Comparison of Low Molecular Weight Heparin and Unfractionated Heparin on Treatment of Obstetrical Antiphospholipid Syndrome

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Objective

The purpose of this research is to determine which method of treatment, low molecular weight heparin (LMWH) or unfractionated heparin (UFH), is more effective in lowering pregnancy losses. Specifically, this poster will demonstrate an understanding of how pregnancy is affected by Antiphospholipid Syndrome (APS) and a comparison of success in lowering recurrent pregnancy loss (RPL) to allow for a successful pregnancy in patients. Also, to define whether heparin is successful in prevention of pregnancy loss in obstetrical APS.

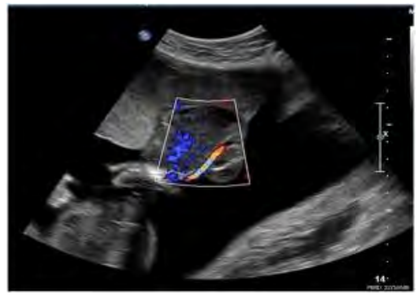

Figure 1. Image of the placental vessel via ultrasound. This image also shows some placental abnormalities not associated with APS; the purpose it to demonstrate the location and physical features. Placenta - Abnormalities. (n.d.). Retrieved August 06, 2016, from https://embryology.med.unsw.edu.au/embryology/index.php?title=Placenta_-_Abnormalities

Abstract

Antiphospholipid Syndrome (APS) is an autoimmune disorder that is associated with pregnancy complications, such as thrombosis, a medical term for an abnormal blood clot that is formed through a series of chemical reactions between special blood cells (platelets) and proteins (clotting factors). In most cases heparin, an effective blood thinner used to prevent thrombosis of the placental vessel (Figure 1), is used to increase chances of a successful conception and pregnancy. There are two types of heparin commonly used and determined safe for pregnant women with APS: low molecular weight heparin (LMWH) and unfractionated heparin (UFH). Both LMWH and UFH contain an essential part within their structure that binds to clotting factors, thus stopping the cascade (Figure 2). Because of LMWH's smaller size, only 5,000 D compared to 10,000-15,000 D, preferentially inhibits clotting factor, Xa, which is higher in the coagulation cascade. The second way that LMWH differs from UFH also relates to the molecule's size. Smaller heparin molecules are less likely to be deactivated by tissue proteins. This results making it last longer in the body. The objective of this research is to compare the outcomes of LMWH to UFH used in pregnant women with APS. A case study evaluated the use of LMWH in combination with low dose aspirin (LDA) for treatment of APS as compared to UFH plus LDA in patients with APS and recurrent pregnancy loss (RPL). Of the 25 patients treated with LMWH, 21 (84%) delivered viable infants and 4 (16%) miscarried. Of the 25 patients treated with UFH, 20 (80%) delivered a viable infant and 5 (20%) miscarried. Limitations in this study include that women treated with LMWH had a tendency towards earlier delivery and the miscarriages occurred significantly later than the UFH group. No patients had any major complications during pregnancy and the differences were not statistically significant, meaning larger trials are required. In another study, an APS trial comparing LMWH to UFH proved LMWH an effective alternative to UFH for treatment of APS in pregnancy. Of the 14 women using LMWH, 1 did not conceive. 9 of the 13 (69%) given LMWH had a successful pregnancy. Of the 14 women using UFH, 1 also did not conceive. 4 of the 13 (31%) given UFH had a successful pregnancy. Limitations include the period of time when the study was conducted, which was 12 years ago (2004). Therefore, a larger multicenter randomized trial is needed to determine the benefit to risk ratios of LMWH to UFH.

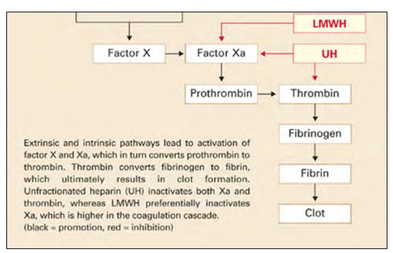

Figure 2. Simplified schematic of the coagulation cascade, the series of steps through the common pathway of blood clotting to the formation of the clot, involved in thrombosis. Emery, S. (2015) [1].

Materials and Methods

Patients were eligible to participate in the following three studies if they had 3 or more recurrent pregnancy losses (RPL). However, the materials and methods differ in each study. In the study conducted about the safety of LMWH at Helsinki University Hospital, Finland (HUHF), 475 patients were administered LMWH, specifically 40 mg/day of Type A or 500 IU/day of Type B. The control group consisted of 622 women (refer to figure 3 for time of entry of patients and dosage of LMWH). At the onset of labor, there was an interruption of LMWH for at least 12 hours[2]. Another study was done at the Health Sciences Center (HSC) in El Paso, Texas, which had extensive experience with the use of LMWH, in collaboration with the University of Tennessee Health Sciences Center, which had extensive experience with the use of UFH. In this study, all patients had positive results at least twice for antiphospholipid antibodies, were screened for previous conditions, and were started on LDA and prenatal vitamins before conception. In the LMWH group, 25 women received enoxaparin (40 mg SC) daily and in the UFH group, 25 women were given 5,000 U SC heparin twice daily. All patients were instructed to stop LDA 3 weeks before due date. UFH was continued until full term and discontinued only when the patient initiated spontaneous labor while LMWH was stopped 5 days before a scheduled induction or cesarean section[4]. In the final study performed at the Reproductive Medicine Program at British Columbia's Women's Health Centre (BCWC), 31 women with consistently positive antiphospholipid antibodies were started on heparin in the luteal phase for a maximum of 3 cycles. Dalteparin, a LMWH, was prescribed at a dosage of 2500 units subcutaneously (SC) every 24 hours. The dosage was increased to 5000 units SC in the second trimester and 7500 units SC in the third trimester. UFH was prescribed at a dosage of 5000 units SC every 12 hours. The dosage was increased to 7500 units SC in the second trimester and 1000 units SC in the third.[6]

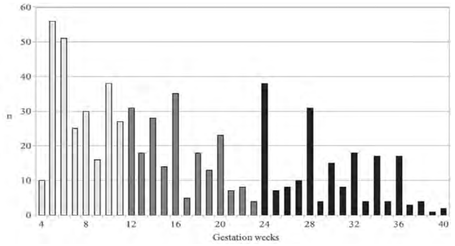

Figure 3. Distribution of time of initiation of low-molecular-weight heparin treatment in patients in pregnancy. The first, second and third trimesters are shown in light gray, dark gray and black, respectively. The dosage of LMWH is represented by n. Kwak-Kim J. (2013) [2]

	LMWH + LDA (n = 25)	UFH + LDA (n = 25)	P
Age at entry (y)	33.1 ± 5.1	32.4 ± 4.2	.60
Total pregnancies	4.5 ± 1.2	5.0 ± 1.4	.18
Prior live births	0.43 ± 0.40	0.36 ± 0.48	.58
Prior miscarriage	4.0 ± 1.3	4.4 ± 1.7	.35
EGA at loss (wk)	7.5 ± 1.7	7.6 ± 1.6	.83

Note: Data are presented as mean ± SD, unless otherwise noted. LMWH = low-molecular-weight heparin; UFH = unfractionated heparin; LDA = low-dose aspirin (81 mg); EGA = estimated gestational age.

Noble. Antiphospholipid antibodies and pregnancy loss. Fertil Steril 2005.

Figure 4. Comparison of women with recurrent pregnancy losses and antiphospholipid syndrome at the time of study entry. Noble, L. S. (2005) [4]

Results

In the HUHF study, incidence rates did not differ between groups (LMWH vs control): 1.56% vs 1.1% for thrombocytopenia, 8.7% vs 6.5% for preterm delivery, 0.7% vs 0.3% for still birth, 1.4% vs 1.0% for severe pre-eclampsia, 2.7% vs 2.2% for fetal growth restriction, and 10.7% vs 7.8% for antenatal bleeding. This study indicates that the use of LMWH is safe during pregnancy. Though, a limitation would be that the data was obtained from patient's electronic hospital records, meaning the researchers had no interaction with the patients. In the HSC study, the 25 patients treated with LMWH, 21 (84%) delivered viable infants and 4 (16%) miscarried. Of the 25 patients treated with UFH, 20 (80%) delivered a viable infant and 5 (20%) miscarried. There were no cases of dee venous thrombosis, thrombocytopenia, pre-eclampsia, gestational diabetes, or bone fractures noted in either group. There were 2 cases of preterm birth and 1 intrauterine growth restriction in both groups. The differences were not statistically significant, meaning larger trials are required. In the BCWC study, of the 14 women using LMWH, 1 did not conceive. 9 of the 13 (69%) given LMWH had a successful pregnancy. Of the 14 women using UFH, 1 also did not conceive. 4 of the 13 (31%) given UFH had a successful pregnancy. No preterm births or fetal demises in either group. Dalteparin group, LMWH, had a live birth rate of 69% whereas the UFH group had a live birth rate of 31%. This study demonstrated that LMWH may be an effective alternative to UFH.

Conclusions

Overall, many researchers have clashed over whether LMWH is more effective than UFH, and vice versa. LMWH and UFH both seem to be effective in lowering RPL. The recent rise in use of LMWH over UFH in most cases is due to the cost, a more predictable pharmacokinetic profile, and ease of use. There is a need for additional studies with larger numbers of subjects, which are not readily available, to determine the benefit to risk ratios of each drug. Perhaps larger trials are not available due to limited access to patients who are willing or available, researchers do not see a significant difference in the smaller trials to continue, and difficulty finding funding. Therefore, I recommend that other well-designed prospective studies be used to complete the understanding of the optimal treatment of patients of APS and attaining the ability to identify women at risk. There is not a significant difference in the treatment of obstetrical APS using LMWH and UFH; the studies presented demonstrate that both, LMWH and UFH have the potential to sustain a pregnancy effectively.

Relevant Application to Biotechnology

Women with APS considering fertility options would have been presented with far less options without the discovery of heparin in 1916 at Johns Hopkins University. Heparin was first discovered by Jay McLean and William Henry Howell. McLean was a second year medical student at Johns Hopkins University who was assisting Howell in the investigation for pro-coagulant preparations. McLean isolated a fat-soluble anticoagulant in canine liver tissue. Though, it was not until May 1935 that heparin was refined to a safe, nontoxic version that could be administered in a saline solution by Connaught Medical Research Laboratories in Sweden. Not only is heparin used to prevent thrombosis in pregnancy, but also is used for a variety of procedures and saves lives very day. Currently, heparin is used to treat and prevent blood clots in the veins, arteries, or lung. Heparin is also used before surgery to reduce the risk of blood clots.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell and Ms. Patricia Winter for their tireless support, for gifting me this amazing opportunity, and for helping me explore exciting and new fields of science. Dr. Chang inspired me in his passion for a better quality of life for women considering fertility options. I would also like to thank my dear neighbor, Dr. Carol Parker, for assisting me in my research and for helping my project come together smoothly. Furthermore, I want to thank my fellow OSA sisters for making this experience unforgettable. I would like to thank my family for believing in me 24/7 and all my scientific endeavors. And last of all, I would like to thank God for leading and guiding me towards future.

References

1) Emery, S. (2015). Anticoagulation in pregnancy: Q&A on low molecular weight heparin. Retrieved August 4, 2016, from http://www.obgmanagement.com/the-latest/past-issues-single-view/anticoagulation-in-pregnancy-q-a-on-low-molecular-weight-heparin/
2) Galambosi, P. J., Kaaja, R. J., Stefanovic, V., & Ulander, V. M. (2012). Safety of low-molecular-weight heparin during pregnancy: a retrospective controlled cohort study. *European journal of obstetrics, gynecology, and reproductive biology, 2,* 154–159.
3) Kwak-Kim J, Agcaoili MSL, Aleta L, Liao A, Ota K, Dambaeva S, Beaman K, Kim JW, Gilman-Sachs A. Management of women with recurrent pregnancy losses and antiphospholipid antibody syndrome. Am J Reprod Immunol 2013; 69: 596–607.
4) Noble, L. S., Kutteh, W. H., Lashey, N., Franklin, R. D., & Herrada, J. (2005). Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low-molecular-weight heparin versus unfractionated heparin. *Fertility and sterility, 3,* 684–690.
5) Di prima FA, Valenti O, Hyseni E, et al. Antiphospholipid Syndrome during pregnancy: the state of the art. J Prenat Med. 2011;5(2):41-53.
6) Stephenson, M. D., Ballem, P. J., Tsang, P., Purkiss, S., Ensworth, S., Houlihan, E., & Ensom, M. H. (2004). Treatment of antiphospholipid antibody syndrome (APS) in pregnancy: a randomized pilot trial comparing low molecular weight heparin to unfractionated heparin. *Journal of obstetrics and gynaecology Canada: JOGC = Journal d'obstetrique et gynecologie du Canada: JOGC, 8,* 729–734.

The Influence of Polymorphisms in Complement Genes on Preeclampsia Risk

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Objective

Preeclampsia is a condition that occurs during pregnancy and is characterized by hypertension and proteinuria. Preeclampsia poses a significant threat to both the mother and the fetus, sometimes leading to morbidity and mortality. Researchers have not yet been able to identify the exact causes of preeclampsia. The objective of this poster is to explore the relationship between polymorphisms in genes that encode complement proteins (complement genes), which play a role in the inflammatory response, and the development of preeclampsia during pregnancy.

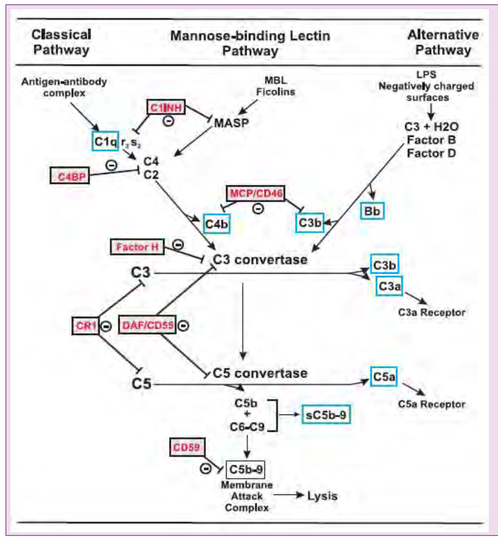

Figure 1: Activation of Three Complement Pathways. The three complement pathways include the classical pathway, the mannose-binding lectin pathway, and the alternative pathway. Blue boxes indicate products of complement pathway activation. Red text indicates regulators of the complement pathway. Regal, J. F., Gilbert, J. S., & Burwick, R. M. (2015).[3]

Abstract

During pregnancy, the complement system, an essential part of the immune response, plays a significant role in regulating the interface between the placenta, the embryo, and the mother's tissues . In women with preeclampsia, hyperactivity of complement proteins has been observed, which suggests that genetic variation in a number of complement genes may have a role in the development of preeclampsia. A study was conducted in Taiyuan, China, among 3166 pregnant women greater than 18 years of age who came to the hospital for delivery (gestational age ≥ 20 weeks). A questionnaire was used to collect data regarding demographics, reproductive and medical histories, behavioral factors, occupational history, physical activity, and diet of the participants. Additionally, information on birth outcomes and pregnancy complications (including birth-weight, whether the birth was a caesarean section, etc.) was extracted from medical records. Blood samples from the women were collected for genotyping. Among the study participants, 203 had been diagnosed with preeclampsia. 51 single nucleotide polymorphisms (SNPs) in six different complement genes, *C3*, *C5*, *C6*, *MASP1, MBL2,* and *CD55*, were analyzed using the blood samples. The study demonstrated that SNPs in the *C6* and *MASP1* complement genes were significantly associated with the risk of preeclampsia. Another previous study demonstrated that mutations in complement genes were associated with the development of preeclampsia and miscarriage. These results demonstrate that there is a relationship between SNPs in complement genes and preeclampsia risk and suggest that the immune response may have a role in the pathogenesis of preeclampsia. Further studies should be conducted to determine whether other genetic variations are associated with the onset of preeclampsia.

Methods and Materials

A case-control study was conducted in Taiyuan, China, among 3166 pregnant women greater than 18 years of age who came to the hospital for delivery (gestational age ≥ 20 weeks). A questionnaire was used to collect data regarding demographics, reproductive and medical histories, behavioral factors, occupational history, physical activity, and diet of the participants. Additionally, information on birth outcomes and pregnancy complications (including birth-weight, whether the birth was a caesarean section, etc.) was extracted from medical records. Blood samples from the women were collected for genotyping. Among the study participants, 203 had been diagnosed with preeclampsia, based on high blood pressure levels (≥140/90 mmHg) and proteinuria (≥ 0.3 g/L in a 24 hr urine collection period or 2 random urine specimens showing at least 1+ protein by dipstick test). The 203 cases were matched with 233 randomly selected controls by age, residence, and time of conception. 51 single nucleotide polymorphisms (SNPs) in six different complement genes, *C3*, *C5*, *C6*, *MASP1, MBL2,* and *CD55*, were analyzed using the blood samples.

	Mild	Severe	Early-Onset	Late-Onset
Systolic BP	140 to 160 mmHg	≥ 160 mmHg	≥140 mmHg	>140 mmHg
Diastolic BP	90 to 110 mmHg	≥ 110 mmHg	≥90 mmHg	≥90 mmHg
Proteinuria	1+	≥ 2+	≥ 1+	≥ 1+
Symptoms of Severity	No	Yes	Yes/no	Yes/no
Gestational age	>20 weeks	>20 weeks	21 to 34 weeks	>34 weeks

Figure 2: Preeclampsia Subtypes. Classification of various subtypes of preeclampsia, including mild, severe, early-onset, and late-onset. Wu, W., Yang, H., Feng, Y., Zhang, P., Li, S., Wang, X., . . . Zhang, Y. (2016).[5]

Results

The preeclampsia cases in the study were further divided into subtypes, including early-onset preeclampsia, late-onset preeclampsia, mild preeclampsia, and severe preeclampsia (Figure 2). When the demographics and other selected characteristics were compared between the cases and the controls, the cases were more likely to be less educated, overweight, and to have had a caesarean section . No significant differences were observed among the cases and control regarding parity and smoking status.

Statistical analysis of data using logistic regression demonstrated that SNPs in the *C6* complement gene were significantly associated with the risk of preeclampsia (*C6* rs7444800: OR=2.40; 95% CI=1.38-4.18; p-value=0.002; *C6* rs4957381: OR=2.44; 95% CI=1.31-3.83; p-value=0.004). SNPs in *C6* were also associated with early-onset preeclampsia (min P=0.017) and severe preeclampsia (min P= 0.011).

An increased risk of late-onset preeclampsia was observed among participants with polymorphisms in the *MASP1* gene (*MASP1* rs1357134: OR=3.16; 95% CI=1.46-6.83; p-value=0.003; *MASP1* rs6908090: OR=3.22; 95% CI=1.47-7.07; p-value=0.001). *MASP1* polymorphisms was also associated with early-onset preeclampsia (min P=0.005) and severe (*MASP1* rs1357134 and *MASP1* rs6908090) preeclampsia (min P=0.021) (Figure 3).

Gene	SNP Number and Database ID	Minimum P test *P*				
		Preeclampsia (PE)	Early-Onset PE	Late-Onset PE	Mild PE	Severe PE
C6	2: rs7444800, rs4957381	0.004	0.017	0.045	0.142	0.011
MASP1	42: rs1357134, rs850307, rs13064893, rs698094 rs698084, rs698090, rs3107215, rs4686864, rs3774275, rs11720718, rs4455312, rs3843010, rs4686870, rs3914010.....	0.006	0.005	0.11	0.431	0.021

Figure 3: Association Between Examined Complement Gene Regions and Risk of Preeclampsia and Subtype. Polymorphisms in the *C6* and *MASP1* genes were shown to be significantly associated with preeclampsia. Wu, W., Yang, H., Feng, Y., Zhang, P., Li, S., Wang, X., . . . Zhang, Y. (2016).[5]

Conclusions

Preeclampsia and related conditions affect about 5 to 8 percent of pregnancies in the United States, but in the developing world, the incidence of preeclampsia is estimated to be seven times higher. If left untreated, preeclampsia can lead to eclampsia and other life-threatening complications.

The results of this study demonstrate that there is a relationship between SNPs in complement genes and preeclampsia risk and suggest that the immune response may have a role in the pathogenesis of preeclampsia. Another study examined the relationship between autoimmune diseases caused by complement-mediated injury and the risk of preeclampsia. It was found that patients with genetic mutations in complement genes were at risk of developing preeclampsia. These findings can facilitate better treatment and management of preeclampsia.

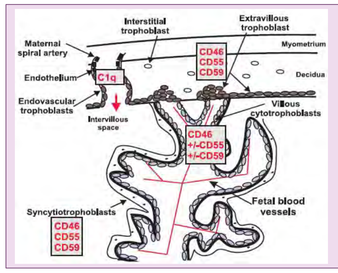

Figure 4: Schematic of Hemochorial Placenta. Subtypes of trophoblasts and associated complement components and regulators that are a part of normal placental development are illustrated here. Regal, J. F., Gilbert, J. S., & Burwick, R. M. (2015).[3]

Relevant Applications to Biotechnology

Single nucleotide polymorphism (SNP) genotyping is used to determine variations of a single nucleotide on a specified location in the genome. SNPs have been increasingly linked to diseases and varying responses to drugs. Thus, the use of SNP genotyping technologies can lead to a better understanding of the role of genetics in disease and allow the development of drugs that are tailored to a patient's specific needs and conditions (as related to genetics).

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I would like to sincerely thank Dr. Ericka Senegar-Mitchell for teaching us, supporting us, and serving as an inspiration for us all. I would also like to thank Dr. Chang, Dr. Su, and all the physicians and scientists who made this program possible. Additionally, I would like to thank Ms. Patricia Winter for coordinating the program . A special thanks goes to my OSA sisters and Kathleen for always motivating me, being by my side, and never failing to have smiles on their faces. Lastly, I am so very grateful to my parents for instilling a love for science in me and for encouraging me to pursue my dreams.

References

1. Derzsy, Z., Prohászka, Z., Jr, J. R., Füst, G., & Molvarec, A. (2010, February 23). Activation of the complement system in normal pregnancy and preeclampsia. *Molecular Immunology, 47*(7-8), 1500-1506.
2. Osungbade, K. O., & Ige, O. K. (2011, January 19). Public Health Perspectives of Preeclampsia in Developing Countries: Implication for Health System Strengthening. *Journal of Pregnancy, 2011*, 1-6.
3. Regal, J. F., Gilbert, J. S., & Burwick, R. M. (2015, March 21). The complement system and adverse pregnancy outcomes. *Molecular Immunology, 67*(1), 56-70.
4. Salmon, J. E., Heuser, C., Triebwasser, M., Liszewski, M. K., Kavanagh, D., Roumenina, L., . . . Atkinson, J. P. (2011, March 22). Mutations in Complement Regulatory Proteins Predispose to Preeclampsia: A Genetic Analysis of the PROMISSE Cohort. *PLoS Medicine, 8*(3).
5. Wu, W., Yang, H., Feng, Y., Zhang, P., Li, S., Wang, X., . . . Zhang, Y. (2016, July 12). Polymorphisms in complement genes and risk of preeclampsia in Taiyuan, China. *Inflammation Research,* 1-9.

The Effect of Polycyclic Aromatic Hydrocarbons (PAH) and Heterocyclic Amines (HCA) Consumption on Breast Cancer for Women

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Objective

The focus of this research is to evaluate Heterocyclic amines (HCA) and Polycyclic aromatic hydrocarbons (PAH) that are commonly found in cooked red meats and demonstrate how a diet high in HCAs and PAHs can increase a women chances for chances for breast cancer. This research will also highlight how a diet such as a Mediterranean or Vegetarian diet that contains higher amounts of fruits and vegetables that contain carotenoids, vitamin C, fiber, and potassium can significantly decrease ones risk for breast cancer.

Abstract

Breast cancer is the second of the leading cause of death in women. The exact causes of breast cancer are still being researched today, yet there is evidence to suggest that certain foods are putting women at an increased risk for breast cancer. This research will demonstrate how a vegetarian diet, Mediterranean diet or, a diet that contains very little red meat will help women decrease their chances for breast cancer. This poster will then help further educate society on how a balanced diet containing fruits and vegetables can greatly decrease one's risk for breast cancer. In 1989, the Nurses' Health Study II performed a prospective cohort study in which 97,813 nurses ages 24-43 completed a food frequency questionnaire that detailed their usually food intake and their medical history in the past year. Every four years the women would come back to complete another questionnaire, resulting in a large study that is still being continued on today. NHSII detailed how women that ate 2.9 servings per day of vegetable and fruits in their adolescence had as much as a 25% decreased the risk of breast cancer, while women that at a meat one serving of red meat on daily basis had as much as a 13% higher risk of being diagnosed with breast cancer. NHSII demonstrated how a diet such as a Mediterranean diet that contained certain fruits and vegetables that hold amounts of substances such as carotenoids, vitamin C, fiber, and potassium have been associated with a decreased risk of breast cancer by 25%, while also resulting in data that provide evidence as to how a daily servings of red meats that contain Heterocyclic amines and Polycyclic aromatic hydrocarbons can increase your risk of breast cancer as an adolescence by 12%.

Method and Materials

In 1991, 97,813 nurses from ages 24-43 agreed to be a cohort study that would evaluate their usually food intake while monitoring their health. Each nurse would complete a food frequency questionnaire that would help the researchers evaluate the nurse's daily food intake and health. Women that had implausible total energy intake left all items on fruit, vegetable, and, red meat section blank, had missing information on age, postmenopausal in 1991 or had pervious cancer besides non-melanoma skin cancer were all excluded from the study. Every four years the nurses would then come back and complete a semi-quantitative food frequency questionnaire that contained approximately 130 food items. Responses to the questionnaire were to evaluate the portion sizes and intake frequency which ranged from "never or less than once per month" to "six or more a day." Meat items were broken into the sections of processed, unprocessed, poultry, fish and other. Vegetable items were broken into sections of green leafy, yellow/orange, tomatoes, cruciferous and other vegetables. While fruit items were broken into groups of citrus, berry, drupes, tropical, grapes, and melon. A similar study was then given to the subjects to evaluate what food items were commonly eaten when the women were adolescents in high school. A follow-up biennial questionnaires were given every two years and asked the women about the diagnosis of breast cancer. When a diagnosis of breast cancer was given, permission was asked to obtain the hospital records and pathology report. Estrogen and progesterone receptor status of the tumors was then collected. From another biennial questionnaire information regarding risk for breast cancer such as age, height, weight, family history, smoking, parity, menopausal status, postmenopausal hormone use, age at menopause and age at menarche were all collected. A woman was considered postmenopausal of they still had periods or had a hysterectomy with one ovary left, yet women that reported going through menopause or undergone a bilateral oophorectomy were considered postmenopausal. All data was processed using a P trend that measures the linear regression.

Results

NHSII's data trend established that a diet that contains 2.9 servings of certain fruits and vegetables such as grapes, tropical, pomes, melons, and kale contain substances such as carotenoids, vitamin C, fiber, and potassium have been associated with a decreased risk of breast cancer by 25% in adolescents. Yet, total fruit intake during early adulthood was not significantly associated with a lower risk of breast cancer. An adult diet that contained high amounts of *α* carotene were associated with a low risk of cancer, yet the trends were not significant. However, fruits (r=0.43) and vegetables (r=0.42) in adolescence and early adulthood were moderately correlated with the risk for breast cancer. A similar risk was seen among all women when viewing the cumulative average of premenopausal fruit and vegetable intake and risk of breast cancer (0.91 95% confidence interval 0.81 to 1.03 p trend 0.05). An inverse association with adolescent fruit intake was stronger for both estrogen and progesterone receptor negative cancer (hazard ratio .70 95% 0.57 to 0.86 per serving/ day), yet vegetable consumption and breast cancer did not differ significantly by estrogen and progesterone receptor positive or negative for adolescents or early adults.

Table 3 | Hazard ratios and 95% confidence intervals for breast cancer in two models* according to fifths of intake of fruit during adolescence among women in Nurses' Health Study II

	Fifth of intake						
	1	2	3	4	5	P_{trend}	Per serving/day
All cases							
Median intake (serving/day)	0.5	0.9	1.4	1.9	2.9	—	—
No of cases/person years	315/134 534	263/134 375	250/134 590	272/134 414	242/134 576	—	—
Model 1†	1	0.83 (0.71 to 0.98)	0.79 (0.67 to 0.93)	0.86 (0.73 to 1.01)	0.79 (0.66 to 0.93)	0.02	0.93 (0.87 to 0.99)
Model 2	1	0.82 (0.69 to 0.96)	0.76 (0.64 to 0.90)	0.82 (0.69 to 0.97)	0.75 (0.62 to 0.90)	0.01	0.91 (0.85 to 0.98)
Premenopausal cases							
Median intake (serving/day)	0.5	0.9	1.4	1.9	2.9	—	—
No of cases/person years	135/69 408	105/69 528	123/69 429	104/69 505	109/69 518	—	—
Model 1	1	0.77 (0.60 to 1.00)	0.87 (0.68 to 1.12)	0.75 (0.58 to 0.97)	0.81 (0.62 to 1.04)	0.15	0.93 (0.84 to 1.03)
Model 2	1	0.78 (0.60 to 1.01)	0.87 (0.68 to 1.13)	0.75 (0.57 to 0.99)	0.80 (0.60 to 1.05)	0.17	0.93 (0.83 to 1.03)
Postmenopausal cases							
Median intake (serving/day)	0.5	0.9	1.3	1.9	2.9	—	—
No of cases/person years	149/53 265	134/53 368	108/53 454	138/53 178	111/53 219	—	—
Model 1	1	0.89 (0.71 to 1.13)	0.72 (0.56 to 0.92)	0.92 (0.73 to 1.16)	0.74 (0.57 to 0.95)	0.05	0.91 (0.82 to 1.00)
Model 2	1	0.85 (0.67 to 1.08)	0.67 (0.52 to 0.87)	0.84 (0.66 to 1.07)	0.69 (0.52 to 0.90)	0.02	0.88 (0.79 to 0.98)

*Model 1: stratified by age in months at state of follow-up and calendar year of current questionnaire cycle. Model 2: as for model 1 and simultaneously adjusted for smoking (never, past, current 1-14/day, current 15-24/day, current ≥25/day), race (white/non-white), parity and age at first birth (nulliparous, parity ≤2 and age at first birth <25, parity ≤2 and age at first birth ≥30, parity 3-4 and age at first birth <25, parity 3-4 and age at first birth 25-<30, parity 3-4 and age at first birth ≥30, parity ≥5 and age at first birth <25, parity ≥5 and age at first birth ≥25), height (<157, 157-<165, 165-<173, ≥173 cm), BMI at age 18 (<18.5, 18.5-<22.5, 22.5-<25, 25.0-<30, ≥30.0), weight change since age 18 (continuous, missing indicator), age at menarche (<12, 12, 13, ≥14), family history of breast cancer (yes, no), history of benign breast disease (yes, no), oral contraceptive use (never, past, current), adolescent alcohol intake (non-drinker, <5, ≥5 g/day), adult alcohol intake (non-drinker, <5, 5-<15, ≥15 g/day), adolescent energy intake (fifth). In postmenopausal women, we additionally adjusted for hormone use (postmenopausal never users, postmenopausal past users, postmenopausal current users), age at menopause (<45, 45-46, 47-48, 49-50, 51-52, ≥53). Among all women, we additionally adjust for hormone use and menopausal status (premenopausal, postmenopausal never users, postmenopausal past users, postmenopausal current users, unknown menopausal status) and age at menopause (premenopausal, unknown menopause, <45, 45-46 years, 47-48, 49-50, 51-52, ≥53).

Figure 1: Table comparing premenopausal and postmenopausal cases based on the amounts of fruit and vegetable intake in one day and the number of breast cancer incidents. Farvid (2016) [3]

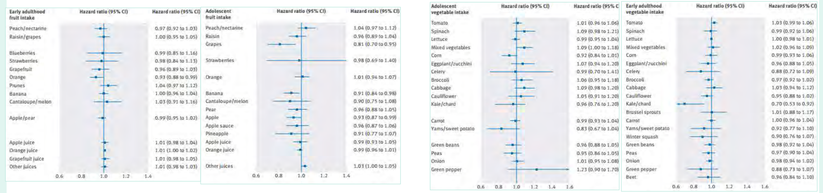

Figure 2: 2 sets of graphs that display fruits and vegetables presented in survey and their increased or decreased risk for breast cancer in premenopausal and postmenopausal women . Farvid (2016) [3]

Among all women a higher total red meat intake was associated with an increased risk of breast cancer. Positive associations between total red meat were still significant after additional adjustment for total red meat intake. Each additional serving/day increase in total red meat increased the risk of breast cancer by 13% among all women, a 12% increase among premenopausal women and 8% increase in postmenopausal women. In a cumulative average of premenopausal intake of poultry was connected with a lower risk of postmenopausal cancer, each additional serving of poultry was associated with a 24% lower risk of postmenopausal breast cancer. Substituting one serving of legumes for one serving of red meat was seen to lower breast cancer risk among all women by 14%. Yet, for postmenopausal women who used oral contractive, each serving of red meat increased their risk for cancer by 54%. Data did not support any significant correlations between red meat intake and tumor status among all women.

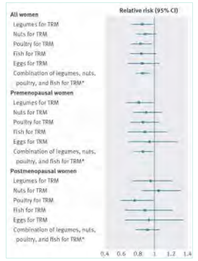

Figure 3: Graph displays legumes, nut, poultry, fish, and, eggs food items found on survey and their increased decreased risk for breast cancer in premenopausal and postmenopausal women and in all women. Graph was adjusted for other factors such as race, age, fat intake etc. Farvid (2015) [5]

Figure 4: Table reviews the amount of red and processes meats against premenopausal and postmenopausal women. Farvid (2015) [5]

Table 2. Relative risk (RR) and 95% confidence intervals (95%CI) for breast cancer according to quintile of adolescent intake of meat and other protein sources in premenopausal women, postmenopausal women and all women

	Quintile of intake						
	1	2	3	4	5	P_{trend}[1]	Per 1 serving increase/day
Total red meat (unprocessed and processed red meat)							
All women							
Median intake, serving/day	0.70	1.13	1.42	1.78	2.43		
No. of cases/person-years	223/120,337	200/120,929	229/120,227	236/120,790	244/120,161		
Age-adjusted RR (95%CI)	1	0.88 (0.72–1.06)	1.01 (0.84–1.22)	1.01 (0.84–1.21)	1.05 (0.87–1.26)	0.29	1.03 (0.95–1.12)
Multivariable RR (95%CI)	1	0.88 (0.73–1.08)	1.04 (0.85–1.26)	1.07 (0.88–1.31)	1.17 (0.94–1.44)	0.048	1.09 (0.99–1.20)
Premenopausal women							
Median intake, serving/day	0.70	1.06	1.42	1.78	2.36		
No. of cases/person-years	104/67,481	98/66,983	104/69,164	115/66,996	125/67,864		
Age-adjusted RR (95%CI)	1	0.94 (0.71–1.24)	0.92 (0.70–1.22)	1.07 (0.82–1.40)	1.13 (0.87–1.47)	0.18	1.08 (0.96–1.22)
Multivariable RR (95%CI)	1	1.01 (0.76–1.34)	1.04 (0.78–1.39)	1.27 (0.95–1.70)	1.43 (1.05–1.94)	0.007	1.21 (1.05–1.39)
Postmenopausal women							
Median intake, serving/day	0.77	1.13	1.49	1.85	2.50		
No. of cases/person-years	103/43,029	88/43,449	95/43,404	93/43,011	104/42,986		
Age-adjusted RR (95%CI)	1	0.83 (0.62–1.10)	0.90 (0.68–1.20)	0.88 (0.66–1.16)	0.97 (0.73–1.27)	0.94	0.98 (0.86–1.11)
Multivariable RR (95%CI)	1	0.80 (0.59–1.07)	0.86 (0.64–1.15)	0.84 (0.62–1.15)	0.99 (0.72–1.37)	0.73	0.99 (0.85–1.15)

Discussion

This research furthers the evidence that a great consumption of red meat that contains HCA and PAH is connected to a higher risk of breast cancer for premenstrual breast cancer, while an early adult diet that contains higher amounts of fruit and vegetable intakes that contain *α* and β carotene can greatly lower a women's risk for cancer. Certain fruits such as apples, bananas, grapes and kale have been seen to provide a significantly lowered risk of breast cancer as they contain more materials, such as carotenoids, vitamin C, fiber, and potassium, that have been seen to cause inverse effects on breast cancer. While the red meat was associated with a 13% increased risk of breast cancer for all women, poultry items were associated with a lower incident of breast cancer in postmenopausal women. Many poultry items are known to have high amounts polyunsaturated fats which change the cooking process of poultry items over red meats. HCAs and PAHs are created while red meat is being cooked at high temperatures, such as wood fire cooking, these two naturally occurring chemicals have been seen to promote estrogenic activity and cause breast tissue carcinogenic activity. HCA and PAH are both chemicals that affect the Aryl Hydrocarbon Receptor (AhR). AhR has been known to handle immunotoxicity in response to new chemicals and environmental contaminants that enter the body and as been known to target Cytochrome P450-1 (CYP1) genes. However, AhR has been known to cause incidents of cancer, autoimmune diseases and other diseases in response to this reaction. With diets such as a Western diet that commonly eats high amounts of red and processed meats, higher amounts of HCAs and PAHs are being products and are affecting the AhR which in return is causing more carcinogenic activity within the breast tissue: thus, increase a woman's risk of breast cancer by 13%. Yet, a diet such as a Mediterranean or vegetarian diet that contains little to no red or processed meats are decreasing the amounts of HCAs and PAHs being consumed and are then replacing meats with fruits and vegetables that are highly enriched in substances such as carotenoids, vitamin C, fiber, and potassium; thus, decreasing a adolescent woman's chance of breast cancer by 25%. With this evidence in mind, a diet that contains little to none red or processed meats, such as a Mediterranean or vegetarian diet, should be encouraged in adolescents and young adults to decrease their chances for premenstrual breast cancer. However, more research should be done to discover more relationships between carotenoids and risk for breast cancer, diet in early life and risk for breast cancer, as well as the impact of timing of dietary exposure and cancer risks.

Relevant Application to Biotechnology

Biotechnology has been used greatly to study the Aryl Hydrocarbon Receptor (AhR) and its impact on mice and humans. Through Biotechnology, scientist were able to use mice to discover that the AhR was responsible for responding to new chemicals and environmental contaminants that enter the body, thus leading to cancer and may other diseases. However, Biotechnology could further studies on cooked meats and HCA and PAH effect on the AhR. Biotechnology could help find further evidence as to how much red or processed meat is needed in order to activate the AhR receptor and start to cause carcinogenic activity within the breast tissue. This research could the help educate society on how their diet could greatly increase or decrease one's chances of breast cancer, and help create a decrease in the number of breast cancer incidents.

References

1. Albuquerque, R. C., Baltar, V. T., & Marchioni, D. M. (2014). Breast cancer and dietary patterns: a systematic review. *Nutrition reviews*, *72*(1), 1-17.
2. Brennan, S. F., Cantwell, M. M., Cardwell, C. R., Velentzis, L. S., & Woodside, J. V. (2010). Dietary patterns and breast cancer risk: a systematic review and meta-analysis. *The American journal of clinical nutrition*, *91*(5), 1294-1302.
3. Farvid, M. S., Chen, W. Y., Michels, K. B., Cho, E., Willett, W. C., & Eliassen, A. H. (2016). Fruit and vegetable consumption in adolescence and early adulthood and risk of breast cancer: population based cohort study. *bmj*, *353*, i2343.
4. Farvid, M. S., Cho, E., Chen, W. Y., Eliassen, A. H., & Willett, W. C. (2014). Dietary protein sources in early adulthood and breast cancer incidence: prospective cohort study. *BMJ*, *348*.
5. Farvid, M. S., Cho, E., Chen, W. Y., Eliassen, A. H., & Willett, W. C. (2015). Adolescent meat intake and breast cancer risk. *International Journal of Cancer*, *136*(8), 1909-1920.

Acknowledgements

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An Analysis of the Anticancer Potential of Malaysian Tualang Honey and its Efficacy as a Cancer Treatment

Hilltop High School
Sarah Esparza

Objective

The most common treatment for patients with cancer is chemotherapy in which cytotoxic drugs are administered. Although treatments using such hormonal therapy has been efficient in reducing the mortality rates of cancer, undesirable side effects arise as a result of the drugs' inability to specifically target cancer cells. Additionally, cancer cells have begun to develop resistance to such treatments. This poster will provide an analysis of the potential for Malaysian Tualang Honey to be used as a natural cancer treatment and alternative to traditional treatments.

Abstract

While many of the causes for cancer remain unidentified, researchers have found the following to assist in its development: accumulation of toxic free radicals, chronic infections, low immune status, chronic inflammation, and chronic non-healing ulcers. Malaysian Tualang Honey (TH) has shown potential of being an anticancer agent against cancer because it is high in antioxidants, is a scavenging agent for toxic free radicals, is a natural antimicrobial, is a natural immune booster, is a natural anti-inflammatory agent, and heals chronic ulcers and wounds. The purpose of this investigation is to research the level of efficacy of TH in the treatment of Breast and Cervical cancers. In order to assess the anticancer potential of TH, human breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cell lines, as well as normal breast epithelial cell line, were treated with increasing dosages of TH (1-10%) for up to 72 hours. The results showed that the TH was cytotoxic and induced significant cell death in all three cancer cell lines, with 93% cell death of MDA-MB-231with 10% TH, 91% of MCF-7 with 5% TH, and 100% of HeLa with 5% TH. The treatment of the normal breast epithelial line did not show clear cytotoxic effects with 1-10% TH, however the maximum cell death was only 28% with 5% TH. In another study, the MCF-& and MDA-MB-231 cell lines were treated with either 1% TH, 2.5, 5, 10, or 15 µM Tamoxifen (TAM) alone, or a combination of TH and TAM for 6-72 hours. This study showed that the combination of TH and TAM significantly increased the percentage of apoptotic cells compared to single treatments (43.8% with TH+TAM, 31.2% with TAM). In conclusion, my research has shown that Malaysian Tualang Honey is an effective anticancer agent. I propose that physicians work to combine chemotherapeutic treatment with TH.

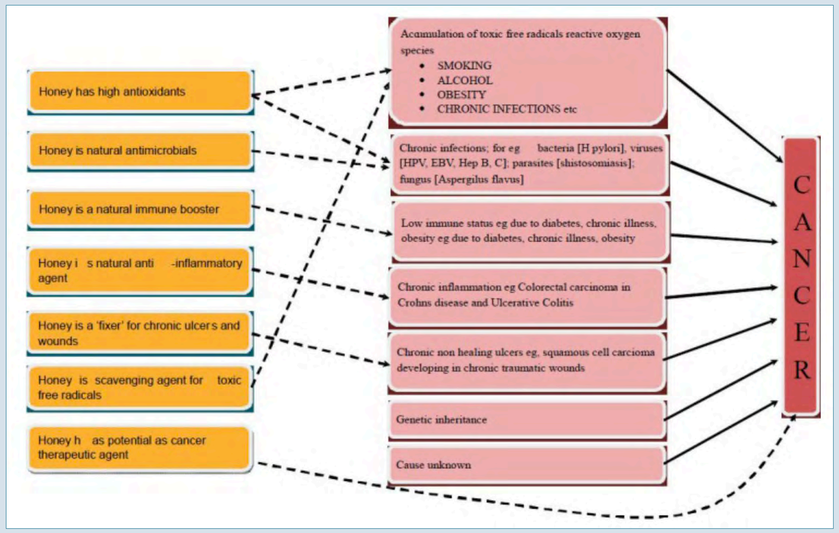


Figure 1: Representation of the relationship between the characteristics of Malaysian Tualang Honey and known causes of Cancer. *Othman, Nor Hayati. "Does Honey Have the Characteristics of Natural Cancer Vaccine?" Journal of Traditional and Complementary Medicine 2.4 (2012): 276-83. Web.*

Methods and Materials

In the first study, human breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cells and normal breast epithelial cells were treated with various concentrations of TH (1-10%) for up to 72 hours. Cells were collected at 6, 24, 48, and 72 hours after treatment. To determine the amount of apoptosis, cells were analyzed using the fluorescent activated cell sorter. The apoptotic activity was then compared to that of Tamoxifen. Three independent experiments were performed. In the second study, human breast (MCF-7 and MDA-MB-231) cancer cells were treated with 1% Tualang Honey or in combination with various concentrations of Tamoxifen (2.5 µM- 15 µM), or Tamoxifen alone. Cells were collected at 6, 24, 48, and 72 hours after treatment and incubated with JC-1 dye the accumulates within intact mitochondria and turns red, but changes to green with a reduction of mitochondrial membrane potential. Three independent experiments were performed.

Results

The results of the first study showed that the TH was cytotoxic and induced significant cell death in all three cancer cell lines, with 93% cell death of MDA-MB-231 with 10% TH, 91% of MCF-7 with 5% TH, and 100% of HeLa with 5% TH. The treatment of the normal breast epithelial line did not show clear cytotoxic effects with 1-10% TH, and the maximum cell death was only 28% with 5% TH. When the cells were stained with Annexin V Fluorescence antibody and Propidium Iodide (**Fig.** 4), it was found that the percentage of apoptotic cells was significantly higher in those treated with TH than untreated cells.
The results of the second study showed that the combination of TH and TAM significantly increased the percentage of apoptotic cells compared to single treatments (43.8% with TH+TAM, 31.2% with TAM), and the difference between the percentage of TH and TH+TAM treated cells was not significant.
Both studies found that there was a reduction in Mitochondrial Membrane Potential. After treatment with TH, it was found that caspase-3/7 and -9 were activated. The second study also identified activation of caspase -8 by TH and only caspase-7 by TAM.

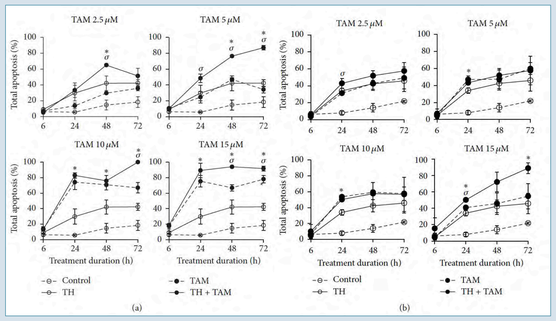
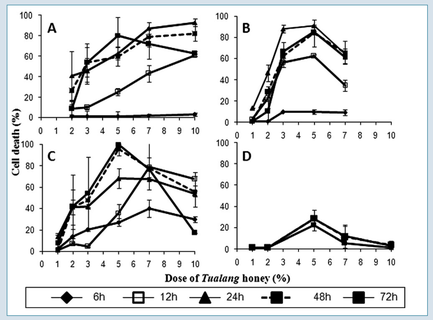


Figure 2: Percentages of Total Apoptosis vs. Treatment Duration for varying dosages of Tamoxifen and Tualang Honey *Yaacob, Nik Soriani, Agustine Nengsih, and Mohd. Nor Norazmi. "Tualang Honey Promotes Apoptotic Cell Death Induced by Tamoxifen in Breast Cancer Cell Lines." Evidence-Based Complementary and Alternative Medicine 2013 (2013): 1-9. Web.*

Figure 3: Cytotoxic effect of varying doses of Tualang Honey on (A) MDA_MB-231, (B) MCF-7, (C) HeLa and (D) MCF-10A cell lines. *Fauzi, Agustine Nengsih, Mohd. Nor Norazmi, and Nik Soriani Yaacob. "Tualang Honey Induces Apoptosis and Disrupts the Mitochondrial Membrane Potential of Human Breast and Cervical Cancer Cell Lines." Food and Chemical Toxicology 49.4 (2011): 871-78. Web.*



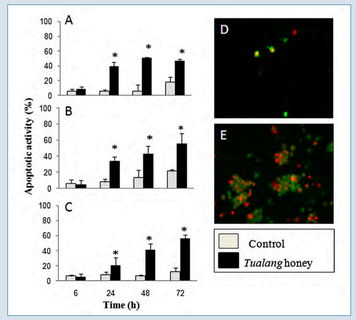


Figure 4: Percentage of apoptotic cells induced by Tualang Honey in (A) MDA-MB-231, (B) MCF-7, and (C) HeLa cell lines. Apoptosis was detected by staining (D) untreated MCF-7 and (E) untreated MCF-7 cells with annexin V Fluos antibody. *Fauzi, Agustine Nengsih, Mohd. Nor Norazmi, and Nik Soriani Yaacob. "Tualang Honey Induces Apoptosis and Disrupts the Mitochondrial Membrane Potential of Human Breast and Cervical Cancer Cell Lines." Food and Chemical Toxicology 49.4 (2011): 871-78. Web.*

Conclusions

Malaysian Tualang Honey has been found to be rich in flavonoids, phenolic acids, hydroxymethylfurfural (HMF) contents, and main fatty acids, all of which either inhibit tumor cell growth or induce cell death. Furthermore, Tualang Honey activated caspases that initiate and execute apoptosis. It proved to be effectively cytotoxic to cancerous cells, while not killing normal cells, making Tualang Honey potentially useful as an anticancer agent and more advantageous than current anticancer agents. It was also discovered that the combination of Tualang Honey and Tamoxifen has a higher efficacy in inhibiting tumor cell growth than Tamoxifen alone. This indicates that the required effective dose of Tamoxifen may be reduced, as well as the side effects of its use.

Applications to Biotechnology

The biotechnology that made this research possible includes the Annexin V Fluorescence antibody, Propidium Iodide JC-1 dye, and the Fluorescent Activated Cell Sorter that were used to differentiate between the apoptotic cells and the living cells. The Annexin proteins and Propidium Iodide work by binding to and detecting the morphological changes of the cell membranes. The JC-1 dye accumulates in varying amounts depending on the membrane potential of the mitochondria of the cells. The Fluorescent Cell Sorter organizes the cells based on the marker that they express.

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References

1. Fauzi, Agustine Nengsih, Mohd. Nor Norazmi, and Nik Soriani Yaacob. "Tualang Honey Induces Apoptosis and Disrupts the Mitochondrial Membrane Potential of Human Breast and Cervical Cancer Cell Lines." Food and Chemical Toxicology 49.4 (2011): 871-78. Web.

2. Othman, Nor Hayati. "Does Honey Have the Characteristics of Natural Cancer Vaccine?" Journal of Traditional and Complementary Medicine 2.4 (2012): 276-83. Web.

3. Othman, Nor Hayati. "Honey and Cancer: Sustainable Inverse Relationship Particularly for Developing Nations—A Review." Evidence-Based Complementary and Alternative Medicine 2012 (2012): 1-10. Web.

4. Premratanachai, Pongsathon, and Chanpen Chanchao. "Review of the Anticancer Activities of Bee Products." Asian Pacific Journal of Tropical Biomedicine 4.5 (2014): 337-44. Web.

5. Yaacob, Nik Soriani, Agustine Nengsih, and Mohd. Nor Norazmi. "Tualang Honey Promotes Apoptotic Cell Death Induced by Tamoxifen in Breast Cancer Cell Lines." Evidence-Based Complementary and Alternative Medicine 2013 (2013): 1-9. Web.

The Correlation Between Higher Levels of Dioxin in the Atmosphere and Decreased Sertoli Cell Proliferation

Esin Gumustekin, Francis Parker School

Objective

The purpose of this poster is to examine the correlation between dioxin in the air and decreased Sertoli cell proliferation, which is causing men to have decreased sperm count as adults. Dioxin is an extremely toxic pollutant which is a by product of many industrial processes including chlorine bleaching of paper, the manufacturing of certain pesticides, incineration of medical waste and plastic, and smelting. Sertoli cells are somatic cells found in the testis that are essential for spermatogenesis. They enable the progression of germ cells to become spermatozoa. The number of Sertoli Cells ultimately determine how many sperm cells adult males will be able to form.

Abstract

In a population of 7 billion, decreasing fertility rates may not be viewed as an urgent issue. However, it is estimated that in 2050 the world population will start to decrease, one main cause of this being decreased sperm count in men. In my poster, I will be analyzing the correlation between dioxin in the air and decreased Sertoli cell proliferation, which is causing men to have decreased sperm counts as adults. Decreasing sperm count throughout the world is an emerging issue. This became evident in Dr. Le Moal's study in France in which over 26,000 men aged 18-70 were sampled at clinics around the country from 1989 to 2005. These men were the partners of women who were known to be infertile and were going through fertility treatments at clinics around the country. The men weren't known to be infertile and thus were a representative sample of the population. The study found that during the 17 years, sperm count decreased by a third. Evidence suggests that one main cause of decreased sperm count is perinatal exposure to dioxins. Dioxins interfere with the proliferation of Sertoli cells, which determine the number of sperm produced in adult life. Dioxin's detrimental effect became evident in the 1976 industrial accident in Seveso, Italy in which a trichlorophenol plant explosion resulted in dioxin contamination in the surrounding area. Thirty nine men who were born near Seveso between 1977 and 1984 were followed through life and had semen samples drawn as an adult. When compared with the 58 men who were used as a control group who were not born in Seveso but in a different city during the explosion, the 39 men who were prenatally exposed to dioxin had significantly lower sperm count, thus demonstrating dioxin's toxic effects.

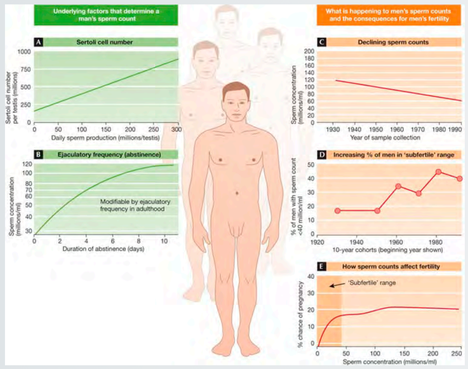

Figure 1. These graphs show that over time men's sperm count is decreasing as the percentage of men in the subfertile range is increasing. Sharpe (2012)[3]

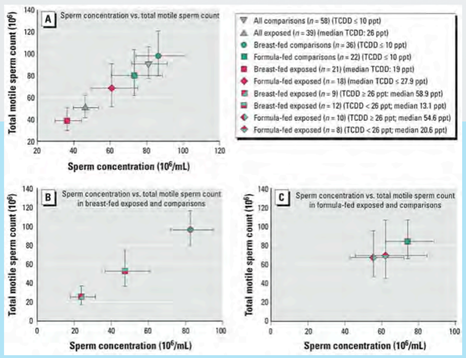

Figure 2. These graphs compare the semen samples of men who were exposed to dioxin in utero and perinatally during the Seveso accident and men who were used as a control group. Mocarelli (2011)[4]

Methods and Materials

From 1989-2005, Dr. Le Moal and fellow researchers studied the sperm count of men aged 18-70 in clinics around France. 26,609 men who were the partners of women who were infertile due to blocked or missing fallopian tubes were part of the study. They came from over 126 clinics and were a a close reflection of the male population as they were not known to be infertile. The men provided fresh ejaculate and sperm concentration, motility and morphology were all analyzed as part of the study. The researchers calculated the mean sperm data for each year group and adjusted the results in order to represent an average 35 year old man. The results from this study are very fascinating and can be applied to many other developed countries in the world. Evidence suggests that one cause of decreasing sperm count is because of increased dioxin in the air. This can best be seen in the trichlorophenol plant explosion in Seveso, Italy in 1976 which released toxic amounts of dioxin into the atmosphere. A study headed by Dr. Mocarelli was conducted in which the sperm of 39 men born in Seveso from 1977 to 1984 were compared to 58 men who were not born in Seveso. Of these 39 men, 21 were breast-fed and 18 were formula-fed. This division allowed the researchers to separate the exposed men into two groups: men who had been exposed in utero only (those formula-fed and not breast-fed) and men who had been exposed perinatally—both in utero and during nursing (breast-fed). The 58 men who were used as a control group were of the same age and similar socioeconomic status as the exposed men but had mothers who had not lived in the dioxin-contaminated area. The men were comparable in terms of breast-feeding and formula feeding. (36 men were breast-fed and 22 were formula-fed). All participants completed a survey on their health, smoking and drinking habits, working conditions, weight at birth and how long they nursed as infants. Additionally they were screened for hidden diseases at clinics. The mothers of the participants had serum samples taken in 1976 and 1977 so that the dioxin levels of the control and exposed groups could be compared. A dioxin level of 19ppt (parts per trillion) and above is considered unsafe.

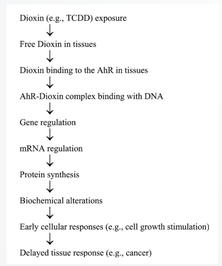

Figure 3. This is a schematic representation of dioxin's interaction inside the human body. Mandal (2005)[1]

Figure 4. This graph shows a few of the countries that contain the most dioxin in the atmosphere. (2008)[5]

Results

During Dr. Le Moal's 17 year study, the mean sperm count of French men decreased by 32.2%, 1.9% each year. The sperm concentration of an average 35 year old man fell from 73.6 million/mL to 49.9 million/mL. This drastic trend of declining sperm count is not only noted in France but in many other industrialized nations as well including India, Japan and Germany. Evidence suggests that one main cause of decreased sperm count is perinatal exposure to dioxins, as seen in the trichlorophenol plant explosion in Seveso, Italy in 1976. The results of this study show that exposure of low concentrations of dioxin perinatally results in permanent impairment of a male's reproductive system (50% reduction in sperm count and 20% decreased sperm motility as seen in Figure 2). For example, the 21 breast-fed sons whose exposed mothers had a median serum dioxin concentration of 19 ppt at conception had lower sperm concentration (36.3 vs. 86.3 million/mL), total count (116.9 vs. 231.1), progressive motility (35.8 vs. 44.2%), and total motile count (38.7 vs. 98 million) than did the 36 breast-fed comparisons. The breast-fed exposed group even had significantly decreased sperm count when compared to the formula-fed exposed group as they were exposed to dioxin for a longer period of time, in which Sertoli Cell proliferation was continuing to occur. Dioxin interacts with the Ah receptor which then causes decreased Sertoli cell proliferation. Dioxin acts as a ligand and binds to the aryl hydrocarbon receptor (AhR)/AhR nuclear translator complex which then binds to the dioxin-responsive element (DRE) on target DNA. Then, the expressed AhR/ARNT, when ligand activated, interacts with many transcription factors that ultimately influence tissue development. AhR/ARNT then directly intervenes in male reproductive system development. Dioxin response in the body can be seen in Figure 3.

Conclusion

In conclusion, there is a strong correlation between dioxin in the atmosphere and decreased Sertoli cell proliferation which is causing decreased sperm count in adult life. Increasing amounts of dioxin in the air due to industrialization are a cause of declining birth rates in the world as dioxin is resulting in decreased sperm count. Japan, for instance, creates 40% of the world's dioxin production and has a radically declining birth rate, one of the lowest in the world. Japan contains 1,800 household-waste incinerators while the U.S. has about 250. The excessive amounts of dioxin in the air and decreasing birth rates are not a coincidence. Other countries that have excessive atmospheric pollution and decreasing birth rates include China and Germany. As countries continue to industrialize they must take into account the effects it has on their citizens' reproductive health. Citizens must become aware that the environment they live in will have an effect on their children's reproductive health later on in life. To facilitate this learning, countries should establish a database that has the dioxin levels in the air of each city. This way, before citizens decide to move to another city they would become aware of the dioxin levels in the atmosphere and the repercussions it would have on their child's reproductive health.

Application to Biotechnology

Through improved technology, researchers have been able to conduct better studies that are more detailed and accurate. Through advancements in microscopy, scientists have been better able to count and determine the morphology of sperm. In the future as technology continues to advance, we will be better able to find the factors that are leading to decreased sperm count throughout the world and understand all of the mechanisms contributing to it.

Acknowledgements

I would like to thank Dr. Ericka for all of her help, support and enthusiasm. She has truly inspired me to become a woman scientist and become a "big sister in science". I would also like to thank Ms. Winter for all of her dedication and hard work to make this amazing program possible. Additionally, I would like to extend my thanks to Dr. Chang and all the doctors for their countless hours of help and for answering all of our questions. I would also like to thank Kathleen and all of my OSA Sisters for all of their support and friendship throughout this academy. I would like to extend my sincerest thanks to my family for their continuous support, encouragement and love. They've supported me through all of my endeavors and have always given me the confidence and courage to pursue my dreams. Finally, I would like to thank my AP Science teachers including Dr. Pierce, Dr. Joshi and Mr. Johnson for inspiring me to pursue a career in science.

References

1) Mandal, P. K. (2005, April 8). Dioxin: A review of its environmental effects and its aryl hydrocarbon receptor biology. Retrieved August 08, 2016, from http://link.springer.com/article/10.1007/s00360-005-0483-3

2) Burton A. Study Suggests Long-Term Decline in French Sperm Quality. *Environmental Health Perspectives*. 2013;121(2):a46. doi:10.1289/ehp.121-a46.

3) Sharpe, R. M. (2012). Sperm counts and fertility in men: a rocky road ahead: Science & Society Series on Sex and Science. *EMBO Reports*, *13*(5), 398–403. http://doi.org/10.1038/embor.2012.50

4) Mocarelli P, Gerthoux PM, Needham LL, et al. Perinatal Exposure to Low Doses of Dioxin Can Permanently Impair Human Semen Quality. *Environmental Health Perspectives*. 2011;119(5):713-718. doi:10.1289/ehp.1002134.

5) World Map - Political. (2008). Retrieved August 08, 2016, from http://geology.com/world/world-map.shtml

Da Vinci System: Improving Hysterectomy Surgery for Endometrial Cancer Patients

Jennifer Harrison ● Castle Park High School

Objective

The intention of my study is to demonstrate what ways technology assists surgeons in laparoscopic surgery. The goal is to publicize the da Vinci system to make robot-assisted hysterectomies available to everyone. Also, how it can further be improved and utilized by more surgeons and hospitals. The main focus of this poster is to study supracervical, total, and radical hysterectomies, since those are the most common treatment strategies in early-stage endometrial cancer. This poster will demonstrate the strengths, along with the weaknesses, of the da Vinci system, in order to portray the advances in technology and ultimately to provide the optimum patient care.

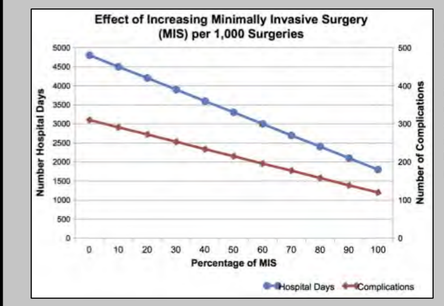

Figure 1. The increased MIS per 1000 surgeries. Based on this, each 10% increase in MIS would result in 41 fewer complications and 600 fewer days in the hospital. Lim P., et al. (2016)[5]

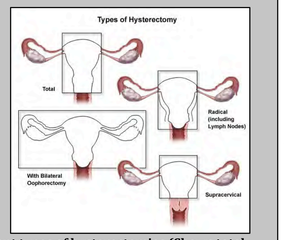

Figure 2. Different types of hysterectomies (Shows total, radical, supracervial, hysterectomies and bilateral oophorectomy) Retrieved from www.hopkinsmedicine.org/healthlibrary/conditions/gynecological_health/hysterectomy_85,P0056

Abstract

Minimally-invasive surgeries (MIS) are transforming the medical community. The focus of this poster is to study supracervical, total, and radical hysterectomies: the most common treatment strategies in early-stage endometrial cancer. This study is considered an unmet medical need because 90% of the women diagnosed with endometrial cancer are post-menopausal and are over the age of 60, therefore, their bodies are significantly weaker. They count on MISs to increase their chance of survival and to reduce recovery time. In 1901, Georg Kelling of Germany invented laparoscopy when he inserted a cystoscope into the abdomen of a dog. Currently, laparoscopy has greatly-improved as surgeons utilize the procedure to perform surgery with minimum pain, blood loss, and recovery time. Introducing technology into the process, laparoscopy has become quicker, more precise, and less painful. This project explores the benefits of robot-assisted laparoscopic surgery, the most common being the da Vinci Surgical System, while also suggesting challenges and needs for improvement. In a study done between January 1st, 2010 and September 30th, 2013, specialists evaluated 32,118 hysterectomies. Comparing the robotic, abdominal, vaginal, and laparoscopic groups, robotic surgery was the only group that prevented the most hemorrhaging, nerve injury, mechanical failures, and more. Along with a decrease in the amount of complications and pain experienced by the patients, there was a decrease in readmission and reoperation from the da Vinci system, as opposed to the other methods of hysterectomies. Robotic surgery shows advancement in comparison to other surgical procedure types, but certain limitations such as cost may prevent many individuals from having that option (traditional laparoscopic surgery remains the cheapest). The da Vinci system has undergone many innovations, such as manipulated robotic arms to prevent clashing, improved docking, with an autofocus universal endoscope. Yet, there are still many improvements that could be made to it. In conclusion, robot-assisted laparoscopy should be universally utilized for endometrial cancer patients, thus many postmenopausal women diagnosed with endometrial cancer can receive optimal care.

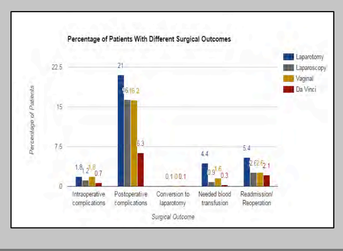

Figure 3. The surgical outcomes from the different surgical methods. Data retrieved from Lim P., et al. (2016)[4]

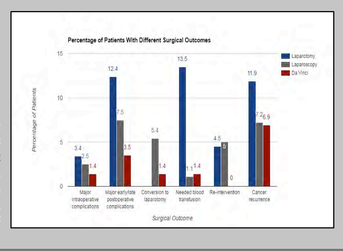

Figure 4. The surgical outcomes from the different surgical methods. Data retrieved from Corrado G., et al. (2015)[2]

Methods and Materials

The Premier Perspective database provided statistics of robot-assisted hysterectomies, for benign indications, from nine centers, and abdominal, vaginal, and laparoscopic hysterectomy data from 405 hospitals. 32,118 patients underwent hysterectomies from January 1st 2010 to September 30th 2013: 9,745 were abdominal, 8,121 were vaginal, 11,952 were laparoscopic, and 2,300 were robot-assisted. Although this study did not measure surgeries done for malignancy, it clearly demonstrates the effectiveness of the da Vinci System over other surgical methods. The surgeons who contributed to this study were required to perform at least 60 benign hysterectomies prior to the study period. Eligible patients were matched by age, body mass index (BMI), indications of past surgeries, hysterectomy type, and size of uterus, and they were studied for readmission/reoperation, conversion to laparotomy, blood transfusions, and length of hospital stay. At the Gynaecologic Oncological Unit, "Regina Elena" National Cancer Institute in Rome, Italy, from January 2001 to December 2013, a study of women with endometrial cancer, FIGO (International Federation of Gynecology and Obstetrics) staged from IA-IVB, was conducted. Out of the 526 patients who underwent hysterectomies to treat their endometrial cancer, 177 of them had an abdominal hysterectomy, 277 had a laparoscopic hysterectomy, and 72 had a robot-assisted hysterectomy, all of which were performed by the same surgical team. The robotic surgery was introduced later in the study, since the FDA did not approve of the system until 2005. The women were matched by the same characteristics and studied for surgical outcomes as the previous study, except for the inclusion of being matched by FIGO (International Federation of Gynecology and Obstetrics) staging and studied for cancer recurrence. From 2005-2013, the Joint Institutional Review Board of Taipei Medical University enrolled 365 women into a study. Their criteria was that they have FIGO stage IA-IIIC endometrial cancer, without previous brachytherapy or chemotherapy, and that they treat it with either laparoscopy, laparotomy, or robot-assisted laparoscopy. 86 patients received robot surgery, 150 received laparoscopic surgery, and 129 received laparotomy. They obtained postoperative pain scores, time of full diet resumption, hospital stay, and long-term survival outcomes.

Results and Interpretation

From the first study, robotic surgery had a 0.7% intraoperative complicating rate, while abdominal surgery had 1.8%, vaginal had 1.8%, and laparoscopy had 1.2%. These complications included hemorrhage, nerve injury, mechanical failure, laceration, and cautery injury. The length of hospital stay was 1.31 ± 1.1 days for the robot group, 3.0 ± 1.6 days for the laparotomic abdominal group, 1.9 ± 1.0 for the vaginal group, and 1.7 ± 1.2 days for the laparoscopic group. In the second study, the operation time for the da Vinci surgery was slightly longer than the laparoscopic surgery by an average of 15 minutes (laparotomy took around 120 min, laparoscopy took about 100 min, and the da Vinci system was around 115 min). The blood loss was the same between the robotic group and the laparoscopic group, both losing a median of 100 mL, and the blood loss was half of the amount as the open abdominal surgery (200mL). There was a 4.5% re-intervention outcome in the laparotomic group, 5% in the laparoscopic group, and 0% in the robot-assisted group, and the hospital stay was the least time in the robotic group with a range of 2-10 days, as opposed to 2-20 days in laparoscopy and 4-34 days in open surgery. The postoperative complications also had a much lower percentage in robot-assisted laparoscopy over the other tested surgical methods (12.4% had complications in open surgery, 7.5% had complications in laparoscopy, and 3.5% had complications in the robotic surgery). The last study, unlike the second one, exhibits data that the operation time for the robot-assisted laparoscopy is lower than the other surgical methods (an average of 155.6 min for the robotic surgery 178.6 for the laparoscopy, and 195.3 for the laparotomy). The blood loss for the da Vinci System was around 94.8 mL, for the laparoscopic group it was about 174.2 mL, and for the laparotomic group it was around 234.4 mL. These results differed from the results in the National Cancer Institute in Rome, Italy, and that is likely caused by the slightly different methods and materials. The other outcomes matched the other studies, for instance the average hospital stay days were 3.1 in the robotic surgery, 3.7 in laparoscopy, and 5.8 in laparotomy. Robotic surgery has shown to be superior to the other methods of performing hysterectomies, but the procedure ended up costing around $2030-$2349 more than a traditional laparoscopic procedure. This factor leads to a lower accessibility rate to the general public. Although the price for the da Vinci surgeries are more expensive, there are still progressions to be made to it, and will eventually have the same price as laparoscopic surgery, or even less.

Conclusion

The da Vinci System needs many improvements before it is the perfect universal surgical method. It definitely has potential to improve tremendously since it has only been readily available for hospital utilization for around 11 years, and new technology generally undergoes endless refinement and improvement. Over the years, the Da Vinci System has undergone many innovations, such as having the docking improved, the robotic arms manipulated to prevent clashing, and adding an autofocus universal endoscope. Yet, there are still many improvements that could be made to it, to lower the mortality, blood loss, pain, surgery complications, operation time, and hospital stay even more. Also, to get the overall cost of the robot-assisted hysterectomy procedure lower than the traditional laparoscopic surgery. Someday, hysterectomies may even be performed without incisions. Robot-assisted laparoscopic surgery should be universally utilized for endometrial cancer treatments, thus many postmenopausal women diagnosed with endometrial cancer can receive optimal care.

Relevant Applications to Biotechnology

In 2014 there was an estimated 52,630 new cases of endometrial cancer and 8,590 deaths from it, making it the most common cancer of the female reproductive organs. Generally, postmenopausal women treat their endometrial cancer with a hysterectomy, regardless of what stage it is at. For laparotomic surgery, the chance of recurrence was 11.9%, for traditional laparoscopy it was a 7.2% chance, and for the robot-assisted laparoscopy the chance was 6.9%. Robotic surgery has only been FDA approved since 2005, therefore these amazing statistics are proof of the potential for a cure to cancer, as the machinery becomes more and more precise and the recurrence levels get lower and lower from upgrades and innovations.

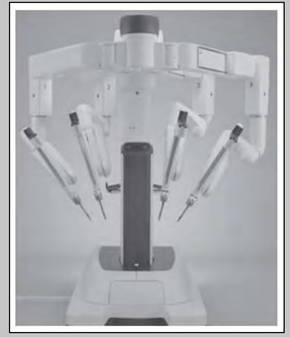

Figure 5. The da Vinci System has improved tremendously over the years. Gettman M., Rivera M., (2016)[3]

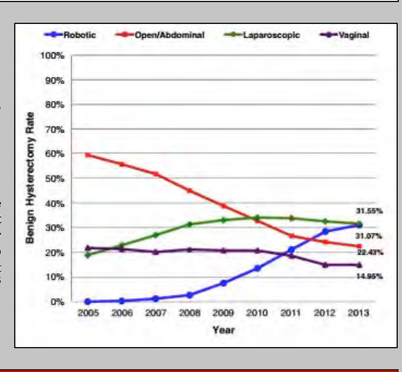

Figure 6. The increased amount of robotic surgery from 2005 to 2013. Scalici J., et al. (2015)[5]

References

1. Chiou H., et al. (2015) Comparing robotic surgery with laparoscopy for endometrial cancer management: A cohort study. Retrieved from www.journal-surgery.net/article/S1743-9191(14)00975-3

2. Corrado G., et al. (2015) Surgical and oncological outcome of robotic surgery compared to laparoscopic and abdominal surgery in the management of endometrial cancer. Retrieved from http://www.sciencedirect.com/science/article/pii/S074879831500428X

3. Gettman M., Rivera M., (2016) Innovations in robotic surgery. Retrieved from http://journals.lww.com/co-urology/pages/articleviewer.aspx?year=2016&issue=05000&article=00011&type=fulltext

4. Lim P., et al. (2016) Multicenter analysis comparing robotic, open, laparoscopic, and vaginal hysterectomies performed by high-volume surgeons for benign indications. Retrieved from http://www.ijgo.org/article/S0020-7292(16)00089-8/fulltext

5. Scalici J., et al. (2015) The trend towards minimally invasive surgery (MIS) for endometrial cancer: An ACS-NSQIP evaluation of surgical outcomes. Retrieved from www.gynecologiconcology-online.net/article/S0090-8258(14)01479-6/fulltext

6. Wright J., etal. (2013) Robotically Assisted vs Laparoscopic Hysterectomy Among Women With Benign Gynecologic Disease. Retrieved from jama.jamanetwork.com/article.aspx?articleid=1653522

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Figure 7. OSA sisters and their first photo together. Taken by Ms. Patricia Winter in July, 2016

The Correlation Between the Zika Virus and Microcephaly

Sapna Mehta • San Marcos High School

Objective

The Zika Virus is a current mosquito-borne pandemic, attacking millions, but especially dangerous when infecting pregnant women, as it is correlated with microcephaly, a neurodevelopmental birth defect, as illustrated in Figure 1. As a public health emergency spreading explosively, having reached over fifty countries, research on this topic and its relation to microcephaly would benefit those who are infected with Zika and are at risk of passing the virus to their future children in the form of microcephaly. The intent of this study is to investigate the connection between Zika and microcephaly using data about the increased incidents of microcephaly during the Zika outbreak, the consistent presence of Zika in the amniotic fluid and the trans-placental penetration of Zika that leads to the fetus contracting microcephaly.

Microcephaly Brain Alterations

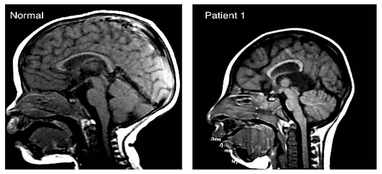

Figure 1. Microcephaly Brain Alterations. Normal brain (left), brain of fetus with microcephaly; whose mother contracted Zika while pregnant. Adapted from Romero Álvarez, D. (2016, January 21). Virus Zika: Más casos, más evidencia, más hipótesis -. Retrieved August 08, 2016, from http://latinamericanscience.org/spanish/2016/01/virus-zika-mas-casos-mas-evidencia-mas-hipotesis/

Abstract

Zika, a pandemic in progress, creates a possibility of deadly consequences when infecting pregnant women, by potentially being linked to a neurodevelopmental birth defect, microcephaly. Is there a correlation? A study from 2013-15 in French Polynesia's Zika outbreak analyzed the risk of microcephaly in Zika infected pregnant women. This trial studied factors corresponding to the relationship. Significant data analyzed includes seroprevalence for Zika and microcephaly's association with Zika during pregnancy stages. Specifically, the blood serum results found suggest that the rate of microcephaly's spread inclined from 0.8% to 66% by the time the outbreak ended and 1% of pregnant women's fetuses contracted microcephaly who were infected with Zika during trimester one. As the outbreak of Zika intensified, the spread of microcephaly dramatically increased as well. Scientists are investigating a deeper connection between the two phenomenons. Two pregnant Zika infected women from Brazil whose fetuses had been diagnosed with microcephaly had amniocenteses performed at 28 weeks, looking for microcephaly's cause. A similar study was completed at the UMC in Slovenia where a woman and her fetus had been infected with Zika. She aborted her fetus, as it had a risk of defects, and it was analyzed using several methods such as an autopsy, electron microscopy, indirect immunofluorescence and microbiologic investigation. Both studies extracted amniotic fluid and found the presence of the Zika genome, illustrating that the virus can infect the fetus through trans-placental and intrauterine transmission. The electron microscope demonstrated viral replication in the brain which correlates with the neurotropicity of Zika, highlighting a possible entryway into the brain of the fetus. In conclusion, Zika and microcephaly have a deep correlation which will guide science along its way towards the cure for Zika to prevent increased incidents of microcephaly, however, scientists are trying to determine whether or not Zika is the direct causality of microcephaly.

Materials and Methods

A study taking place from September 2013 to July 2015 in French Polynesia, the risk of microcephaly in Zika infected pregnant women was analyzed. This study had proven information on this correlation by researching factors corresponding to this relationship. Data analyzed includes: seroprevalence for Zika antibodies with thousands of test subjects and microcephaly's association with Zika during pregnancy stages with eight women who are pregnant with microcephaly infected fetuses with a twenty-three month period as well. The association between microcephaly and Zika was characterized by a mathematical model, used to determine which pregnancy stage is riskiest for microcephaly. This is the equation used:

$$P_1(W_1) = Y\frac{I_{W_1}}{\sum_W I_W}$$

P¹(w¹)=probability that these women are infected with Zika during the week
Y = final attack rate and strength of the tie between Zika and microcephaly
Iw=number of consultations

Two pregnant Zika infected women from Paraíba, Brazil, whose fetuses had been diagnosed with microcephaly had amniocenteses performed at twenty-eight weeks gestation to find microcephaly's cause. Using viral metagenomics and phylogenetic analysis, amniotic fluid was centrifuged at 21,130xg and 15,000 rpm for 90 min at 4 degrees Celsius to concentrate the virus particles. A similar study was completed at the UMC in Slovenia where a woman and her fetus had been infected with Zika. Her was given a poor prognosis for neonatal health, therefore she chose abortion. Several methods were used to analyze the fetus and placenta including an autopsy, electron microscopy, indirect immunofluorescence and microbiologic investigation. The situations for both of these studies had studied contents of the womb of Zika infected pregnant women whose children were found to have contracted microcephaly.

Results and Interpretations

From my research, the data has shown that there is a connection between Zika and microcephaly, possibly a causality. The results found in the first study suggest that the attack rate of Zika has changed from 0.8% before the outbreak to an overwhelming 66% afterwards. The rapid speed that Zika had spread at highlights the significance of the need to cease the transmission of this global pandemic. The risk of microcephaly infection in pregnant women is highest when infected during the first trimester at 1%, with a p value of 0.0007, rendering this data to be statistically significant, compared to the end of the pregnancy where the risk is about 0.53%, with a p value of 0.05, rendering this data to be statistically significant as well. As the outbreak of Zika intensified, the spread of microcephaly inclined as well. From an alternate study examining the first diagnoses of intrauterine transmission of Zika and the first study to support the hypothesis that Zika can spread through transplacental transmission, both patients had negative results for Zika but amniocentesis and RT-PCR, performed after fetal diagnosis of microcephaly, was positive for Zika in both patients. The fact that Zika has been found in the amniotic fluid provides support for the hypothesis that it is transmitted in utero and/or transplacental. A similar study had found microcephaly within the aborted fetus of a Zika infected pregnant woman through an autopsy, revealing the brain weight of 84 g (4 SD below average), widely open sylvian fissures and a small cerebellum and brain stem. The electron microscopy found ruptured and lysed cells in the brain tissue and the microbiological investigation had resulted in a positive result for Zika on the RT-PCR. These damaged brain cells and the occupation of the virions is displayed in Figure 2. Additional results from this study suggest the strong neurotropism of Zika, as viral damage is only present in the brain, no other fetal organs. The localization of immunofluorescence signals and the morphologic appearance of the calcification resembled destroyed neuronal structures. This indicates the probable presence of the virus in the neurons of the fetus which would indicate the likely pathway that this virion takes from the mosquito to the brain of the fetus.

The Effect of Zika on the Fetal Brain

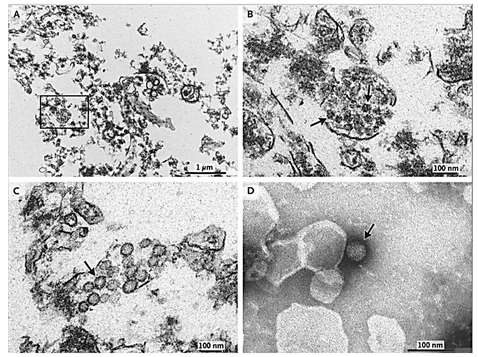

Figure 2. Effect of Zika on Fetal Brain. Part A shows a damaged brain cell with a cluster of virions in the endoplasmic reticulum. Part B provides a magnified view of Part A, virions clearly visible (arrows). Part C shows a group of enveloped structures (arrows) with a lighter interior, indicating viral replication. Part D highlights a viral particle (arrow), negatively stained with morphological characteristics consistent with Flaviviruses. Mlakar (2016)[4]

Zika Infects the Fetus via Placenta – Direct Transfer Hypothesis

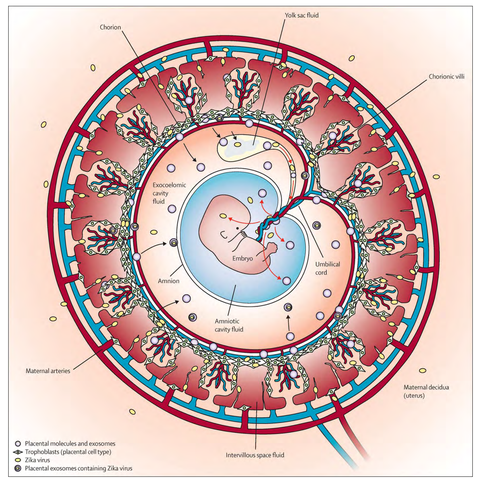

Figure 3. Zika Infects the Fetus through the Placenta as shown in the Direct Transfer Hypothesis. It is possible that Zika accesses the brain of the fetus through the placenta when maternal flow begins through the umbilical cord and could potentially enter by: direct transfer of free virus through the trophoblast layers, placental exosome-mediated transfer, or minimal to no transfer; all displayed here. The Zika virus enters through the maternal arteries, past the trophoblasts or placental cells, through exocoelomic cavity fluid and into the amniotic fluid of the embryo. A potential theory is that the virus travels through the umbilical cord after it crosses the placenta and is distributed directly into the blood flow of the fetus. Adibi (2016)[1]

Discussion

The Zika Virus and microcephaly have a correlation that will guide science along its way towards the cure for Zika to prevent increased incidents of microcephaly but scientists are trying to determine whether or not Zika is a causality of microcephaly. Zika has been proven to have a surface level connection with microcephaly, displayed by the increasing incidents of microcephaly during the Zika outbreak. Further research revealed that amniotic fluids of fetuses with microcephaly in Zika infected pregnant women was found to have traces of Zika within it, displaying the route that this disease possibly took to travel from the mosquito bite into the brain of the fetus. Scientists are very close to finding out the way in which Zika travels from the blood stream into the brain of the fetus. Scientific possibilities about how Zika infects the fetus that are currently being contemplated such as the direct transfer hypothesis, as shown in Figure 3, suggesting different possible ways that Zika could penetrate the placental barrier such as entering through the uterine gland secretions among many more scientific ideas. Put quite simply, the Zika virus spreads from the mother's bloodstream, through her uterus, into the amniotic fluid and brain of the fetus, causing microcephaly. I personally believe that one of many causes of microcephaly is Zika, however, more microbiological research to support the hypothesis that Zika causes microcephaly is needed to draw conclusions on this topic. The current Zika outbreak and its teratogenic effects will soon be explained as the epidemiology and pathology of this viral infection are confirmed, providing scientific achievement that can help those facing this crisis as well as future similar epidemics. Understanding that Zika is potentially passed to the fetus, scientists can further look into a special vaccination or medication to inhibit Zika from crossing the placental barrier. Moreover, the vaccination that scientists are currently creating will prevent Zika from even infecting the victim and many others will avoid this infection including its horrendous side effects while pregnant. As a current pandemic that is effecting millions of lives, a vaccination for Zika is necessary to improve the quality of life for those living with Zika and to prevent illness and life-impacting changes such as contracting microcephaly.

Relevant Applications to Biotechnology

Advancements in biotechnology have played a significant role in the research behind Zika and microcephaly because the approaches used to find data utilized technology to create statistics such as: the use of an electron microscope to detect abnormalities in the brain tissues of the fetus, as illustrated in Figure 2 and the use of viral metagenomics, the PRINSEQ software for example, separating the amniotic fluid from the Zika genome that inhibited scientists to analyze the findings of Zika within amniotic fluid including which strain it came from. These were just a few examples of the information provided to the researchers as a result of the use of these biotechnologies. If scientists were to draw a conclusion to identify Zika as a cause of microcephaly, biotechnology would be impacted in finding the way to inhibit Zika from infecting the fetus and creating a vaccination for those infected with Zika, impacting the options for those infected with similarly transmitted diseases such as rubella or herpes simplex.

Acknowledgements

I would like to express my gratitude towards Dr. Ericka and Dr. Chang for all of their enlightening teaching that helped me get where I am from the start of the academy. I would like to thank Ms. Winter for all her time and effort in organizing everything. Thank you to Dr. Dave for inspiring me to research the Zika virus. Thanks to my OSA sisters for their support and for making this an experience that I will never forget. I would like to extend my thanks to my club advisor, Mr. O'Neill, for always believing in my abilities and cheering me on. Thank you to my family and friends for their consistent encouragement, support and help in achieving the goals that I aspire to complete. Thank you to UCSD Health Sciences and all of the doctors involved in the creation of this academy for inspiring and sparking interest in young women in science to pursue our dreams and scientific interests.

References

1. Adibi, J. J. (2016, April 9). Teratogenic effects of the Zika virus and the role of the placenta. Retrieved August 7, 2016, from http://thelancet.com/journals/lancet/article/PIIS0140-6736(16)00650-4/fulltext
2. Calvet, G., PhD, & Aguiar, R. S., PhD. (2016, February 17). Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: A case study. Retrieved July 30, 2016, from http://www.thelancet.com/journals/laninf/article/PIIS1473-3099(16)00095-5/fulltext
3. Cauchemez, S., PhD, & Besnard, M., MD. (2016, March 15). Association between Zika virus and microcephaly in French Polynesia, 2013–15: A retrospective study. Retrieved July 31, 2016, from http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(16)00651-6/fulltext
4. Mlakar, J., MD, & Korva, M., Ph.D. (2016, March 10). Zika Virus Associated with Microcephaly — NEJM. Retrieved July 27, 2016, from http://www.nejm.org/doi/full/10.1056/NEJMoa1600651#t=article
5. Solomon, I. H., Milner, D. A., & Folkner, R. D. (2016, June 2). Neuropathology of Zika Virus Infection. Retrieved July 27, 2016, from http://www.omicsonline.com/open-access/neuropathology-of-zika-virus-infection-2314-7326-1000220.pdf

Maternal Caffeine Consumption and the Risk of Select Neural Tube Defects

Claudia Monarrez
High Tech High Chula Vista

Objective

The purpose of this poster is to define select Neural Tube Defects, to highlight insufficient results concluding any teratogenic properties of caffeine, and to show how ingesting excessive amounts of caffeine during pregnancy could possibly harm fetuses by possibly developing select birth malformations.

Abstract

Ever since the popularity of caffeinated drinks arose, 90% of American women that consumed caffeine before pregnancy continued ingesting caffeine through beverages, bringing concern to the risk of developing Anencephaly, Spina Bifida, Encephalocele, and other select Neural Tube Defects (NTD). Since the amount of research on caffeine containing teratogenic properties is restricted to very few studies, excessive amounts of caffeine may become a threat for developing fetuses. A 2010 research study conducted an evaluation of 133 case mothers with children with NTDs and 273 control mothers with children for possible effects of substances consumed before and during pregnancy, including caffeine, and found mothers ingesting caffeine before and during pregnancy did have an increased risk for developing spina bifida, a common NTDs. A daily average of caffeine consumption was recorded a year prior and during pregnancy for mothers who gave birth to children between 2000 through 2008. After collecting all data, the study concluded more extensive research should be completed to find whether consuming caffeine could increase the risk of NTDs. A Similar study observed caffeine to not hold any effect to the risks of developing any select NTDs, from a lack of any convincing changes. Overall, the evaluations did not find a convincing connection between excessive maternal caffeine consumption during pregnancy and NTDs, due to caffeine lacking teratogenic properties that may trigger select birth malformations. Due to few research studies resulting in similar conclusions, the connection between caffeine and NTDs is not available. More financial support, willing clients, and awareness to caffeine's effect to fetuses may bring possible correlation between caffeine and birth malformations.

Table 4 Multivariate models of the association between maternal risk factors and NTD occurrence

Risk factors	OR (95% CI)	*P* value
Folic acid supplements		
Before and after conception	Ref.	
After conception	2.38 (0.64–8.88)	
Never	20.54 (5.41–77)	0.0001
Coffee		
None	Ref.	
<3 cups/day	1.98 (0.87–4.50)	
>3 cups/day	10.82 (3.78–31)	0.0001
Fruit/vegetable consumption[a]		
Regular	Ref.	
Occasional	3.38 (1.67–6.82)	0.001
Diet[b]		
Balanced	Ref.	
Low calorie	5.15 (1.79–14)	
High calorie	1.12 (0.37–3.37)	0.01
Alcohol		
Less than half a liter	Ref.	
More than half a liter	3.05 (1.24–7.50)	.01
Birth order		
1	Ref.	
2	1.16 (0.54–2.47)	
≥3	6 (1.55–23)	.03

OR odds ratio, *CI* confidence interval, *Ref.* reference group
[a]Healthy diet, 1,200–2,000 calories/day; low calorie diet, less than 1,200 calories/day; high calorie diet, more than 2,000 calories/day
[b]Regular, 3 or more times/week; occasional, less than 3 times/week

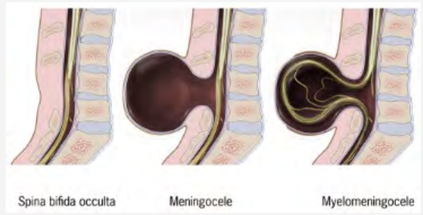

Figure 1. A visual representation of Spina bifida occulta, Meningocele, and Myelomeningocele. (Facts. (2015, December 30). Retrieved August 08, 2016, from http://www.cdc.gov/ncbddd/spinabifida/facts.html)

Table 1. A table depicting viewed associations between maternal habits and consumption factors with the development of Neural Tube Defects. (De Marco, P., Merello, E., Calevo, M., Mascelli, S., Pastorino, D., Crocetti, L., . . . Capra, V. (2011, January 5). Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: An Italian case-control study. Retrieved August 03, 2016) [4]

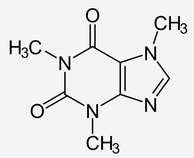

Figure 2. A depiction of the molecule Caffeine. (Caffeine. ((n.d.). Retrieved August 08, 2016, from https://pubchem.ncbi.nlm.nih.gov/compound/caffeine#section=Top))

Methods and Materials

A 2010 research study conducted an evaluation of 133 case mothers with children with NTDs and 273 control mothers with children for possible effects of substances consumed before and during pregnancy. Out of 850 initial case mothers , 133 who participated were Caucasian Italians, gave information within 24 months after the delivery of the index pregnancy, and their child gave birth to a child that had non-syndromic open or closed spinal dysraphism. While 332 control mothers were inquired to participate in the study, only 273 mothers agreed to participate. Eligible control mothers were Caucasian, and gave birth to healthy children, reassured by checking birth registration forms prior to the evaluations. Before any were conducted, interviewers obtained all mother's history prior to pregnancy, including lifestyle habits and exposure information 3 months before until 3 months after conception. Information solicited about all mother's history included: mother's birth date, date of delivery, country of birth, educational level, annual family income, marital status, reproductive history, pregnancy history. Lifestyle and exposure information included: Caffeine, alcohol, fruit, and vegetable consumption, smoking and stress levels, medication use, multi-vitaminic and iron therapies, radiation, toxics, and pollutant exposure, and residency to waste cites. All NTD cases were questioned 20 months in total, while all case mothers were questioned for 18 months in total.

Table 3 Periconceptional and first-trimester lifestyle of the mothers and NTD risk

Variable	Cases (*N*=133; %)	Controls (*N*=273; %)	OR (95% CI)	*P* value
Smoking [a]				
No	97 (72.9)	226 (83.7)	Ref.	
Yes	36 (27.1)	44 (16.3)	1.91 (1.16–3.14)	.012
Coffee[b]				
No	26 (19.7)	99 (36.4)	Ref.	
<3 cups/day	59 (44.7)	150 (55.1)	1.50 (0.88–2.54)	
>3 cups/day	47 (35.6)	23 (8.5)	7.78 (4.02–15.05)	<.001
Alcohol[c]				
Less than half a liter	95 (71.4)	240 (90.2)	Ref.	
More than half a liter	38 (28.6)	26 (9.8)	3.69 (2.12–6.42)	<.001
Diet[d, e]				
Healthy	83 (63.4)	215 (87)		
Low calorie	25 (19.1)	14 (5.7)	4.63 (2.29–9.33)	
High calorie	23 (17.6)	18 (7.3)	3.31 (1.70–6.45)	<.001
Fruit and vegetable consumption[f,g]				
Regular	47 (48.5)	220 (81.5)	Ref	
Occasional	50 (51.5)	50 (18.5)	4.68 (2.83–7.74)	<.001
Emotional stress[h]				
Low	50 (37.6)	85 (35)	Ref.	
Moderate	33 (24.8)	125 (51.4)	0.45 (0.27–0.75)	
High	50 (37.6)	33 (13.6)	2.58 (1.47–4.52)	<.001

OR odds ratio, *CI* confidence interval, *Ref.* reference group
[a]Data were missing for 3 control women
[b]Data were missing for 1 NTD mother and 1 control mother
[c]Data were missing for 7 control mothers
[d]Data were missing for 2 NTD mothers 26 control mothers
[e]Healthy diet, 1,200–2,000 calories/day; low calorie diet, less than 1,200 calories/day; high calorie diet, more than 2,000 calories/day
[f]Data were missing for 36 NTD mothers and 3 control mothers
[g]Regular, 3 or more times/week; occasional, less than 3 times/week
[h]Data were missing for 30 control mothers

Table 2. A table depicting the connection between lifestyle habits and consumption before conception and during the first-trimester. (De Marco, P., Merello, E., Calevo, M., Mascelli, S., Pastorino, D., Crocetti, L., . . . Capra, V. (2011, January 5). Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: An Italian case-control study. Retrieved August 03, 2016) [4]

Results

Overall, the evaluation did find some types of lifestyles that increases the risk of select Neural Tube Defects (spina bifida), and concluded that excessive amounts of caffeine leads to increased risks of developing select NTDs. Alongside caffeine consumption, a low calorie diet and occasional consumption of fruit and vegetables was observed to have the strongest association to the development of NTDs. Caffeine consumption was recorded by the amount of coffee consumed daily, excluding any other caffeinated beverages. Measurement was more than 3 cups of coffee to less than 3 cups of coffee, posibliy leaving a gap to the connections of other caffeinated beverage consumption, and the development of other select NTDs.

Conclusions

Due to few research studies resulting in similar conclusions, the connection between caffeine and NTDs is not available. Since this study was one of the first of it's kind conducted in Italy, a feasible conclusion whether specific exposure to some elements and consumption habits, including caffeine could raise the risk of developing select NTDs. Similar studies concluded with the same findings, that caffeine may possibly hold a connection to the development of select NTDs, but with the lack of research following the effects of caffeine, convincing data is still yet to be found. More financial support may bring more willing clients, and awareness to caffeine's effect to fetuses. Due to the lack of a confident conclusion connecting NTDs and caffeine consumption, the potential danger of consuming caffeinated products could bring risk to developing infants before and during pregnancy. Because of the lack of data collection for other caffeinated beverages (caffeinated drinks, sodas), the connection to NTDs is only researched through the consumption of coffee. More awareness to excessive caffeine consumption, daily lifestyle habits, and exposure to some materials may cause more studies to be conducted, in order to possibly find a connection to NTD development.

Relevance to Biotechnology

Within the past few decades, methods to finding any potential effect from substances consumed was restricted due to the technological status at that time. Because of the advancement of biotechnology, more clinical trials may be conducted due to the methods of collecting such data. For example, most clinical trials were recorded from communication based data, through interviews that may not be accurate. Now, data can be more accurate from the means of collecting data with their respective instruments.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell, Ms. Patricia Winter, and my OSA sisters for their support, patience, and energy to allow the Oncofertility Science Academy to become an enjoyable and unique experience. I would also like to thank my family, Mr. Nick, and Mrs. Sara for supporting my endeavors that will propel me to new places.

References

1. Benedum, C. M., Yazdy, M. M., & Mitchell, A. A. (2013, August 2). Risk of Spina Bifida and Maternal Cigarette, Alcohol, and Coffee Use during the First Month of Pregnancy (M. M. Werler, Ed.). Retrieved August 03, 2016
2.Brent, R. L., Christian, M. S., & Diener, R. M. (n.d.). Evaluation of the reproductive and developmental risks of caffeine. Retrieved July 30, 2016
3.Chen, L., Belle, E. M., Browne, M. L., Druschel, C. M., & Romitti, P. A. (n.d.). Exploring maternal patterns of dietary caffeine consumption before conception and during pregnancy. Retrieved July 29, 2016
4.De Marco, P., Merello, E., Calevo, M., Mascelli, S., Pastorino, D., Crocetti, L., . . . Capra, V. (2011, January 5). Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: An Italian case-control study. Retrieved August 03, 2016
5. Rosenberg, L., ScD, Mitchell, A., MD, & Stone, D., MD. (n.d.). Selected Birth Defects in Relation to Caffeine-Containing Beverages (S. Shapiro MB, Ed.). Retrieved July 31, 2016
6. Schmidt, R. J., Romitti, P. A., Burns, T. L., Murray, J. C., Browne, M. L., Druschel, C. M., & Olney, R. S. (2010). Caffeine, Selected Metabolic Gene Variants, and Risk for Neural Tube Defects. Retrieved July 29, 2016

Effects of Ooplasmic Transfer to Revitalize Oocytes in Middle-Aged Women

Vanessa Nyawabila
Mt. Carmel High School

Objective

The aim of the present study is to augment the development and viability of embryos by improving the quality of unfertilized eggs suspected of having deficiencies by supplying them with ooplasm from normal donor oocytes. Demonstrated will be how the transfer of cytoplasm from youthful oocytes will help rejuvenate oocytes in elder women, with the expectation of improving the ability to impregnate. The performance of ooplasmic transfer is to replace damaged mitochondria located in the cytoplasm that have withered away with time or subsided to genetic defects, thus leading to a variety of disorders. New techniques towards this area of IVF will increase the ability of obtaining mature oocytes to be used in various IVF procedures.

Abstract

Ooplasmic transfer, also known as cytoplasmic transplant, is an emerging treatment procedure in IVF, In Vitro Fertilization, that was first conducted by embryologist Jacques Cohen. Transfer of mitochondria from good quality oocytes to poor amplifies the sanctioned amount of energy, which then, enables the receiving oocyte to create sufficient, metabolic products for normal development. This research poster will focus on the use of cytoplasmic transfer to assist older women, significantly after the age of 35, who are probable to suffer from infertility due to deficient or damaged mitochondria. The methods used to conduct ooplasmic transplantation is performed by transferring 5-15% of donor ooplasm to recipient oocytes either by cytoplast construction of the donor oocyte or by electrofusion of the cytoplast to the patient oocyte, followed then by ICSI, Intracytoplasmic Sperm Injection. The results from these studies revolving the different techniques were broken down into two categories. These methods were conducted 30 times on 27 couples. The injection technique culminated in 10 single births, 1 set of twins, 1 quadruplet birth, 1 miscarriage, and 1 ongoing pregnancy. The electrofusion technique, only tested on 3 patients, resulted in no improvement in embryo development or pregnancy. The occurrence of chromosomal irregularities was 1/17, or 5.9%, which is slightly higher than the populations congenital abnormalities. The increasing factor of maternal age and upsurge in sex chromosome aneuploidy after ICSI is a possible correlation to the high statistics of indiscretions chromosomally. Results show that patients aged 35-37 years old after transplantation of ooplasm resulted in singletons pregnancies (5%), similarly equal to the amount of pregnancies from women >35 after the same ooplasm procedure. From older women over 40 where damaged cytoplasmic levels reached over 0.003%, implantation rates were decreased which correlated to high amounts of damaged mitochondria. The data suggests that high levels of impaired components of cytoplasm results in increase embryonic implantation failures that correlates to maternal age, aneuploidy, and embryonic implantations. Thus, inter-cytoplasmic transfer in conjunction with ICSI can be presumed to have a positive effect in the improvement of oocyte development. Furthermore, data from studies with increased numbers are essential for future evaluation in order to gather information towards the energy necessary for oocyte maturation and fertilization for embryo implantation.

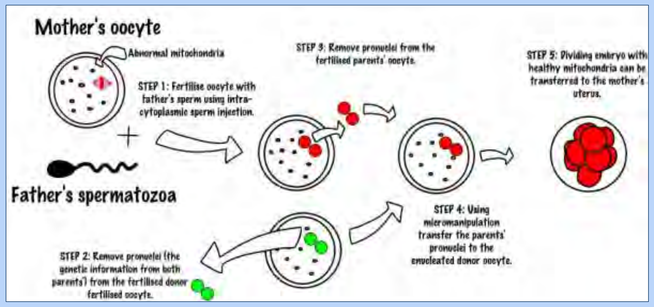

Figure 1(A).This technique involves the use of a fertilized egg from the parents. Genetic information within the egg and the sperm is fused prior to removed. The pronuclei are then put into a donor's enucleated, fertilized egg which can be then transferred to mother's uterus[5].

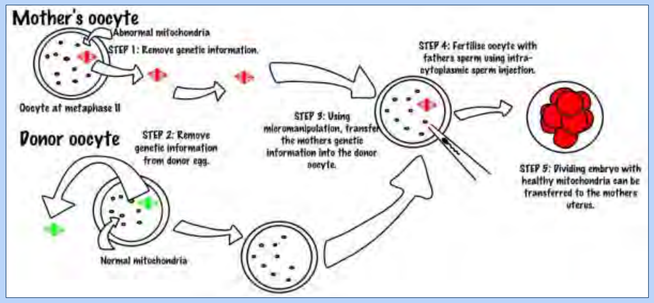

Figure 1(B). The nucleus from the egg of the biological mother is transferred into a healthy donor egg that has had the nucleus removed (enucleated). This creates a new egg, with the nuclear DNA of the mother and the mitochondrial DNA from the donor. The first technique involves taking the nucleus from the mother's egg and putting it into the enucleated donor egg. IVF is then used to fertilize the egg[5].

Methods and Materials

The methods used to conduct ooplasmic transplantation is performed by transferring 5-15% of donor ooplasm to recipient oocytes either by cytoplast construction of the donor oocyte or by electrofusion of the cytoplast to the patient oocyte, followed then by ICSI, Intracytoplasmic Sperm Injection. The two approaches were inspected to transfer ooplasm from donor eggs at metaphase II (MII) stage into patient MII eggs; electrofusion of a ooplasmic donor fragment into each patient egg consisted of three cycles, and direct injection of a small amount of ooplasm from a donor egg into each patient egg consisted of five cycles. Some donor eggs were used numerous times. Donor eggs were divided into two groups, one being used for ooplasmic extraction and the other one for egg donation. Cleaved embryos resulting from the latter were cryopreserved, where numbers and satisfactory development permitted. A second control group consisted of embryos derived from patient eggs after intracytoplasmic sperm injection without ooplasmic transfer. This was performed when sufficient number of eggs were available (n = 5). Donor eggs (n = 40) were evaluated cytogenetically after micromanipulation in order to confirm the presence of chromosomes.

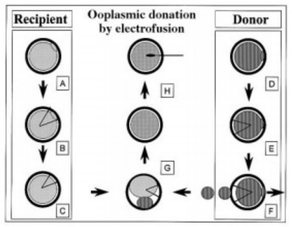

Figure 2(A).Ooplasmic transplantation by electrofusion with donated ooplast[4].

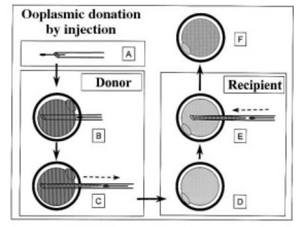

Figure 2(B).Scenario for ooplasmic transplantation by injection[4].

Results

The results from these studies revolving the different techniques were broken down into two categories. These methods were conducted 30 times on 27 couples. The injection technique culminated in 10 single births, 1 set of twins, 1 quadruplet birth, 1 miscarriage, and 1 ongoing pregnancy. The electrofusion technique, only tested on 3 patients, resulted in no improvement in embryo development or pregnancy. The occurrence of chromosomal irregularities was 1/17, or 5.9%, which is slightly higher than the populations congenital abnormalities. The increasing factor of maternal age and upsurge in sex chromosome aneuploidy after ICSI is a possible correlation to the high statistics of indiscretions chromosomally. Results show that patients aged 35-37 years old after transplantation of ooplasm resulted in singletons pregnancies (5%), similarly equal to the amount of pregnancies from women >35 after the same ooplasm procedure. From older women over 40 where damaged cytoplasmic levels reached over 0.003%, implantation rates were decreased which correlated to high amounts of damaged mitochondria. The data suggests that high levels of impaired components of cytoplasm results in increase embryonic implantation failures that correlates to maternal age, aneuploidy, and embryonic implantations.

Table II. Distribution of mature donor and patient eggs for ooplasmic transfer and use of homologous (patient eggs without ooplasmic transfer) and heterologous (donor eggs used for egg donation) control groups

Patient	Donor eggs for transfer	Recipient patient eggs	Ratio of donor/ patient eggs	Enucleated donor egg injected with patient PB	Homologous patient control eggs	Heterologous donor control eggs	Donor embryo frozen
Electrofusion with ooplast							
A	4	12	0.3	0	4	6	5
B1	2	5	0.4	2	0	22	13
C	3	5	0.6	0	0	6	5
Ooplasmic injection							
D	7	14	0.5	0	6	8	4
E	6	12	0.5	0	3	20	0
F	5	9	0.6	0	2	11	6
B2	7	7	1.0	0	0	17	3
G	6	4	0.7	0	0	14	0

PB = polar body.

Figure 3.In all, 26 patient and 45 donor eggs were mature. Only 9/45 (20%) of the donor eggs were needed for ooplast production. Two to five ooplasts were produced from each of the nine eggs. The ratio of donor eggs used for each recipient egg ranged 273 from 0.3 to 0.6. Control donor eggs were injected with the husband's spermatozoa, and embryos from each patient were cryopreserved for later use[4].

Application to Biotechnology

The direct injection approach was relatively simple and entailed minimal disturbance of the usual ICSI routine with even more encouraging clinical electrofusion is technically more complex and radical of the two; although it is by far the more versatile. For the future of biotechnology, however, it may become the method of choice in future attempts to redress ooplasmic imbalance and deficiencies. Exploration involving the two techniques for future medicine can increase the chances of having full terms of pregnancies as the advancement in data of how to eradicate the right amount of cytoplasm to input into oocytes to obtain the maximum amount of full term pregnancies.

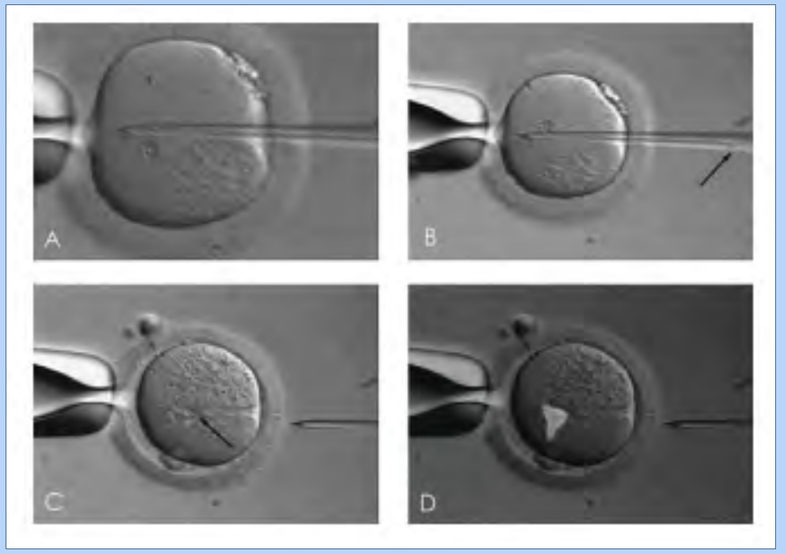

Figure 4. Ooplasmic transfer in a human egg using injection. (A)The membrane is broken and the spermatozoon is positioned at the tip of the needle. (B) Ooplasm is extracted. Arrow indicates sperm cell in needle. (C) The ooplasm and spermatozoon (arrow) are deposited into the recipient's egg. (D) The same as image C, but the position of the injected ooplasm is highlighted[4].

Discussion

Inter-cytoplasmic transfer in conjunction with ICSI can be presumed to have a positive effect in the improvement of oocyte development. Furthermore, data from studies with increased numbers are essential for future evaluation in order to gather information towards the energy necessary for oocyte maturation and fertilization for embryo implantation. Maternal age is an influencing factor on chromosomal status. In conclusion, this is believed to be the first instance where ooplasm donation has been used with the aim of augmenting embryo viability in humans. While such treatment is tentative at this time, it seems to imply that these or similar procedures, such as nuclear transplantation at the germinal vesicle stage, will eventually prove valuable in assisted human conception. The fact that donor eggs or spermatozoa introduce a completely new set of mtDNA and donor chromosomes, and that for most prospective parents the relative importance of third party mtDNA is an unknown issue, may overshadow the demand for these new procedures.

Acknowledgments

I would like to thank my high school, Mt. Carmel, for giving me an academic foundation where I have been able to apply my learnings outside of the classroom. I would also like to thank all the professionals involved in this program that have given not only myself, but the rest of my OSA sisters an eye opening experience with quality support and knowledge. In addition, I would like to thank Dr. Ericka especially for all her help and guidance through this academy process. I would like to say thanks to Ms. Winter for coordinating this exceptional program. My incredible family who has supported me in everything I have ever strived to pursue, and giving me the encouragement and push I needed to move forward. My brilliant OSA sisters, who I have grown close to in just a few weeks and will forever cherish to have been welcomed into my life. Above all, I need to thank God for opening doors for me and giving myself the strength and courage to be the best me he had planned me to be.

References

1.Barritt, J. A., Willadsen, S., Brenner, C., & Cohen, J. (2001). Cytoplasmic transfer in assisted reproduction. Human Reproduction Update, 7(4), 428-435. doi:10.1093/humupd/7.4.428

2.Blerkom, J. V. (n.d.). Human Reproduction. Retrieved July 27, 2016, from http://humrep.oxfordjournals.org/content/16/4/719.full

3.Bredenood, A. L. (n.d.). Human Reproduction Update. Retrieved August 03, 2016, from http://humupd.oxfordjournals.org/content/14/6/669.long

4.Cohen, J. (1998). Ooplasmic transfer in mature human oocytes. *Molecular Human Reproduction, 4*(3), 269-280. doi:10.1093/molehr/4.3.269

5.Conceiving the inconceivable. (n.d.). Retrieved August 03, 2016, from https://conceivingtheinconceivable.wordpress.com/tag/three-parent-ivf/ n

The Increase of Lifespan Using siRNA Assisted Chemotherapy Treatment

Shelby Richards
West Valley High School
2016 Oncofertility Science Academy

Objective

To see which method is most effective, the research poster will compare Monochemotherapy and siRNA assisted treatment. Additionally, the data will show the percentages of tumor decline with the given treatment and look at the benefits and side effects of the treatment.

Abstract

Combining chemotherapeutics with siRNA using Nano Carrier Platforms is much more effective than Monochemotherapy. SiRNA (small interfering RNA) attaches to the miRNA (micro RNA) and the Argonaut will slice the miRNA resulting in the piece being recognized as abnormal and destroyed. Scientists have discovered a way to take a Nano Particle core containing a chemotherapeutic drug and cover it with the siRNA endosomal escape mechanism for the drug to exit later. The last stage is to cover the siRNA with a molecular tumor targeting mechanism for the Nano Particle to reach its final destination. The drug will be diagnosed in multiple doses depending on the patient. Half of the drug will be released within the first couple of days and then the rest will be dispersed over a period of 2-4 months. The purpose is to stop the tumor from growing so the clinician can do further therapy for the patient. Dr. Paula Hammond and her team of researchers at MIT (Massachusetts Institute of Technology) have successfully tested the process. The team tested the procedure on a mouse with DOX that had triple negative breast cancer. When injected with dox + mrp1 siRNA the tumor decreased in size by ~20% within a 15-day period. Many scientists are testing different siRNA assists to find the best one with the best dosage. With 160 companies and 65 universities testing ~250 cancer based drugs, RNA therapeutics have grown to 12% CAGR (Compound Annual Growth Rate) and is expected to reach $1.2 billion by 2020. In Conclusion, the results from the studies have shown that siRNA assisted chemotherapy procedures will be a beneficiary towards the fight against aggressive forms of cancer.

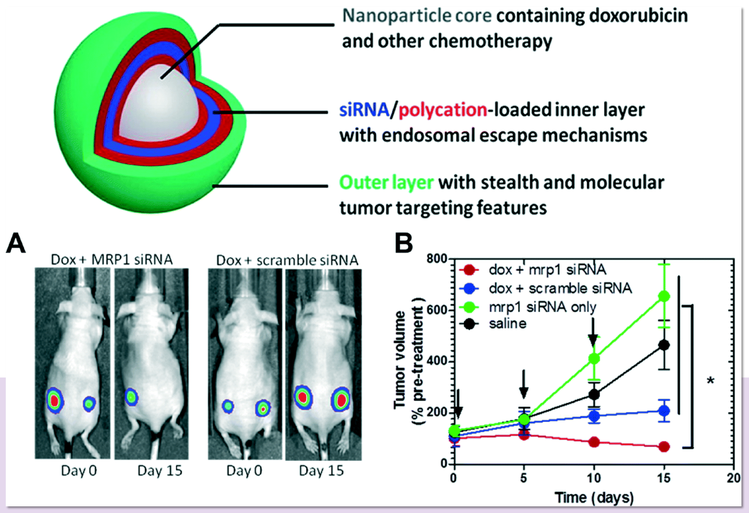

Figure 1. The structure of a Nano Carrier platform and the results of the drug when being tested on mice. The nanoparticle has two additional layers to allows the molecule to be successful. The mice was injected with MRP1+siRNA and scramble siRNA to see which is most successful. Gadde, S (2015)[1]

Methods and Materials

There has been many different procedures and trials being tested to find the best formulas and methods on how to undergo the procedure. Some of the procedures have been done on rats and then on humans. Three rats were injected with dox + mrp1 siRNA and the tumor decreased in size (figure 1) which shows that the siRNA assisted chemotherapy can be successful in humans. Up until 2010, no RNAi trial has moved past stage three trial until siRNA was introduced into the therapy because the injection of chemotherapy is >70% silencing. The siRNA bonds to each of the RISC and unwound the ATP-dependent manner as well as mediating the sequence of mRNA and catalyzes the cleavage and slices the mRNA for the antisense agent to sequence. The first half of the 121 mg/kg dose of the drug is rapidly released for the first 1-2 weeks, then the rest is slowly delivered within a four month period. This procedure is the framework for a lot of scientific testing where the results show in favor of siRNA assists. Twelve patients took 1 mg of siRNA and there was no tumor growth within a 8-12 week span after all the siRNA was released. Another trial was given where the scientists were trying to figure out what dose of medication is most effective. The experiment lasted 26 months with 50 doses total. The first dose was 1 mg but the serum knocked down by 40% with infusion treatment. The dose then exculpated where 32 patients and 17 healthy volunteers were given a dose of .15-.3 mg per kg and there was a 85% serum knockdown. The remaining amount of serum reduced to 60% after four weeks. During the next phase the patients took a .3 mg per kg dose every three weeks for six months. There was a 80% sustained knockdown of serum. Only 15% of the patients had a mild to moderate TTR (transthyretin) but they stabilized and the patients returned to normal. There is another phase that was initiated in 2013 and should be completed in 2017.

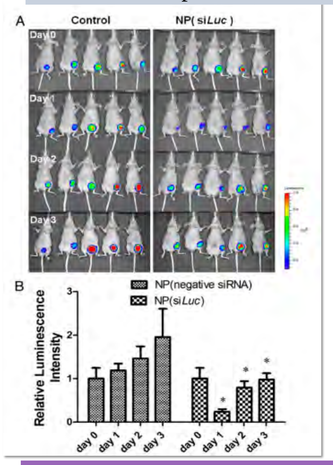

Figure 2. Luminescence tumor response to siRNA and siLUC in comparison to mice without the drug. The mice were injected with serum and the size of the tumor within a three day period. Gousseinov, E. (2015)[2]

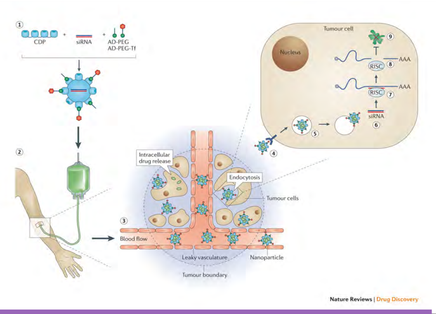

Figure 3. Diagram of the siRNA procedure being used on a human. The serum is injected into the patient and drug will attack the tumor cells. Xu, X (2013)[3]

Results and Interpretations

Based on the diagnosis of the 15 patients in the first trial, each patient was only supposed to live from 8-10 months. As a result of the siRNA induced drug, the patients lived for about half a year longer. When the scientists tried figuring out the correct quantity of medication to give to the patients, each time there was a larger percentage of sustained knockdown of the serum. Sustained knockdown is important because the gathered information shows an indication that the method is working. There are 21 siRNA therapeutics that have been made for a variety of different diseases, ranging from cancer, viruses and genetic disorders.

Conclusion

Combining chemotherapeutics with siRNA carrier platforms have been shown to have some positive correlation with tumor size. Using innovative procedures on a molecular scale allows scientist to understand cells and target the root of the problem. RNA therapeutics are becoming more prominent now that clinical trials are collecting positive information. The function of siRNA and the benefits of the process in science is not only being studied on Cancer Biology but is being tested on all types of diseases and infections such as different forms of STD/I's. If scientists can interact and do more experiments with siRNA and other molecular science then the root (cells) of the problem can be addressed and understood better.

Biotechnology Attributions

Being able to understand the cell is critical to all molecular sciences. For this particular therapeutic, understanding the Central Dogma will allow scientists to understand how to use the process to target specific malfunctions that occur. In this study, the cell become abnormal because an error occurs as the cells divides and the DNA within the cell is damaged. SiRNA treatment targets the root cause of DNA mutations by stopping the production of protein so the cells cannot continue to multiply. Using biotechnology to manipulate the process to fight against cancer is a crucial part of the procedure. Biotechnology is essential to all sections of science and the more advanced the biotechnology becomes, the more scientists are able to discover.

Acknowledgements

I would like to thank Dr. Ericka for constantly supporting everything I do in the program and going above and beyond to teach me, Ms. Winter for always answering every question I have and working with me throughout the entire program., Kathleen Pulvers for always answering every question I ask and helping me a lot with my project, all the presenters and speakers that have taught me more than I could ever think about knowing at this age and my OSA sisters for being wonderful and growing a bond that we will last forever.

References

1. Gadde, S. (n.d.). *Multi-drug delivery nanocarriers for combination therapy*. Retrieved August 18, 2015.
2. Gousseinov, E. (2015, September 15). RNA-Based Therapeutics and Vaccines | Insight & Intelligence" | GEN (M. Kozlov, Ed.). Retrieved September 15, 2015, from www.genegnews.com/insight-and-intelligence/rna-based-therapeutics-and-vaccines/;77900520/
3. Xu, X. (n.d.). (2013, March 1). *Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug*. Retrieved from PNAS.
4. SiRNA delivery systems for cancer treatment ☆. (n.d.). Retrieved August 10,2009,fromhttp://www.sciencedirect.com/science/article/pii/S0169409X09001495
5. A new superweapon in the fight against cancer. (2015, November 21). Retrieved August 07, 2016, from https://www.ted.com/talks/paula_hammond_a_new_superweapon_in_the_fight_against_cancer?language=en

The Effects of Intravenous Chemotherapy on the Prefrontal Cortex in the Development of Major Depressive Disorder in Adolescent Cancer Patients

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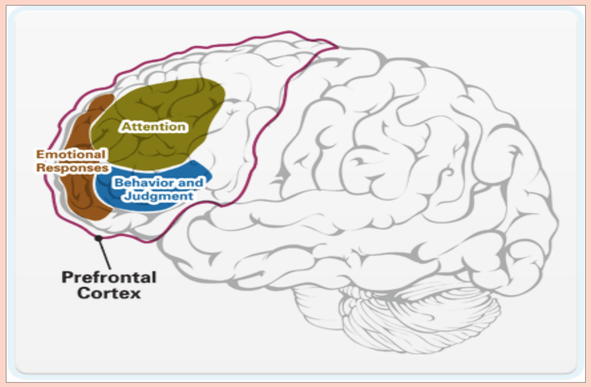
Objective

The objective of this investigation is to determine how decreased brain activity in the prefrontal cortex may lead to adverse psychological changes in adolescent and young adult cancer patients. This poster will demonstrate that decreased activity of the prefrontal cortex causes major depressive disorder (MDD), that is most common in adolescent and young adults due to lack of maturation in that part of the brain. Evidence will show that symptoms recorded in adolescents and young adults undergoing intravenous chemotherapy mirror those of MDD and occur due to disturbances of the prefrontal cortex functionality. MDD, which causes pervasively depressed mood and disinterest in social interaction, more devastatingly effects cancer patients because they are torn away from the normalcy of life and placed in a hospital where they must fight to survive every day, and in addition, battle the overwhelming negativity they feel inside. Forms of therapeutic stimulation of brain activity to encourage positive emotions in preparation for intravenous chemotherapy is a possible solution to hinder the devolvement of MDD in adolescent and young adult cancer patients.

Abstract

Cancer is identified as being among the leading causes of death in adolescents and young adults and chemotherapy has become a routine part of treatment. Intravenous chemotherapy results in many known physical symptoms but also presents risks to mental health, especially in adolescent and young adult patients. The prefrontal cortex is the site of the most prolonged and dramatic brain development until adulthood, and lesions or disturbances to its activity can affect its ability to properly regulate emotional and behavioral expression and can result in psychological disorders. The objective of this investigation is to determine how decreased brain activity in the prefrontal cortex may lead to adverse psychological changes in adolescent and young adult cancer patients. Ten lung cancer patients among 154, ranging from age 20-29, undergoing intravenous chemotherapy were evaluated using the Quality of Life Index-Cancer Version that numerically analyzes different aspects that affect a patient's satisfaction with life. In a separate study, 11 adolescent cancer patients aged 10-17 were measured for symptom distress using the Symptom Disease Scale (SDS) before, during, and after intravenous chemotherapy treatment to determine the impact of the symptoms of chronic illness on emotional distress. In the first study, the average Quality of Life received a score of 23.84+3.24 and the psychological and spiritual subscale score was 24.73+ 4.34. In the second study, SDS was the highest just prior to chemotherapy and 48 hours after chemotherapy treatment. The first chemo treatment mean score was 24.0 out of 25 before treatment and 23.0 after, round two was 18.0 before and 18.0 after, and round three was 19.5 before and 20.0 after. In a third study, 10 adolescent cancer patients, seven undergoing chemotherapy treatment and three in remission, were evaluated and all exhibited symptoms of MDD, some of whom had already received treatment. The data from the first study displays low average QoL scores, and correlated low psychological subscale scores that were calculated based on the experience of sadness, anxiety, and restlessness in patients, all symptoms of MDD. The second study presents that the stress that adolescent patients felt from their symptoms was the highest before and after their intravenous chemotherapy treatments, and that negative psychological changes evaluated in the patient's SDS were indicators of MDD. This proves that intravenous chemotherapy for adolescent and young adult cancer patients can result in the psychological symptoms of major depressive disorder and also suggests, based on brain anatomy and pathology, that these symptoms are attributed to decreased activity of the prefrontal cortex.

Figure 1. An image displaying the prefrontal cortex in relation to the entire anatomy of the brain, located in the frontal lobe. The brain develops from the occipital lobe, or back end of the brain, to the front, making emotional behavior and response underdeveloped in an adolescent. Bio Geo Nerd. (n.d.). Retrieved August 07, 2016, from http://biogeonerd.blogspot.com/2015/03/the-teen-brain.html



Methods and Materials

The Istanbul Institute of Oncology in Turkey studied the adverse neural changes of 154 lung cancer patients undergoing intravenous chemotherapy, 10 of whom were between the ages of 20-29 . The Quality of Life Index- Cancer Version (QLI) was utilized to measure quality of life in terms of satisfaction with various aspects of life according to importance. The scores calculated to assess overall QoL was measured in four subscales: Health and Functioning, Psychological/ Spiritual, Social and Economic, and Family, with a total of scores ranging from 0 to 30. Data was collected over 10 months and analysis was performed using SPSS software. A 14 question survey was administered to patients before treatment to evaluate socio-demographic background and also supplied the cycle and stages of lung cancer. The survey also evaluated ECOG performance, which is criterion used by oncologists to determine the progression of the cancer and how the disease and its treatment affects the living abilities of the patient, ranging from 0 indicating no restrictions to highest (worst) score being 5 .The entirety of the study utilizes descriptive statistics, means, median, frequencies, and percentages to asses personal characteristics and scale scores. In a separate study, at Case Western University in Cleveland Ohio, 11 cancer patients ranging in ages 10-17 were measured for symptom distress in regards to their intravenous chemotherapy treatment. The study utilized the Symptom Distress Scale (SDS) to measure distress caused by symptoms in chronically ill patients. The instrument is a 13 item Likert-type scale that measures a variety of physical and psychological symptoms, one of the most prevalent symptoms tested being anxiety, an indicator of MDD. The scale takes 5-10 minutes to administer with a higher score representing greater symptom distress. The participants of the study were in their first diagnosis of cancer and within the first four months of their initial chemotherapy treatment, and required at least three additional phases of intravenous chemotherapy. They were administered a questionnaire modeling SDS and State-Train Anxiety Inventory for Children (STAIC-1) criterion before each cycle to determine any pre-chemotherapy symptoms. Immediately following intravenous chemotherapy treatment and 48 hours after chemotherapy treatment the SDS instrument scale was used again to determine the impact of the symptoms of a patient's chemotherapy on emotional distress.

The mean scores of "Memorial Symptom Assessment Scale" and "Quality of Life Index" Scales (n = 154).

		Mean	Median	±SD	Minimum	Maximum
Memorial symptom assessment scale	MSAS_Global Distress Index	0.80	0.65	0.60	0.00	2.40
	MSAS_Physical Symptom Subscale	0.77	0.64	0.51	0.00	2.47
	MSAS_Psychological Symptom Subscale	0.76	0.61	0.68	0.00	2.78
	Total MSAS	0.74	0.69	0.45	0.10	2.43
Quality of life index	Health and Functioning Subscale	20.33	21.74	5.59	4.62	29.50
	Social and Economic Subscale	22.64	23.14	4.18	10.14	30.00
	Psychological/Spiritual Subscale	24.73	25.71	4.34	4.29	30.00
	Family Subscale	27.66	28.80	2.77	16.50	30.00
	Quality of Life Index_total scale	23.84	24.46	3.24	13.50	29.41

Figure 2. Study one presents a low mean QoL score of 23.84±3.24 based on the measurements of the Quality of Life Index-Cancer Version. The data also presented a correlated low psychological/spiritual mean subscale score of 24.73±2.77. Akin, S., Can, G, Ayinder, A., Ozidilli, K., & Durna, Z. (2010)[1]

Results

In the first study, as represented by figure two, the quality of life of lung cancer patients (n=154) received a mean score of 23.84± 3.24 with a psychological and spiritual subscale score of 24.73± 4.34.). The data displays low average QoL scores, and correlated low psychological subscale scores that were calculated based on the experience of sadness, anxiety, and restlessness in patients, all symptoms of MDD. Patients with an ECOG score of 2 or higher also suffered from worse psychological subscale scores ($p<0.05$). This indicates that patients who were evaluated as highly debilitated by their intravenous chemotherapy treatment, experienced more adverse psychological changes. The first study shows that intravenous chemotherapy negatively affects the quality of life of cancer patients, specifically in the psychological subscale, where symptoms examined in patients indicated the development of MDD. In the second study, SDS was the highest just prior to chemotherapy and 48 hours after chemotherapy treatment, as represented by figure three. For the first round of intravenous chemotherapy, SDS scored 24.0 before treatment, 21.5 immediately following or post treatment, and 23.0 48 hours after treatment. For the second cycle SDS scored 18.0 before treatment, 16.0 post, and 18.0 48 hours after chemotherapy. For the third and final cycle SDS score was 19.5 pre-intravenous chemotherapy, 19.0 immediately after, and 20.0 48 hours after treatment. The results must account that virtual reality intervention was received by participants in round 2, which significantly dropped average symptom distress levels, however cycle one of intravenous chemotherapy, without virtual technology administered displayed high SDS scores, and cycle three, though lower than one, presented a higher score than round two. The second study presents that the stress that adolescent patients felt from their symptoms was the highest before and after their intravenous chemotherapy treatments, and that negative psychological changes evaluated in the patient's SDS were indicators of MDD.

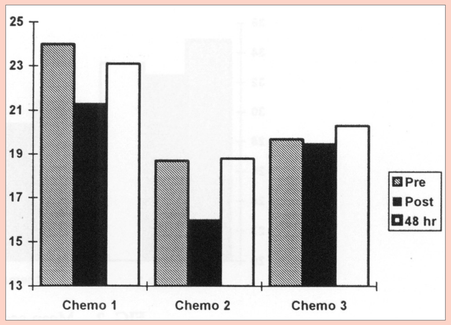


Figure 3. Results from study two present higher SDS scores pre-intravenous chemotherapy and 48 hours following treatment. Though VR was administered to participants in chemo two, levels of distress experienced by patients rebounded 48 hours following in each cycle, and in cycles two and three of chemotherapy, reaching the equivalent or exceeding symptom distress prior to treatment. Schneider, S.M, & Workman, M (1999)[3]

Applications to Biotechnology

Developments in technology have had a major impact on research and diagnostics within the medical community. Statistical Package for the Social Science (SPSS) is a statistical software which manipulates and analyzes highly complex and large portions of data. This advancement in biotechnology has made statistical analysis of studies and clinical trials more efficient and specifically in medicine, it has aided in supporting or disproving correlations between experimental factors and diseases, making diagnostics more accurate. Further progression in biotechnology could lead to possible solutions of MDD in adolescent cancer patients through virtual stimulation of the pre-frontal cortex activity that promotes positive emotion.

Discussion

From analysis of the study results, intravenous chemotherapy for adolescent and young adult cancer patients can result in the psychological symptoms of major depressive disorder and also suggests, based on brain anatomy and pathology, that these symptoms are attributed to decreased activity of the prefrontal cortex. The prefrontal cortex of an adolescent is most vulnerable to damage and lesions due to its delayed stage in brain development and data from the studies suggests that intravenous chemotherapy interferes with neural activity and delays that part of the brain from regulating emotion. MDD has been linked to decreased activity of the prefrontal cortex and data from the results shows high symptom distress scores and low quality of life scores in the psychological subscale, meaning they suffered from adverse psychological changes such as sadness, anxiety and restlessness, all symptoms of MDD. Results from the studies present possible solutions for maintaining normal brain activity of the prefrontal cortex include virtual stimulation, which presented a decrease in symptom distress immediately following chemotherapy treatment in the second study. Further developments of this technique could further lower the score of SDS and lessen the likelihood of MDD symptom appearance in adolescents.

Acknowledgements

I would first like to thank my parents, whose endless love and support have pushed me to maximize my potential and provided me with the platform I need to become successful . I would also like to express my sincerest appreciation to Dr. Ericka Senegar-Mitchell, Patricia Winters, and Kathleen Pulvers for guiding me in my exploration of oncofertility and being inspiring examples of female figures who have impacted the scientific world. Without their enthusiasm, this research would not be possible. Last but certainly not least, I would like to express how grateful I am for my OSA sisters, who I have grown to think of not as classmates, but as great friends. Thank you for being my support unit and making the experience all the more memorable.

References

1. Akin, S., Can, G., Aydiner, A., Ozdilli, K., & Durna, Z. (2010). Quality of Life, Symptom Experience, and Distress of Lung Cancer Patients Undergoing Chemotherapy. European Journal of Oncology Nursing, 400-409. Retrieved July 11, 2016, from PubMed.
2. Pinquart, M., Fröhlich, C., & Silbereisen, R. K. (2007). Optimism, pessimism, and change of psychological well-being in cancer patients. Psychology, Health & Medicine, 12(4), 421-432. doi: 10.1080/13548500601084271
3. Schneider, S. M., & Workman, M. (1999). Effects of Virtual Reality on Symptom Distress in Children Receiving Chemotherapy. CyberPsychology & Behavior, 2(2), 125-134. doi:10.1089/cpb.1999.2.125
4. Thuné-Boyle, I. C., Myers, L. B., & Newman, S. P. (2006). The Role of Illness Beliefs, Treatment Beliefs, and Perceived Severity of Symptoms in Explaining Distress in Cancer Patients During Chemotherapy Treatment. Behavioral Medicine, 32(1), 19-29. doi:10.3200/bmed.32.1.19-29
5. Wu, L., Chin, C., Haase, J. E., & Chen, C. (2009). Coping experiences of adolescents with cancer: A qualitative study. Journal of Advanced Nursing, 65(11), 2358-2366. doi:10.1111/j.1365-2648.2009.05097.x

The Correlation Between Use of Hormonal Contraception and the Impairment of the Ovarian Reserve

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Background

Hormonal contraception (HC)works by increasing the levels of estrogen and progesterone through artificial products, estradiol and progestin as commonly used in most contraceptives. The use of hormonal contraception thereby disrupts the natural biochemistry of the woman's body and feedback cycle of hormone synthesis and essentially tricks the body into believing it's pregnant. Once pregnant, levels of estrogen and progesterone remain high in the normal ovulating woman. The hormones FSH (follicle stimulating hormone) and LH (luteinizing hormone) are prevented from being released by the pituitary which prevents ovulation and therefore pregnancy. On a separate note, the ovarian reserve is evaluated based on two distinct characters: levels of AMH (Anti-Müllerian Hormone) and AFC (Antral Follicle Count). AMH is produced by the granulosa cells mostly during the early antral follicle stage. Therefore it can be used to estimate the volume of early follicles that cannot be detected on ultrasound. The AFC is typically determined using an ultrasound. These two factors together along with sometimes using the size of the ovaries themselves based on ultrasound determine the ovarian reserve and reproductive lifespan.

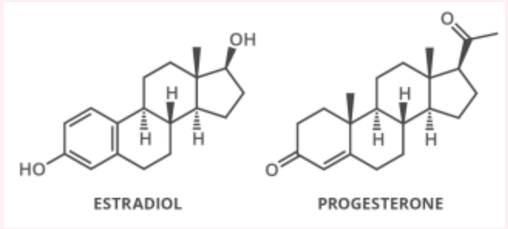


Figure 1. The chemistry of the hormones Estradiol and Progesterone, which both play key roles in the female reproductive system.
The Chemistry of Oral Contraceptives. (2015). http://www.compoundchem.com/2015/02/03/oral-contraceptives/

Abstract

Historically, birth control pills have opened up unprecedented opportunities for women on a global scale. However, in recent studies, data being collected can potentially link oral contraception to decreased ovarian reserves in users. Participants in three studies were placed in categories based on either usage of hormonal contraception or no usage of hormonal contraception. The participants underwent transvaginal ultrasound, blood testing, and were evaluated on full medical history to determine data and demographic for each patient. Studies showed when comparing the ovarian reserve based on AMH levels AFC, and the ovarian volume, women with prolonged uses compared to non-users had significantly decreased counts for each (50% lower volume, 19% lower AMH, and 18% lower AFC). Similar studies found the ovarian reserve to be affected in women taking oral hormonal contraceptives, but when compared to those same women's measurements after cessation, levels were more typical. The studies showed synthetic compounds, estradiol and progestin, components in oral contraceptives could effect the ovarian reserve due to the role these hormones have in inhibiting the pituitary gland from releasing LH and FSH which are essential to the development of oocytes. Limited research is available for the long term use of oral contraception and this limits the ability of researchers to draw conclusions as to the reversibility of the effects. The importance of quantification is to avoid the medical need for future. A 2011 study by the CDC shows approximately half of all pregnancies in the U.S. were unplanned, meaning there is an immense need, however there is always room for the improvement of such drugs.

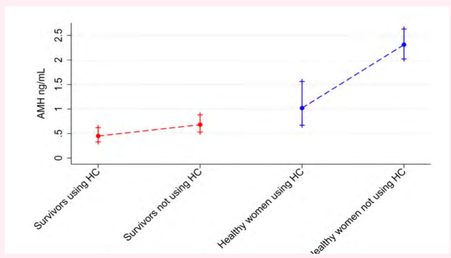
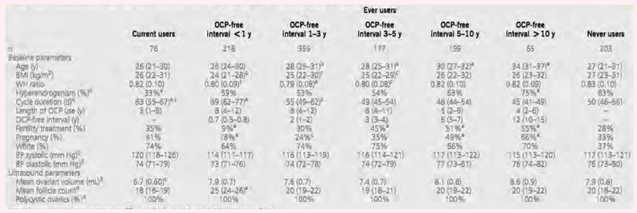


Figure 2. (Left) Graph showing the levels of AMH based on the two populations: cancer survivors and normal ovulatory women, both for those using HC and those without exposure. Johnson LN, e. (2016)[4]

Figure 3. (Right) Table showing the levels of hormones associated with the reproductive system for women with and without HC.
Deb S, e. (2016)[2]

Methods and Materials

In a case controlled study by the University of Nottingham, 34 healthy women who had been taking combined oral contraceptive pills (COCP) within the last year and 36 normo—ovulatory, age matched women who had not taken hormonal birth control within the last year underwent several areas of testing, including a series of 3D ultrasounds to evaluate antral follicle counts. These tests were done in the first few days of the early follicular phase (days 2-5 of menstrual cycle).
In a similar study by the VU University Medical Center, 25 women between the ages of 18 and 40 and had been taking hormonal contraception regimens for a least 3 months volunteered for vaginal ultrasounds to analyze both ovary size and number of antral follicles. Testing for these participants was done in the hormone free interval and measured for comparison during the two subsequent natural menstrual cycles.
In all cases, participants also submitted to blood testing to monitor levels of the AMH, FSH, LH, and Estradiol.
Furthermore, a study by the National Institute of Health compared 126 cancer survivors with at least one functional ovary and a uterus and one year of cancer therapy with a control group of 123 similarly aged women with normal cycles. Data was collected over a course of approximately 2 years and the control group was frequently exposed to several forms of hormonal contraception, including the pill.



	Current users	Ever users: OCP-free interval <1 y	OCP-free interval 1–3 y	OCP-free interval 3–5 y	OCP-free interval 5–10 y	OCP-free interval >10 y	Never users
n	76	218	359	197	199	65	203
Baseline parameters							
Age (y)	26 (21–30)	26 (24–30)	28 (25–31)[a]	28 (25–31)[a]	30 (27–32)[a]	34 (31–37)[a]	27 (21–31)
BMI (kg/m²)	26 (22–31)	24 (21–28)[a]	25 (22–30)[a]	25 (22–29)[a]	25 (22–32)	25 (23–32)	27 (23–31)
WH ratio	0.82 (0.10)	0.80 (0.09)[a]	0.79 (0.08)[a]	0.80 (0.08)[a]	0.82 (0.10)	0.82 (0.09)	0.83 (0.10)
Hyperandrogenism (%)[b]	33%[a]	55%	53%	56%	69%	75%[a]	63%
Cycle duration (d)[c]	63 (55–67)[a,c]	59 (52–77)[a]	55 (49–62)[a]	49 (45–54)	48 (44–54)	45 (41–48)	50 (46–56)
Length of OCP use (y)	3 (1–8)	8 (4–12)	8 (4–12)	8 (4–11)	5 (2–9)	4 (2–6)	–
OCP-free interval (y)	–	0.7 (0.5–0.8)	2 (1–2)	3 (3–4)	6 (5–7)	12 (10–15)	–
Fertility treatment (%)	35%	9%[a]	30%	45%[a]	51%[a]	53%[a]	28%
Pregnancy (%)	61%	18%[a]	24%[a]	35%	49%[a]	66%[a]	33%
White (%)	74%	64%	74%	75%	56%	70%	37%
BP systolic (mm Hg)[d]	120 (118–126)	114 (111–117)	116 (113–119)	116 (114–121)	117 (113–122)	115 (113–120)	117 (113–121)
BP diastolic (mm Hg)[d]	74 (71–79)	73 (71–76)	74 (72–78)	74 (72–79)	77 (73–81)	78 (74–82)	76 (73–80)
Ultrasound parameters							
Mean ovarian volume (mL)[e]	6.7 (0.60)[a]	7.9 (0.7)	7.6 (0.7)	7.4 (0.7)	8.1 (0.8)	8.6 (0.9)	7.9 (0.6)
Mean follicle count[e]	18 (16–19)[a]	25 (24–26)[a]	20 (19–22)	19 (18–21)	20 (19–22)	20 (19–22)	20 (18–22)
Polycystic ovaries (%)[e]	100%	100%	100%	100%	100%	100%	100%

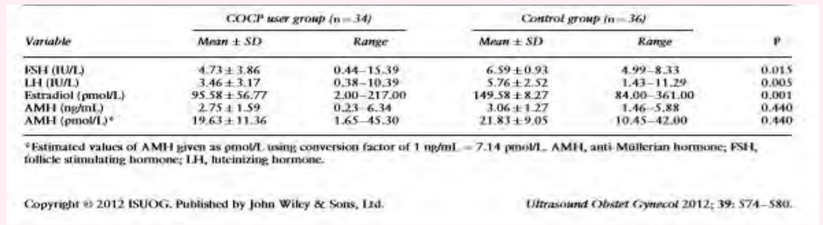
Figure 4: Data collected showing the demographics for each population. Mes-Krowinkel MG, e. (2016)[5]

	Antral Follicle Count		Unadjusted RR[δ]
	HC users	HC non-users	All subjects
Cancer Survivors	12.5 (10.1-15.5)	16.3 (13.7-19.4)	0.73 (0.61-0.87) <0.001
Healthy Controls	22.4 (15.1-33.2)	27.1 (24.8-29.6)	

Figure 5. Table showing the two populations, the cancer survivors and the normal ovulatory women when using HC and after cessation. Johnson LN, e. (2014)[4]

Results

The study by the National Institute of Health found AMH levels were 55% lower than the normal for the control group HC compared to the 22% for the cancer survivors. The AFC for the control was 20% lower as was the AFC for survivors. Based on the individual, the control group during their use of HC had a 17-35% decreased AMH level and a 11% lower AFC.
The University of Nottingham found that there was a significant difference in the size of ovaries (P<0.001) and also significantly decreased amounts of follicles greater than 6mm (P<0.001). There was no significant change in the levels of AMH according to this particular study.
VU University Medical Center produced the following results: significant increases in AMH (P<0.001) and AFC values (P=0.004).



Variable	COCP user group (n = 34) Mean ± SD	COCP user group (n = 34) Range	Control group (n = 36) Mean ± SD	Control group (n = 36) Range	P
FSH (IU/L)	4.73 ± 3.86	0.44–15.39	6.59 ± 0.93	4.99–8.33	0.015
LH (IU/L)	3.46 ± 3.17	0.38–10.39	5.76 ± 2.52	1.43–11.29	0.005
Estradiol (pmol/L)	95.58 ± 56.77	2.00–217.00	149.58 ± 8.27	84.00–361.00	0.001
AMH (ng/mL)	2.75 ± 1.59	0.23–6.34	3.06 ± 1.27	1.46–5.88	0.440
AMH (pmol/L)[a]	19.63 ± 11.36	1.65–45.30	21.83 ± 9.05	10.45–42.00	0.440

[a] Estimated values of AMH given as pmol/L using conversion factor of 1 ng/mL = 7.14 pmol/L. AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone.

Ultrasound Obstet Gynecol 2012; 39: 574–580.

Discussion

Based on the data it is reasonable to assume there is a connection between the use of hormonal contraception and an impairment in the ovarian reserve. In each study, there was a significant difference in levels of AMH, FH, LH, and quantity of antral follicles. Based on the three studies, it can be concluded that while using HC, the follicle size and count is decreased compared to woman at that same moment in their cycle. Because the pituitary is inhibited by the levels of estrogen and progesterone in the body. While this change appears to be reversible, the availability of data and research available for long term use of hormonal contraception is limited. The purpose of this poster is to avoid the possibility of an unmet medical need that may result due to prolonged use of synthetic hormones. In addition there is a limited faction of the population that truly understands how hormonal contraception works and that in itself is a problem.

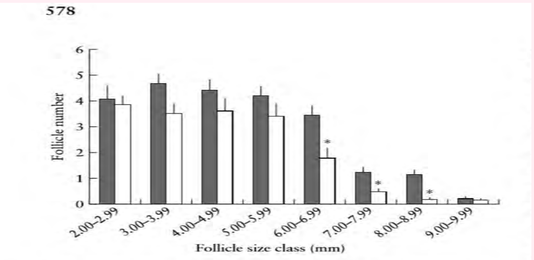


Figure 6. Graph depicting follicle size based on the two populations being studied, women using HC (white) and those without exposure (grey). Johnson LN, e. (2014)[4]

Connections to Biotechnology:

HC is a great example of the innovation and advancements to science that biotech allows for. The ability to accurately mimic natural hormones by isolating similar ones from plants. The future possibilities for advancements in the field of biotechnology for contraceptives is vast. There are so many different paths to travel down from the mechanical device currently being developed for men which consists of a temporary vasectomy, to more effective long term hormonal birth control that could potentially increase the reproductive lifespan for women.

References

1. Bentzen JG, e. (2016). *Ovarian reserve parameters: a comparison between users and non-users of hormonal contraception. - PubMed - NCBI. Ncbi.nlm.nih.gov*.
2. Deb S, e. (2012). *Quantifying effect of combined oral contraceptive pill on functional ovarian reserve as measured by serum anti-Müllerian hormone and small antral f... - PubMed - NCBI.Ncbi.nlm.nih.gov*.
3. Freour T, e. (2016). *Ovarian reserve and in vitro fertilization cycles outcome according to women smoking status and stimulation regimen. - PubMed - NCBI. Ncbi.nlm.nih.gov*.
4. Johnson LN, e. (2014). *Antimüllerian hormone and antral follicle count are lower in female cancer survivors and healthy women taking hormonal contraception. - PubMed - NCBI. Ncbi.nlm.nih.gov*.
5. Mes-Krowinkel MG, e. (2014). *Influence of oral contraceptives on anthropomorphometric, endocrine, and metabolic profiles of anovulatory polycystic ovary syndrome patients. - PubMed - NCBI.Ncbi.nlm.nih.gov*.
6. Van den Berg MH, e. (2010). *Comparison of ovarian function markers in users of hormonal contraceptives during the hormone-free interval and subsequent natural early follicular... - PubMed - NCBI. Ncbi.nlm.nih.gov*.
7. *You Can Now Turn off Your Sperm Flow with the Flip of a Switch*. (2016). *Discovery*. Retrieved 27 July 2016, from http://www.discovery.com/dscovrd/tech/you-can-now-turn-off-your-sperm-flow-with-the-flip-of-a-switch/

Acknowledgements:

Special thank you to Dr. Chang for being an ongoing inspiration to my scientific career, Dr. Ericka for encouraging my perseverance and showing me how to not only be a scientist but also how to fail and keep going. Thank you also to Ms. Winter for her passion and ability to organize effectively.

The Effect of Luteal Phase Support with Vaginal Progesterone in Patients with Unexplained Fertility Undergoing Intrauterine Insemination Treatment

Bethlehem Wolkeba

Eastlake High School

Objective

Progesterone plays a vital role in preparing the endometrial lining for pregnancy by stimulating a rapid increase in hormones in response to human chronic gonadotropin (hcG) that is produced by the corpus luteum in the luteal phase of the menstrual cycle. Embryonic implantation occurs in the luteal phase and is dependent on the efficacy of the corpus luteum. In Luteal Phase Defect, progesterone is produced in low amounts, or the endometrium is not responding the progesterone being produced. Therefore, ART, such as IUI, supply women with progesterone in order to improve implantation of the egg and pregnancy rates. The objective of this poster is in order to determine the impact of luteal phase support with vaginal progesterone on pregnancy rates in ovarian stimulation and intrauterine insemination (IUI) in couples with unexplained infertility.

Abstract

Progesterone plays a vital role in preparing the endometrial lining for pregnancy by stimulating a rapid increase in hormones in response to human chronic gonadotropin (hcG) that is produced by the corpus luteum in the luteal phase of the menstrual cycle. In Luteal Phase Defect, progesterone is produced in low amounts, or the endometrium is not responding the progesterone being produced. Therefore, ART, such as IUI, supply women with progesterone in order to improve implantation of the egg and pregnancy rates. The objective of this poster is in order to determine the impact of luteal phase support with vaginal progesterone on pregnancy rates in ovarian stimulation and intrauterine insemination (IUI) in couples with unexplained infertility. A prospective randomized trial was done that included 214couples with unexplained infertility that were treated with ovarian stimulation and IUI using recombinant FSH. Semen samples were taken by masturbation and after the procedure, sperm concentration, sperm motility, and total motile sperm number were evaluated and the semen was stored in an incubator at 37°C until the time of insemination. The IUI was performed 36 hours after hcG administration. In the experimental group, luteal phase was supported with vaginal progesterone gel once a day beginning 2 days after insemination until pregnancy testing, and then continuing onto the 12th week of pregnancy if the patient conceived. Patients in the control group did not receive any luteal phase support. Pregnancy tests were performed, and the primary outcome measures were clinical and live births; there were no patients with a poor response to ovarian stimulation with gonadotropin stimulation. Fifty-six pregnancies were observed in the experimental group and28 in the control group. Clinical pregnancy and live birth rate per cycle were significantly higher in the experimental group (21.1%and 17.4%, respectively) compared with that of the control group (12.7% and 9.3%). The application of luteal phase support with vaginal P was clearly associated with significantly higher clinical as well as live birth rates compared with patients without luteal phase support. In conclusion, outcomes would suggest that luteal phase P support can significantly improve the likelihood of clinical pregnancy and live birth in IUI cycles where ovulation induction was achieved with gonadotropins. The study also indicated that luteal support is should be encouraged in ovulation induction cycles with gonadotropins where a multifollicular response has been achieved.

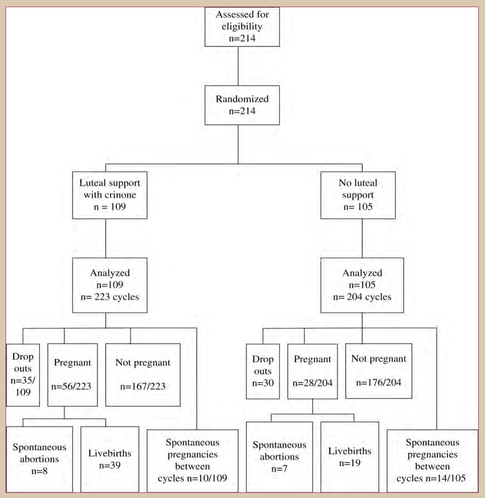


Figure 1. Flow chart through trial of luteal phase progesterone support versus no support for ovarian stimulation in intrauterine insemination cycles. Eredem (2009)[2]

Methods and Materials

A prospective randomized trial was done at the Department of Obstetrics and Gynecology, Gazi University School of Medicine, Ankara, Turkey that included 214 couples with unexplained infertility that were treated with ovarian stimulation and IUI using recombinant FSH from November 2004 to 2006. The duration of infertility was at least 1 year for each subject who had regular menstrual cycles with midluteal P levels of >10 ng/mL, bilateral tubal patency confirmed with a hysterosalpingography, and normal semen analysis. Once a patient was randomized in the first cycle, she remained in the same treatment group during the entire study; a placebo was not used. Patients in the study group received vaginal P gel (Crinone 8% vaginal gel) for luteal support beginning 2 days after IUI. Semen samples were taken by masturbation and after the procedure, sperm concentration, sperm motility, and total motile sperm number were evaluated and the semen was stored in an incubator at 37°C until the time of insemination. The IUI was performed 36 hours after hcG administration in the experimental group, luteal phase was supported with vaginal progesterone gel once a day beginning 2 days after insemination until pregnancy testing, and then continuing onto the 12th week of pregnancy if the patient conceived. Patients in the control group did not receive any luteal phase support. All patients underwent baseline transvaginal ultrasonography (TVU) on day 3 of the menstrual cycle and were then treated with a starting dose of 75 IU recombinant FSH. Cycles were triggered with 10,000 IU hCG when at least one dominant follicle had reached 18 mm in diameter. Pregnancy testing was performed by determining the quantitative serum hCG level at 14 days after hCG administration, and intrauterine pregnancy was confirmed by using TVU 2 weeks after a positive pregnancy test. A clinical pregnancy was defined as the presence of a gestational sac on TVU or by histologic examination of products of conception in patients who were aborted. Live birth was defined as having a child who was living at 1 week after birth.

Figure 2 - Cycle characteristics of patients undergoing treatment with (study group) or without (control group) vaginal progesterone gel. Eredem(2009)[2]

	Study group	Control group	
Duration of therapy (days)	8.7 ± 2.4	9.1 ± 3.1	NS
Total amount of gonadotropins (IU)	985.2 ± 511.3	937.9 ± 417.6	NS
No. of follicles 9-16 mm	2.9 ± 2.1	2.8 ± 2.1	NS
No. of dominant follicles (>16 mm.)	1.6 ± 0.6	1.5 ± 0.9	NS
Endometrial thickness on the day of hCG	10.9 ± 1.9	10.9 ± 2.0	NS
Total progressive motile sperm number after sperm preparation ($\times 10^6$/mL)	37.2 ± 45.6	48.8 ± 58.0	NS
Type of gonadotropin			NS
rec alpha	116	107	
rec beta	107	97	
Total pregnancy rate per cycle (%)	56/223 (25.1)	28/204 (13.7)	*P*=.002
Clinical pregnancy rate per cycle (%)	47/223 (21.1)	26/204 (12.7)	*P*=.028
Live birth rate per cycle (%)	39/223 (17.4)	19/204 (9.3)	*P*=.016
Clinical pregnancy rate per patient (%)	43/109 (39.4%)	25/105 (23.8%)	*P*=.01
Live birth rate per patient (%)	39/109 (35.8%)	19/105 (18.1%)	*P*=.003
Multiple pregnancy rate per cycle	3/223 (1.34%)	4/204 (1.96%)	NS

Results

Pregnancy tests were performed, and the primary outcome measures were clinical and live births. Eighty-six patients had only one cycle; 46 patients had two cycles, and 82 patients had 3 cycles. During the study period, 65 patients (29.8%) dropped out for various reasons but 30 were in the study group and 35 were in the control group, therefore, the difference was not significant. there were no patients with a poor response to ovarian stimulation with gonadotropin stimulation. Of 427 cycles, a total of 84 pregnancies occurred; 58 pregnancies ended with delivery, others ended with biochemical pregnancy (11 patients) and clinical abortion (15 patients). Of the 84 pregnancies, 36 were after the first cycle, 19 after the second, and 29 after the third cycle. Fifty-six pregnancies were observed in the experimental group and 28 in the control group. Clinical pregnancy and live birth rate per cycle were significantly higher in the experimental group (21.1%and 17.4%, respectively) compared with that of the control group (12.7% and 9.3%). Clinical pregnancies and live birth rates were observed per patient and it was seen that these rates were significantly higher in the study group (39.4% and 35.8%, respectively) compared with the control group (23.8% and 18.1%, respectively). When all live births, including patients who conceived spontaneously between cycles and had live births, were compared, the live birth per patient rate were also in the study group than in control group (44.9% vs 31.4%; P<.05). Analysis of the first cycle of the procedure also revealed that clinical pregnancy and live birth rates were higher in the study group compared with the control group (22.9% and 21.1% vs. 9.5% and 6.6%, respectively; P<.001). The results suggested that the application of luteal phase support with vaginal P is clearly associated with significantly higher clinical as well as live birth rates compared with patients without luteal phase support.

Conclusions

In conclusion, outcomes would suggest that luteal phase P support can significantly improve the likelihood of clinical pregnancy and live birth in IUI cycles where ovulation induction was achieved with gonadotropins. The study also indicated that luteal supports should be encouraged in ovulation induction cycles with gonadotropins where a multifollicular response has been achieved. Hormonal changes after ovarian stimulation for multifollicular response in ovarian stimulation and IUI cycles might reveal abnormal endometrial changes. It has long been known that in cycles with multifollicular growth, an advanced endometrium in the early luteal phase was observed in approximately 50% of women. These observations suggest that multifollicular response in ovarian stimulation and IUI cycles seems similar to superovulation in IVF cycles, and that high steroid environment in the luteal phase is responsible for the luteal insufficiency. It is suggested that luteal support is mandatory in ovulation induction cycles with gonadotropins where a multifollicular response has been achieved.

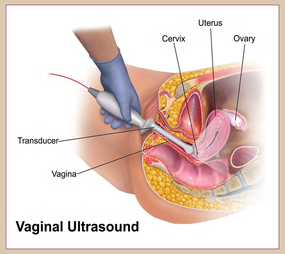


Figure 3 – A cartoon demonstration of a vaginal ultrasound Vaginal ultrasonography. (n.d.). Retrieved August 08, 2016, from https://en.wikipedia.org/wiki/Vaginal_ultrasonography

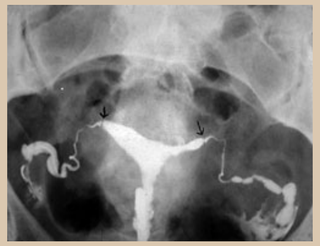


Figure 4- An X-ray of a uterine cavity (arrows pointing to the two fallopian tubes) using the hystersalpingogram Treatments and investigations. (n.d.). Retrieved August 08, 2016, from http://www.infertility-guidance.co.uk/fertility_treatments/hysterosalpingography.html

Relevant Applications to Biotechnology

A couple of significant technologies used in the commencement of this trial was a vaginal ultrasound and a hystersalpingogram. Both the vaginal ultrasound and the hystersalpingogram were important in detecting any endocrine abnormalities and confirming the bilateral tubal patency in the women before undergoing the trials (Figures 3 and 4 from left to right). This was significant in eliminating any potential errors that could make the test subjects different from one another altering the data collected. Benefits that emerge from these technologies is that the ultrasound is non-invasive and safe to use where as the hytsersalpingogram is commonly used and is known for its efficacy in detection of abnormalities within the fallopian tubes. These advancements in medical technology are essential in improving women fertility because they have the potential to work in ways unimaginable.

Acknowledgements

I am very grateful to everyone who supported me throughput this academy. I give my greatest acknowledgments to Dr. Ericka Senegar-Mitchell and the UCSD facility for putting in so much time into helping us OSA sisters achieve success in all our work. I would also like to specially thank Ms. Patricia Winter for coordinating this program for us and giving us the opportunity to become sisters in science. I would like to also thank all the doctors who gave us seminars to help solidify our knowledge of Oncofertilty; especially Dr. Jeffrey Chang and Dr. Irene Su who inspired me on this topic. Special thanks to my family for supporting me through this rigorous academy and their constant words of encouragement. Finally, thank you to my OSA sisters! I couldn't have enjoyed this experience as I did without every single one of them.

References

1. Daya, S., & Gunby, J. (2004). Luteal phase support in assisted reproduction cycles. *Protocols Cochrane Database of Systematic Reviews. doi:10.1002/14651858.cd004830*
2. Erdem, A., Erdem, M., Atmaca, S., & Guler, I. (2009). Impact of luteal phase support on pregnancy rates in intrauterine insemination cycles: A prospective randomized study. *Fertility and Sterility,91(6), 2508-2513. doi:10.1016/j.fertnstert.2008.04.029*
3. Hill, M., Whitcomb, B., Lewis, T., Levens, E., Decherney, A., & Propst, A. (2013). Progesterone luteal support following ovulation induction and intrauterine insemination cycles: A systematic review and meta-analysis. *Fertility and Sterility,100(3). doi:10.1016/j.fertnstert.2013.07.1028*
4. Maher, M. A. (2011). Luteal phase support may improve pregnancy outcomes during intrauterine insemination cycles. *European Journal of Obstetrics & Gynecology and Reproductive Biology,157(1), 57-62. doi:10.1016/j.ejogrb.2011.03.022*
5. Nieto, M. I., González, J. L., Arjona-Berral, J. E., Muñoz-Villanueva, M. D., & Castelo-Branco, C. (2014). Luteal phase support with progesterone in intrauterine insemination: A prospective randomized study. *Gynecological Endocrinology,30(3), 197-201. doi:10.3109/09513590.2013.859242*

The Effects of Anti-CD19 CAR-T Cell Therapy on Relapsed and Refractory B-cell Acute Lymphoblastic Leukemia (B-ALL)

Connie Yu
Pacific Ridge School

Background

This poster intends to investigate an emerging immunotherapy, chimeric antigen receptor (CAR) T-cell therapy, as a therapeutic treatment for relapsed and refractory B-cell acute lymphoblastic leukemia (r/r B-ALL). It focuses on two of a growing number of clinical trials evaluating the efficacy of CAR-T cell therapy to induce durable remission with minimal toxicity. With current salvage therapies unable to rescue most patients, novel modalities are greatly needed.

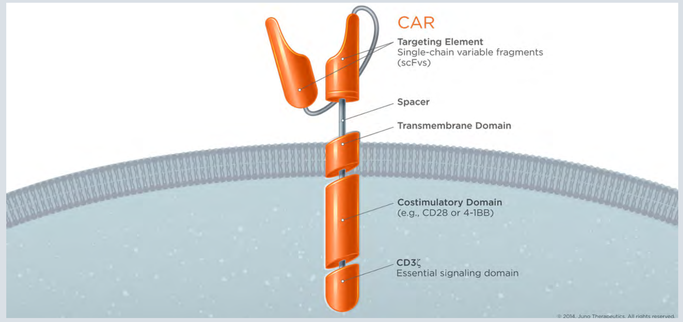


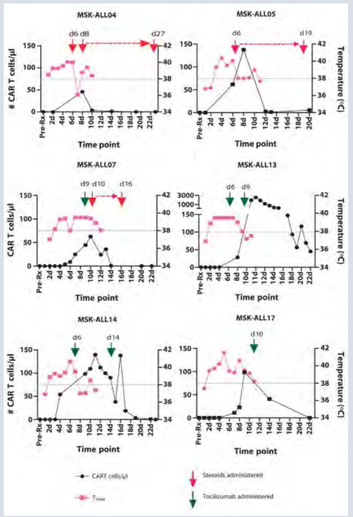
Figure 1 CAR design CAR technology [Digital image]. (n.d.). Retrieved from https://www.junotherapeutics.com/our-science/car-technology/

Abstract

B-ALL is an aggressive disease as the leading cause of cancer-induced pediatric death with a dismal prognosis in adults. It is distinguished by uncontrolled growth and accumulation of immature B-lineage lymphoblasts in the bone marrow (BM). The leukemic cells prove rapidly fatal, invading the body and impeding production of healthy blood cells. Decades-worth of advances in upfront therapy have escalated initial complete remission (CR) up to 90% in children and 80% in adults and overall survival (OS) rates around 80% and <40%, respectively.[3,5] However, contemporary first-line treatment is confined to prolonged intensive chemotherapy regimens that bring significant short and long-term toxicities. Moreover, patients are highly susceptible to relapse (20% of children and >50% of adults), which sees a cycle of greater resistance (refractory) or recurrence (relapse) and fewer options: incidence of relapse drags OS rates to <30% and <10%, respectively, with median OS <6 months.[4,5] Although allogeneic stem cell transplant (allo-SCT) is presently viewed as the greatest prospect for long-term survival with r/r B-ALL, only 5% of patients transition to allo-SCT with many ineligible for the risky procedure.[1] Recently, scientists have approached the challenge of r/r B-ALL through harnessing the body's inherent power. By reprogramming T-cells, lymphocytes that secrete cytokines to direct, regulate, and attack cancerous cells in immune responses, to overcome tolerance through antigen-specific activation, scientists have conducted clinical research to study CAR-T cell therapy. Phase 1 trials from Children's Hospital of Philadelphia and University of Pennsylvania (CHOP/Penn) and Memorial Sloan Kettering Cancer Center (MSKCC) have resulted in 90% and 88% CR, respectively, and strong evidence of sustainment.[1,2] Responses encourage continued follow-up to comprehensively explore long-term efficacy and toxicity, while further research is needed to better understand CAR-T cell therapy.

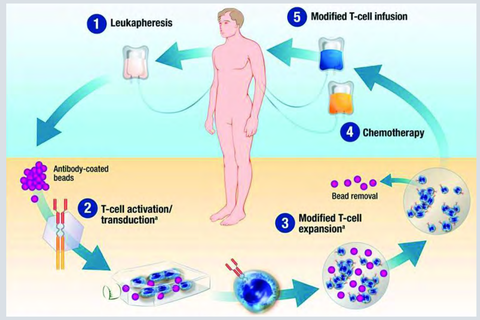
Figure 2 (below) Clinical outcomes Davila et al., 2014; Maude et al., 2014[1,2] **Figure 3 (right) Effect of steroids and tocilizumab on in vivo expansion of CAR-T cells in patients with sCRS** Steroids suppressed proliferation while tocilizumab did not. Rapid reversal of CRS was observed with both. Davila et al., 2014[1]

	CHOP/Penn (N=30)	MSKCC (N=16)
NR (%)	3 (10)	2 (12)
CR (%)	27 (90)	14 (88)
MRD+	2 (7)	2 (13)
CRm	23 (77)	12 (75)
N/A	2 (7)	
Post-therapy (%)		
None	25 (83)	9 (56)
allo-SCT	3 (10)	7 (44)
Other	2 (7)	
CRS (%)		
Not sCRS	3 (10)	9 (56)
sCRS	8 (27)	7 (44)



Methods and Materials

The cohorts comprised patients of different age groups and genders with varying treatment history and status. Baseline characteristics were unfavorable even in the context of an already poor prognostic population (Figure 6). Patients began treatment with leukapheresis followed by salvage therapy at their physician's discretion. Meanwhile, autogenous T-cells were genetically engineered via lentiviral (CHOP/Penn) or retroviral (MSKCC) transduction to express CARs that target the CD19 antigen of B-ALL through an anti-CD19 single chain variable fragment (scFv), enhance T-cell function through a costimulatory domain (CD137 CHOP/Penn; CD28 MSKCC), and activate a cytotoxic response through a CD3-zeta signaling domain (Figure 1). Patients then underwent individualized lymphodepleting chemotherapy (main agent, cyclophosphamide) and within a week were subsequently infused over 1-3 days with their respective CTL019 cells (CHOP/Penn) or 19-28z CAR-T cells (MSKCC). T-cells were administered at 1-10x10^7 CTL019 cells/kg (5-50x10^8 CTL019 cells for patients ≥ 50 kg) and 1-3x10^6 19-28z CAR-T cells/kg. Patients were monitored by follow-ups spanning up to 2 years to assess disease response alongside persistence of the infused cells through BM and blood assays: flow cytometry, quantitative polymerase chain reaction (qPCR), deep sequencing, and cytokine quantification (first month). Responses were classified as no response (NR), CR (<5% blasts in BM), molecular residual disease (MRD+; CR with residual disease detected by assay), or CRm (CR with MRD-).



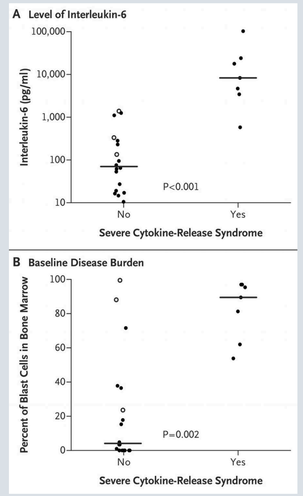


Figure 4 (above) CAR-T cell production Maude, S. L., Shpall, E. J., & Grupp, S. A. (2014). Chimeric antigen receptor T-cell therapy for ALL. *Hematology, 2014*(1), 559-564. doi:10.1182/asheducation-2014.1.559 **Figure 5 (right) Correlates of sCRS** (Panel A) Peak levels of IL-6 in patients with versus without sCRS (Panel B) Severity of CRS according to baseline disease burden after salvage chemotherapy and before infusion (●=CR, ○=NR, ——=median) Maude et al., 2014[2]

Results

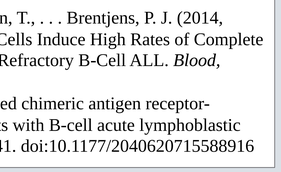
Outcomes demonstrated therapeutic potential for r/r B-ALL treatment with anti-CD19 CAR-T cell therapy. In the CHOP/Penn cohort, 27 of 30 patients achieved CR. 19 remained in remission, with 15 receiving no further treatment and 4 withdrawing to receive other therapy: 3 transitioned to allo-SCT and have since sustained remission while 1 with MRD+ underwent additional therapy, achieving and sustaining CRm. Median follow-up was 7 months (range 1-24). In the MSKCC cohort, 14 of 16 patients achieved CR. 7 transitioned to allo-SCT, with no incidence of relapse. In an updated report almost a year later, 8 additional patients have been treated. In 22 patients evaluable for response, 20 (91%) have achieved CR, 18 (82%) with MRD-. With median follow-up of 7.4 months (range 1-34), 6 patients have sustained CRm beyond a year (range 12.6-34), and the median OS is 9 months. In vivo activity in both trials saw high-levels of expansion but differed in persistence. Median duration of 19-28z CAR-T cells was 30 days (range 0-120) whereas CTL019 probability of persistence at 6 months was 68% with duration so far lasting up to 2 years. Scientists noted a correlation amongst CAR-T cell persistence, B-cell aplasia (for which IgG replacement was administered to maintain levels >500 mg per dL), and sustained remission (in the context of no post-therapy treatment). Sustained remission was also seen with subsequent allo-SCT. A substantial byproduct of "large-scale, synchronous T-cell activation"[1] and concomitantly elevated cytokine levels (Figure 5) was a systemic inflammatory response, cytokine-release syndrome (CRS), symptomized by fever, myalgia, hypotension, respiratory insufficiency, encephalopathy, and/or cytopenia. CRS was treated with either steroid therapy (glucocorticoid-based) or tocilizumab, an interleukin-6 (IL-6) receptor antagonist, and toxicities were fully reversible within 1-3 days. Correlation was drawn between initial disease burden and severity of CRS (Figure 5). For the 7 (CHOP/Penn) and 3 (MSKCC) patients who relapsed, recurrence was tied to lack of CAR-T cell persistence (early loss or steroid interference; Figure 3) or CD19- relapse. Although data is limited to the extent of Phase 1 cohorts and follow-up, progressive clinical phases will help to expand upon these preliminary findings.[1,2,4]

Discussion

Despite improved survival for de novo B-ALL, r/r B-ALL remains an immense challenge to induce and sustain remission in: salvage chemotherapy drugs deliver <25% CR and short persistence.[2] However, both trials highlight potency and durability of CAR-T cells: clinical outcomes are remarkable for such a fatal disease compounded by high-risk populations. Mature follow-up and investigation is necessary to identify factors to maximize efficacy and minimize toxicities: optimal CAR construct and manufacture, infusion dosage, preconditioning therapy, CRS prevention and management, CD19- relapse mechanisms, and post-treatment action. Greater insight would allow scientists to not only provide more innovative CAR-T cell therapy for r/r B-ALL patients, but also apply this technology to patients with other malignancies as well.

	CHOP/Penn (N=30)	MSKCC (N=16)
Female (%)	12 (40)	12 (75)
Male (%)	18 (60)	4 (25)
Age at infusion – years		
Median	14	50
Range	5-60	23-74
Relapse (%)		
1	5 (17)	9 (56)
≥2	22 (73)	7 (44)
Previous allo-SCT (%)	18 (60)	4 (25)
Primary refractory (%)	3 (10)	
Baseline burden (%)		
Morphologic disease	24 (80)	8 (53)
MRD+	1 (3)	5 (33)
MRD-	5 (17)	2 (13)
High-risk cytogenetics (%)	8 (27)	10 (63)
Extramedullary disease (%)	2 (7)	3 (19)

Figure 6 (left) Baseline characteristics of patients Davila et al., 2014; Maude et al., 2014[1,2] **Figure 7 (below) Histology of B-ALL** Acute lymphoblastic leukemia [Digital image]. (n.d.). Retrieved from http://pathology.jhu.edu/cytopath_tut/Atlas/Index.cfm



Relevant Applications to Biotechnology

From agriculture during the Neolithic Revolution to modern day and beyond, biotechnology has and will continue to open, amalgamate, and exponentially progress fields in science that provide useful, and sometimes controversial, services and solutions. In the discipline of medicine, biotech has allowed better understanding of diseases, providing the infrastructure for vast improvements and breakthroughs in diagnosis, prevention, and treatment. CAR-T cell therapy is no exception. From the conceptualization and construction of the cells themselves to the morphologic, molecular, and cytogenetic assays used to analyze, CAR-T cell therapy is a direct product of biotechnology and will continue to revolutionize r/r B-ALL and other diseases to follow.

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Mom, thank you for your perpetual love and sacrifice. Dad, I wish you could see the impact your dedication to science has had on my own growing passion for medicine and healthcare. Dr. Ericka, Ms. Winter, and Kathleen, I want to extend my upmost gratitude to you all for your time and commitment. You have provided me and my OSA sisters with such an invaluable summer opportunity, and the academy will be a keystone of our path as female scientists. Thank you for challenging us to venture, to learn not only about oncofertility but also ourselves.

References

1 Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., . . . Brentjens, R. (2014, December 20). Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. *Science Translational Medicine, 6*(224). doi:10.1126/scitranslmed.3008226
2 Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., . . . Grupp, S. A. (2014, October 16). Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. *New England Journal of Medicine N Engl J Med, 371*(16), 1507-1517. doi:10.1056/nejmoa1407222
3 Maude, S. L., Teachey, D. T., Porter, D. L., & Grupp, S. A. (2015, June 25). CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood, 125*(26), 4017-4023. doi: 10.1182/blood-2014-12-580068
4 Park, J. H., Riviere, I., Wang, X., Bernal, Y. J., MS, Yoo, S., Purdon, T., . . . Brentjens, P. J. (2014, December 4). CD19-Targeted 19-28z CAR Modified Autologous T Cells Induce High Rates of Complete Remission and Durable Responses in Adult Patients with Relapsed, Refractory B-Cell ALL. *Blood, 124*(21), 382. doi:dx.doi.org/
5 Tasian, S. K., & Gardner, R. A. (2015, October 06). CD19-redirected chimeric antigen receptor-modified T cells: A promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). *Therapeutic Advances in Hematology, 6*(5), 228-241. doi:10.1177/2040620715588916