yet significant, cytokine response (Pincus et al 2015). This is a potential roadblock in the implementation of phage therapy. The only way this treatment could work is if the scientists find a way to inhibit the human body's negative response to the phage itself.

The next step involved investigation of the impact of phage SATA-8505 on human blood in both healthy and immuno-deficient individuals. Unfortunately, the phage did not seem to affect bacterial growth or colony morphology. The bacteria cells that were exposed to phage in blood and were then re-cultured grew just as well as bacteria that were unexposed to bacteriophage. The researches further investigated the phage and bacterium used and found that the bacterial cells were killed by the phage in a culture plate. This negates the possibility that a phage-resistant strain of MRSA was used to inoculate the blood sample. In order to elucidate why the bacteria is unaffected by phage in the presence of human blood, further research is necessary.

The last part of this research included the study of the efficiency of SATA 8505 on Vancomycin Resistant Staphhylococcus Aruerus (VRSA). The researchers determined that this phage is incapable of killing out the VRSA strains (Pincus et al., 2015). This observation highlights the strain-specificity aspect of phage therapy, since the phage worked fairly well on the MRSA but did not affect the VRSA growth.

Strain Specificity

The strain specificity of phage has both positive and negative aspects to it. While it is true that phage therapy successfully avoids destroying normal flora, one must first determine the exact strain of the infection prior to beginning the treatment. Unfortunately, this determination often takes time, and while treating MRSA infection, time is often of essence. Therefore, phage therapy may not yet serve as the first line treatment against infections (Pincus et al., 2015).

Thus, possibility of using phage cocktails holds much promise. Similar to multivalent vaccines, phage cocktails combine

a few different strains of bacteriophage. This would increase the chances that one of the strains would cover the unknown pathogen in the human host. Although this solution seems scientifically sound, there is a financial drawback. Each phage used in the cocktail must be separately tested in isolated therapy. Then research must be conducted on the combination to ensure that the combined effects are positive or synergistic. The added cost of all this research may be an issue (Pincus et al., 2015).

Phage Lysins: Pathogen Directed Treatment

One major drawback to phage therapy is the bacteria's ability to develop resistance to phage attachment. Recently researchers have developed ways to purify the lysins from the bacteriophages. The lysins can be used on their own as an antibacterial agent by causing bacterial lysis on contact. The use of lysins is generally limited to bacterial organisms with a cell wall, although researchers are searching for lysins with Gram-negative bacterial activity (Pastagia et al., 2013).

Lysin Mechanism

The lysins have two main functions: substrate recognition and enzymatic hydrolysis. The N-terminal domain enzymatically severs specific peptidoglycan bonds. This can be accomplished in multiple ways. They can hydrolyze the glyosidic bonds in the glycan strand of the cell wall, or the enzymes can hydrolyze the cross-bridge. The enzymes may also cleave the amide bond which is located between the glycan moiety and the main peptide. There is a level of specificity of lysin binding which is based on binding ligands and the specificity of cell wall binding domains.

Additionally, the lysins bind to their substrates with an affinity constant similar to antibodies (Ka = 6x108). This suggests that lysins do not disengage from the receptors after they bind and cleave, just like antibodies. This also explains the speed of lysin action. Their high affinity constant accounts for their speedy identification of their target bacterial cells (Pastagia et al., 2013).

Lysin Clinical Potential

The primary benefit of phage lysins is that, to date there has been no documentation of bacterial resistance to phage lysins. Additionally, bacteria with thick capsules and bacteria in biofilms do not inhibit lysin activity. These reasons are enough to aim research toward harnessing bacteriophage lysin antibacterial potential.

Lysins generally exhibit bactericidal activity against the bacterial host of the phages they were harvested from. Like bacteriophages, lysins are strain specific, and therefore do not affect normal flora. One specific lysin, chimeric lysin (ClyS) has been proven to kill MRSA strains along with other staphylococci. Some lysins have been proven to affect a broader range of bacterium (Pastagia et al 2013). These kinds of lysins would be a great first defense method against infections in which the exact strain of pathogenic bacteria is unknown.

Phage Lysin and MRSA Biofilm Eradication

Phage lysins have potential to fight biofilms, a serious health problem. All biofilms are harder to treat than planktonic bacteria; specific biofilms of antibiotic resistant pathogens, such as MRSA are even more difficult. Biofilms exhibit antibiotic resistance up to 1000 times more than planktonic bacterial cells (Chopra et al., 2015). These biofilm infections develop in patients that have prosthetic objects implanted in their bodies. Phage lysins seem to be the optimal way to combat MRSA infections because of the speed of their bactericidal activity, the small chances of bacterial resistance, and the low probability of it affecting the normal flora. Additionally, the usage of lysins allows for all the positive aspects of phage therapy without the negative possibilities such as resistant mutations.

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Research was conducted on the efficiency of phage lysins in eradicating MRSA biofilms. The object of this study was to evaluate the success of a phage lysin in eradicating old and new biofilms alone and in conjunction with antibiotics. The MRSA bacteria strain and the antibiotic, Minocycline, were obtained commercially. The phage lysin was extracted from phage MR-10 that had been previously been characterized by the same research group. In order to isolate and confirm their results, the team followed a published method for isolating phage enzymes (Chopra et al., 2015). This method of obtaining the phage lysin left some room for potential error. The research team may have made some errors in isolating the original phage, and the methods that they used originally are not documented in their paper. Thus, the whole project could have operated with impure or misnomered materials. Conversely, the method they utilized to isolate the enzyme leaves little room for error because they followed a proven method of isolating the endolysin. They did not rely solely on their observations and research. Additionally, they confirmed their results with SDS-PAGE analysis after every purification step (Chopra et al., 2015).

The biofilms were grown in the lab, and their morphological characteristics were identified by scanning with electron microscopy. The biofilms were developed on coverslips. Two different types of biofilms were formed; ica-positive and ica-negative. For the first part of the research, both types of biofilms were treated with the endolysin. The ica-negative biofilms were initially treated with 9 µg/ml. After three hours no significant reduction was observed. Even once they increased the duration of the treatment, there was no significant reduction. However, when the ica- negative biofilms were treated with 18µg/ml of lysin a statistically significant reduction was observed after six hours. This seemed to be the highest effective dosage for any increase in phage lysin concentration did not create a significant decrease in bacterial count. Contrarily, the ica-positive MRSA biofilm showed a reduction in bacterial cell count with an application of 36µg/mL of phage lysin after six hours. Increasing the duration to 24 hours did not make a difference in the bacterial reduction (Chopra et al., 2015). Although the two biofilms were very similar, the difference in the intra-cellular adhesion clearly affected the optimum concentration of lysin needed to eradicate the biofilms.

Phage Lysin and Antibiotics

The next step in this research was to examine the combined effects of phage lysin with antibiotics in treatment of biofilms. They were subjected to both minocycline (a tetracycline antibiotic,) and MR-10 endolysin. A significant decrease was observed in the first three days of the treatment. After that there was no more significant reduction in both forms of the biofilms The researchers also conducted this experiment on old biofilm This strong unit did not exhibit significant reduction in the cell count. They researchers theorize that this observation is a result of the two antibiotic agents acting primarily on the top layer of the thick biofilm. Other literature clearly states that antibiotics cannot penetrate deep into the biofilm because of the complicated nature of the biofilm matrix. Since the lysins are one time use enzymes, it is possible that they acted on the same cells as the antibiotic at different receptor sites. This could be the reason for the lack of reduction in the very thick biofilms (Chopin et al., 2015). Based on this theory, one way to combat thick biofilms would be continuous application of lysins. Once they kill out the upper layers, the new lysins can kill the cells in the deeper layers of the matrix.

The next step of this research team was to study how sequential treatment of phage lysin in conjunction with minocycline would affect the biofilms. There were two different sequences studied with different results. The first time the researchers applied endolysin for six hours and then added minocycline before incubating overnight. The next time they applied the minocycline first and then the phage endolysin. In comparison to both the controls and each other, the second method of sequential treatment delivered the strongest significant decrease in bacterial cell count. Figure 1 below compares the difference in total bacterial cell concentration from the control group and the two separate sequential treatments. Using the Live/Dead backlight kit, the researcher's stained the biofilms to see the total live and dead cells in the biofilm. After the second sequential treatment the staining confirmed that the majority of the live cells were killed (Chopin et al., 2015).

The positive results of biofilm treatment with three hours of minocycline followed by overnight incubation with endolysin MR-10 has a two-fold explanation. First, the antibiotic, minocycline eradicated the metabolically active cells. According to literature, the active cells are in the upper layers in the biofilm. Since the antibiotics cannot penetrate the layers and it is only affective on active cells, using it first ensured that there were active cells in the biofilm that the antibiotics could access. Next, the endolysins were applied. These lysins are small, low molecular weight proteins. Additionally, they act on both growing and stationary cells. Therefore, adding the lysins second enabled the smaller molecules to penetrate the biofilm and act on the metabolically inactive cells (Chopin et al., 2015). This reasoning also explains why the first sequential treatment was not nearly as successful. The endolysins probably killed the uppermost cells first, so subsequent treatment with antibiotics did not have a very strong additive benefit. By the time the antibiotic was applied there was not much more metabolically active cells present in the uppermost layer where the antibiotics were able to access. Therefore, they did not do as much as they could when they were applied first.

MRSA biofilms are another manifestation of the dangers posed by this antibiotic resistant bacterium strain. Instead of using the whole bacteriophage, this study provided solid research that supports the idea that an isolated enzyme from the phage will work in eradicating these biofilms. Since the lysins attack the peptidoglycan of the bacterial cell, it is a great solution for the hard to treat metabolically inactive cells found in biofilms. With the foundation of this research, many more studies can be conducted in order to isolate other endolysins for all sorts of biofilm infections.

Conclusion

Although many are discouraged by the growth of MRSA infections and the lack of treatment options, research suggests that bacteriophages may serve as the solution to this medical dilemma. Researchers have explored multiple aspects of bacteriophages and they have classified numerous strains of anti-MRSA phages. In order for the medical world to harness the natural powers of these viruses, more clinical studies must be

performed. Scientists must first continue to experiment on animals, in order to rule out all possibilities of the occurrence of negative side effects of phage inoculation. After the preliminary experimentation on animals has been completed, human trials can establish phages as tried and true medicinal option. If scientists continue to diligently explore this topic, there may be reason to believe that the world will be rid of the inherent dangers of multi-resistant pathogens.

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Stem Cell Therapy and Macular Degeneration

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Abstract

Macular degeneration is the leading cause of vision loss in Americans sixty years and older. Currently, it is an incurable disease. Stem cell therapy is the idea of transplanting stem cells to replace damaged cells in the body. As the demand for transplantable organs far outweighs the supply, stem cells are an encouraging alternative to replace damaged cells. Can stem cell therapy be the first cure for macular degeneration? Many experiments have been done on transplanting stem cells into the eyes of rats with macular degeneration yielding promising results. The first transplantation of retinal pigmented epithelial stem cells into humans to treat macular degeneration was done in 2012. Stem cells were differentiated and inserted into two patients suffering from macular degeneration. Both patients that partook in the study displayed significant visual improvement, and no abnormal growth was observed. In another study, the use of retinal epithelial cells vs. the use of other types of eye cells to treat macular degeneration was studied. Each cell type has the same potential for use in stem cell therapy. Stem cell therapy is a hopeful option for treatment of macular degeneration. Further research is needed before it can be used as a widespread cure.

Introduction

Two important characteristics distinguish stem cells from other cells. Firstly, they are unspecialized cells that have the ability to renew themselves through cell division, and secondly under certain conditions they can be induced to become tissue or organ-specific cells with special functions.

Until recently, scientists have mainly worked with embryonic stem cells and somatic stem cells. Embryonic stem cells are from the blastocyst of the embryo. The blastocyst is composed of an inner cell mass of stem cells that differentiate into different types of cells, giving rise to the entire body of the organism.

Embryonic stem cells are donated for research purposes by eggs that have been fertilized in vitro, and they are not derived from eggs fertilized in a women's body. The donated stem cells are transferred to a culture dish that contains a nutrient broth called a culture medium where they divide and spread over the surface of the culture dish. When stem cells are grown under specific conditions, they can remain unspecialized. If the stem cells are allowed to clump together to form embryoid bodies, spontaneously they will begin to differentiate. Scientists can modify the stem cells by inserting specific genes or by changing the chemical make-up of the culture. Figure 1 illustrates the process of modifying stem cells to become gene specific stem cells.

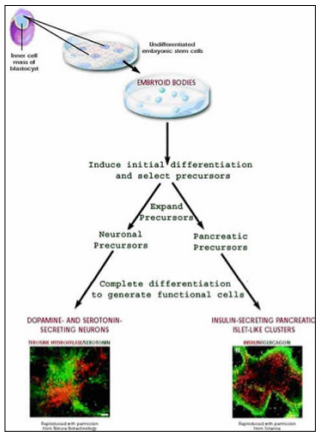


Figure 1: Stem cells clump together to form embryoid bodies, and spontaneously they will begin to differentiate. The stem cells can be modified by inserting specific genes. (Bethesda, 2016)

Adult stem cells are different from embryonic stem cells because they are found in mature tissues or organs. Adult stem cells are undifferentiated cells that can develop into specific cells of the tissue or organ where it is found. Adult stem cells are found in a specialized region called the stem cell niche. When needed the stem cells will divide and differentiate. This can occur when there is tissue damage or under normal wear and tear conditions – the stem cells will divide in order to replenish the supply of cells. There are various types of adult stem cells that can differentiate into numerous types of cells hematopoietic stem cells (develop into blood cells), mesenchymal stem cells (develop into bone cells, cartilage cells and fat cells), and neural stem cells (develop into cells of the nervous system).

Human induced pluripotent stem cells are an additional type of stem cell. This stem cell is an adult cell that has been genetically reprogrammed to express the characteristics of an embryonic stem cell. The method used to reprogram these cells help researchers learn more about the possibility of reprogramming damaged or diseased cells in the human body.

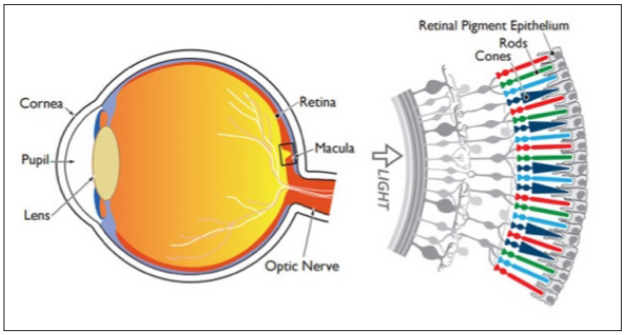
Human stem cells have many uses in research and clinic. The most important use being cell-based therapy. Cell-based therapy is the potential to direct the differentiation of stem cells in order to transplant them as replacement cells or tissue. Today, tissue and organs are being donated, but the need for transplantable organs and tissue far outweighs the supply. Cell-based therapy can offer a renewable source of replacement cells which can be used to treat different diseases. (Bethesda, 2016).

There are several drawbacks of using transplanted stem cells to treat disease including rejection and long-term side effects are not known. Therefore, a lot of research is still being done before stem cell therapy is used as an established treatment for disease. (Ladock, 2016).

One disease scientists believe stem cell therapy can treat is macular degeneration. Macular degeneration is the most common cause of vision loss affecting more than 10 million Americans.

The retina is the part of the eye that records the images we see and sends them via the optic nerve to the brain. The macula is located in the central area of the retina and is responsible for focusing central vision, seeing fine details, and recognizing faces

Figure 2: Anatomy of the human eye showing the location of the macula. (ISSCR, 2015)



and color. The anatomy of the human eye is illustrated in figure 2 with the location of the macula shown. Macular degeneration is a disease which causes the deterioration of the cells of the macula. At the present time it is an incurable disease.

Age-related macular degeneration is the most common cause of blindness in people over the age of 60 years old. Age-related macular degeneration gets progressively worse as one ages. It starts with the dysfunction and death of retinal pigment epithelial (RPE) cells. It continues with photoreceptor loss, and insufficiencies in high acuity vision. There are 3 stages of age-related macular degeneration. Early age-related macular degeneration is the first stage where vision loss is not experienced. It can be diagnosed by the presence of small yellow droplets beneath the retina called drusen. The second stage is called intermediate age-related macular degeneration where some vision loss may be experienced. A comprehensive eye exam will look for drusen or pigment change in the retina. The last stage is called late age-related macular degeneration. At this stage vision loss is noticeable. (Buchholz, 2009)

It is known that the causes of macular degeneration are both genetic and environmental, but the exact cause is not known. Age is the biggest risk; there is more of a chance one will develop macular degeneration as one ages. (Akpek, 2013).

Methods

Literature for this article was obtained primarily using Touro College Online library. Other databases such as PubMed were used. Additionally, Google Scholar was valuable for finding necessary and relevant articles.

Discussion

Only 5.5 millimeters in diameter, the macula is a part of the retina that is responsible for central vision. Researchers have been studying the possibility of using stem cells to treat macular degeneration. The macula is made up of photoreceptor cells called rods and cones. Rods and cones respond to light by sending electrical impulses to the brain through the optic nerve. The brain then interprets these impulses. Behind the rods and cones is a layer of cells called retinal pigmented epithelial cells. (ISSCR, 2015).

Functions of Retinal Pigmented Epithelium

Retinal pigmented epithelial cells have many functions in the eye. Firstly, these cells are responsible for transport in two directions. In one direction they transport glucose and other nutrients from the blood to the rods and cones. In the other direction the transport electrolytes and water from the subretinal area to the choroid. Another function of retinal pigmented epithelial cells is to absorb and filter entering light. The retina is made up of various pigments that are sensitive to different wavelengths of light. Additionally, retinal pigmented epithelial cells are responsible for phagocytosis. (Simó Et. Al. 2010)

The eye is an excellent part of the body for researchers to test stem cell therapy on because the eye is well contained by its many barriers. It is hard for the stem cells to move to other parts of the body. Additionally, researchers can assess the differences between a treated and untreated eye on the same patient. There is equipment available that allows one to see the interior and exterior of a person's eye.

The focus of scientists has been on using retinal pigmented epithelial cells for stem cell therapy. This is because it is a lot harder to ensure the proper placement of rods and cones in a patient's eye. Rods and cones connect with nerve fibers, and it is extremely complex to correctly integrate these photoreceptor cells with the nerve fibers. Retinal pigmented epithelial cells do not connect to nerve fibers; therefore they are a better option for stem cell therapy.

It is the goal of scientists to be able to transplant retinal pigmented epithelial cells before the disease has progressed such that the photoreceptor cells have died. In this way, the transplanted retinal pigmented epithelial cells can take on some function of the damaged retinal pigmented epithelial cells and prevent the rod and cone cells from dying, thereby stopping the progression of the disease. (ISSCR, 2015).

Delivery of Stem Cells into the Eye

The following method was used to deliver retinal epithelial cells into rats. The rat was put under anesthesia. A sharp sterilized needle was inserted to make a hole right beneath the limbus, which is the border between the cornea and the sclera

of the eye. The tip of the syringe was inserted into the hole, injecting the retinal pigmented epithelial cells. The syringe was then taken out slowly, and eye moisturizing drops were given. (Westenskow Et. Al. 2015).

A study of rats with macular degeneration, treated with human embryonic stem cells has shown evidence of rescued photoreceptors and vision loss prevention. Three different levels of pigmented cells were used; light, medium and heavy. Different amount of cells were used in each group of rats. Visual acuity improved in the groups of rats treated with a dose of between 5,000 – 100,000 retinal pigmented epithelial cells. The cells injected into the rats sustained visual function for at least 60 days. The cells survived in the rats for at least 220 days. All the groups of rats showed deterioration of visual acuity over time. There is no evidence that showed any group having a slower deterioration over time. The deterioration of the visual acuity of the rats in this study may be related to the inadequate use of immunosuppressive drugs to treat the rats. Another hypothesis of why the cells didn't last is that cell transplantation may need to be repeated several times in order to sustain their therapeutic effect. The study confirmed the long-term safety of retinal pigmented epithelial cells for treatment of macular degeneration in rats. It is a promising step in the research of stem cell therapy to treat specific types of macular degeneration in humans. (Lu Et. Al. 2009).

In one study researchers tested whether human embryonic stem cells can safely be used to treat patients with macular degeneration. Although human embryonic stem cells were first discovered in 1998, this was the first study to report transplanting human embryonic stem cells into human patients. The study tested for signs of hyperproliferation, carcinogenicity, abnormal tissue formation, and rejection. Two patients were selected for the study. One patient had dry age-related macular degeneration and the other had Stargardt's macular dystrophy (most common pediatric macular degeneration). (Schwartz Et. Al. 2012).

In the experiment human embryonic stem cell culture MA09 (classification of human embryonic stem cells) were used to generate a master cell bank. (Schwartz Et. Al. 2012) Cell line MA09 has contact with the environment before transplanted into the patient and therefore is classified as a xenotransplantation. Xenotransplantation is any procedure that involves the transplantation, infusion, or implantation to humans of nonhuman live cells. (Samdani, 2014) After embryoid bodies were formed and there was multiplication of the cells, retinal pigment epithelial patches were isolated. Embryoid bodies are the first phase of embryonic stem cell differentiation. There are various ways to form embryoid bodies, but it has been a challenge to form embryoid bodies uniform in size. (Xu Et. Al. 2011) The retinal pigmented epithelial cells were tested for pathogens and phagocytosis. They were also tested for RPE and hESC markers.

Human embryonic retinal pigment cells were successfully cultured. The cells showed typical retinal pigmented epithelial cell behavior by losing their pigmented cobblestone morphology during proliferation and re-differentiating into a monolayer of polygonal cuboidal pigmented epithelium.

One hundred and fifty nanoliters of retinal pigmented epithelial cells were injected into the patients' eyes through a cannula that delivered a dose of 500,000 viable retinal pigmented epithelial cells into the subretinal space. Immunosuppressive drugs; tacrolimus and mycophenolate mofetil were given to each patient a week before the surgery and continued for six weeks after the surgery. Tacrolimus was not given after week six and the mycophenolate mofetil was continued for an additional six weeks. (Schwartz Et. Al. 2012).

There was no hyperproliferation or abnormal growth in either of the patients following surgery as determined by biomicroscopic and ophthalmoscopic clinical examinations. (Schwartz Et. Al. 2012).

Anatomical evidence showed survival of human embryonic stem cells in the patient with Stargardt's macular dystrophy. The transplanted cells were localized to exactly the correct anatomical location. Increased pigmentation was seen in the retinal pigmented epithelial cells beginning from week one after the surgery up until month three after the surgery.

The patient with age-related macular degeneration did not take the immunosuppressive drugs after the operation; therefore anatomical evidence was difficult to confirm.

Both patients displayed functional visual improvement. The

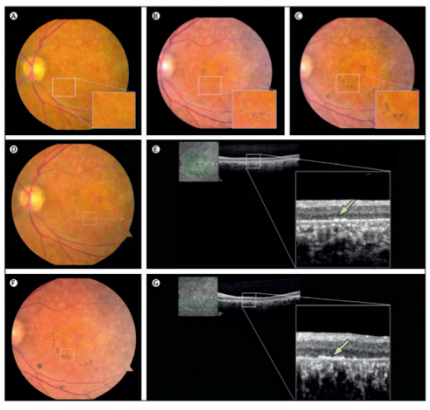


Figure 3: A series of images showing the macula before the operation until after the operation. Increased RPE are seen post operation. (Schwartz Et. Al. 2012)

patient with Stargardt's macular dystrophy was able to read study letters of 20/500 immediately following surgery. By week six, the patient was reading letters of 20/320 which remained stable through the postoperative period. The patient with age-related macular degeneration showed mild visual improvements.

The series of images in figure 4 illustrate the area in the eye called the macula from before the operation until after the operation. Increased retinal pigmented epithelial cells are seen post operation.

In this study human embryonic stem cells were safely transplanted into 2 patients. (Schwartz Et. Al. 2012).

A second study compared the effectiveness of using human induced pluripotent cells with using human embryonic stem cells. Both types of stem cells have the ability to differentiate, but it is unclear if they both have the same potential for use in stem cell therapy. The results were determined by finding differentiating ocular cells called retinal pigmented epithelium (RPE).

Transcription factors, protein arrangement, and gene expression were looked at when comparing the two types of stem cells. The study concluded that human induced pluripotent stem cells are a feasible candidate for cellular therapy since they have similar cellular function and possess proper gene expression. (Buchholz Et. Al. 2009).

Where we are now

There are still several difficulties that must be overcome before stem cell therapy is used as a widespread treatment. Many continued years of research are needed.

There are legal and ethical issues with using embryonic stem cells for stem cell therapy; therefore, a lot of effort is being put into generating induced pluripotent stem cells. There is a risk, however, that the induced pluripotent stem cells will turn into cancer cells, and that is a risk not worth taking. Therefore, it has been a challenge for researchers to obtain a readily available bank of stem cells to be used for stem cell therapy.

Can the stem cell therapy be effective if the condition that caused the cells to die in the first place is still present? Most likely, stem cell therapy will need to be combined with additional treatments in order to limit further damage in macular degeneration patients. These additional treatments are still being researched and developed.

An additional difficulty that must be overcome is to find out what exactly is the correct dosage of cells to transplant. There have only been a limited number of clinical procedures done. In order to figure out the correct dosage more clinical studies must be performed.

Scar tissue is present in the damaged eyes of patients with macular degeneration. When new cells are transplanted there is a barrier between the host retina and the grafted cells. This can affect the way the light is transmitted, and the way the patients will see. Treating patients before scar tissue has formed, or discovering ways to get rid of scar tissue is crucial before stem cells can be transplanted. (Barnstable).

Conclusion

There is promising research for use of retinal pigmented epithelial cells to treat macular degeneration. Continued research is needed before stem cell therapy can be used as a widespread treatment for macular degeneration. It is a goal of researchers to treat patients earlier on in the progression of the disease, before complete visual function has been lost.

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Is There a Link between Zika Virus and Microcephaly In Neonates?

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Abstract
With Zika virus spreading worldwide, a lot of attention is drawn to researching its pathogenesis and etiology. It has also been noticed by various research groups and such health agencies such as CDC and WHO that there might be a connection between ZIKV and microcephaly, due to the spiking number of cases of microcephaly reported in areas with affected patients. Temporal and geographical data from the affected ZIKV areas, including Brazil and French Polynesia, suggests a connection between microcephaly and the virus. Tests of amniotic fluids of pregnant women with reported Zika virus infection and microcephalic fetuses revealed the presence of viral RNA. Zika was also found in placenta and fetal brain tissue collected from two miscarriages and two newborns. Evidence presented in this review is sufficient to prove an existent causal relationship between Zika virus and prenatal and neonatal microcephaly.

Introduction

Zika virus (ZIKV) is an emerging disease that has gone from a mild endemic, circulating only locally in Africa and parts of Asia, to a worldwide pandemic, spreading rapidly throughout the Americas. (Lupton, 2016; Paixão, et. al. 2016).

ZIKV is an arbovirus which belongs to the genus Flavivirus, family Flavivirdae. It is known to be transmitted by mosquitoes of the Aedes species – such as Ae. luteocephalus, Ae. aegypti, Ae. africanus, Ae. alboictus, and Ae. hensilli, which are usually found in areas with warm climate; however, Ae. albopictus inhabits as far north as the Great Lakes in the U.S. (Fellner, 2016) Ae. aegypti is considered to be the most efficient vector for ZIKV, which creates a bigger threat of spread of the virus, because this specie is characterized by an easy adaptation to human environments, indoor and daytime feeding, preference of feeding on humans, and an ability to breed in extremely small amounts of water. (Bell, et. al. 2016) As all other flaviviruses, including Dengue virus, Yellow fever virus, West Nile virus, Japanese encephalitis virus, Tick-Borne encephalitis virus, ZIKV carries a positive single-stranded RNA, which encodes a polyprotein that is then processed into three structural proteins: the envelope, the capsid, and the precursor of membrane. It also encodes seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. (Al-Quahtani, et. al. 2016).

ZIKV was discovered in 1947, in Uganda's Zika forest, near Entebbe, during regular surveillance for sylvatic yellow fever in Macacamulatta rhesus monkeys. (Paixão, et. al. 2016) Shortly after, in 1952, the first case of ZIKV infection in humans was reported in Uganda and the United Republic of Tanzania. For the next 4 decades, ZIKV had spread to equatorial Asia (India, Pakistan, Malaysia, Indonesia, etc.), but infections were mild, sporadic, and without large epidemics. (Al-Quahtani, et. al. 2016) However, in 2007 the first major outbreak of ZIKV infection occurred on Yap Island in the Federated States of Micronesia, where well-nigh 75% of population was infected with the virus. (Fellner, 2016; Al-Quahtani, et. al. 2016) Next major epidemic of ZIKV was reported in 2013 in French Polynesia. The most recent outbreak of ZIKV happened in Brazil in 2015, with first confirmed case reported in May 2015. (Al-Quahtani, et. al. 2016).

Due to an increasing number of microcephaly cases being reported in the ZIKV affected areas, WHO and Brazilian Ministry of Health have confirmed possibility of a link between ZIKV outbreaks and such severe fetal neurological defects as microcephaly and recommended that all pregnant women residing in or traveling to the affected areas should take precautions to avoid contact with possible vectors. (Calvet, et. al. 2016) Having a virus as a cause of birth defects is not surprising (for example, cytomegalovirus or rubella virus can be a source of microcephaly and other anomalies), but there has never been a time when a virus caused an epidemic of congenital birth anomalies. (Rasmussen, et. al. 2016) The CDC has advised that all infants, whose mothers traveled or resided in areas affected by ZIKV during pregnancy, should be suspected for congenital ZIKV infection if either intracranial calcifications or microcephaly were detected at birth or prenatally or if mother's ZIKV testing results were positive. (Fleming-Dutra, et. al. 2016) Can there really be a connection between Zika virus and neonatal microcephaly?

Methods

This systematic review was composed after reviewing and evaluating clinical data reports of Zika virus outbreaks in Haiti, French Polynesia, Philippines, and Cambodia, case-studies of infants and fetuses with presumed or confirmed ZIKV infection, and review articles; this data was obtained via searching engines as MEDLINE and ProQuest, or directly from published sources.

Discussion

Zika Virus: Diagnosis and Treatment

ZIKV infection starts in dendritic cells near the site of inoculation (if mosquito-mediated), which is followed by spreading to lymph-nodes and the bloodstream. (Al-Quahtani, et. al. 2016) Replication of flavaviral RNA occurs in both cellular cytoplasm and nucleus. Infectious ZIKV appears in human blood as early as day 1 of illness onset, and can stay in blood as late as day 11 after onset. (Hayes, 2009) The incubation period for ZIKV has been determined to be 3-12 days. The outcome of infection depends

on competition between viral replication and the host immune response. (Al-Quahtani, et. al. 2016) Usually, human infections with ZIKV are mild, and lethal outcomes are rare; common symptoms include fever, arthralgia and muscle pain, conjunctivitis, rash, and headaches, lasting up to one week. (Pastula, et. al. 2016) However, 80% of ZIKV are asymptomatic, which makes it extremely hard to diagnose and treat.

In addition to diagnosis based on epidemiological circumstances and clinical symptoms, there are not so many laboratory diagnostic tests available, because ZIKV as a pandemic is very recent. Existent assays for ZIKV diagnosis include use of reverse transcriptase-polymerase chain reaction (RT-PCR) to detect viral nucleic acid in the blood and other body fluids, such as saliva or urine. This test is useful only if performed on serum collected within day one to day three of symptom onset or on saliva or urine collected up to day five of symptom onset. Serological tests using enzyme-linked immunosorbent assays (ELISA) are able to detect the presence of anti-Zika virus immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, which develop in human organism by the end of the first week of infection. (Al-Qahtani, et. al. 2016; Fellner, 2016) However, as specific as these two test are (compared to NAT or reduction neutralization assay, for example), cross-reaction with related flaviviruses, such as West Nile and yellow fever, and dengue, is very common; therefore, additional tests should be performed to exclude those options.

Microcephaly

Microcephaly (micro- small; cephal- head) is a congenital condition which is diagnosed when newborn children have a smaller brain and skull compared to those of others of the same sex and age (See Fig. 1). (NINDS, 2016) Microcephaly can be a result of both environmental and genetic causes. Most common environmental factors that lead to microcephaly in infants are radiation, lead and mercury intoxication, in utero exposure to alcohol and drugs, and infections, such as TORCHES. (Calvet, et. al. 2016; Teixeira, et. al. 2016) Common genetic causes are autosomal recessive microcephaly, Aicardi-Goutières syndrome, Rett syndrome, and chromosomal trisomy. (Calvet, et. al. 2016) Frequently, microcephaly is associated with mental retardation, Down's syndrome, and neurometabolic syndromes.

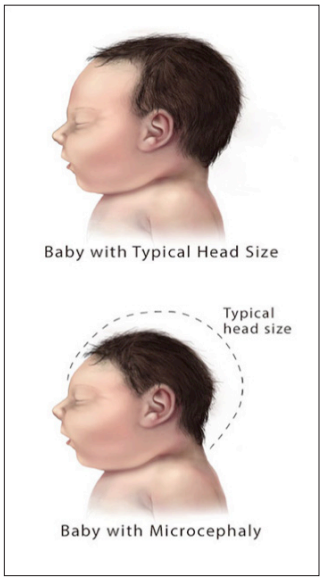

Figure 1 Microcephaly in an Infant (Bottom) Compared With an Infant With a Normal Head Size (Top) Source: CDC

There is no treatment available in order to return a baby's head to a normal size and shape; and all treatment techniques focus on decreasing the impact of accompanying disabilities. (NINDS, 2016) Therefore, understanding whether there's indeed a relationship between maternal infection with ZIKV and congenital microcephaly and the mechanism, in which the virus affects prenatal development, is extremely important for decreasing the number of microcephaly cases.

Evidence for a Link between ZIKV and Microcephaly

After the emergence of ZIKV in Brazil in 2015, a 20-fold average annual increase of microcephaly cases was reported. In 2015, there has been 1248 new suspected cases – prevalence of 99.7 per 100 000 livebirths. (Ventura, et. al. 2016) On November 28, 2015 Brazil declared congenital ZIKV to be responsible for the microcephaly epidemic. The very first alert was raised by the State Secretariat of Health of Pernambuco, Brazil in October 2015 after analyzing the obvious spike in microcephaly cases: from January to July 2015 there had only been 6 cases reported, 6 cases in August, 11 in September, and 39 in October. Mothers of 38 newborns were interviewed, 24 of whom reported having a rash during pregnancy. They also reported not to have had any common exposure to pesticides, drugs, alcohol abuse, radiation, or any other possible teratogenic factors. (Teixeira, et. al. 2016) As ZIKV has spread to other states in Brazil, 49th epidemiologic week had the highest rate of cases of microcephaly reported – 900 cases in a week; women delivering then would have been in the first trimester of pregnancy during the peak of ZIKV outbreak. (Kleber de Olivera, et. al. 2015; Teixeira, et. al. 2016) This observation suggests a very strong association, both geographic and temporal, between ZIKV and microcephaly epidemics.

In addition, a retrospective study, using serologic and statistical data, of 2013-2014 outbreak of ZIKV disease in French Polynesia identified that 1% of mothers infected with ZIKV in the first trimester, had given birth to neonates with microcephaly, which suggests a prevalence to be 50 times as high as the estimated baseline. (Rasmussen, et. al. 2016) Yet, this study was based on small numbers of patients, fetuses were not tested for ZIKV, and other brain anomalies were not included in the study.

Eight-eight pregnant women in Brazil, who reported having an onset rash for the previous 5 days, were tested and evaluated for ZIKV RNA. There were 72 with positive test results and 16 negative. Ultrasonography of 42 of those testing positively and the 16 negative ones revealed fetal brain anomalies were present in 29% of the former and 0% of the latter. (Rasmussen, et. al. 2016) Yet, this study is very limited by lack of tests for other viruses in mothers' serum and amniotic fluid for ZIKV or other viruses. Also, there were no postnatal observations of the patients.

Link between congenital ZIKV and microcephaly is strongly supported by histopathologic evaluation of 4 formalin-fixed, paraffin-embedded tissue samples from two newborns, born at 36 and 38 weeks of gestation, who died shortly after birth and had microcephaly, parenchymal calcification, microglial nodules, gliosis, brain cell necrosis, and two miscarriages (lost at 11 and 13 weeks gestation) with chorionic villi with calcification, fibrosis, and focal villitis. All four mothers reported having clinical signs of Zika during the first trimester of pregnancy – fever and rash. Laboratory testing was performed by the CDC; the samples included brain tissues from both newborns, placenta of one newborn, and products of conception from the two miscarriages. These samples were tested by ZIKV RT-PCR and all four cases turned out positive for NS5 and envelope genes. Further sequence analysis revealed the highest matches with ZIKV strains isolated from Brazil in 2015. The same samples were also tested for viral antigens by immunohistochemistry using a mouse polyclonal anti-Zika virus antibodies, and viral antigens were found in glial cells and neurons of one newborn and in chorionic villi of one of the miscarriages. RT-PCR for dengue was performed and tests results were negative, which eliminates the possibility of cross-reaction of the virus. (Martines, et. al. 2016) Also, the mothers' tests for rubella, herpes simplex, HIV, toxoplasmosis, and cytomegalovirus were negative, which points at ZIKV infection as the cause of microcephaly and fetal demise.

Another piece of evidence that supports the suggestion that ZIKV can negatively affect fetal CNS and cause microcephaly was obtained by a research team at Hospital Geral Roberto Santos in Salvador, Brazil. There, tissues and fragments of a female fetus, delivered after fetal demise in the 32nd week of gestation, were examined and tested for congenital ZIKV. Fetus had signs of microcephaly and arthrogryposis. Samples of cerebral cortex, medulla oblongata, cerebrospinal and amniotic fluids tested positive by ZIKV-specific RT-PCR, which targets viral NS5. Samples from other organs, such as extracts from the heart, lungs, and liver, didn't test positive and appeared to be undamaged. Also, the mother's tests for HIV, HTLV, hepatitis C, toxoplasmosis, rubella virus, and cytomegalovirus during the 4th gestational week were negative, which leaves responsibility for fetal brain anomalies to the virus. (Sarno, et. al. 2016) However, this study is limited by not performing histopathological analysis of the sample tissues.

In addition, a different group of researchers tested the amniotic fluid of two pregnant women with microcephalic fetuses. Both women had normal fetal ultrasounds in the first trimester, but ultrasounds done later on in weeks 25 and 27 revealed severe microcephaly (head circumference below the third percentile), hypoplasia of the cerebellar vermis, and posterior fossa enlargement in one fetus and asymmetry of hemispheres, and hypoplastic cerebellum with the cerebellar vermis completely missing. Both mothers did not report being on any medications during pregnancy, alcohol and drug use, or smoking. Tests for autoimmune and immunodeficiency diseases, and TORCHES were negative as well. However, they both reported having ZIKV infection symptoms at 10 and 18 weeks of gestation, respectively; also, they haven't reported travelling outside of Brazil during the previous years, or being in contact with any infected individuals. The amniotic fluid from each patient was then tested for any non-human genomic sequences, and ZIKV genome was identified in both samples. (Calvet, et. al 2016; Schuler-Faccini, et. al. 2016) The samples were also tested with RT-PCR for dengue and chikungunya, and the results were negative. ELISA test identified the presence of anti-ZIKV IgM in both samples as well. (Calvet, et. al. 2016) This study proves that ZIKV infection can cross the placental barrier. And since the ultrasounds at earlier gestation stages (during the first trimester) appeared to be normal but during the second trimester the fetuses started to develop signs of microcephaly and other brain anomalies (after the mothers being infected in the first trimester), the link between ZIKV and microcephaly is evident.

Also, according to Shepard, a pioneer in the field of teratology, in order to consider something a teratogen, there has to be an association of a rare environmental exposure with a rare defect. (Rasmussen, et. al. 2016) Considering the stats for microcephaly obtained in Brazil would not qualify because exposure to ZIKV wasn't rare. Yet, ZIKV is a rare exposure for the people that spent only a short time in the ZIKV affected areas and gave birth to infants with such brain anomalies. Considering microcephaly statistics in the United States of America, it can be assumed to be a rare defect, with only 6 cases in 10000 live-born infants. One of the recent reports in the U.S. illustrates this connection: a pregnant woman traveled to Mexico, Belize, and Guatemala during her 11th gestation week. She was tested positive for ZIKV IgM after her return. Ultrasonography and MRI of the fetus was performed during gestation weeks 19 and 20 and severe brain anomalies were diagnosed; the head circumference had dropped from the 47th percentile during the 16th week of gestation to the 24th percentile at gestation week

20. The pregnancy was terminated in the 21st gestation week, but considering the trend of the head circumference decrease, microcephaly would have developed if the pregnancy continued. (Rasmussen, et. al. 2016).

Mechanisms of infection of the fetal brain by Zika virus

The most recent research suggests that ZIKV is able to infect human neural progenitor cells, dysregulate transcription and stunt their growth. Human induced pluripotent stem cells (hiPSCs) were used as an in vitro model to investigate the mechanism of ZIKV infection and impact on neural cells. This study was able to determine that cells of different cell lines have variation in their susceptibility to the virus; hiPSCs showed one of the highest permissiveness levels. Further investigation of hiPSCs established that human neural progenitor cells (which can further be differentiated into cortical neurons, astrocytes, and oligodendrocytes) are a direct target of a ZIKV. Within 72 hours after being infected with ZIKV, total number of viable cells was observed to be reduce by 29.9 +/- 6.6%. Levels of caspase-3, involved in apoptotic pathway regulation, in infected hNPCs were found to be significantly higher than those in control group. (Tang, et. al. 2016) However, it is hard to conclude if all strains of ZIKV affect human embryotic brain cells in the same manner, for only one strain of virus was used in this research. In addition, assessing the level of neurotropism expressed by ZIKV is intricate because Zika infects other types of cells as well.

Conclusion

Evidence linking frequent occurrence of microcephaly in regions with ongoing ZIKV is deficient and scarce, for the pandemic is very recent and emerging. There are still many questions that remain unanswered, such as whether there's a range of associated birth defects, how this range is affected by the timing of fetal exposure to the virus and severity of maternal infection, if such range exists, and whether severe anomalies are only limited to the CNS.

However, based on the data available for analysis, it may be concluded that ZIKV can cross the placental barrier and exhibit higher tropism to a specific range of tissues. The accumulation of geographical and temporal and rare exposure-rate defect associations between maternal ZIKV infection and microcephaly in fetuses and neonates, and multiple case-study findings support a causal relationship present between the two events. Yet, more research must be conducted to have a better understanding of the effects and mechanisms of infection and to be able to propose possible treatment solutions.

List of Acronyms

Arbovirus	Arthropod-borne virus
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-Linked Immunosorbent Assays
HIV	Human Immunodeficiency Virus
hiPSC	Human Induced Pluripotent Stem Cell
hNPC	Human Neural Progenitor cell
HTLV	Human T-Cell Lymphotropic Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NAT	Nucleic Acid Amplification Test
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
TORCHES	Toxoplasmosis, rubella, cytomegalovirus, herpesvirus, and syphilis
ZIKV	Zika Virus

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How does High Intensity Interval Exercise Affect Fat Loss?

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Abstract
US survey trends show that since the 1970's there has been a decline in energy intake, (consumption of food) and a rise in the prevalence of obesity. This suggests that there must have been a dramatic decrease in total energy expenditure. Energy expenditure is the sum of energy (or calories) the body needs to carry out physical functions such as breathing and digestion, as well as the energy used in physical activity. As the rate of obesity in the US increases, it is becoming more and more important to find ways to increase energy expenditure, specifically through physical activity. Physical activity is often thought of as the key to fat loss because it is the component of energy expenditure that is most variable and can lead to the greatest total energy expenditure. The issue that follows is that many overweight individuals lack the motivation to invest time and energy into a substantial amount of exercise. To overcome this problem and to help make exercise more manageable, high interval intensity exercise (HIIE) can be a practical solution. HIIE is an exercise strategy that prescribes short periods of intense exercise alternating with less intense recovery periods. HIIE can potentially, with little time investment, result in energy expenditure equivalent to that of lower intensity continuous exercise done for a longer period of time. The purpose of this review is to examine the effects of HIIE on body composition and fat loss according to the current literature. If HIIE proves to be an effective way to burn fat without the time investment exercise routines typically require, it may make fat loss goals more attainable and lead to better results if implemented into weight loss programs.

Introduction

Fuel Source During Exercise

While the human body is at rest, skeletal muscle is responsible for 30% of oxygen consumed. During strenuous exercise, skeletal muscle can account for more than 90% of the total metabolism. ATP is required in order for skeletal muscles to contract and relax during exercise. As such, the purpose of muscle metabolism is to generate ATP via oxidative phosphorylation. Skeletal muscle can use free fatty acids, glucose, or ketone bodies as fuel for ATP production (Garrett et al., 2012). Since cellular respiration involves several complex metabolic pathways, the relative utilization of fat and carbohydrate during exercise can vary enormously and depends strongly on exercise intensity (Loon et al., 2001). Generally, as intensity of exercise is increased, the amount of oxygen available decreases and the muscle carries out anaerobic respiration. This refers to glycolysis, a metabolic pathway that primarily uses carbohydrates as substrate. The increased carbohydrate metabolism (glycolytic flux) limits the transport of long chain fatty acids into the mitochondria where fat oxidation generally occurs. Consequently, increased carbohydrate usage as fuel will inhibit the use of fat as fuel (Christmass et al., 2000). In high intensity intermittent exercise it's often found that carbohydrate breakdown decreases, and fat oxidation increases as the exercise progresses (Talanian et al., 2006). This can be attributed to the interval nature of HIIE and is known as the "substrate-shuttle" effect. (EG Trapp et al., 2007).

The "Substrate-shuttle" Effect

Intramuscular triglycerides are an important supply of fuel for skeletal muscle. Each one is composed of three fatty acids esterified to a glycerol backbone. When glycerol levels are elevated, it's indicative of an increased reliance on fats for fuel. (Trapp et al., 2007).

Lactate is a waste product of glycolysis, generated from the anaerobic breakdown of glycogen. While exercising steadily, there is normally a balance between lactate production and lactate removal. When the intensity of exercise reaches a point at which there is an abrupt increase in lactate levels, lactate threshold has been reached. If lactate levels are above threshold, the excess blood lactate will inhibit carnitine- acyl CoA transferase, an essential enzyme which enables beta-oxidation to occur. (Trapp et al., 2007) (explain beta oxidation).

Lactate and glycerol levels were analyzed in one particular study of HIIE. 16 women, 8 trained and 8 untrained, participated in two 20 minute short sprint sessions (8 second sprint/12 second recovery) and two 20 minute long sprint sessions (24 second sprint/36 second recovery). After exercising, they found that plasma glycerol levels went up dramatically for both groups ($P<.0001$). Lactate levels rose as well; they were higher during long sprints for trained women than for short sprints ($P<.01$). (Trapp et al., 2007).

The elevated glycerol levels indicate that there was an increased fat oxidation for fuel. This enhanced fatty acid oxidation, seems contradictory to the fact that lactate levels were elevated above threshold (3.5mmol/L - 4.0mmol/L). The solution proposed for this contradiction is the intermittent nature of HIIE. The intervals in HIIE create a "substrate shuttle" with alternating anaerobic and aerobic energy sources. During the high intensity periods, ATP and creatine phosphate are broken down to produce energy. During the rest periods, they are resynthesized aerobically. However, since the rest interval isn't that long, it is likely that the resynthesis is incomplete and anaerobic glycolysis provides the rest of the needed energy. This is verified by

Bangsbo et al. 1991, who found that glycogen, the substrate for anaerobic glycolysis, was shown to be progressively diminished over the course of HIIE. Eventually, repeated exercise results in an accumulation of cytosolic citrate which inhibits phosphofructokinase activity and impairs glycolysis. It is suggested that the progressive depletion of glycogen and phosphocreatine along with the increased levels of cytosolic citrate, act together to inhibit glycolysis, and allow for repeated bouts of high intensity exercise, while reducing lactate accumulation. The aerobic metabolism that accompanies lower lactate levels may enhance fatty acid oxidation. (Trapp et al., 2007).

Methods

This comprehensive review is based on critical analysis of literature obtained using the Touro College Online library and Google Scholar. References in these articles were retrieved and served as additional points of reference for further research. Review articles were useful in helping provide additional references to source material.

Discussion

Many studies have been performed in an effort to establish the relationship of HIIE with increased fat loss. The studies examine whether HIIE causes enhanced fat loss, and via which mechanisms this may occur. Since the mechanisms of carbohydrate metabolism, fat oxidation, and fat loss in general are complex and detailed, there are many different ways to determine if fat loss occurred as a result of exercise. The study of energy expenditure, the analysis of skeletal muscle, the catecholamine response, and the respiratory exchange ratio can all provide useful information regarding whether enhanced fat oxidation occurred as a result of HIIE.

Energy Expenditure and Fat Loss

The volume of physical work done is often the factor that plays the greatest role on energy expenditure (EE). This volume is based on the duration and intensity of the exercise done. For example, more energy is expended when walking 5 miles than when walking 3. Generally, as long as the activity is done at the same intensity, there is a linear relationship between EE and work carried out. However, when exercise intensity is changed, it affects how efficiently work can be performed. There is a negative relationship between exercise intensity and efficiency (Hunter et al., 1998). For example, for the same amount of cycling, EE was 22% greater when it was carried out at a high intensity than at a low intensity (Treuth et al., 1996). The exact reasons behind this inverse relationship between efficiency and exercise intensity are unknown. In any case, it seems to be that the best way to increase EE is to carry out a high volume of work at high intensity. However, this kind of exercise can bring about fatigue very quickly. Therefore, high intensity exercise interspersed with rest intervals seems to be an ideal way to increase EE by combining high intensities with multiple rest intervals (Hunter et al., 1998).

The question that follows is if two exercises have equal EE, yet one was reached via HIIE and the other via low intensity continuous exercise, will HIIE generate more fat loss?

One study that compared results of high and moderate intensity exercise programs with equivalent EE found that this was indeed the case. This 15 week intervention study was performed on 34 healthy women aged between 18 and 30 years old. They were divided into an HIIE group, a continuous exercise group, and a control group. The HIIE protocol consisted of 8 seconds sprinting on an ergometer(?), alternating with 12 seconds of turning the pedals slowly. As each subject was able to complete 20 minutes of HIIE at a resistance of .5kg, the resistance for that subject was increased by increments of .5kg. The continuous exercise protocol consisted of a five minute warmup and cooldown, and a 15-20 minute (later increasing to 40 minute) work session pedaling at a continuous intensity of 60% VO_2 peak. VO_2 peak is the point when a subject's oxygen consumption plateaus at ≤150 ml/min VO_2 as the test progressively gets more difficult. When this happens, it's indicative of the fact that the subject is close to his actual VO_2max capacity, or maximal oxygen intake. In the continuous exercise group as well, the resistance was raised by incremnets of .5kg as the fitness of each subject improved. The control group was instructed to maintain their usual diet and physical activty. Total EE for the continuous exercise group and HIIE group was 36.3 ± 3.4 MJ and 41.5 ± .81 MJ, respectively. This is considered a non-significant difference. Nevertheless, there was a substantial decrease in total body mass for the HIIE group compared to the other groups. The HIIE group also displayed a significant decrease in fat mass while the other two groups had a fat mass gain. (Trapp et al., 2008). Not only did the HIIE group expend the same amount of energy in a shorter amount of time, that equivilent EE caused more fat loss when reached via higher intensity exercise.

It should be noted that studies have indicated a direct correlation between initial adiposity and amount of fat lost. Research has shown that subjects with higher fat levels to begin with, tend to lose more fat (Trapp et al., 2007), (Trapp et al., 2008). This would support the notion that HIIE would generate a more significant loss of fat in overweight individuals than in thinner subjects. That being said, there were four lean women in the HIIE group of the aforementioned study who possesed significantly lower fat mass than the rest of the group. With these four women removed, the HIIE group displayed a 4.3% decrease in total body mass, a 14.7% decrease in total fat mass, and a 9.5% decrease in central abdominal fat. Conversely, the continuous

exercise group displayed a 10.5% increase in central abdominal fat. (Trapp et al., 2008). These results futher demonstrate that for a given expenditure of energy, fat loss is greater when intensity is high.

Another study was performed on 21 middle aged obese women with the metabolic syndrome, displayed similar results. During this 16-week exercise intervention program, the women were divided into 3 groups – control, low intensity exercise training (LIET), and high intensity exercise training (HIET). The LIET group trained five days a week at an intensity at or below their lactate threshold. The duration of each session was adjusted so that the participants expended a specific amount of energy per week. The HIET group also exercised 5 days a week, with high intensity sessions for three of the days and moderate intensity exercise sessions for the remaining two. Participants exercised at an intensity between their lactate threshold and VO_2peak on the high intensity days and at an intensity at or below their lactate threshold on the lower intensity days. Again, the duration of sessions was modified based on the progression of caloric expenditure as the training intervention proceeded. The findings indicate that exercise of higher intensity generates greater fat loss compared to exercise of lower intensity under isocaloric training conditions. (Irving et al., 2008).

It should be noted that the HIET group in this study likely had a total EE that was slightly greater than that of the LIET group. This is because the total EE per session was a combination of the kcal expended during training as well as resting metabolism. Therefore, on HIET days when the duration of exercise was slightly shorter, the resting metabolism would contribute to a lower fraction of the total EE. This caused a 25 kcal difference between the HIET and LIET groups per week, totaling a difference of 400 kcal over the course of the training intervention. (Irving et al., 2008).

The previous studies examined the difference in fat loss when EE was equal between groups. Overall, greater fat loss was noted when exercise was done at higher intensites. Research has also been done to compare HIIE and continuous exercise when EE is not equivalent.

In one study, the mean estimated total energy cost was 120.4 MJ for the continuous/endurance training (ET) exercise program, and 57.9 MJ for the HIIE program. Nevertheless, the reduction in the sum of six skin folds was greater after HIIE. Since there is an association between exercise duration and fat loss, the changes in subcutaneous fat were corrected based on the total energy cost of training. As demonstrated when the decrease in the sum of the skinfolds was divided by energy expenditure and expressed as changes in subcutaneous skinfolds per MJ, the participants in the HIIE program lost nine times more subcutaneous fat than those in the ET program (Tremblay et al., 1994).

However, not all studies show HIIE to be the superior to ET. Very often, when the EE is not equivalent, HIIE will not bring about more favorable results than ET. Nevertheless, if HIIE brings about the same results with a smaller time investment, it is still extremely worthwhile. In the present study is an example of HIIE that brought about results copmarable to ET. Sprint interval training is a form of HIIE where the high intensity intervals involve 30 second "all-out" sprints on an ergometer. 16 active men were separated into a sprint interval training (SIT) group and a high volume endurance training (ET) group. In this study, the SIT protocol consisted of 6 sessions of 4-6 repeats of 30 seconds "all-out" cycling at about 250% VO_2peak with 4 minute recovery periods. The ET program consisted of 90-120 minutes of continuous cycling at 65% of VO_2peak. Total EE was 630 KJ for the SIT group, and 6500 KJ for the ET group. Despite the fact that EE for the ET group was 10 times more than the EE for the SIT group, the results showed that SIT brought about similar improvements in muscle oxidative capacity. (Gibala et al., 2006).

Another SIT study yielded similar results. 20 young men and women were separated into SIT and ET groups for 6 weeks of training. The SIT consisted of 4-6 30 second "all-out" cycling tasks (at 500W) three times a week, and the ET group consisted of 40-60 min of continuous cycling at about 65% VO_2peak five days a week. Total weekly exercise volume was 90% lower in the SIT group. Moreover, since most of SIT exercise time is actually spent in recovery from the 30 second bouts of cycling, actual weekly exercise time was only about ten minutes in SIT, compared to 4.5 hours in ET. Nevertheless, results between the two groups were very similar: whole body fat oxidation was increased and carbohydrate oxidation was decreased. (Burgomaster, 2008).

Skeletal Muscle Analysis

As mentioned earlier, the "substrate-shuttle" effect supports enhanced fat oxidation by activating aerobic metabolism and enabling the mitochondria to produce ATP. In line with this, it's likely that HIIE will result in increased mitochondrial enzyme activity. Therefore, when studying the effects of HIIE on skeletal muscle, many researchers focus on changes in mitochondrial enzymes. Based on increased activity of mitochondrial enzymes and the fact that aerobic metabolism favors enhanced fatty acid oxidation, it's possible to surmise that an increase in mitochondrial enzyme activity during HIIE will correlate with fat loss.

Citrate synthase and β-HAD (Hydroxyacyl- Coenzyme A dehydrogenase) activity, are important indicators of enhanced fatty acid metabolism, that were significantly increased in an HIIE

intervention in just 2 weeks. In this study, skeletal muscle enzyme activity was measured to confirm an increase in the use of fats as fuel. 8 healthy recreationally active women participated in this study. Each of them performed a VO_2peak test before and after 2 weeks (7 sessions) of HIIE. The VO_2peak tests involved a 60 minute cycling trial at about 60%VO_2peak. The seven HIIE sessions consisted of 10 cycles of 4 minutes of high intensity cycling at 90%VO_2peak alternating with 2 minute resting periods. Whole body fat oxidation and total fat oxidation were both significantly increased during the 60 minute cycling session after HIIE. Accordingly, there was a significant decrease in whole body carbohydrate oxidation and a 23% decrease in total carbohydrate oxidation. These results were then supported with a skeletal muscle analysis. (See Table 2)

ANALYZED	IN 60 MINUTE SESSION AFTER HIIE
β-HAD activity	increased by 32%
citrate synthase activity	Increased by 20%.
HSL protein content	Increased by 13%
FABPpm	increased 25%
FAT/CD36	remained unchanged
Resting muscle glycogen content	Unchanged
Net muscle glycogen utilization	Decreased by 12%
Intramuscular triglycerides	Decreased by 17%
(before HIIE, by 12%)	

Table 2 Summary of skeletal muscle analysis conducted after a 2 week HIIE intervention study (Talanian et al., 2006).

Usually, exercise training results in increased fat oxidation from fatty acids stored in adipose tissue and intramuscular triglyceride (IMTG) stores. In this study, however, HIIE did not cause a significant increase in IMTG utilization. HSL (hormone sensitive lipase) protein content was also not seen to be significantly increased. HSL is a key regulatory enzyme in lipolysis of IMTG stores. It's possible that the HIIE protocol in this study was too short to bring about significant adaptations in skeletal muscle (Talanian et al., 2006).

FABPpm (plasma membrane fatty acid-binding protein) and FAT/CD36 (fatty acid translocase) are two proteins that transport long chain fatty acids (LCFA) across the plasma and mitochondrial membranes. They are useful in determining if skeletal fat oxidation was enhanced or limited during exercise. In this study, FABPpm concentration was increased by 25% after HIIE,, and FAT/CD36 concentration remained unchanged. It is true that an inhibition of FABPpm will reduce the uptake of LCFA, but an unchanged FAT/CD36 doesn't necessarily mean the same thing. It's possible that there was a shift in the fractional concentrations of FAT/CD36 on the mitochondrial and plasma membranes that could have increased LCFA uptake without a notable increase in FAT/CD36 concentration. (Talanian et al., 2006).

The elevated citrate synthase and β-HAD levels indicate increased use of fats for ATP. Citrate synthase is a critical enzyme in the citric acid cycle, a cycle which favors aerobic metabolism, and likely the use of free fatty acids as fuel. β-HAD, or 3-hydroxyacyl-CoA dehydrogenase, is an enzyme that catalyzes an important step in fatty acid metabolism. (Talanian et al., 2006)

In another SIT program, citrate synthase and β-HAD were analyzed as well. The results after 6 weeks of training showed that both citrate synthase and β-HAD increased after SIT in a similar manner to that of ET (See Figure 1), suggesting that fatty acid oxidation was occurring. This was further proven by the tremendous increase in whole body fat oxidation noted after training (Burgomaster, 2008).

Malate dehydrogenase (MDH) is another enzyme that is needed in the citric acid cycle. Increased MDH is indicative of aerobic conditions, and likely increased fat oxidation. In one particular study that compared HIIE to ET, increased MDH was found in both groups. 27 men and women partook in this study. 8 men and 9 women participated in a 20 week ET intervention, and 5 men and 5 women participated in the 15 week HIIE intervention. The ET program included a 30 minute (increasing to 45 minutes) cycling session, 4 times (increasing to 5) times) a week. Intensity corresponded to 60% (and increasing to 85%) of the maximal heart rate reserve. The HIIE intervention consisted of 25 sessions with 30 minutes of continuous exercise at an intensity similar to the endurance training program. Half of these were completed by the fifth week of the program. It also included 16 short interval sessions (10-15 bouts of 15-30 seconds of high intensity intervals, alternating with recovery periods to allow heart rate to return to 120-130 beats per minute), and 19 long interval sessions (4-5 bouts of 60-90 seconds of high intensity intervals alternating with recovery periods to allow heart rate to return to 120-130 beats per minute). The results showed that participants in the HIIE program lost nine times more subcutaneous fat than those in the ET program. These results were supported by the increased MDH activity. Furthermore, there was a substantial increase in β-HAD in the HIIE group alone, another indication of the fact that HIIE leads to better lipid utilization (Tremblay et al., 1994).

Catecholamine Response

Epinephrine is a catecholamine known for stimulating lipolysis. As such, elevated epinephrine concentrations after exercise would indicate an increased use of fat as fuel. Trapp et al., 2007 demonstrated this in their study when examining the different responses of trained and untrained women during HIIE.

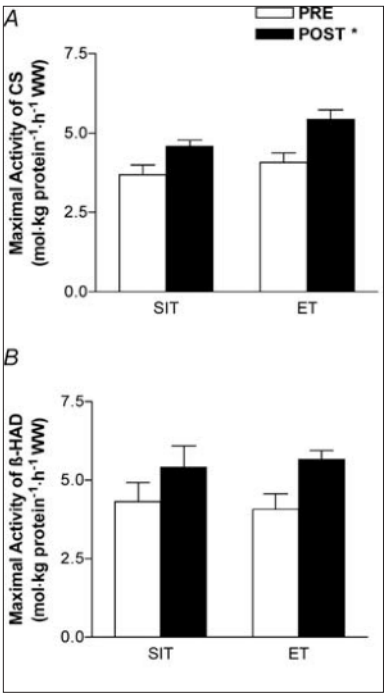

Figure 1 Activity of citrate synthase and β-HAD before and after 6 weeks of SIT training (Burgomaster, 2008).

It was found that epinephrine and norepinephrine levels both increased after short sprint and long sprint exercise (see Figure 8). The elevated catecholamine levels serve as another explanation of why the glycerol levels in that previously mentioned study were increased despite lactate levels above threshold.

Respiratory Exchange Ratio (RER)

RER is the ratio of carbon dioxide produced to oxygen consumed. Based on the RER, it is possible to estimate which particular fuel is being oxidized to produce energy. An RER close to .7 is indicative of fat as the energy source, and an RER close to 1.0 is indicative of carbohydrate as the energy source. An RER value between the two indicates a mixture of fat and carbohydrate. Therefore, by measuring RER after an exercise intervention, one can deduce what fuel was oxidized in order to produce ATP.

In one study, 10 obese males participated in 6 sessions of sprint interval training over a 2 week period. After the 2 week intervention, it was noted that the RER decreased from .78 +/- .01 to .73 +/- .01, suggesting that fat was the primary substrate used for oxidation. This was further confirmed by the fact that resting carbohydrate oxidation was significantly lower after training and that resting fat oxidation was increased by 18.2%. Even though significant weight loss wasn't noted in this study, (body mass was somewhat lower (P=.055), and waist and hip circumference were reduced by ≈1 %,) the magnitude of increase in resting fat oxidation is used in order to predict the extent of exercise-induced fat loss. This estimate is based on resting fat oxidation alone and is independent of exercise energy expenditure and change in resting metabolic rate. The fact that no noteworthy weight loss was noted in this study is explained by the fact that the intervention was short-term. Based on the increased fat oxidation, it can be inferred that a longer-term intervention would likely yield more substantial weight loss results (Whyte et al., 2010).

Can HIIE really help the overweight population?

The actual benefits of interval training can be seen when comparing it to another weight loss method. 62 overweight adolescents were divided into two groups- aerobic interval training group (AIT) and multidisciplinary approach group (MTG). The AIT group did HIIE on a treadmill twice a week for 3 months, and were then encouraged to perform at least 2 interval sessions for the next 9 months. The HIIE sessions included 4, 4 minute bouts at 90% of maximal heart rate, alternating with 3 minute lower intensity sessions at 70% maximal heart rate. The MTG were subjected to a 12 month regimen which included meeting every 2 weeks with a doctor, a clinical nutritional physiologist, and a nutritional psychologist. After 3 and 12 months, only the subjects in the AIT group had a decreased percentage of body fat. It's interesting to note that the subjects in the AIT group followed a better diet than those in the MIT group even though they were not given clear guidelines regarding caloric intake. This is possibly because AIT brought about a motivational side effect of improving their aerobic capacity. Indeed, some members commented that they became more conscious of their eating habits after 3 months of the AIT program. (Tjonna et al., 2009).

Limitations

When analyzing fat loss over an extended period of time, it is difficult to isolate the cause of it. Even though subjects in the HIIE studies were instructed to maintain their regular diets, it's not guaranteed that they did. As a result, it's possible that some of the fat loss displayed after an exercise intervention was partly due to dietary modifications.

Another limitation the study of HIIE poses, is the varying intensities and duration of intervals. Most studies mentioned in this paper prescribed a different HIIE "formula." Since there hasn't yet been a study that tests every combination of intensity and duration of exercise, it is difficult to determine the minimum length and intensity needed in order to bring about the benefits of HIIE. It's possible that the results discussed in this review would not be successfully replicated if done at a slightly lower intensity, or for a shorter amount of time.

Conclusion

HIIE is an arduous method of exercising which requires subjects to push themselves to their maximum capacity for short intervals of time. Most studies conducted regarding HIIE involve high

intensity intervals that are extremely difficult and demanding. Although HIIE has been shown to enhance fatty acid oxidation and increase fat loss, it may be dangerous for an obese, non-physically active person to attempt.

That being said, for individuals who could handle the workload of HIIE, the short duration of intense intervals might encourage exercise because there's only a small span of time where they are required to push themselves. Moreover, more people might be motivated to exercise daily since only a few short sessions of HIIE are needed to improve whole body fat oxidation. As mentioned earlier, it took only 7 sessions of HIIE to increase total fat oxidation by 36% in previously untrained women (Talanian et al., 2006), and only 6 sessions of sprint training for overweight men to increase resting fat oxidation by 18.5% (Whyte et al., 2010).

	MTG			AIT		
Variable	Baseline	3 Months ($n = 22$)	12 Months ($n = 14$)	Baseline	3 Months ($n = 20$)	12 Months ($n = 13$)
Body composition						
Weight (kg)	94.3 ± 15.3	95.4 ± 1.4	96.1 ± 1.7	94.1 ± 23.3	94.4 ± 1.4	94.4 ± 1.7
Height (cm)	168 ± 8.6	169 ± 0.4**	171 ± 0.5**	169 ± 10.6	170 ± 0.4**	173 ± 0.5**
BMI (kg/m²)	33.3 ± 4.5	33.1 ± 0.4	32.9 ± 0.5	33.2 ± 6.1	32.5 ± 0.4**	31.4 ± 0.5
Waist (cm)	101.4 ± 10.8	104.2 ± 1.6	100.1 ± 2.3	105.3 ± 10.5	104.9 ± 1.7	98.1 ± 2.2*
Total fat (%)	41.1 ± 4.7	40.8 ± 0.5	39.1 ± 0.6	40.6 ± 5.3	39.3 ± 0.5**	38.6 ± 0.7**
Fat weight (kg)	34.8 ± 7.8	35.1 ± 1.1	34.7 ± 1.3	34.5 ± 12.2	33.6 ± 1.1**	32.1 ± 1.5
Fat weight trunk (kg)	17.7 ± 5.3	17.9 ± 0.6	17.1 ± 0.8	18.3 ± 7.2	17.0 ± 0.6**	16.8 ± 0.8
Lm_t (kg)	56.1 ± 8.7	57.9 ± 0.6**	58.8 ± 0.8	55.9 ± 11.8	57.9 ± 0.6**	60.0 ± 0.8

Table 2 Variables related to the overweight adolescents during the experimental period. Values are means±S.E.M., except baseline values which are means±S.D. *P<0.05 and **P<0.01, significantly better compared with baseline within the group; §P<0.05, significantly better compared with the respective time in the MTG group. 1RM; one repetition maximum; nm, not measured (Tjonna et al., 2009).

Further research is needed in order to determine the minimum intensity and duration required for HIIE-induced fat loss. Because there are a large number of potential variables regarding the kind of exercise, the intensity and duration of the exercise, and the population exercising, it is difficult to create an exact guideline for HIIE. Consequently, more research is needed before HIIE is prescribed, to help decrease fat in overweight individuals.

Abbreviations

AIT – aerobic interval training group
EE – energy expenditure
ET – endurance training
FABPpm (plasma membrane fatty acid-binding protein)
FAT/CD36 (fatty acid translocase)
HIET – high intensity exercise training
HIIE – high intensity interval exercise
HSL – hormone sensitive lipase
IMTG – intramuscular triglycerides
LCFA – long chain fatty acids
LIET – low intensity exercise training
MDH – malate dehydrogenase
MTG – multidisciplinary approach group
RER – respiratory exchange ratio
SIT – sprint interval training
β-HAD – Hydroxyacyl- Coenzyme A dehydrogenase

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3D Organ Printing

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Abstract

The global shortage of donor organs is a cause for countless fatalities across the world. Although, diseases can be treated through organ transplantation it can come along with many complications. Not only is there a high demand for donor organs, there is also the risk of the body's rejection of the newly implanted organ. Through the method of 3D printing organs, many lives could be saved as well as reducing the need for donor organs. Finding materials to create a suitable scaffold is the focus of many experiments. Materials that are used in organ printing are made from soft materials, therefore, suspended hydrogel techniques are utilized for printing organs and for creating vascularization systems in the printed organs. The vascularization level of 3D printed organs is the most complicated because of its vast and detailed preciseness. Detailed magnetic resonance imaging is taken to generate the 3D image of the structure and consequently print the image layer by layer as opposed to the older method of manually applying cells onto scaffolds. While many research teams have made progress with 3D organ printing, complex organs such as the heart and kidney are currently undergoing research to be implemented into clinical settings.

Introduction

Transplanting new organs is a way of trying to deal with life threatening diseases. Instead of the chronic maintenance of diseased organs medical professionals would be able to replace them, thus curing the condition. One leading major health crisis is the shortage of organs available for transplants. Although medicine's advancements help people live longer, organs tend to fail more often as a result of longer lifespans. For instance, in the United States, a name is added to the Transplant List on an average of every ten minutes. Additionally, the number of patients needing organs have doubled while the amount of transplant procedures has barely increased (OPTN, 2016). 3D organ printing technology is a promising step to help solve the organ transplantation crisis.

The field of regenerative medicine deals with replacing, engineering, or regenerating human cells, tissues, or organs in order to help achieve normal function. Regenerative medicine is not a new field; Alexis Carrel, a Nobel Prize winner, writes in his book, "The Culture of Organs" (Carrel, Lindbergh, 1938) about technologies that are still used today for blood vessel grafting.

Thomas Boland, self-described as the "grandfather of bioprinting," took an old Lexmark computer, emptied the ink cartridge and filled it with collagen instead. He then glued a thin, black silicon sheet onto blank paper and inserted it into the printer. He opened a Word document on his PC, typed his initials, and hit print. The paper came out with "TB" clearly presented in off-white proteins. By 2000, Boland and his team had reconfigured a Hewlett-Packard DeskJet 550C to print with E. coli bacteria. Then they expanded to larger mammalian cells obtained from Chinese hamsters and lab rats. Once printed, 90 percent of the cells remained viable, which meant the product was useful, not only art. Boland introduced 3D printing for cellular construct when he patented the process for using the ink-jet printer for printing cells. Boland's process used a system where cells were deposited into 3D matrices placed on a substrate. Instead of using ink, 3D printers use cells in a cartridge where the cells are being layered in order to create a three-dimensional structure that can lead to functional organs and tissues (Wilson, Boland, 2003). This paper will review the current progress in organ printing technology with its promises and challenges and evaluate whether functional organs can be produced.

Methods

The information in this paper was obtained by the analysis of scientific articles and research papers obtained from the Touro College Online Library, specifically, the Health Science related databases such as Pubmed, Proquest Medical Library, and EBSCO multisearch. Additionally, websites like NIH.gov, Webmd.com, Mayoclinic.com and OPTN.com were used to gain general knowledge and information about the subject. Keywords such as "3D organ printing" and "regenerative medicine" were used to search for scientific articles.

Discussion: The Bladder

At first, researchers were pipetting cells into petri dishes by hand without the use of 3D printers. To do so, researchers would seed the cells from the petri dishes onto artificial scaffolds; a suitable base for the organs. The scaffolds were made from biodegradable polymers or collagen which provided a temporary matrix for the cells to cling to until they were able to stand on their own. Dr. Anthony Atala, the lead researcher at Wake Forest Institute for Regenerative Medicine, implanted the first lab grown bladder organs into seven patients at Boston Children's Hospital between 1999 and 2001 (Atala et. al., 2006).

There are a variety of injuries that can lead to damage or loss of the bladder, requiring eventual replacement or repair of the organ. Children with congenital anomalies such as: bladder exstrophy, myelomeningocele, or posterior urethral valves, can develop high-pressure and hypertonic low compliant bladders. Often times, these patients require cystoplasty when drug treatment fails. Gastrointestinal segments are frequently used as donor tissues for cystoplasty. However, when such tissues are incorporated into the urinary tract, several complications

can ensue; such as metabolic disturbances, urolithiasis, increased mucous production, and malignant disease (Emedicine, 2016).

Atala, along with his research team, explored an alternative approach using autologous engineered bladder tissue for reconstruction. Seven patients with myelomeningocele, aged 4-19 years old, with poorly compliant bladders were identified as candidates for cystoplasty. Urethral and muscle cells from the patients were grown in a culture, and seeded on a biodegradable bladder shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid. About seven weeks after biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted into patients. Tests such as serial urodynamics, cystograms, ultrasounds, bladder biopsies and serum analyses were performed. The bowel function of the patients returned to normal promptly after surgery with no metabolic consequences and renal function was preserved (Atala et. al., 2006).

Researchers soon adopted 3-D printers to make scaffolds more precisely because manually placing the cells onto scaffold remained a time-consuming and arduous process. Engineered bladders were made possible because they can be made with just two cell types however, an organ such as a kidney consists of thirty cell types. When attempting to engineer more complex tissues, there is no way to manually place different cell types into different locations that can replicate the native tissue structures. Manual placement is not the optimal method for delivering cells (Murphy, Atala, 2014).

The Heart

The human heart is a complex biological machine. It pumps blood to all parts of the body, begins to beat three weeks after conception and does not stop until the day of death. It is remarkable how the heart can last that long and function with the same redundancy and efficacy. The heart operates for billions of cycles, it is chemically powered and electrically synchronized. The heart is composed of over a hundred billion cells including cardiomyocytes, conduction system, fibroblasts, endothelial cells, smooth muscle cells and neurons. The main reason researchers are trying to 3D print the heart is because the heart is unable to regenerate itself and for this reason heart disease is the leading cause of death in first world countries (Mayo Clinic, 2016).

A heart transplant is a lifesaving procedure which removes a damaged or diseased heart and replaces it with a new one. Heart failures come as a result of different conditions such as coronary heart disease, damaged heart muscle or valves, congenital heart defects, or viral infections of the heart. Once the human heart is damaged, there is only a limited amount of regeneration that can occur and may require a heart transplant. A heart transplant comes with many risks and complications such as infection or the recipient's body attempting to reject the newly placed heart. To prevent rejection, the patients must immediately be given immunosuppressants as a lifelong treatment (Mayo clinic, 2016). There are currently 4,138 candidates who are on the UNOS list for a heart transplant (OPTN, 2016). The high number of candidates results in an agonizing wait for a new heart which usually does not have a positive ending. This long waiting list can be reduced through the possibility of 3D printing of the human heart.

The hierarchical structure and function in the heart has levels that scales from the nanometer level up to the macroscopic level. Every heart beat is powered by molecular motors, the actin and myosin, which are only a few nanometers in size and generate pico newtons amount of force. The actin and myosin are built into larger structures called the sarcomeres, the contractile unit of a muscle cell. Then, these are built into myofibrils which form tissue and surrounds the ventricles of the heart. At this present day, technology is unable to engineer something this complex starting from the nanometer scale and up. Yet, scientists are able to figure out where they are able to interface with the system, by providing information into the biology that can guide the living system to do the work of producing the components of the microstructures. Once the cells are formed, they themselves manufacture the microstructures necessary and the 3D bioprinting scaffolds, the suitable base, are used to build larger structures up to the size of a whole organ.

The embryo is a perfect example of biological manufacturing, it starts off with one cell and those cells divide and build an extracellular matrix around themselves until there is a full functioning organism. The extracellular matrix is a nanofiber network of protein and other molecules. It mechanically integrates cells into tissues, acts as an insoluble signaling network and functions as a scaffold that fills the space between the cells and tissues. This matrix acts as information because it provides cells with instructive cues on how to organize and form tissue. It signals between the cells using physics, chemistry and mechanics and is integral to tissue formation and function (Rozario, Desimone, 2010). A technique called Bottom Up Engineering of the Extracellular Matrix (ECM) was an attempt to build the ECM in the same way cells do; from single molecules into a complex 3D fiber with tissue specific structure and function according to the organ that is being engineered. This technique thereby accelerates the system of tissue formation (Szymanski et. al., 2014).

The idea of using the extracellular matrix is utilized in the following technique for 3D bioprinting organs and other structures. Soft materials such as collagen and fibrin are difficult for scientists to work with because they collapse under their own weight and do not have the mechanical strength to be printed

in air. To overcome this problem, a gel in gel technique dubbed Freeform reversible embedding of suspended hydrogels is used. This approach involves soft materials such as fibrin, collagen and the polysaccharide alginate. The materials are inside a thick slurry of gelatin microparticles and water in a petri dish. The semiliquid mixture of gelatin microparticles is able to flow around the printing needle as it operates. At the same time, the soft biomaterials being released are firmly implanted during the layer by layer printing technique. Once the printing of the scaffolds is completed, the dish is warmed up to 37 degrees Celsius thereby removing the gelatin and revealing the scaffold replica of an organ or tissue (Hinton et. al., 2015, Figure 1).

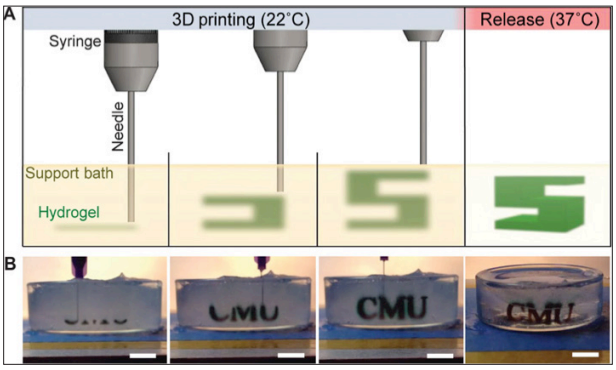

Figure 1 (Hinton et. al., 2015)
Freeform reversible embedding of suspended hydrogels technique

One application of this technique is using magnetic resonance imaging (MRI) to print patient specific scaffolds to engineer tissues. Through magnetic imaging the right coronary arterial tree is 3D printed. The right coronary artery along with the left coronary artery supplies blood to the heart. The right coronary artery is specifically responsible to supply blood to the right atrium, right ventricle, bottom portion of the left ventricle and the back of the septum. Additionally, the coronary arteries become blocked during a heart attack. The 3D printed artery was made of ECM materials such as fibrin, hyaluronic acid and alginate with solid walls and an open lumen. On a makerbot 3D printer the artery took about one hour to print which is the timeframe within the viability of cells. The wall thickness, lumen diameter and density can be tailored based on patient anatomy. To evaluate whether the 3D printed arterial tree was manifold it was set up in a custom made 3D printed perfusion fixture. A solution of $CaCl_2$ and 0.1% black food coloring was injected into the root of the tree. Perfusion was successful and captured on camera. The printed coronary artery has not been used for transplants such as a coronary artery bypass, yet this technique is a step up from the current printing of just straight tubes. This technique creates more complex architecture like the real coronary artery tree that can hold a better potential graft (Hinton et. al., 2015, Figure 2).

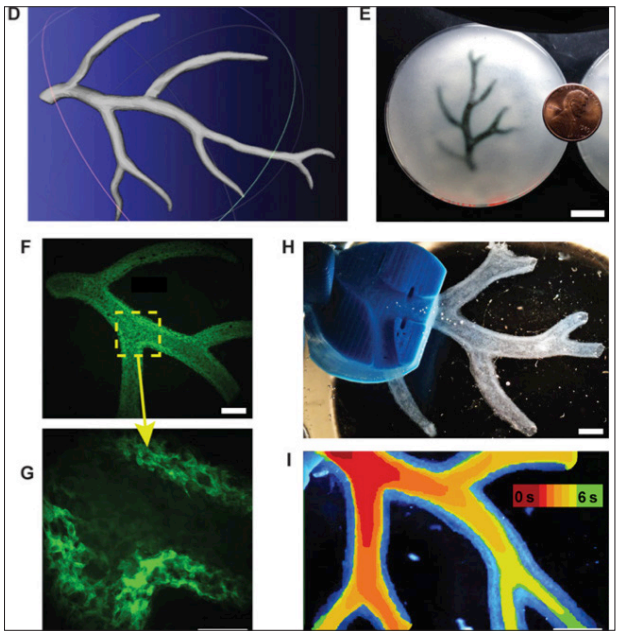

Figure 2 (Hinton et. al., 2015)

(D) A model of a section of a human right coronary arterial tree from 3D MRI is processed at full scale into machine code for FRESH printing. (E) An example of the arterial tree printed in alginate (black) and embedded in the gelatin slurry support bath. (F) A section of the arterial trees printed in fluorescent alginate (green) and imaged in 3D to show the hollow lumen and multiple bifurcations. (G) A zoomed-in view of the arterial tree shows the defined vessel wall that is <1 mm thick and the well-formed lumen. (H) A dark-field image of the arterial tree mounted in a perfusion fixture to position a syringe in the root of the tree. (I) A time-lapse image of black dye perfused through the arterial tree false-colored at time points of 0 to 6 s to show flow through the lumen and not through the vessel wall. Scale bars, 4 mm (B), 10 mm (E), 2.5 mm (F), 1 mm (G), and 2.5 mm (H and I). (Hinton et. al., 2015).

Another application of the Freeform reversible embedding of suspended hydrogels technique is used to build the embryonic heart. The 3D model of the embryonic chick heart was generated from 3D optical imaging data of a fluorescently labeled 5-day-old heart. The embryonic stage is the time when the heart muscle forms. It is the only time where the human body has the potential to form new heart muscle. In order to accurately 3D print an organ, there needs to be accurate data input to the system. The first step is the 3D imaging of the embryonic heart to identify the ECM macrostructure and microstructure associated with myogenesis and vascularization. 3D imaging goes through the embryonic heart and images the entire heart, ventricles, and trabeculated tissue together with all the exquisite details. A 3D computer model of the trabeculated embryonic heart is generated based on confocal imaging of cells and the extracellular matrix. Then, based on the computer model the 3D heart is printed and compared with the computer generated image (Hinton et. al., 2015, Figure 3).

One step further in 3D cardiac printing is the ability to print cardiac cells called organoids. This research called "Body on a Chip" is currently ongoing at Wake Forest Baptist Medical Center. The research converted human skin cells into a network of functioning heart cells, and also fused them with lab grown liver cells using a 3D printer. The heart organoid is able to beat because it contains specialized cardiac cells and the cells receive correct environmental cues such as keeping them at

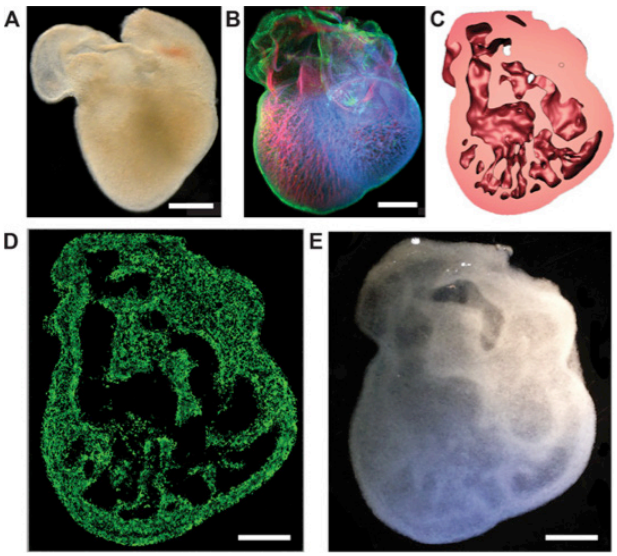

Figure 3
Printed embryonic heart scaffolds with complex internal and external architectures based on 3D imaging data from whole organs

the same temperature as the human body. The organoid heart cells are made by genetically modifying adult human skin cells into induced pluripotent stem cells. Then, these cells are reprogrammed to produce the organoids which have a diameter of 0.25 millimeters. The organoids grow and form balls which then are 3D printed into different forms and sizes. These cells will create tiny organ-like structures that mimic the function of the real organ. The organ structures are then placed on a chip to provide for an online monitoring. The ultimate goal is to create mini organs to test them with different biological and chemical

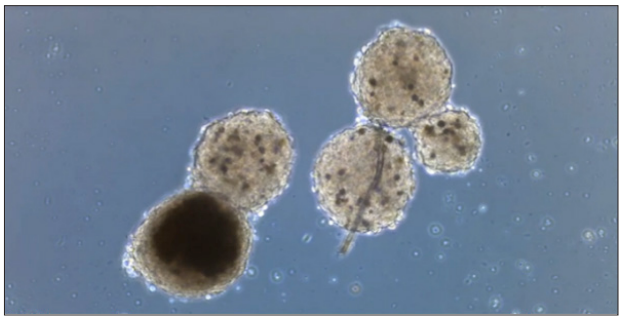

Figure 4 (Wakehealth.edu
3D printed beating, cardiac cells called "organoids)

agents and the effectiveness of various treatments against diseases (Wakehealth.edu 2016, Figure 4).

The Kidney

In addition to the great need of heart transplants there is even a greater number of patients waiting for a kidney transplant. Currently, there are over a hundred thousand people anxiously waiting for a lifesaving kidney (OPTN, 2016). The kidneys are responsible for removing excess fluid and waste from the blood. When the kidneys lose their filtering ability, there is a dangerously high level of fluid and waste accumulating in the body. This condition is known as kidney failure or end stage kidney disease. A kidney transplant procedure is often the best way to treat a kidney failure. Only one kidney is necessary to replace two failed kidneys, thus making a living donor kidney transplantation possible (Kidney.org, 2014).

A kidney transplant can pose risks to both the donor and the recipient. There can be possible surgical complications such as pain, infection, blood loss, blood clots, allergic reactions to anesthesia, pneumonia, injury to surrounding tissue and organs, or even death. There are also long term risks for donating a kidney such as hypertension, large amounts of protein in the urine, hernia, organ impairment or failure that can lead to the need for dialysis or transplantation and can even cause death. Additionally, there is the problem of the recipient's body rejecting the kidney as a foreign invader. Just as in a heart transplant, the patient will need to remain on immunosuppressants every day to prevent rejection of the new kidney. These anti-rejection medications have a large number of possible side effects because the body's immune system is suppressed (kidney.org, 2014).

Dr. Anthony Atala demonstrated an early stage experiment that could solve the kidney organ donor problem. The experiment uses a 3D printer that contains live cells and kidney shaped structure of collagen to output a transplantable kidney. A blueprint is created through a CT scan that goes layer by layer using computerized morphometric imaging analysis and 3D reconstruction which leads to actually imaging the kidneys. The image can even do a full 360-degree rotation to analyze the kidney in its full volumetric characteristics. This information is then taken and scanned into a printing computerized form. The printer is then guided by the computer imaging and drips the cells layer by layer over the scaffold causing the inert mold to come to life. Overtime, the millions of cells begin to communicate and function as one organ. It took the research team about seven hours to print the 3D kidney structure (Murphy, Atala, 2014, Figure 5, Xu et al., 2013).

Vascularization

The kidney represents a challenge more than other organs due to the kidney's detailed, tiny structures that allow the organ

to perform its sophisticated filtration job by removing waste chemicals from blood and turning the waste into urine. The function of removing waste is processed by roughly one million nephrons which are tiny vessels made up of even smaller urine collecting structures called tubules. The kidneys are also responsible to measure chemicals such as sodium and potassium that are then released back into the bloodstream in healthy dosages. The kidney's complex vascular structure especially poses a challenge. The vascular tree of the kidney can come down to the size of a capillary about eight microns in diameter. Due to the kidney's complexity, it has not been printed for transplant in humans at this present time, however researchers are avidly looking for ways to make it a reality and help people suffering from dialysis treatments while being on a multiple year waiting list for a kidney (Murphy, Atala, 2014).

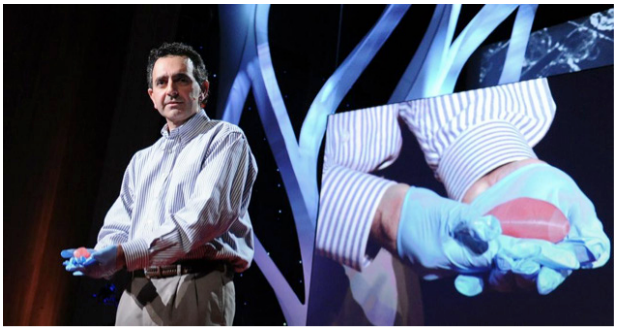

Figure 5 (Murphy, Atala, 2014) (TED.com 2011)
Dr. Atala holding his printed kidney during a TED talk called "On Printing a Human Kidney," in March 2011.

For a transplant tissue to thrive, a complex level of vascularization must be achieved. To some extent, capillaries can branch out from already existing blood vessels and into transplanted tissue on their own. Researchers have seen signs of spontaneous vascularization in small areas of engineered tissue such as in healed rat bone defects around three millimeters in diameter (Fielding, Bose, 2013). Rather than letting blood vessels spontaneously branch off and expand to engineered tissue, researchers are crafting templates for more orderly vascular growth. The idea is to create hierarchical microvascular networks that will guide the endothelial cells that line blood vessels to form tubes along predetermined courses. However, capillaries are small, measuring just a few microns in diameter. Therefore, even with high resolution printers, such small vascular structures would most likely collapse especially when printed into soft, biocompatible gel.

Jennifer Lewis researched the vascularization problem using a customized, high resolution 3D printer that can form microchannels in biocompatible gels. Lewis's research group is able to print hydrogel materials down at the micron length scale. The smallest microvascular channel the group was able to print was about ten microns in diameter. To solve the problem of collapsing channels, she prints them in fugitive ink which is a substance designed to melt away forming the channels pattern. The fugitive ink used is called Pluronic 4127, a gel that is often used in eyeglass lense cleanser and cosmetics. Pluronic 4127 is made up of three parts, the two poles of the molecule are hydrophilic while the middle segment is hydrophobic. The ink also liquefies when it is cooled down as opposed to most materials that solidify when they are cooled down (Wu et. al.,2011).

Lewis also used Pluronic F127 as the matrix for the printed channels, but the matrix molecules were modified so that they polymerize, and thus solidify, in the presence of UV light. This allows the matrix to become firm before cooling the gel so that the fugitive ink melts away. Taking advantage of the printer's fine-tipped nozzle, the research team printed a capillary network of fluorescently labeled fugitive ink into the Jello-like matrix. Through this technique, a way to pattern hydrogels with vascular channels was accomplished (Wu et. al., 2011). The next step is to take advantage of the self-organizing quality of endothelial cells in the 3-D–printed constructs, seeding the printed vascular structures with these blood vessel–lining cells. The self organizing quality is the tendency of the finest capillaries to grow spontaneously out of larger microvascular structures which is the work of biology when the cells are given a reasonable environment (Perryn et. al., 2008).

Inspired by some of Lewis's lab work with "fugitive inks," Jordan Miller, at Rice University, created a technique for 3D printing of vasculature-mimicking channels. Using a simple open-source 3D printer, Miller and his team constructed a carbohydrate lattice made from a combination of simple and complex sugars. There is a major difference between Miller's experiments and those done by Jennifer Lewis. Lewis uses machines that are very high-end and have incredible precision, but they are not easily duplicated by others. Miller saw potential in a cheaper printer called the Frostruder, a printer originally used to extrude sugar frosting for printing fancy designs onto edible treats. Miller adapted a printer to incorporate elements of the Frostruder printer's design and was soon able to print dissolvable lattices of carbohydrate filaments (Miller et. al., 2012). The experiment used a process called "3-D sacrificial molding" that is similar to the lost-wax method used by sculptors. His printer deposits filaments of carbohydrate on top of each other in sequence so they are self-supporting. Then the entire lattice structure is covered in a protective layer of a biodegradable polymer. After pouring and cross linking a cell-filled gel over the carbohydrate lattice, the lattice is dissolved with an aqueous solution (Miller et. al., 2012).

Miller's channels are not as small as Lewis' channels. His channels range from 150 microns to around a millimeter in diameter. However, when he and colleagues seeded the channels with endothelial cells, they lined the interiors of the channels and even began to penetrate the surrounding cell-gel mixture. By guiding blood cells into the larger channels, he can set the stage for endothelial cells to spontaneously form their own capillary networks. Miller has successfully pumped human blood through his constructs in vitro, and the research team plans to cooperate with a surgeon to connect one of his printed tissues to the vascular system of a rat to see how long he can get blood to flow through his channels (Miller et. al., 2012).

Vascularization procedures done by Miller and team take a short amount of time to complete which benefits the large scale tissues, like liver cells. Large scale tissue cannot survive the several hours it takes in the extruder nozzle long enough to build something the size of the human liver. Additionally, quickly pouring the cells and gels over the 3-D–printed lattice is easier on fragile cells than the arduous process of printing. The one disadvantage is that the researchers cannot control the exact placement of the cells. Therefore, this may not be the optimal method for experiments involving multiple cell types. In the versions of the constructs printed with rat liver cells or with human embryonic kidney cells, the cells near the channels survive longer than the cells deeper in the gel, suggesting that the experiment is leading in the right direction (Miller et. al., 2012).

Conclusion

At this present time, a 3D printed organ is unable to be inserted into a patient with the same efficacy as a normal organ. Many more years of research need to be conducted. There are constantly many advances being made by countless numbers of research teams who are devoted to help suffering patients and increase the availability of organs for transplant. However, even though 3D printed organs cannot be inserted into patients, they can be used to assist doctors and surgeons in surgery. Advanced models of organs that are 3D printed are able to prepare surgeons on what they might encounter in the operating room. Simply by looking at models, doctors are able to avoid potential complications that could have been unforeseeable without the physical model. 3D printed organs can also help train medical students and professionals. Researchers and Doctors are able to replicate the part of the patient with the complicated problem so students can learn how to go about saving lives. It also allows healthcare professionals to better explain situations to the patient. The healthcare provider can physically hold the model and explain what is wrong and the different procedures available for the patient. Currently, the main use of the 3D printed organs has been for screening new medications and modeling various diseases.

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CRISPR/Cas9 as a Therapeutic Approach to Duchenne Muscular Dystrophy

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Abstract
Transhumanism, designer babies, gene therapy, and super-soldiers are founded upon the same concept—genetic engineering. Clustered Regularly-Interspersed Short Palindromic Repeats (CRISPR) is a natural bacterial immune response method that takes advantage of gene manipulation to prevent an infection from mobile genetic elements. Since Mojica et al. (2005) first suggested the relationship between the CRISPR/Cas system and prokaryotic immunity, significant advancements have been made in understanding the mechanism and subsequent applications of CRISPR. CRISPR, has three main subtypes based on unique proteins and interference pathways and serves as an accurate and effective method for gene editing. Its mechanism consists of spacer acquisition, crRNA production, and interference. This highly dynamic form of genetic modification generates significant CRISPR sequence differences in species that are almost identical when comparing the rest of their genome. CRISPR/Cas9 demonstrates the simultaneous alteration of multiple gene loci in individual cells with a high degree of specificity and precision. Duchenne muscular dystrophy (DMD) is a genetic neuromuscular disorder characterized by progressive muscle loss and eventual death in the late teens to early twenties. DMD affects calcium homeostasis, vasculature, genetic regulation, muscle movement, glycosylation, tissue remodeling, and inflammatory response mechanisms. Current treatments include antifibrotic pharmaceuticals, calcium maintenance, myostatin inhibitors, upregulation of uthrophin, nonsense suppression drugs, vector-mediated gene therapy, and cell transplantation. This review describes the mechanism of CRISPR/Cas9 and its application as a therapeutic approach to treating Duchenne muscular dystrophy.

Keywords: CRISPR, genomic engineering, Duchenne Muscular Dystrophy, Cas protein, interference mechanism, PAM recognition

Introduction

DNA sequencing assays are highly efficient and economical and dramatically increase our understanding of genetic diseases. Once we identify and comprehend the cause of the disease, the quandary is to figure how to reverse the mutation and cure it. The ability to modify genes in mice serves as a crucial function to model human diseases (Yang et al., 2014). Conventional approaches to study and develop disease models utilize homologous recombination, such as retroviral insertion (Kuehn et al., 1987), in embryonic stem cells, to knockout the gene of interest (Thomas and Capecchi, 1987). Genetic variations among embryonic stem cells (Ledermann, 2000) coupled with relatively long time requirements (Markel et al., 1997) cause significant limitations to this approach (Carbery et al, 2010). Other methods used in gene editing include zinc-finger nucleases (ZFNs) (Kim et al., 1996; Geurts et al., 2009) and transcription activator–like effector nucleases (TALENs) (Nanjidsuren et al., 2016; Tesson et al., 2011).

Plasmids, bacteriophages, and transposons promote adaptation and survival by inducing the development of toxic compound degradation, antibiotic resistance, and other evolutionary advantages (Frost el at., 2005). Among mechanisms used to resist harmful infections and monitor the entry of genetic material, bacterial species utilize clustered regularly interspersed short palindromic repeats (CRISPR) (Labrie et al., 2010). CRISPR, a defense mechanism ensuring resistance against viral invasion, is exhibited by an estimated 40 percent of eubacterial and 90 percent archaeal species (Hatoum-Aslan et al., 2011). Kunin et al. (2007) used PILAR-CR, an algorithm that identifies CRISPR repeats, and found 561 arrays in 44 percent of the genomes tested. A population of ~1031 viruses coupled with ~1025/s rates of infection promoted the evolution of defense pathways that effectively identify foreign genetic elements and mount a response to degrade harmful foreign nucleic acids (Hendrix, 2003; Richter et al., 2012). These repeats are ubiquitous in archea and evident in some bacteria (Lillestøl et al., 2006).

While sequencing the Escherichia Coli iap gene in 1987, a researcher identified an "unusual structure" at the 3' end flanking the gene, noting five repetitive sequences of 29 nucleotides each having unique 32 bp spacer segments (Ishino et al., 1987). In 2005, Francisco Mojica was first to suggest a relationship between the CRISPR-Cas system and bacterial immunity. This observation later turned out to be extremely significant, yielding a dramatic benefit to genetic research. CRISPR, an adaptive bacterial immunological response mechanism, consists of alternating sequences, one repetitive and the other a segment of a viral genome or a plasmid sequence. CRISPR utilizes CRISPR associated (Cas) proteins and small non-coding RNAs for its function. Abutting each CRISPR loci, CRISPR-associated (CAS) genes encoding for various enzymatic proteins couple with CRISPR to form a multitude of different CRISPR/CAS pathways (Horvath and Barrangou, 2010). Incorporation of phage DNA into the spacer portion of a CRISPR array in Streptococcus thermophilus yielded resistance towards viral infection of the corresponding phage (Barrangou et al., 2007). CRISPR can be involved in several processes such as; replicon partitioning in

halo bacteria (Mojica et al., 1995), DNA rearrangements within a replichore (Deboy et al., 2006), and thermal adaptation in E. Coli (Riehle et al., 2001). There is a direct correlation between an increased sensitivity to viral infection and a mutation to the Cas genes or the spacer sequences of the corresponding virus (Barrangou et al., 2007; Brouns et al., 2008; Oost et al., 2014). CRISPR/Cas systems can be used to manipulate genes with substantial precision and accuracy, effectively giving researchers the ability to develop causal linkages between known mutations and observed phenotypes (Hsu et al., 2014). This review article will assess the application of the CRISPR/Cas system as a therapeutic approach to Duchenne muscular dystrophy.

Methods

An analysis of scholarly articles with a focus on papers published in peer-reviewed journals with high impact factors were performed through access to databases of the Touro College Online Library, Medline, Proquest, NCBI Pubmed, and Google Scholar. In-print articles were obtained from the Touro College library in the Avenue J campus. An analysis of both review and experimental research articles were conducted to delineate the mechanism and outline recent applications of CRISPR in a clinical setting. In each database, the search word "CRISPR" prompted recent publications on that topic. Articles that were labeled as "similar" to papers published recently were also used. Proteins associated with CRISPR discussed in this paper were analyzed using the uniprot database. Original research papers describing aspects pertaining to the discovery, mechanism, and applications of CRISPR were found on the webpage of Dr. Lluís Montoliu's Lab at Centro Nacional de Biotecnología.

Results

Spacer Acquisition

The genetic interference pathway of the CRISPR/Cas system is initiated with spacer acquisition upon entry of foreign genetic material (Swarts, 2012; Richter et al., 2012; Marraffini, 2010a). This step is highly dynamic and involves the recognition of foreign DNA by the host as well as its first integration into the spacer portion of a CRISPR array. Identification of the foreign genetic element is essential to the CRISPR mechanism. Proto-spacer adjacent motifs (PAMs) or spacer precursors are important components of the CRISPR systems. Each CRISPR-CAS variant can correspond to a specific spacer precursor or proto-spacer that will be evident on the foreign DNA particle. The interference target is determined by a specific short motif sequence that corresponds to each CRISPR variant. Using the classification of CRISPR variants determined by Kunin et al. (2007), a sequence of either two or three nucleotides abutting each proto-spacer was found to be conserved in six main groups (Mojica et al., 2009). This finding suggests a correlation between PAMs and each CRISPR-CAS system.

Various Cas genes are found adjacent to all CRISPR arrays with the exception of Thermoplasma acidophilum (Marraffini and Sontheimer, 2010a). More than 45 distinct CRISPR associated protein families have been identified using Hidden Markov models (Haft, 2005). Each subtype of CRISPR is classified based on associated Cas genes as well as its distinct repeat characteristics (Gesner, 2011). Three main subtypes are classified based on the presence of a unique Cas protein: type 1 has Cas3, type 2 has Cas9, and type 3 has Cas10 (Gleditzsch et al.; 2016, Makarova et al., 2011a; Richter et al., 2012). A fourth subtype, with its mechanism and function still uncharacterized, is called CRISPR type U (Koonin and Makarova, 2013).

Cas1 and Cas2, a metal-dependent nuclease (Wiedenheft et al., 2012) and a pH-dependent nuclease (Ka et al., 2014), respectively, are necessary in initiating spacer acquisition by incorporating non-self DNA into the leading end of the CRISPR array (Mojica et al., 2009). Strains of E. Coli lacking the endogenous cas genes prevented spacer acquisition from occurring without affecting further steps in the pathway, outlining their importance in the first step. Both Cas1 and Cas2 are evident among almost all CRISPR systems (Makarova et al., 2011b), possess a crucial role in spacer acquisition (Yosef et al., 2012), and contain highly conserved motifs. CRISPR type U is the only known form of CRISPR that does not possess a CRISPR array or Cas1 (Koonin and Makarova, 2013). These findings outline the importance of Cas1 and Cas2 in spacer acquisition (Oost et al., 2014). Cas1 and Cas2 form a complex determined by a 2.3Å resolution crystal structure (Nuñez et al., 2014).

Both DNA recognition and spacer acquisition will be prevented if the Cas1-Cas2 complex formation is disrupted by a mutation (Nuñez et al., 2014). In addition to its role in the CRISPR/Cas system, Cas1 is believed to be involved DNA repair (Babu et al., 2010). The recognition of a variant-specific adjacent short sequence on the foreign DNA particle prompts the incorporation of a spacer precursor. This completes the first step in the CRISPR defense mechanism (Mojica et al., 2009). Replication of the inserted repeat begins with the repeat most proximal to the leader portion of the array (Yosef et al., 2012). The CRISPR response is amplified through the increase in spacer sequences corresponding to a specific foreign DNA element (Swarts et al., 2012).

crRNA Expression

The successful incorporation of a foreign DNA segment into the spacer region of the CRISPR array and the production of a multiunit precursor (Koonin, 2006) permits the subsequent processing of the precursor CRISPR RNA (pre-crRNA) (Oost et al., 2009; Wiedenheft et al., 2012). The crRNAs specific to each CRISPR array are integral to the CRISPR pathway. Analysis

of the CRISPR/Cas system in Escherichia Coli K12 determined that both the repeats and the spacers within the CRISPR are transcribed into a long precursor RNA (Marraffini and Sontheimer, 2010a). The crRNA transcript transcribed from the CRISPR array requires cleavage prior to activation.

Cas proteins catalyze the conversion of precursor RNAs (pre-crRNA) into small crRNAs (Gleditzsch et al., 2016; Marraffini and Sontheimer, 2010b). Eight Cas genes were identified in this strain: cas123 and casABCDE (Brouns et al., 2008). After knocking out each individual Cas gene using in-frame single-gene deletions (Baba and Mori, 2008), the resulting transcript determined the position of each gene on the CRISPR array. Further, the RNA cleavage assays did not require ATP or divalent metal ions to progress. CasE, an endoribonuclease resembling an RNA-binding protein, fused together with the maltose binding protein (MalE) in Escherichia coli K12, did not require any other Cas proteins to cleave the pre-crRNA. Northern blot analysis determined His20 residue to be essential for catalysis of the pre-crRNA. A casE knockout prevents the processing of pre-crRNA in Escherichia coli K12 outlining its importance in the pathway (Brouns et al., 2008). Cascade (CRISPR-associated complex for antiviral defense), a ribonucleoprotein, is a 405 kDa undecamer made of five different Cas proteins. Cascade is coupled with a 61 nucleotide crRNA structure spanning the length of the protein complex that has a 5'-hydroxyl and 2',3'-cyclic phosphate termini forming a seahorse configuration prior to target DNA binding (Jackson et al., 2014, Jore et al., 2011).

Cas3, essential to all type I CRISPR systems, functions both as an ATP-dependent type A superfamily 2 helicase and a ss-DNA nuclease (Brouns et al., 2008; Gesner, 2011; Huo et al., 2014; Sinkunas et al., 2011). Cas3, together with the mature crRNA as a guide and Cascade as a targeting complex, catalyze the degradation of double stranded DNA elements (Huo et al., 2014). Brouns et al. exposed Escherichia coli to virulent Lambda phage in various scenarios to determine the role of Cascade and Cas3 in resisting phage infection. Two Escherichia coli strains each contain a CRISPR variant that targets four important lambda genes: the coding strain produced crRNAs complimentary to both the coding and non-coding strand of the four genes, while the template strain produced crRNAs complementary to the proto-spacer regions. With both Cascade and Cas3 present, results showed a hundred-fold and ten million-fold decrease in sensitivity to phage infection with the coding strain and template strain, respectively. Based on the aforementioned experiment, the presence of Cascade and Cas3 is crucial to phage resistance in the CRISPR defense mechanism (Brouns et al., 2008).

High resolution X-ray structure analyses of Cse3, a component of Cascade, both before and after cleavage of pre-crRNA, suggests a molecular basis for the mechanism of crRNA recognition by the Cascade. Three structures of Cse3 bound to different RNA products all displayed a stem loop complex, suggesting the involvement of Cse3 in RNA recognition (Gesner et al., 2011). Processing of pre-crRNA into mature crRNA allows effective interference by the CRISPR-Cas system.

Interference

The interference stage differs mechanistically among the three main subdivisions of CRISPR: type I, II, and III. Each subtype accomplishes the same goal of foreign DNA degradation, differing only in the route of interference. The goal of CRISPR interference is to degrade the foreign genetic elements that correspond to the acquired spacer sequences in the CRISPR array.

CRISPR type I, evident in both bacteria and archaea, exploits Cas3 for target degradation. Cascade is the multi-subunit crRNP (CRISPR ribonucleoprotein) complex that is unique to CRISPR type I. Different routes, depending on the subtype of CRISPR type I, can induce a conformational change in the crRNP complex which may cause the recruitment of Cas3 for degradation (Oost et al., 2014). The interference in CRISPR type I is initiated when the mature crRNA binds to a variant of Cas6, an endoribonuclease. The variant will depend on the subtype of CRISPR type I (Richter et al., 2012). The crRNA forms a stem loop within each repeat and bind to the corresponding Cas6 protein. Cas5d, the Cas6 variant of type I-C/Dvulg, processes the pre-crRNA. Further, Cas5d binds to the mature crRNA (Nam et al., 2012) and recruits the Cascade which induces a conformational change in the complex (Oost et al., 2014). In type I-E, CasA or Cse1, functions to discriminate between self and foreign DNA through the recognition of a proto-spacer adjacent motif (PAMs) (Sashital et al., 2012; Westra et al., 2013) as well as induce interactions between DNA and Cascade (Jore et al., 2011). The initial interaction with the foreign DNA employed by a short loop on Cse1 recognizes a sequence of seven nucleotides near the 5' end in addition to the PAM (Richter et al., 2012).

The CRISPR Type II system uses trans-activating crRNAs (tracrRNA) in crRNA processing. Strains lacking tracrRNA did not yield mature crRNA, demonstrating the importance of tracrRNA in crRNA processing. The tracrRNA base pairs with 24 nucleotides (Deltcheva et al., 2011) of the crRNA and recruits RNase III for cleavage. The fusion of tracrRNA and crRNA to become a single-guide RNA (sgRNA) can be easily programmed and is used in the modification of multiple DNA sequences simultaneously (Bolukbasi et al., 2016; Cho et al., 2013; Mali et al., 2013). The process of cleaving tracrRNA and crRNA also

requires a Cas protein called Csn1 determined by in-vivo in-frame deletions of the gene. The subsequent appearance of mature crRNA and cleaved tracrRNA after the induced expression of Csn1 further supports the hypothesis that Csn1 is vital to the processing of crRNA and tracrRNA (Deltcheva et al., 2011). However, Csn1 only serves to stabilize the interaction between pre-crRNA and tracrRNA without a direct contribution to the catalysis, further emphasizing catalytic role of RNase III (Fonfara et al., 2013). CRISPR-associated endonuclease Cas9 or Csn1 is Mg2+-dependent and contains two endonuclease domains. One is a RuvC-like nuclease domain and the other is a HNH nuclease domain cleaving the target DNA non-complimentary and complimentary to the crRNA, respectively (Jinek et al., 2012; Anders et al., 2014; Nishimasu et al., 2014). Csn1 undergoes a conformational change upon binding to the tracrRNA and mature crRNA, effectively activating its nuclease activity (Jinek et al., 2014). Although both type I and type II use PAM recognition to bind to DNA, there are two technical differences. Firstly, the PAM motif is adjacent to the 5' end of the crRNA in type I and the 3' end in type II. Secondly, the PAM motif of the target DNA in type I is the strand that directly interacts with the crRNA whereas it is on the displaced strand in the mechanism of type II. After the ribonucleoprotein complex is in its active form, the subsequent activation of the nuclease domains mediates site-specific double stranded breaks of the foreign DNA via Cas9 (Oost et al., 2014). Sternberg et al. classified the interaction between the RNA-Cas9 and the target DNA to be through a three-dimensional collision outlining the specificity of the CRISPR mechanism (2014).

The crRNP complexes in CRISPR type III are structurally alike and have similar roles to type I. Type III-A and type III-B are associated with csm and cmr complexes, respectively. The type III-A system displays the ability to degrade double-stranded DNA and single-stranded RNA (Niewoehner and Jinek, 2016), whereas the type III-B system targets RNA (Hale et al., 2009). Type III-A, present in staphylococcus epidermidis RP62a, contains nine csm genes (Hatoum-Aslan et al., 2014) and does not rely on PAM recognition for target degradation (Marraffini and Sontheimer, 2010b). Instead, the csm complex uses the csm3 and cas10 subunits to target and degrade single-stranded RNA and double-stranded DNA, respectively. Csm6, a single-stranded RNA-specific endoribonuclease noted for its higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain, is integral to the function of the csm complex. Ribonuclease activity was determined to be a common feature among orthologs of csm6, outlining its importance in the type III pathway. Although the mechanism of csm6 interference is currently inconclusive, a notable theory posits that when the csm complex is unsuccessful in resisting foreign invasion, csm6 somehow activates and targets its own nucleic acids inducing apoptosis. Such regulatory methods serve as an important defense in preventing further infection. Structural analysis of csm6 in Thermus thermophilus describes multiple binding domains which could indicate that there are ligand-dependent levels of catalytic activity (Niewoehner and Jinek, 2016). Type III-B in Thermus thermophilus uses the cmr complex, an 11 subunit protein complex comprised of six distinct proteins aptly labeled cmr1-6, for RNA degradation. The cmr complex is a Mg2+-dependent endoribonuclease that targets the RNA strand at multiple sites complementary to the crRNA. Degradation begins at the 3' end of the target RNA strand and cleaves toward the 5' end with each cleavage separated by six nucleotides. The distance between two cmr4 subunits is consistent with the six nucleotide intervals present in the cleavage mechanism which suggests characterizing cmr4 as a ribonuclease within the cmr complex (Staals et al., 2013). A significant distinction in the molecular mechanism of CRISPR type III is that it relies on Cas10/Csm or Cas10/Cmr complex for target interference whereas type I and type II require a recognition sequence (Samai et al., 2015).

It is important to note the ability of the CRISPR/Cas system to differentiate its own genetic material from foreign nucleic acids. Without the basic capacity to distinguish self versus non-self, all CRISPR mechanisms would act on their own DNA and subsequently induce autoimmunity and cell death. The basic mechanism behind preventing self-degradation relies on a genetic distinction between target and self. Since the sequences incorporated in the spacers are meant to assist in the recognition of foreign genetic elements, they are the same and should cause self-interference. However, analysis of the CRISPR array in Staphylococcus epidermidis yielded dissimilarities between sequences of target DNA and the CRISPR DNA that are not in the spacer region. The higher degree of base complementarity between CRISPR DNA and crRNA in regions flanking the spacer sequences prompts evasion of interference. Adding fifteen base pairs matching both sides of the spacer's flanking sequences onto a target strand showed that interference was unsuccessful in the plasmid that had the 5' flanking sequence. The exact "protective region" was determined to be eight base pairs flanking the spacer sequence. It is the variable complementarity within sequences abutting the spacer region that prevents autoimmunity in all CRISPR systems (Marraffini and Sontheimer, 2010b).

CRISPR/Cas9 Genome Engineering

Jinek et al. (2012) was the first to propose the application of CRISPR/Cas9 for genomic manipulation. CRISPR/Cas9 is a form a CRISPR type II and is well understood (Ran et al., 2013). The purpose of CRISPR/Cas9 is to induce double-stranded breaks at specific locations within a chromosome. Cong et al.

(2013) investigated the application of CRISPR/Cas9 in genomic engineering by reconstructing the CRISPR type II locus of Streptococcus pyogenes SF370 in mammalian cells. A spacer sequence that resembles a specific portion of the EMX1 gene adjacent to the proper PAM sequence was designed and then transfected into 293FT cells along with RNase III, Cas9, tracrRNA, and pre-crRNA. Notably, cleavage activity was prevented when a base mismatch between the protospacer and the guide RNA sequence was within eleven nucleotides of the PAM sequence, demonstrating the specificity and accuracy of CRISPR/Cas9. Results yielded effective cleavage even without the addition of RNase III. This finding outlines three essential components required for this method of genetic engineering; Cas9, mature crRNA, and trans-activating crRNA.

Cas9 is an endonuclease that requires a Mg cofactor in order to bind to target DNA (Jinek et al., 2012) and is inhibited by EDTA (Jiang et al., 2016). The target sequence needs to abut the 5' end of a protospacer adjacent motif. Each ortholog of Cas9 requires a specific PAM sequence. The crRNA contains the twenty nucleotide sequence used to target the gene of interest that leads to the PAM. Furthermore, the crRNA requires tracrRNA for its activation into discrete units. When used in genome editing, the crRNA and tracrRNA are fused together to become a complex commonly called single-guide RNA (sgRNA) or guide RNA (gRNA) (Ran et al., 2013).

Mali et al. (2013) described how the CRISPR/Cas9 system can be used to both stimulate homologous recombination and modify a locus. They developed human embryonic kidney HEK 293T cells that contain a green fluorescent protein (GFP) sequence with an interruption that prevented functionally fluorescing GFPs. Two gRNAs were designed to target the region that disrupted florescence. After transfection of a donor to repair the sequence, Cas9, and a gRNA, flow-activated cell sorting (FACS) began detecting fluorescing cells at ~20 hours, elucidating the efficiency of CRISPR/Cas9 in inducing homologous recombination with a repair donor. To demonstrate how CRISPR/Cas9 can modify genomic loci, Mali et al. (2013) introduced two gRNAs that would each target nineteen base pairs with one base pair in-between them and then a double stranded donor sequence to take its place at the AAVS1 locus. PCR and Sanger sequencing assays confirmed the integration of a foreign sequence in a genome. These findings demonstrate important applications of CRISPR in genomic engineering.

Another important application of CRISPR/Cas9 is the single-step induction of mutations in multiple genes. Developing disease models through targeted deletions and engineering in multiple chromosomes gives extensive insight into the formation of various illnesses. Maresch et al. (2016) exploited electroporation to introduce CRISPR/Cas9 vectors in pancreatic cells and demonstrated optimal results when targeting a "few hundred cells" in each organ. To investigate pancreatic tumorigenesis, sgRNAs for thirteen tumor-suppressor genes and two neutral genes were transfected into a mixture of C57BL/6J and 129S mice strains. Magnetic resonance imaging determined the average time for tumor development to be 10.7 weeks and a 54% tumor incidence at 24 weeks. Next generation sequencing analysis confirmed a significant number of mutations in the target sites of tumor tissue samples. Furthermore, it did not find any significant mutation rates in the target sites of the tissue surrounding the tumors which is explained by the electroporation protocols that target only a few hundred cells in relatively small area (Maresch et al., 2016). All the aforementioned capabilities of CRISPR/Cas9 amalgamate to produce a highly efficient and effective method of genomic engineering with a short time period required to develop disease models. Prospective applications of CRISPR/Cas9 can involve the introduction of targeted mutations that can prevent the acquisition of diseases such as Human Immunodeficiency Virus (Lombardo et al, 2007). The future applications of CRISPR engineering are limitless and serve as an extremely viable option for use as a therapy for Duchenne Muscular Dystrophy.

Duchenne Muscular Dystrophy

In 1836, Gaetano Conte was the first to describe a case of Duchenne muscular dystrophy (DMD) (Nigro, 2010), an X chromosome linked recessive disorder that is classified as a severe progressive muscle wasting disorder. This neuromuscular disease is caused by a mutation in the DMD gene that codes for dystrophin (Hoffman et al., 1987), a 427 kDa rod-shaped cytoskeletal multi-domain protein made of 3,685 amino acids (Koenig et al., 1988) that is expressed in all human muscle cell types. Dystrophin interacts with dystrobrevin alpha (Sadoulet-Puccio et al., 1997), alpha-1-syntrophin (Ahn et al., 1996), and beta-1-syntrophin (Ahn and Kunzel, 1995). The primary function of dystrophin is to connect a cytoskeleton component, actin, with the extracellular matrix (Norwood et al., 2000). DMD occurs when mutations affecting the open reading frame cause premature termination of dystrophin during translation, resulting in a protein with complete loss of function (Yiu, 2015). The end result of a deficiency in dystrophin is muscle fiber degeneration and is believed to be secondary to factors such as sarcolemma impairment, structural damage to the cytoskeleton, and an aberrant calcium homeostasis. Patients with DMD require respiratory, cardiac, orthopaedic, and nutritional management throughout their lives (Yiu, 2015). Life expectancy for DMD patients is usually 25 years with the cause of death commonly being cardiomyopathy or lung issues (Long et al., 2014).

Pathophysiology

DMD, being a neuromuscular disorder, detrimentally affects the body's mechanical abilities, calcium homeostasis, vasculature, genetic regulation, glycosylation, tissue remodeling, and inflammatory response mechanisms. The absence of dystrophin or other proteins within the dystrophin associated complex could significantly decrease normal contractions that increase tension as the muscle lengthens, or eccentric contractions, thereby damaging the membrane of the muscle fibers. A muscle biopsy will show muscle fiber degeneration or necrosis. Eventually, the continual attempts to regenerate the muscle fibers leads to a burnout and begin substituting muscle for connective and adipose tissues (Deconinck and Dan, 2007).

DMD induces a leak within the calcium channels of the cell. The lack of dystrophin affects the structure of the membrane causing compensatory mechanisms that maintain calcium levels to eventually become ineffective. When there is a prolonged influx of extracellular calcium, proteases are activated, causing further degradation of the membrane, which leads to a further increase in intracellular calcium. This glut of calcium can presumably lead to cellular death.

Neuronal-type NO synthase (nNOS) is normally localized to the membrane of white muscle fibers and produces nitric oxide, which is a short-lived highly reactive signaling molecule with important biological functions (Nelson and Cox, 2013). The absence or deficiency of dystrophin causes a reduction in nNOS activity as well as its delocalization from the sarcolemma (Brenman et al, 1995; Crosbie et al., 2002). Dabiré et al. (2012) demonstrated a link between vascular endothelial dysfunction and the expression of endothelial and neuronal nitric oxide synthases in DMD patients.

The conversion of mechanical stimuli into electrical or chemical signals is known as mechanotransduction and is involved in genetic expression and other important physiological processes (Katsumi et al., 2004). Goldspink (1998) demonstrates the effects of muscle activity on mechanotransduction. The progressive loss of muscle tissue occurs secondary to the lack of autocrine insulin-like growth factor-1 production, which is used to repair muscle tissue.

CRISPR/Cas9 Therapy

The human dystrophin gene contains seventy-nine exons and seventy-eight introns (Kole and Krieg, 2015) with at least seven promoters. Alternative splicing yields different variants of dystrophin depending on the stage of development and type of tissue (Im et al., 1996). A spontaneous mdx gene mutation in a colony of C57BL/10ScSnJ mice resulted in increased serum levels of specific proteins as well as histological similarities compared to that of human muscular dystrophy (Bulfield et al., 1984). This spontaneous point mutation yielded a stop codon affecting exon 23 in the dystrophin gene (Sicinski et al., 1989). Biochemical similarities coupled with cross-breeding analysis of mutant and normal mice set the precedent to use the mdx mouse as a model for muscular dystrophy (Bulfield et al., 1984). Although not entirely equivalent to the human disease, researchers utilize the mdx mouse as the predominant model to investigate pathogenic mechanisms of DMD (Partridge, 2013).

The primary goal is to cure DMD by correcting any harmful mutations. Treatments either alleviate the symptoms or aim to cure the disease itself. Pharmacological approaches improve muscle function with corticosteroids (Mendell et al., 1989), maintain calcium homeostasis (Zhao et al., 2012), inhibit the IKK/NF-κB signaling pathway (Acharyya et al., 2007), reduce inflammation and induce the upregulation of uthrophin as a surrogate to dystrophin (Gordon et al., 2013). Glucocorticoids, such as prednisone or deflazacort, are beneficial to muscle function and are the only accepted drug therapy of DMD (Matthews et al., 2016). Van Deutekom et al. (2001) describes a form of gene therapy that attempts to correct the reading frame through the induction of skipping an additional exon. An exon 45 deletion exhibited in DMD patients causes a stop codon in exon 46. The open reading frame is restored when exon 46 is deleted. The resulting protein is still dysfunctional yet displays a milder form DMD, outlining a method of treating the disease. Other attempted strategies of treatment include antifibrotic pharmaceuticals, myostatin inhibitors, nonsense suppression drugs, vector-mediated gene therapy, and cell transplantation (Shimizu-Motohashi, 2016).

The aforementioned therapeutic approaches to treat DMD focus on either reestablishing the expression of or compensating for a deficiency in dystrophin. The efforts to treat DMD are, in many instances, relatively transient and compensatory without any curative effects. CRISPR/Cas9 is revolutionary for its attempt to treat the underlying cause of the disease— mutation or mutations in the dystrophin gene. Long et al. (2014) demonstrated the application of CRISPR/Cas9 to repair the genetic defect in an animal model of DMD. The zygotes of the mdx mouse, containing a nonsense mutation in exon 23 of the dystrophin gene (Sicinski et al., 1989), were injected with a 20 nucleotide single-guide RNA containing a PAM sequence, Cas9, and 90 base pair single stranded template. This template strand incorporates four silent mutations as well as a TseI restriction site for data analysis. After a double-stranded break was induced by Cas9, the strands were repaired by either homology directed repair (HDR) or nonhomologous end-joining (NHEJ). The optimized condition involved injecting the Cas9, sgRNA, and template into the zygote and then performing re-implantation into a female

mouse. The offspring of that mouse determined the results of the experiment. Analysis of eleven repaired mdx progeny revealed adult development of all mice without signs of abnormal phenotypes. Control groups were used to test for off-target effects and yielded data consistent with previous genome-wide studies outlining the specificity of Cas9. Using histological analysis of different muscles, the results of this experiment demonstrated the capability of CRISPR/Cas9 to repair the primary mutation that causes DMD, thereby preventing the symptoms associated with the disease. The determined threshold for sufficient repair was 17% which effectively displayed a dystrophin level comparable to the wild-type mouse. This finding suggests a mechanism of selective advantage for the repaired skeletal myocytes. Soles and heart tissue immunostaining of a three-week old mdx repaired mouse of 40% displayed myofibers without dystrophin while the nine-week old mdx repaired mouse of 41% did not reveal any myofibers without dystrophin. The dystrophin expression levels between the three and nine week old mice were insignificant, suggesting a compensatory mechanism of rescue by the repaired nuclei in a myofiber. Immunostaining revealed myofibers containing dystrophin secondary to a fusion between a repaired cell and dystrophic muscle, providing further evidence of a rescue mechanism. Additionally, the serum creatine kinase levels were inversely related to the percentage of genomic repair, which is consistent with previous data in this paper. A higher level of serum creatine kinase signifies muscle breakdown. The repair of only a percentage of cells can induce a total rescue, suggesting an unknown mechanism that induces muscle regeneration in mice treated with CRISPR/Cas9. The results of this experiment yield a breakthrough in our approach to cure previously incurable diseases by effectively correcting the underlying cause of the disease.

Discussion & Conclusion

Analysis of the CRISPR/Cas system, both in terms of its mechanism and bioengineering applications, yields a plethora of data on various diseases. The short time requirements (Markel et al., 1997) coupled with the simultaneous manipulation of multiple genes (Bolukbasi et al., 2016) characterizes CRISPR as an advanced and highly efficient approach to modeling and thus understanding maladies. Applying a form of CRISPR type II to induce double stranded breaks provides researchers the ability to target a portion of DNA with high specificity and then stimulate homologous recombination to modify a specific locus (Mali et al., 2013). Duchenne muscular dystrophy is a debilitating neuromuscular disease commonly affecting males and generally leads to death before the age of 25 (Long et al., 2014). DMD is a prime example to be used in the application of CRISPR/Cas9 for its well defined mutations that cause the disease. The data presented in experiments using CRISPR/Cas9 to modify a gene seem to be consistent and display accuracy and efficiency (Cong et al., 2013; Mali et al., 2013; Maresch et al., 2016). Based on current data, CRISPR/Cas9 genomic engineering is a promising and hopeful route to effectively reverse disease-causing genetic mutations such as Duchenne muscular dystrophy.

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