The Effects of High Cortisol Levels on Fertility in Women With a History of Anxiety and Depression

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Objective

This poster will demonstrate the correlation between the stress hormone cortisol and fertility in patients with a history of depression and anxiety. This study is vital in the study of reproductive biology in that abnormal amounts of cortisol in patients could potentially be an explanation for patients with unexplained infertility.

Abstract

Infertility affects approximately 11% of reproductive age women (ages 15-44). Cortisol is the primary stress hormone in humans that is secreted from the cortex of the adrenal glands, and then released into the bloodstream for various functions; it is also a major glucocorticoid (steroid hormone) in the body. Research has revealed that stress causes the release of cortisol from the adrenal glands and can make women infertile. Modifications of the hypothalamo-pituitary-adrenal axis and associated changes in circulating levels of glucocorticoids form a key component of the response of an organism to stressful challenges. ***Figure 2.*** It is found that women under constant stress tend to ovulate irregularly. As a result of the increase in cortisol concentration, the concentration of LH decreases in the ovaries disrupting and sometimes halting ovulation. Levels of cortisol above 450-650nmol/L have been reported to be associated with miscarriages. In a study by the International Health Foundation, 24.9% of women who had been experiencing infertility were proven to have depressive issues compared to only 6.8% of fertile women. Further research seems to confirm a link between stress, anxiety, and infertility.

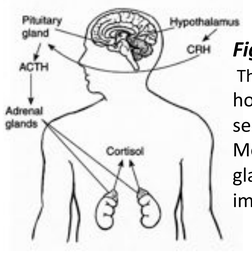

Figure 2 Hypothalamo-Pituitary-adrenal axis. The pituitary gland secretes Adrenocorticotropic hormone into the adrenal glands, telling them to secrete cortisol into the body for various functions. Modifications of this system can cause the adrenal glands to over produce cortisol causing an imbalance and therefore leading to infertility.

Materials and Methods

A study by the University of Western Australia was conducted using 13 women volunteers who were actively attempting to conceive. Each participant was examined for the association between miscarriage and levels of maternal urinary cortisol during the first 3 weeks after conception if they were successful in conceiving. All women were Caucasian. It was requested that each participant fill out a questionnaire twice per menstrual cycle leading up to conception. 10 of these women collected a 12 hour overnight urine sample to be tested for cortisol excretion. A second study was conducted using 264 women who came for the first cycle of IVF or ICSI. Only women with regular menstrual cycles and using no hormonal contraceptives could participate. All smokers were excluded. Women, who agreed to participate, were asked to complete standardized psychological questionnaires and were given blood tests on four time points shown in *figure 1*. Each participant also underwent a comprehensive medical exam prior to the study.

Results and Interpretation

It was found that pregnancies characterized by increased maternal cortisol during this period were more likely to result in spontaneous abortion. The women who became pregnant reported feeling more elated, composed, and agreeable. As a result of the increase in cortisol concentration, the concentration of Luteinizing Hormone (LH) decreases in the ovaries disrupting and sometimes halting ovulation. 24.9% of women who had been experiencing infertility were proven to have depressive issues compared to only 6.8% of fertile women. Levels of cortisol above 450-650nmol/L are also associated with miscarriages. State Anxiety scores were positively associated with cortisol values. In particular, a positive association was found between the State Anxiety scores and cortisol levels in serum before IVF treatment.

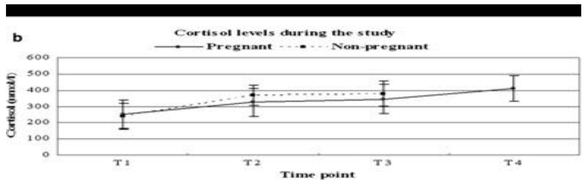

Figure 1: T1: before the start of treatment, T2: day of oocyte retrieval, T3: day of pregnancy detection, T4: 5–8 weeks of gestation for 92 pregnant women

Conclusion

High stress causes a woman's adrenal glands to secrete cortisol; with cortisol levels above 450-650nmol/L a woman's LH level in her ovaries will drop causing disruptions in ovulation therefore making this woman infertile. However, this is reversible. In women undergoing IVF, cortisol release from stress is associated with negative success in both pregnancy rate and live birth rate in IVF patients cortisol may be an important factor in the relationship between psychosocial stress and outcome after IVF/ ICSI.

Relevant Applications to Biotechnology

Advancements in biotechnology have made urinalyses possible. Since 1967, Urinalysis has been used to detect hormone levels, in drug tests, and to detect pregnancy. Today urinalysis can be performed manually with a dipstick or via an automated machine. Using urine analyzers make it possible for cortisol and other hormone levels to be counted in a sample of urine. Urine analyses are used worldwide. Researchers today can now test hormone levels in blood serum as well.

Acknowledgements

A huge thank you is extended to Dr. Ericka Senegar-Mitchell for always being an encouragement, teacher, and mentor. To Mrs. Patricia Winter for her dedication to making this academy available to young women in San Diego County, Dr. Chang for his support and insight. And to Dr. Doug Saunders for coming from Australia to be a mentor and teacher. Also to Nina Caudill for her help, encouragement, and friendship.

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Correlation Between the Stress-Related Hormone Cortisol, and Luteinizing Hormone in Sheep

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Objective

Stress is a prevalent issue individuals in the United States face on a day to day basis. Therefore, it is important to study the effects stress can have on fertility. Luteinizing hormone (LH) is produced by the pituitary gland and produces the LH surge to induce ovulation which is vital to conception. It is the intent of this study to expose the effects the hormone cortisol, a hormone produced by the adrenal cortex in response to stress, has on the fertility of sheep, specifically, the luteinizing hormone. By observing how cortisol can affect LH in sheep, it can be applied to research on human females. Thus, identifying a detectable cause of infertility in women.

Abstract

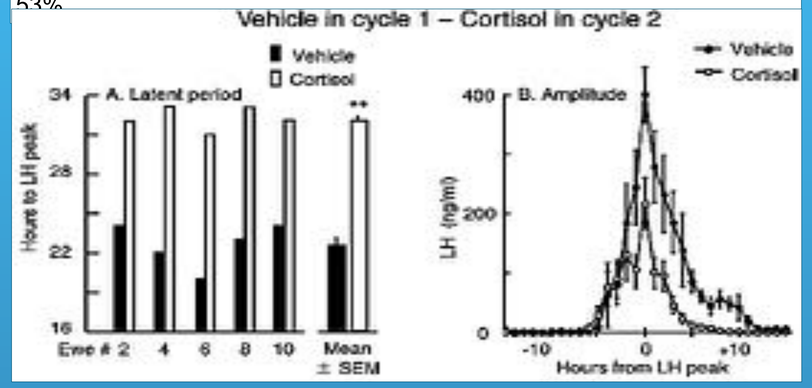
As stress continues to be an increasing issue in our society, it is of significant importance to study how this issue affects fertility. It is the intent of this study to expose the effects the hormone cortisol, a hormone produced by the adrenal cortex in response to stress, has on the fertility of sheep, specifically, the luteinizing hormone (LH). A study completed by researchers from the University of Michigan in partnership with Monash University in Australia, tested the hypothesis that cortisol interferes with the positive feedback action of estradiol, the hormone that provokes the LH surge. This study was conducted on sexually mature ewes who underwent ovariectomy, or removal of the ovaries, prior to the initiation of the experiments. Artificial estrous cycles were simulated using estradiol implants on each ewe and two different intravaginal devices that released progesterone to simulate concentrations of plasma during the luteal phase. In addition, estradiol implants were also used to simulate the estradiol rise. During this stage, LH and GnRH rises occurred. Two catheters were inserted into the jugular vein to infuse cortisol or vehicle (saline) and withdraw blood samples for testing. Based on this study cortisol was found to cause a delay in the surge of LH as well as a reduction in the amplitude in sheep. Further studies can be conducted on to confirm similar findings in women.

Methods and Materials

A study has tested the hypothesis that there is an interference of cortisol with the positive feedback action of estradiol that provokes the LH surge. This study was conducted on sexually mature ewes who were fed hay and alfalfa with access to water and mineral licks. The ewes utilized in these experiments had undergone ovariectomy 2 months prior to the initiation of the experiments. The experiments occurred during non-breeding season. Artificial estrous cycles were simulated using estradiol implants on each ewe and two different intravaginal devices that released progesterone to simulate concentrations of plasma during the luteal phase. Progesterone was withdrawn after 7-9 days, which simulated the length of progesterone secretion of the luteal phase. Such effect was caused by the implantation of estradiol implants with the objective to simulate the follicular phase plasma estradiol rise. During this stage, LH and GnRH rises occurred. Furthermore, two catheters were inserted into the jugular vein in order to infuse cortisol or saline and withdraw blood samples for testing. In the first experiment, two consecutive artificial estrous cycles were used from two groups of ewes. The first group was induced with vehicle (saline) for the first artificial estrous cycle and cortisol for the second cycle. The other group of ewes received the same substances in opposite order. Each hour, blood was withdrawn beginning 2 hours prior the stimulus of estradiol and ended 4 hours after.

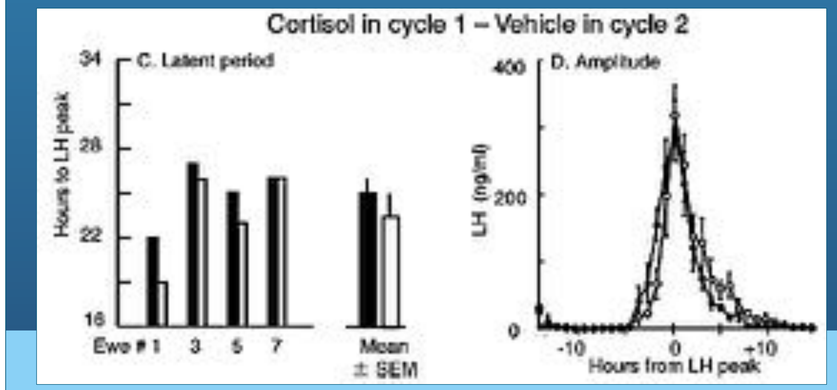
Results

In experiment 1, the mean concentrations of cortisol during infusion of saline were 11.3 +/- 1.4 ng/ml. During infusion of cortisol, values remained at 172.5 +/- 6.9 ng/ml during the cortisol infusion in experiment 1 and didn't differ between cycle 1 and cycle 2. When vehicle was infused in the first cycle, and cortisol in the second, the LH surge delayed in all ewes. The latent period increased by a mean of 10 hours, 22.6 h in cycle 1 and 32.2 h in cycle 2. The amplitude between infusions decreased by 53%.



Graphs show the influence of cortisol on LH in experiment 1. As shown in A and B, vehicle was infused in the first cycle followed by cortisol infusion in cycle 2. A represents the latent period from estradiol stimulus to LH peak for each ewe and the mean SEM of all ewes. B shows the mean SEM plasma LH concentrations. [1]

In experiment 2, the mean concentrations of cortisol during infusion of saline were 13.0 +/- 1.6 ng/ml. In this experiment, cortisol SEM surge duration of vehicle vs. cortisol was 12.4 +/- 0.8 h (vehicle) and 11.6 +/- 0.5 h (cortisol), thus cortisol didn't affect the mean SEM surge duration in the first cycle. In the second cycle, the mean SEM surge duration was not affected by cortisol showing 11.4 +/- 0.9h (vehicle) and 10.9 +/- 0.6 h (cortisol). During cortisol infusion, the mean SEM plasma cortisol concentration in the first cycle was 168. 0 +/- 11.6 ng/ml, more than cycle 2 with 136.0 +/- 7.7 ng/ml. It was also shown by this experiment that cortisol delayed the LH surge by an average of 4.3 h in cycle 1 and 4.2 h in cycle 2. The amplitude was reduced only in cycle 2 by 30% less in cortisol infused ewes in comparison to vehicle infusions.



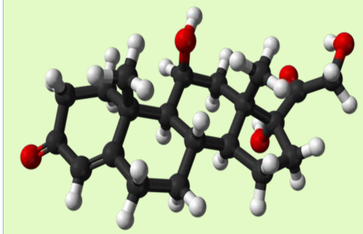
The graphs above demonstrate the results of experiment 2. C demonstrates the latent period from the estradiol stimulus to the LH peak. D shows the mean SEM plasma LH concentrations normalized to the peak of the LH surge. In this experiment, cortisol was infused in the first cycle and in cycle 2, vehicle was infused. [1]

Discussion

It is of great significance to clarify that according to this study, cortisol does not block the LH surge. It has been demonstrated by this study that cortisol does have detrimental effects on the LH surge in sheep by decreasing the amplitude and delaying the time it takes for the surge to occur. Such effects have been caused by the increase in plasma cortisol and interference with the positive feedback action of estradiol. The conclusions demonstrate that cortisol affects the estradiol feedback necessary for the LH surge, therefore, creating an obstacle for ovulation. Thus, fertility would not be impossible, however, it would be more difficult to occur. Because according to this study cortisol can affect ovulation, it can be concluded that stress can be a detrimental factor as a woman attempts to conceive.

Applications to Biotechnology

Stress-reducing drugs such as ketoconazole (Nizoral), mitotane (Lysodren) and metyrapone (Metopirone) have been used to control cortisol levels in patients who have Cushing's disease, a disease caused by high cortisol levels. It could be possible to use these as cortisol reducers for women seeking to conceive.



Cortisol molecular model. [6]

Acknowledgements

I would like to thank SDEMC and SDCC for giving me an academic foundation where I have been able to express my talents and learn in a culturing and exciting environment. I would also like to thank all the professionals involved in this program that opened not only the doors to their realms of study, but also to their experiences and support. In addition, I would like to thank Dr. Ericka for all her help and guidance throughout the academy. Dr. Saunders is also a deserver of my thanks for his sympathy and for being a gentleman. Quisiera agradecerles a mis padres por confiar en mi y hacerme creer en mi misma. Los quiero! Above all I need to thank God for opening doors for me and holding my hand every step of the way.

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The Potential Application of Stem Cells in the Restoration of Fertility in Males

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the Oncofertility™ Consortium Saturday Academy

Objective

The objective of this poster is to demonstrate how SSCs (spermatogonial stem cells) can make new sperm cells for males. This will be shown by studies on mice and zebrafish, which will open up new possibilities for humans.

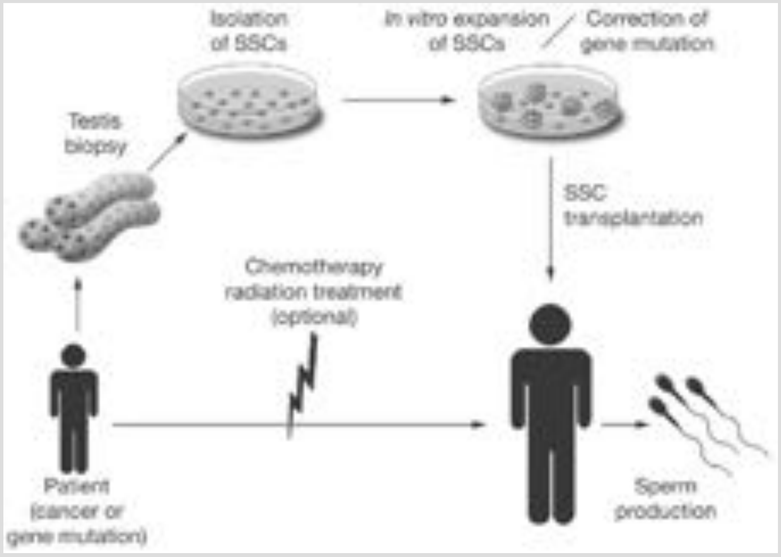

Figure 1: Process through which SSCs can restore fertility following chemotherapy; source: http://www.nature.com/nrendo/journal/v2/n2/fig_tab/ncpendmet0098_F4.html

Abstract

Spermatogonial stem cells(SSCs) are the cells behind spermatogenesis in men. SSCs are among the unipotent stem cell types, due to their ability to produce specifically sperm cells.[i] In some men, however, spermatogenesis has been impeded due to the effects of things such as cancer treatment.[2 5] If SSCs are transplanted, these men can create their own sperm, eliminating their dependence on things such as sperm banking or sperm donors.[6] The transplantation of SSCs has already been done successfully in mice and zebrafish; [3 4] all that's needed is to perfect the technique in humans. In mice, the growth factors that are needed for self-renewal and expansion of SSCs have been identified for successful transplantation. These growth factors include an anti-Thy-1 antibody that has been cultured on SIM mouse embryo-derived thioguanine and ouabain resistant feeders, and glial cell line-derived neurotrophic factor. Stem cell activity was maintained constantly in the germ cells that had been cultured in Thy1+. When these cultured SSCs were transplanted into infertile mice, progeny were produced in the mated females. [4] The zebrafish study showed how transplantation had been successful by locating the niche of the SSCs and developing a transplantation technique. Although the transplantation efficiency was low, it's still significant in that it did work.[3] Both of these studies showed positive results: when the environment for the SSCs is right, they will restore spermatogenesis to the studied organisms. In conclusion, SSCs can restore fertility to human males, should this research become successful on humans.

Methods and Materials

SSCs have already been transplanted successfully in mice and zebrafish. In mice, the growth factors that are needed for self-renewal and expansion of SSCs have been identified for successful transplantation. These growth factors include an anti-Thy-1 antibody that has been cultured on SIM mouse embryo-derived thioguanine and ouabain resistant feeders, and glial cell line-derived neurotrophic factor. Stem cell activity was maintained constantly in the germ cells that had been cultured in Thy1+.[4] When the testes are biopsied, the SSCs are taken out and frozen. Upon thawing, they are exposed to the aforementioned growth factors, and later transplanted. If this is done with success, spermatogenesis will occur.[7]

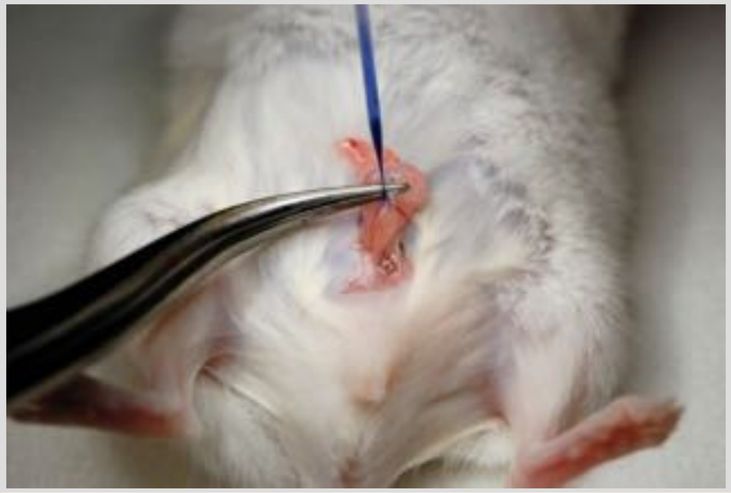

Figure 2: SSCs being transplanted into mouse testis; source: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3987472/

Results

When these cultured SSCs were transplanted into congenitally infertile mice, they were then mated with normal females; progeny were later produced in the mated females.[4] The zebrafish study showed how transplantation had been successful by locating the niche of the SSCs and developing a transplantation technique. Although the transplantation efficiency was low(about a 30% success rate), it is still significant in that it did work.[3] Both of these studies showed positive results: when the environment for the SSCs is right, they will restore spermatogenesis to the studied organisms.

Conclusions

In conclusion, the growth and transplantation of SSCs in mice and zebrafish have been successful. The subjects produced viable offspring, and spermatogenesis continued uninterrupted. SSCs can restore fertility to human males, should this research become successful on humans. If this is to be done in humans, research should take place on the growth factors needed for human SSCs and proper transplantation techniques, modeling the mouse and zebrafish experiments. In the mouse experiment, it was shown that the germ-lines of individuals who have undergone SSC transplants may be extended beyond a typical lifetime. The zebrafish experiment showed how the niche is significant in transplantation success. Although it may be difficult, it will surely be worth it for those who have no other fertility options.

Applications to Biotech

The mouse SSC study was made possible due to the advances in biotechnology that allows scientists to breed transgenic mouse lines that express reporter genes.[4] And in the zebrafish SSC study, a green fluorescent protein is used to identify sexually mature male transgenic zebrafish. This study also utilizes the Image J software and an inFlux cell sorter.[3]

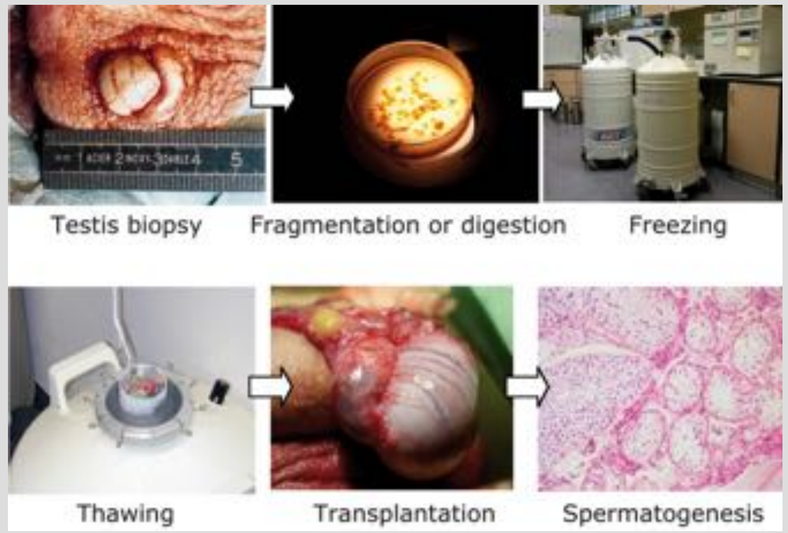

Figure 3: A flowchart showing the steps of SSC transplantation spermatogenesis; source: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3991415/

Acknowledgements

I would like to acknowledge Mrs. Winter, Dr. Senegar-Mitchell, all the doctors we worked with, and my OSA sisters, who all supported and educated me throughout the period of the academy. I would also like to thank Dr. Saunders and Dr. Chang for helping us out on so many of our sessions.

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Reversing Platinum Resistance in Ovarian Cancer Patients Using Hypomethylating Agents

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Introduction

Platinum agents are the most commonly used drugs in chemotherapeutic treatment of ovarian cancer. They form adducts in DNA that are then recognized by DNA repair system proteins, which eventually leads to apoptosis.[1] However, one of the major difficulties in successfully treating ovarian cancer is the acquisition of platinum resistance. This can be caused by multiple factors; abnormal DNA methylation brought about by platinum-induced DNA damage is one of them. DNA methylation is the addition of a methyl group to DNA that can reduce or completely silence the expression of a gene. Under normal conditions, DNA methylation helps stabilize gene expression in cells that are undergoing differentiation. Abnormal methylation can cause disorders in gene expression, leading to diseases like cancer. Hypomethylation plays a role in the activation of oncogenes, while hypermethylation is associated with silencing of tumor suppressor genes in cancer. Hypermethylation, in particular, has also been shown to contribute significantly to platinum resistance.[1] This poster aims to analyze the ability of epigenetic therapies, specifically hypomethylating agents, in reversing platinum resistance in ovarian cancer patients.

Abstract

Ovarian cancer is the deadliest of all gynecological cancers. One of the main issues associated with its high mortality rate is drug resistance after initial chemotherapy. While most patients originally respond to platinum- or taxane-based treatment, about 80% of them eventually relapse; this is often due to acquired resistance to these drug regimens.[2] Resistance generally involves the upregulation of antiapoptotic oncogenes and/or downregulation of tumor suppressor or proapoptotic genes, which can be brought about by both genetic mutations and epigenetic modifications.[2] However, due to the reversible nature of epigenetic modulation, researchers have had an increasing interest in using it as a target for cancer treatment. Multiple *in vitro* and preclinical studies have shown that hypomethylating agents such as decitabine (5-aza-2'-deoxycytidine), azacitidine, and zebularine can reverse platinum resistance in ovarian cancer cell lines and xenografts. They have also linked the demethylation (and subsequent reexpression) of certain genes, such as *hMLH1*, *RASSF1A*, *HSulf-1*, *p16*, and *DR4*, to resensitization to platinum.[234] The purpose of this study is to assess the possibility of hypomethylating agents to resensitize patients with platinum-resistant ovarian cancer. Two early clinical trials demonstrated that administering a hypomethylating agent prior to platinum treatment resulted in patient response. One group used azacitidine in combination with carboplatin, while another chose decitabine with carboplatin. In the first trial, an overall response rate (RR) of 13.8% (4 of 29) and a median progression-free survival (PFS) of 3.7 months were achieved.[5] The second clinical trial, using decitabine, produced a RR of 35% (6 of 17) and a PFS of 10.2 months.[6] Overall, these two studies provide early evidence that hypomethylating agents may be able to reverse resistance in platinum-resistant ovarian cancer. Larger studies in the future should be conducted to further investigate the effectiveness of hypomethylating agents in resensitizing ovarian cancer to platinum.

Materials & Methods

Characteristic	Clinical Trial 1	Clinical Trial 2
Hypomethylating agent	Azacitidine	Decitabine
Dosage	75mg/m^2 for 5 days	10mg/m^2 for 5 days
Carboplatin dosage	AUC of 4 or 5 on day 2	AUC of 5 on day 8
Patient population	18 platinum-resistant 12 platinum-refractory*	15 platinum-resistant 2 platinum-refractory
Response evaluation	WHO criteria	RECIST/Rustin criteria

Figure 1: Summary of materials & methods from the 2 clinical trials. *Data adapted from text content by Fu, S., et al. (2011)[5] and Matei, D., et. al. (2012).*[6]
*One patient voluntarily withdrew from the study during the first cycle and so was not eligible for antitumor evaluation (n=11).

Two clinical trials were conducted to assess the ability of hypomethylating agents in reversing platinum resistance in ovarian cancer. In one phase1b-2a study, 18 platinum-resistant ovarian and 12 platinum-refractory patients were administered azacitidine in combination with carboplatin, with one early withdrawal. Patients received 75mg/m^2 doses of azacitidine subcutaneously for 5 days and carboplatin at either an area under curve (AUC) of 4 or 5 intravenously on Day 2 every 28 days.[5] DNA was extracted from peripheral blood mononuclear cells (PBMC) obtained from patients before and after treatment and methylation was analyzed using a methylation kit. Tumor response was characterized using the World Health Organization (WHO) criteria of complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). All patients were treated until PD and observed until the end of the trial or until death.[5] In a second phase 2 clinical trial, 15 platinum-resistant and 2 platinum-refractory patients were given 10mg/m^2 doses of decitabine intravenously for 5 days. Carboplatin was then administered intravenously on day 8 at an AUC of 5. Patients also received peg-filgastrim on day 9 to prevent prolonged myelosuppression.[6] Blood, tumor biopsies, and/or ascites were collected from each patient before and after decitabine treatment for DNA extraction and methylation analysis by pyrosequencing. Response rate was determined by Response Evaluation Criteria in Solid Tumor (RECIST) or modified Rustin criteria. Patients continued treatment until PD or intolerable toxicity.[6] In both studies, toxicity and adverse side effects were carefully monitored.

Results

The results of the two clinical trials indicate the potential of hypomethylating agents in treating platinum-resistant ovarian cancer patients. In the first study, the overall RR was 13.8%, the median PFS was 3.7 months, and the median overall survival (OS) was 14 months.[5]

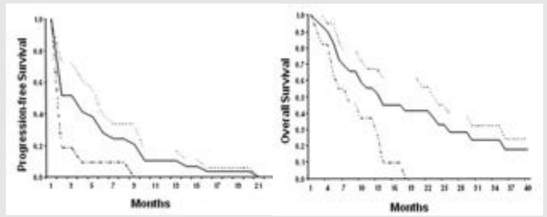

Figure 2: PFS and OS estimated by Kaplan-Meier curves and the log-rank test. Straight line indicates all patients (n=29), dotted line represents platinum-resistant patients (n=18), and dotted and dashed line denotes platinum-refractory patients (n=11). *Adapted from Fu, S., et al. (2011).*[5]

Further analysis also revealed that there was a significant difference in the clinical outcomes between platinum-resistant and platinum-refractory patients. The results are summarized in Figure 3.

Clinical Outcome	Platinum-Resistant	Platinum-Refractory
CR	1	0
PR	3	0
SD	9	1
PD	5	10
Overall RR	22%	0%
Median PFS	5.6 months	1.9 months
Median OS	23 months	10 months

Figure 3: Clinical outcome summary of platinum sensitivity-specific groups. This table indicates that platinum-resistant patients responded more favorably to hypomethylation strategies. *Data adapted from text content by Fu, S., et al. (2011).*[5]

In the second trial, a RR of 35% (6 of 17), a median PFS of 10.2 months, and a median OS of 13.8 months were achieved. One patient reached a clinical CR and an additional 6 patients had SD. Furthermore, at the time the article was written, one patient was still receiving treatment 2.5 years after the start of therapy.[6] An analysis of demethylating activity was also conducted. Methylation of *LINE1* (long interspersed) repetitive elements in PBMC DNA was assessed in place of global demethylation activity. All patients had reduced ($p<0.001$) DNA methylation on day 8 as compared with day 1, although they eventually returned to baseline values by the end of the study (usually coinciding with PD).

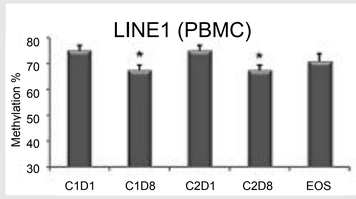

Figure 4: Methylation levels of *LINE1* in PBMCs on days 1 and 8 of cycles 1 (C1D1, C1D8) and 2 (C2D1, C2D8) and at the end of the study (EOS). Significant demethylation occurred during cycles 1 and 2 of treatment, demonstrating promising evidence that hypomethylating agents may be a viable option to treat platinum-resistant ovarian cancer. *Adapted from Matei, D., et. al. (2012).*[6]

It was also shown that the number of demethylated genes was greater in responding patients who had a PFS of more than 6 months than in those who had a PFS of less than 6 months (311 vs. 244 genes in core biopsies and 630 vs. 474 genes in PBMCs, PFS > 6 vs. PFS <6). Finally, baseline and decitabine-altered tumor methylation levels of 5 genes were found to differ in patients with a PFS longer than 6 months versus those with a PFS shorter than 6 months in a manner that was statistically significant ($p<0.05$).[6] This data supports the hypothesis that treatment-induced hypomethylation can help reverse platinum resistance in ovarian cancer patients.

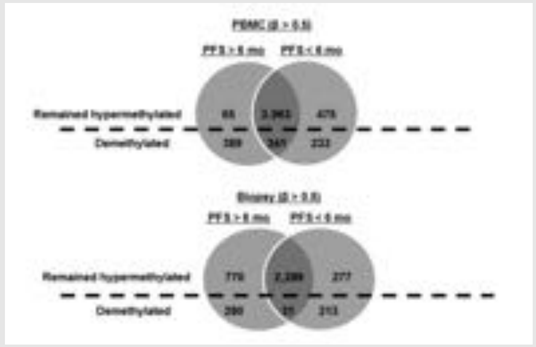

Figure 5: The Venn diagrams show the numbers of similar demethylated and remaining hypermethylated genes in PBMCs and in biopsies before and after decitabine treatment in patients with PFS > 6 months and PFS < 6 months. The dotted line divides the numbers of demethylated genes and those that remain hypermethylated. This figure reveals that there was a large number of shared decitabine-induced demethylated genes in responding patients, providing biological data supporting hypomethylation strategies in resensitizing platinum-resistant ovarian cancer patients. *Adapted from Matei, D., et. al. (2012).*[6]

Conclusion

These two studies provide early evidence that hypomethylating agents may be able to reverse resistance in patients with platinum-resistant ovarian cancer. While results from studies evaluating the use of single-agent hypomethylating and single-agent carboplatin in treating platinum-resistant solid tumors have been disappointing, combination therapies appear to be promising.[6] Each trial produced a relatively high RR (22% for azacitidine, 35% for decitabine) compared to the <10% RR reported by studies re-treating platinum-resistant ovarian cancer patients with single-agent carboplatin,[5] as well as a prolonged PFS (5.6 and 10.2 months, respectively). However, it was shown in the first study that platinum-refractory patients achieved no clinical response after being treated with successive rounds of a hypomethylating agent and carboplatin regimen.[5] This suggests that the different underlying biological mechanisms driving platinum-resistant and platinum-refractory cancers cause them to behave differently when treated with a hypomethylating agent; platinum-resistant patients are more likely to benefit from hypomethylating treatment strategies. The second trial correlated the number of demethylated genes and the level of global demethylation to patient response. In addition, it identified a rudimentary list of candidate genes and methylation profiles predictive of patient clinical outcome that can be used to individualize hypomethylating therapies to each patient, thus improving prognosis.[5] In summary, both studies demonstrate the potential of hypomethylating agents in reversing resistance in platinum-resistant patients and offer preliminary evidence that warrant further investigation of hypomethylating strategies in treating platinum-resistant ovarian cancer.

Relevant Applications to Biotechnology

While the two clinical trials presented above reported overall favorable clinical activity that resulted from treatment by hypomethylating strategies, it is clear that there was heterogeneity in the response to the hypomethylating agents.[5] It is imperative to take patient disparities into account to maximize treatment benefits and optimize clinical outcome. The latest advancements in biotechnology, including genome sequencing and analysis, assays, and PCR allow researchers to generate more individualized treatment regimens for patients based not only on their phenotypic characteristics and patient history, but also on their genetic makeup. In addition, as demonstrated by the second study, methylation profiling and analysis can be used to create a panel of genes that may help predict patient response to certain hypomethylating agents.[6] As medical practitioners begin to increasingly turn to targeted therapy in cancer treatment, epigenomic information will prove to be vital in creating the best treatment plan.

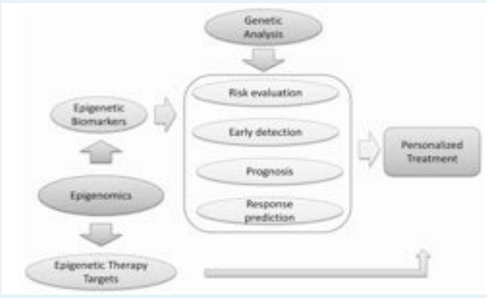

Figure 6: Role of epigenomic studies in cancer prevention, diagnosis, and treatment. Epigenetic biomarkers can be used in risk evaluation, early detection, prognosis, and response prediction. In addition, investigation of epigenomic pathways can reveal novel epigenetic therapy targets. This information, along with genetic studies, will be important in creating optimal personalized treatment plans for cancer patients. *Adapted from Chen, H., et. al. (2011).*[1]

Acknowledgements

I would like to extend a sincere thanks to Dr. Ericka Senegar-Mitchell for her continuous support and guidance throughout the course of the project and academy, Dr. Saunders for his knowledge and advice, Ms. Winter for her organization of the program, and Miss Nina Caudill for her nonstop encouragement and assistance. I would also like to acknowledge Dr. Chang, Dr. Su, and all the other professors, doctors, medical practitioners, and presenters that came to talk to us for taking the time to share their invaluable knowledge and experiences. I would like to recognize my wonderful family for all their support and advice. Finally, I would like to thank all my OSA sisters for their love, insights, and laughter and for making this summer truly amazing.

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Potential Risks of Human Papilloma Virus Vaccine

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Objective

Few medical discoveries can compete with vaccines for their overall impact on human health, lifestyle, and population. A vaccine can produce a total of 30 antibodies that combine chemically with substances in the body to fight foreign matters. Vaccines are designed to generate an immune response that will protect the vaccinated individual during exposures to the disease, however immune responses vary, and in some cases a person's immune system will not generate an adequate response.

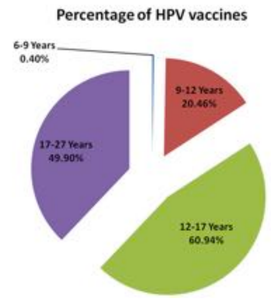

Figure 1 June of 2006 the HPV vaccines contribute to an over 50% increase in reports in the 12-27 age groups. The largest percentage is in the 12–17 age groups with 60.94% difference between the two HPV vaccines and all the other vaccines combined.[4]

Research has questioned, in the case of the Human Papilloma Virus (HPV) vaccine, whether the body's immune system is unable to respond to the vaccine, or the vaccine itself is flawed. The objective of this poster is to identify the potential risk factors of the HPV vaccine and present the role of the vaccine in the context of its medical consequences.

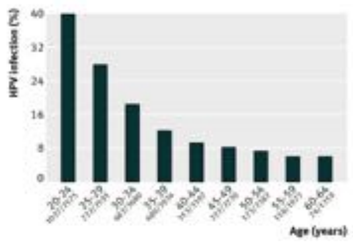

Figure 1 Prevalence of high risk HPV according to five year age groups. The numbers below each bar represent the number of people testing positive over the total number of people tested within each age group.[1]

Abstract

Human Papilloma Virus (HPV) is the most common sexually transmitted infection in the world, affecting 20 million people. There are 100 types of HPV: 40 infect the genital tract causing genital warts and cervical and pharyngeal caner. Half of these infections occur among young adults, ages 15 through 24. The HPV vaccine, known as Gardasil, stimulates an immune response in the body, causing antibodies to attack the protein. Over the past few years, however, the efficacy of the HPV vaccine has been questioned, as research has uncovered potential side effects of the vaccine. In a study (Slade), reporting the effects of the HPV vaccine, 6.2% of cases described serious adverse events following immunization, including 32 death reports, syncope, and infection, but these findings were underreported.[5] Another study tested females with 1-3 doses of HPV4, then compared the immediate and long-term risks, comprising of syncope and skin infection, caused by vaccination.[2] Another study recording the rate of the vaccine's 3-dose follow-through showed that only 17.2% of those reminded to receive their vaccines did, in comparison to the 18.9% in the control group.[3] The participants who completed the series on time were older. The HPV vaccine is encouraged at 10 years old, before most begin sexual activity, and thus follow-through rates are low, causing the vaccine to prove ineffective. In conclusion, studies haven't confirmed quantitative side effects of the HPV vaccine. The results vary case-to-case and although medical risks aren't agreed upon, the public health problems have not been addressed.

Materials and Methods

Two studies analyzed the HPV vaccine's adverse events following immunization (AEFIs). The first study, reported to the vaccine adverse event reporting system (VAERS), was conducted from June 1, 2006, through December 31, 2008. The number of doses distributed in the United States, 23,051,336, provided the denominator to estimate reporting ratios. Additional analyses were performed for AEFIs of uncommon severity or those that had received public attention at prelicensure trials. Statistical data mining, involving proportional reporting ratios (PRRs) and empirical Bayesian geometric mean methods, were used to detect the ratios in reporting. To code reported symptoms, internationally standardized terminology, the Medical Dictionary for Regulatory Activities (MedDRA), was used. VAERS reports were classified as serious according to the FDA regulatory definition of a serious AEFI, as one that is life threatening; results in death, permanent disability, congenital anomaly, hospitalization, prolonged hospitalization; or requires medical or surgical intervention. Case reviews and separate analyses for syncope, dizziness, nausea, headache, local injection site reactions, hypersensitivity reactions, deaths, and pregnancy outcomes were also performed.[4] The second study was an observational cohort study within the health care systems of Northern and Southern California Kaiser Permanente. This study included all females who received at least 1 dose of HPV4 during routine clinical care. Subject increase began following the first administration of HPV4 (August 2006) and continued until 44,000 female members ages 9 to 26 years at first dose had received 3 HPV4 doses within 12 months, completed in March 2008. A larger safety population was evaluated, comprising females of any age who received at least 1 HPV4 dose between August 2006 and March 2008.[2]

Results

From June 1, 2006, through December 31, 2008, VAERS received 12, 424 reports of AEFIs following receipt of qHPV, an overall reporting rate of 53.9 reports per 100 000 vaccine doses distributed. The majority of reports (68%) were submitted by the manufacturer, compared with an overall rate of 40% for VAERS reports on other vaccines. During the same time period, manufacturer reports accounted for 14.5% of the meningococcal conjugate vaccine reports and 7.5% of the reports for tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine submitted to VAERS. Of the 8471 manufacturer reports for qHPV AEFIs, 7561 (89%) had insufficient identifying information to permit clinical follow-up or review the most frequently reported AEFIs included syncope (n = 1847, 15%), dizziness (n = 1763, 14%), nausea (n = 1170, 9%), headache (n = 957, 8%), and injection site reactions (n = 926, 7.5%).[4] In the second study, 189,629 females received at least 1 dose and 44, 001 received 3 HPV4 doses. Fifty categories had significantly elevated odd ratios (ORs) during at least 1 risk interval. Only skin infections during days 1 to 14 and syncope on day of vaccination were noted by an independent Safety Review Committee as likely associations with HPV4. Of the 50 Healthcare Cost and Utilization Project (HCUP) categories with elevated ORs, 40 were in the days 1 to 14 risk interval and 26 were in the days 1 to 60 risk interval, with some overlap, for a total of 47 HCUP categories during the days 1 to 14 and 1 to 60 risk intervals. Overall, 265 HCUP categories were analyzed and 7551 comparisons were conducted. The HCUP categories corresponding to attention-deficit disorder, ear conditions, and congenital anomalies among vaccines were the only significantly elevated ORs prior to multiple-comparison adjustment during both the days 1 to 60 and days 1 to 14 risk intervals. Taking into account all the analyses, subanalyses, and relevant record reviews, the safety committee noted that there may be an association between HPV4 vaccination and both day 0 syncope and skin infections during the 2 weeks after immunization.[2]

Conclusion

Vaccination with qHPV has the potential to decrease the global morbidity and mortality of HPV-associated diseases, including cervical cancer. Immunization and injections in general have a known association with syncope, and thus the skin infections and syncope were not surprising. Studies haven't confirmed quantitative side effects of the HPV vaccine. The results vary case-to-case and although medical risks aren't agreed upon, the public health problems have not been addressed. The study that recorded the rate of the vaccine's 3-dose follow through showed that the rate in young children is low.[3] Most adults who are able to follow through have already had experience with HPV and thus the vaccine cannot be preventative. This issue is prevalent and must be addressed as although the statistical side effects are uncertain, we can deal with the public health inefficiencies.

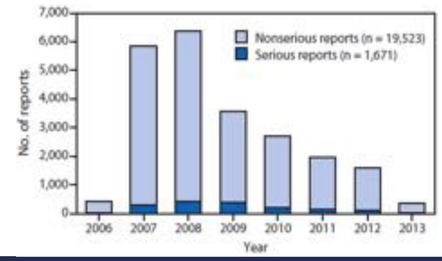

Figure 3 Number of serious and nonserious reports of adverse events after administration of quadrivalent HPV4 vaccine in females, by year. Vaccine Adverse Event Reporting System, United States, June 2006–March 2013.[4]

Relevance to Biotechnology

HPV is a current topic and should be researched further, as if severe side effects are found, an improved vaccine should be developed. Biotechnology has played an incredibly significant role in the development of vaccination thus far and will only continue to advance in the coming years. Vaccine-like particles (VLPs) have been extremely crucial in the creation of Gardasil. The ability to modify particles like these has enabled a variety of vaccines as well as drugs to successfully fight a foreign substance in the body. Due to progress in the biotech field, scientists have been able to develop synthetic vaccines. The gene that encodes an antigen is isolated, and the precise sequence of amino acids that make up the antigen is determined.

Acknowledgements

I would like to thank Dr. Ericka, Ms. Winter, and all of my OSA sisters who have allowed me to grow and have taught me so much along the way. This program would not be what it is without the doctors and medical professionals that have guided us and taken out time to let us experience such significant parts of their lives, so thank you for your dedication.

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The Role of the Inhibition of Tyrosine Kinase in the Impediment of Chronic Myelogenous Leukemia

Elena Kharitonova • University City High School

Introduction

Chronic Myelogenous Leukemia is a cancer that originates in hematopoietic stem cells due to a mutation. The Breakpoint Cluster Region (BCR) gene from Chromosome 22 and the Abelson (ABL) gene from Chromosome 9 translocate and fuse together forming the Philadelphia Chromosome. This new BCR-ABL gene codes for a nonspecific cytoplasmic tyrosine kinase that increases the amount cell proliferation, reduces the amount of apoptosis, and alters the adhesion properties of the cell. [1,5] Too many white blood cells with deficient adhesion to the bone marrow form, break off immaturely, and continue to grow at a faster rate than they die off. [3] This phenomenon causes cancer. Imatinib mesylate and nilotinib are tyrosine kinase inhibitors, or drugs specifically targeting cancer cells by binding to the enzyme and stabilizing it. This poster will determine whether or not the inhibition of tyrosine kinase can impede the progression of Chronic Myelogenous Leukemia.

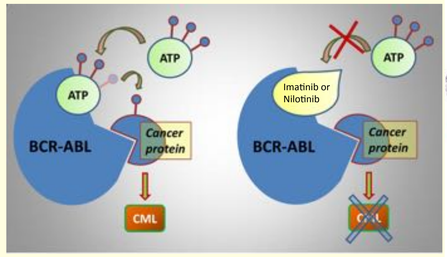

Figure 2: Imatinib and Nilotinib Mechanism of action. Retrieved from http://www.intechopen.com/books/leukemia/modern-therapy-of-chronic-myeloid-leukemia

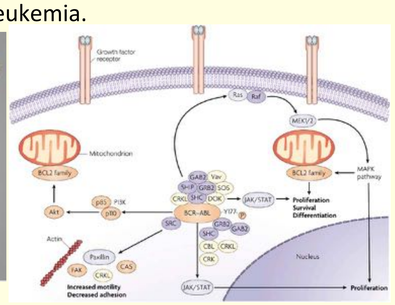

Figure 1: How the BCR-ABL tyrosine kinase protein causes cancer. Retrieved from http://www.nature.com/nrc/journal/v7/n5/fig_tab/nrc2126_F1.html

Abstract

Chronic Myelogenous Leukemia is caused by the translocation and fusion of the BCR and ABL genes in hematopoietic stem cells. This new BCR-ABL gene creates a mutant form of nonspecific cytoplasmic tyrosine kinase, which alters cell proliferation, apoptosis, and adhesion properties of the newly formed white blood cells, leading to leukemia.[1,3,5] The focus of this study is to see if the inhibition of tyrosine kinase impedes the progression of Chronic Myelogenous Leukemia. 846 adult patients of both genders who had been diagnosed within the past 6 months with Philadelphia chromosome-positive Chronic Myelogenous Leukemia participated in a phase 3, multicenter, open-label and randomized study named ENESTnd.[2,4] The patients were randomly assigned to be orally administered one of three drug regimes, nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, or imatinib mesylate 400 mg once daily in a 1:1:1 ratio. After an average of 24 months, the cumulative incident of complete cytogenetic response, meaning there are no Philadelphia chromosome-positive cells left in the blood or bone marrow, was 87% of the patients who received nilotinib 300 mg twice daily, 85% of the patients who received nilotinib 400 mg twice daily, and 77% of the patients who received imatinib mesylate once daily. This study proves that there is a clear correlation between the inhibition of tyrosine kinase and the impediment of Chronic Myelogenous Leukemia, since the tyrosine kinase inhibitors helped treat Chronic Myelogenous Leukemia and inhibit the cascade of events that lead to its progression.

Methods and Materials

The efficacy of the tyrosine kinase inhibitors was tested in a phase 3, multicenter, open-label, randomized study named ENESTnd. 846 adults of both genders who were diagnosed with chronic phase Philadelphia chromosome-positive Chronic Myelogenous Leukemia within the past six months through conventional cytogenetic analysis were randomly assigned to orally receive nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, or imatinib 400 mg once daily in a 1:1:1 ratio. [2,4] Patients were excluded if they did not have adequate organ function and if they had received tyrosine kinase inhibitors previously. The patients were assessed by a real-time quantitative PCR (Polymerase Chain Reaction) at base line for molecular responses monthly for 3 months, and every 3 months after that. Cytogenetic analysis of the bone marrow was conducted every 6 months. Complete blood counts were measured at baseline, at weeks 1, 2, and 4, then every month until 6 months, then every 3 months. [2,4]

Results

The results of this study show that tyrosine kinase inhibitors are an effective means to treat Chronic Myelogenous leukemia. The median time to major molecular response (when the amount of BCR-ABL protein in the bone marrow is very low) was 8.3 months for each nilotinib group and 11.1 months for the imatinib group. For those that achieved a major molecular response at 12 months, the response was maintained at 24 months in 93% of patients taking nilotinib 300 mg twice a day, 91% taking nilotinib 400 mg twice a day, and 92% for patients taking imatinib 400 mg once a day. The number of patients who achieved a complete molecular response was 44% in the nilotinib 300 mg twice daily, 36% in the nilotinib 400 mg twice daily, and 20% in the imatinib 400 mg once daily. [4] Also, 87% of patients taking the nilotinib 300 mg twice a day experienced a complete cytogenetic response after 24 months, 85% for the patients taking Nilotinib 300 mg twice a day, and 77% for the patients taking imatinib 400 mg once a day. Only 17 patients had the Chronic Myelogenous Leukemia progress to the next phase: 2 in the nilotinib 300 mg twice daily treatment, 3 in the nilotinib 400 mg twice daily, and 12 in the imatinib 400 mg once daily. [2]

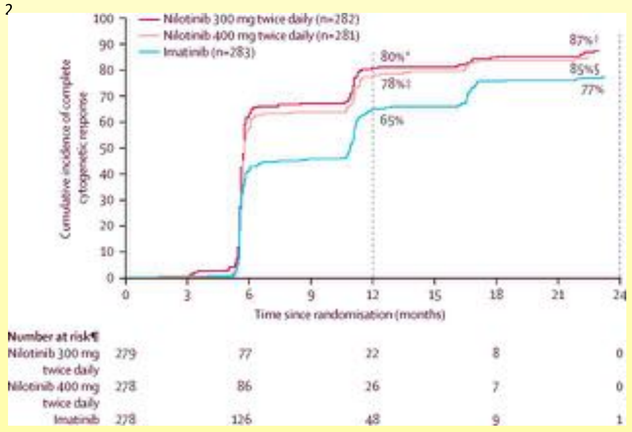

Figure 3: Graph of percentage of complete cytogenetic response after receiving tyrosine kinase inhibitors. (Kantarjian, H., et al, 2011)

Conclusion

There is a clear correlation between tyrosine kinase inhibitors and the impediment of the progression of Chronic Myelogenous Leukemia. The tyrosine kinase inhibitors lengthen the life span of the patients and improve their quality of life. By inhibiting the tyrosine kinase protein, the disease does not progress to the next phases, giving more than the standard 9-24 months to live. Tyrosine kinase inhibitors are also a more effective long term treatment than chemotherapy, since one of the most commonly used chemotherapeutic agent, hydroxyurea, relieves symptoms but causes cytogenetic responses in 5% of patients. Overall, tyrosine kinase inhibitors appear to be the next breakthrough for Chronic Myelogenous Leukemia. They should be studied more and a tyrosine kinase inhibitor with a higher complete molecular response should be found and developed.

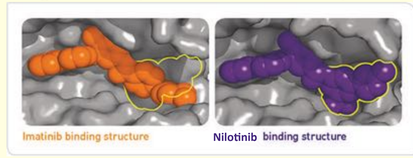

Figure 4: Difference between Imatinib and Nilotinib in binding structure. Retrieved from http://www.us.tasigna.com/index.jsp

Applications to Biotechnology

The test and study would not have been able to be completed without the Polymerase Chain Reaction software to test genes.[4] These advances in the Biotechnology field allowed for the cytogenetic molecular tests to detect the Philadelphia chromosome and the BCR-ABL gene. This discovery led to the idea of tyrosine kinase as the cause of Chronic Myelogenous Leukemia and the development of tyrosine kinase inhibitors. These tyrosine kinase inhibitors save thousands of lives of patients diagnosed with Chronic Myelogenous Leukemia annually.

Acknowledgements

I am very thankful for Dr. Senegar-Mitchell, Dr. Saunders, Dr. Chang, and Dr. Su for their wonderful teaching. I would like to thank Ms. Winter for all the time she put into this course. I would also like to thank all my OSA sisters for all their help and support. I am very grateful for all my past science teachers who helped me find my passion for science.

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Goserelin Drug Used to Preserve Fertility in Breast Cancer Patients

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Objective

The objective of this research poster is to determine whether or not the goserelin drug can be used to preserve fertility in breast cancer patients who undergo chemotherapy. The results of the studies are based on the return of menstrual cycles and/ or the occurrence of menopause in breast cancer patients.

Abstract

Fertility preservation is a major issue for cancer patients. Cytotoxic chemotherapy often causes the eggs to die off prematurely. Unfortunately, fertility preservation options are not typically expressed to patients prior to chemotherapy treatment and 29% of women in a Web-based survey stated that infertility concerns influenced their treatment decisions. One option for fertility preservation is using Gonadotropin-releasing hormone (GnRH) Analogues to temporarily suppress the functions of the ovaries. However, this is still in clinical trials despite the potential. The objective of this study is to see whether or not goserelin (a GnRH Analogue) can preserve fertility in breast cancer patients undergoing chemotherapy, using the marker of menstrual return.

Image A

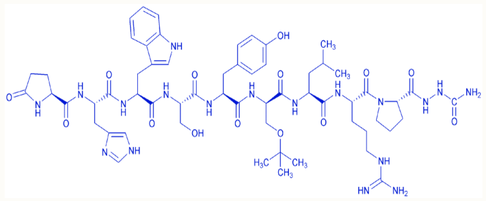


Image A: Goserelin is a GnRH Agonist that suppresses ovarian function by occupying the GnRH receptor on the pituitary and stops the production of Luteinizing Hormone (LH) and Follicle stimulating hormone (FSH)[4].
Image B: The degree of gonadal failure form chemotherapeutic agents[2].* Used in study [2]

Image B

High Risk
Cyclophophamide*
Melphalan
Busulfan
Nitrogen mustard
Cholarambucil
Procarbazine
Intermediate Risk
Cisplantin
Adriamycin
Low or No Risk
Methotrexate*
5-Flourouracil*
Vincristine
Bleomycin

In a study, goserelin was administered to patients throughout the triple therapy, cyclophosphamide, methotrexate, 5-fluorouracil (CMF) chemotherapy; the study showed that goserelin can potentially prolong fertility in stages I-III breast cancer patients who are estrogen receptor-positive. This subject should be further explored due to the importance and potentiality of the drug. Goserelin can be given before and during chemotherapy to possibly prevent premature menopause. Breast cancer patients could potentially have more noninvasive options for fertility preservation.

Methods and Materials

Thirty estrogen receptor-positive premenopausal patients with stage I-III breast cancer were administered in a phase II study (A larger pool of people in clinical trial). 3.6 mg of Luteinizing hormone-Releasing Hormone (LH-RH) analogue goserelin was administered to the patients every four weeks throughout the triple therapy cyclophosphamide, epirubicin, 5-fluorouracil (CEF) chemotherapy. Fertility options were given to each patients and the risks of failure was communicated to each patient. In a separate study, goserelin was given monthly for a year to 64 premenopausal patients with early breast cancer. Eighteen patients were given the triple therapy CMF and 46 patients were given an anthracycline based regimen.

Image C

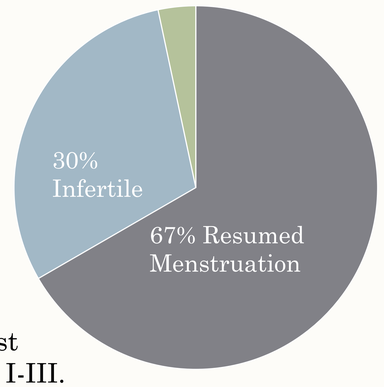
Image C: Patients given goserelin throughout chemotherapy shows the potential success of fertility preservation through goserelin.

Results

In the first study, the menstrual activity was resumed in 21 out of 30 patients (Image C).
In the second study, thirty patients were enrolled and 29 were evaluable. The ages ranged from 29-47. All but one patient received CEF regimen. Menstrual activity returned in 21 patients and menses returned in 16 out of the 17 patients younger than 40 years of age and 5 out of 12 patients over 40 years old[1]. (Image D).
It is prominent that goserelin is only proved effective in estrogen-receptor positive breast cancer patients between stages I-III. Nevertheless, due to the fact that younger patients have a larger ovarian reserve, they might be able to afford some loss in the follicular pool, and immediate ovarian function might not be affected in the short term. However, all patients who receive high-dose chemotherapy will eventually suffer from premature ovarian failure.

Image D

Breast Cancer Patients who received goserelin during CEF Chemotherapy[1]

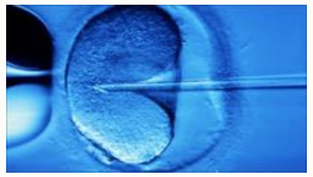


Conclusion

Although there is no concrete proof that goserelin can be used effectively to preserve fertility, it has the potential to provide another option for breast cancer patients undergoing chemotherapy. More clinical studies should be conduced to determine whether or not goserelin can be modified to have a higher efficacy. Normally, patients who undergo chemotherapy face infertility due to the cytotoxicity of the treatment. This discovery, if/when it is successful, can lead to the research of other GnRH Agonist like leuprolide and nafarelin that could annihilate the infertility caused by chemotherapy completely.

Relevance to Biotechnology

If goserelin were to be administered to breast cancer patients throughout chemotherapy, the In Vitro Fertilization (IVF) processes would be a more viable option to all chemo-therapy patients. These options would expand to hysterectomy patients when uterus is no longer viable. Cryopreservation using IVF will be widely exercised with this new technology[3]. Thanks to assisted reproductive technologies (ART's) these trials are possible to conduct. There is a bright future for biotechnology.



IVF as an option for patients[3]

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell for encouraging me as a female scientist. Along with Ms. Patricia Winters and Nina Caudill for organizing every event I participated in. I would like to give a special thank you to my mentor Mrs. Berman and my best friend Julia Padilla for being by my side while I follow my dreams. Most importantly I am thankful for my parents and I give all the glory to God.

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Comparison of Radiation Therapy alone with Combined Therapy of Radiation and chemotherapy in Patients with Advanced Cervical Cancer

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Introduction

This poster will indicate the differences between treatments used in patients diagnosed with advance cervical cancer. The objective of this research is to demonstrate how combined therapy of radiation and chemotherapy has a higher survival rate than radiation therapy alone in patients with advanced cervical cancer.

Abstract

About 12,360 women in the United States are estimated to be diagnosed with cervical cancer this year, and 4,020 (33%) are estimated to die due to the cancer. Many of these women will luckily be diagnosed at an early stage and can undergo surgery or radiation therapy (RT) to destroy the cancerous cells. If diagnosed in later stages, the cancer could have already invaded the surrounding organs such as the upper/lower vagina, rectum, bladder, and upper organs if it has reached the bloodstream. Due to late detection or a patient's age, the patient cannot undergo surgery and due to it being spread, other treatments are necessary. RT was the main treatment for women with late stage cervical cancer but it was only helpful in treating local areas. Chemotherapy treats the cancer with drugs through the bloodstream to eliminate cancerous cells. Combined therapy (CT) is a new treatment that uses both radiation and chemotherapy. In The Radiation Therapy Oncology Group's (RTOG) clinical trial of 386 patients, of these 258 had advanced cervical cancer. 128 were treated with RT and 130 were treated with CT for 43 months. The results are significantly different in which, the survival rate for patients treated with CT was 73% while, the patients treated with RT alone had a survival rate of 58%. Overall, many researchers have discovered through a series of clinical trials has resulted that RT combined with chemotherapy has benefited women with invasive cervical cancer stages IIB - IV, rather than RT alone.

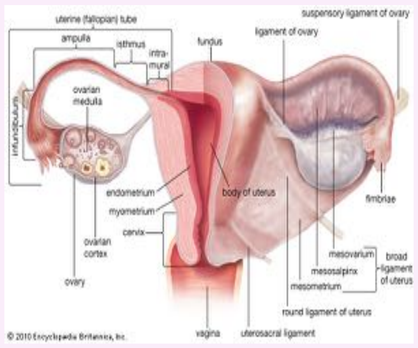

Figure 1: Illustrates the female reproductive system. Encyclopedia Britannica. (2010)

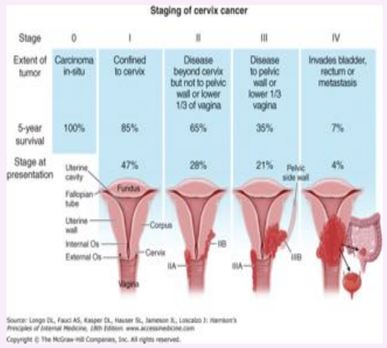

Figure 2: Staging of cervical cancer.

Materials and Methods

One study performed by The Radiation Therapy Oncology Group (RTOG) correlates the survival rates between patients treated with pelvic radiation alone and patients treated with pelvic radiation and concurrent chemotherapy. Out of 388 patients with cervical cancer stages IIB – IVA; 195 were treated with combined therapy and 193 were treated with pelvic radiation. All patients treated with radiation received 85 Gy, 2 cm lateral and 2cm superior to the cervix, and 75 Gy towards the bladder, 70 Gy towards the rectum, and 130 Gy towards the surface of the vagina. In addition to the same radiation dose as the patients treated with radiation alone, patients treated with combined therapy, 16 hours after their radiation dose, they received an intravenous infusion of 75 mg of cisplatin per-square meter of body-surface area over a 4 hour period. Following the cisplatin infusions, 4000 mg of fluorouracil per-square meter over a 96- hour period was infused. This processes occurred in two cycles, from days 1 through 5 and 22 through 26.

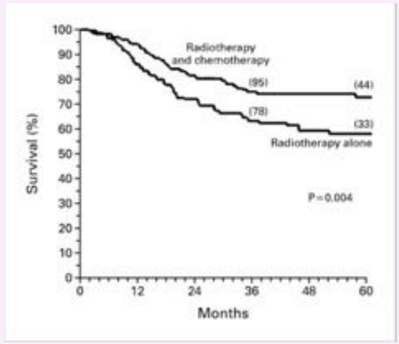

Figure 3: Demonstrates the correlation survival rates between CT and RT alone treatment groups. [3]

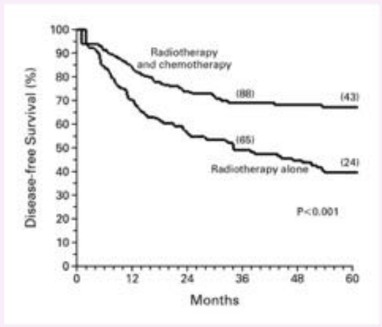

Figure 4: Demonstrates the correlation of the Survival Rates that are disuse-free between the CT and RT alone treatment groups.[3]

Results and Interpretation

The clinical trial took place from 1990 to 1997. The follow up after the patients were given treatment was 43 months. 19 out of the 193 of the patients in the CT group and all 193 of the RT alone group were traced. On the last time of analysis, 147 patients in the combined group and 122 patients in the radiotherapy group were still alive. Only 13 patients from the CT group and 32 from the RT group had recurrent cervical cancer. The data showed that the overall survival rates were 73% with patients treated combined of both chemotherapy and radiation as compared to 58% of the patients who received RT alone. The disease-free survivors that were treated with CT after 5 years was 67% while the patients that were treated with RT was 40%.

Discussion

CT consisting of RT and chemotherapy is more effective at treating advanced cervical cancer than RT alone. Various clinical trials have demonstrated that CT has a higher survival rate and can help a patient have a longer life. CT is a great opportunity for patients with cancer, and its discovery has helped many. Further trials comparing different types of chemotherapy drugs, can be more effective than others when treating cancer. For instance, cisplatin and radiation is more efficient than the use of radiation with hydroxyurena in treating patients with advanced cervical cancer. [5] In the end, it has been demonstrated that RT alongside chemotherapy can be more efficient than radiation therapy alone in patients with advanced cervical cancer.

Relevant Applications to Biotechnology

Radiation has been a great discovery since 1896 that has allowed us to learn more of the human body, and help diagnose and treat patients. Technology had been advancing throughout the years, and new discoveries have made it possible to cure different kids of illnesses. RT became one of the first treatments to eliminate cancerous cells and help cure cancerous patients. As years passed chemotherapy became a treatment that uses drugs in order to destroy cancerous cells and help cure caner. Both of these treatment are great ways to treat cancer individually, but if the cancer is advanced, using both can be more effective. Combined therapy has made a great breakthrough in cancer and is used now to treat patients, in hope of being cured.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell, Dr. Chang, Dr. Saunders, Ms. Winter and many other instructors who have given me the great opportunity to learn so much more science this summer. I would also like to thank my parents and older brother who have supported and helped me through my great journey in The Oncofertility Consortium.

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Analysis of Bioethical Implications on the Applications of Three-Parent In Vitro Fertilization (I.V.F)

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Objective

Mitochondrial diseases have been found to be increasingly involved in several chronic age-related diseases. Since the year of 1999, almost a dozen genes linked to mitochondrial depletion syndrome have been discovered. The purpose of this research is to analyze and understand the influence of bioethics on preventing the use of this assisted reproductive technique (ART). Along with this information, the procedure and benefits of this technique will also be addressed.

Abstract

These rare diseases are formed when mitochondrial DNA (mtDNA) becomes damaged and mutations occur. Three-parent I.V.F takes the pro-nuclei from a fertilized egg that carries mutated mtDNA and transfers it into a fertilized donor egg with "healthy" mitochondria. In 2012, this technique had yet been approved by the Human Fertilization and Embryology Authority to be used on patients, and studies were conducted through polls by *Life* Magazine and other organizations. Some religions don't approve of I.V.F. entirely, saying it's a form of "unnaturalness." People fear the unknown and when hearing this procedure can possibly create "designer babies," are furiously opposed to it. However, researchers argue that the mtDNA contains no critical blueprint that can control how we look, just how healthy we are; and these diseases have been linked to affect high energy using organs such as the heart, muscles, and brain. Theoretically, three-parent I.V.F could eliminate these diseases in certain individuals rather than a population, as many people fear. Thus, seeing how people react, the idea of three-parent I.V.F is opposed, but this technique can potentially save the lives of future generations.

Photo from *Draft Regulations on 'Three Parent' Ivf Published*

Shown here is the photo of an egg with a needle used for ICSI. The needle contains sperm and is injected into the egg.

Methods and Materials

Currently, there are two methods to three-parent I.V.F: pronuclear transfer and spindle transfer. Pronuclear transfer involves the use of a couple's fertilized egg, a fertilized donor egg, and both sets of pronuclei. The pronuclei of the couple's egg, which contains "unhealthy" mtDNA, is transferred to the donor egg that has no mutated mtDNA. The donor egg's pronuclei have been removed and terminated prior to transfer. This method raises more controversy due to the fact embryos are being created specifically for research and financial compensation of ooycte donors. Spindle transfer involves the use of the mother's unfertilized egg and an unfertilized donor egg. This method is less ethically controversial. The nucleus of the mother's egg containing the mutated mtDNA is transferred to the healthy donor egg. Prior to nucleus transfer, the nucleus of the donor egg is removed and terminated. Then, after the transfer, the now healthy egg can be fertilized. But, since the mitochondrial genome contains 37 genes, they are recognized from a third parent, raising many different issues.

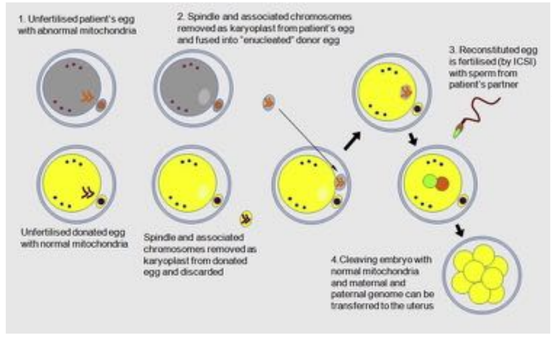

Photo from *Amato, P., et al. S. (n.d.). Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases.*

Shown here is a brief explanation and overview of how the spindle transfer procedure works.

Results and Interpretations

Much controversy is raised regarding these two methods of three-parent in vitro fertilization. Most are centered around the popular view of negative eugenics: discouraging reproduction by persons carrying genetic defects or presumed to have inheritable undesirable traits. This technology is applied to treatment of couples with severe transmittable diseases. This notion can be used to justify the ethnic cleansing that took place in Europe during the Third Reich. Another popular, but negative association, revolves around the eugenics movement in the 1880s, which is orientated with Sir Francis Galton. His focus during the eugenics movement was "through selective breeding, the human species should direct its own evolution." People fear this technique will lead to the procedure of selecting specific traits for non-medical reasons or known as creating "designer babies." However, researchers argue they are too far away to determine whether they can select certain genes located in the nucleus. Their intent is to not have the idea of eugenics associated with classism and racism, but to have the idea of eugenics based on creating a disease-free society. Now, a six-month analysis was conducted by The Nuffield Council on bioethics on issues surrounding this new technique. A few ethical considerations raised were anonymity of donors, relationships between donor parents and children, issues of child identity and sex-selection. The anonymity of donors is being treated the same way as tissue donation, therefore the child wouldn't have the right to know the donor's identity. The Human Fertilization and Embryology Authority stated donor should not have any parental or involvement in the child's life. As for sex selection, any mitochondrial mutations carried with the mother are directly passed on to the child. It is apparent that only girls can pass on these genetic abnormalities. So, in the case mistakes are made during any of the two methods, it should be made that only male embryos are selected, raising more ethical debate.

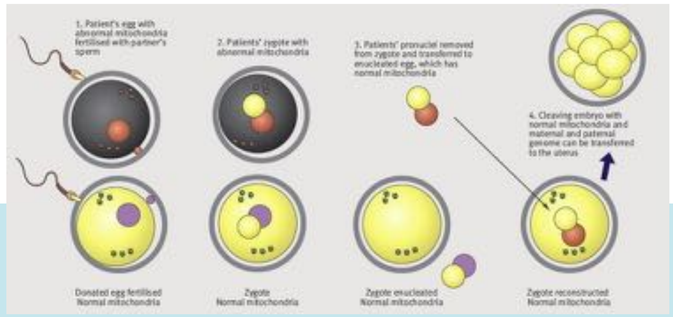

Photo from *Amato, P., et al. S. (n.d.). Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases.* This diagram briefly shows and explains the procedure of pronuclear transfer.

Applications to Biotechnology

Without venture, ARTs would have not been attained or further developed. Three-parent I.V.F has the potential for improving the health and lifestyles of future generations. Using this technique, the prevention of serious, although rare, mitochondrial diseases will significantly increase. These egg cells are undergoing mitochondrial replacement therapy (MRT), causing the germline to change and having the goal of these changes to be passed on the offspring. The procedure of in vitro fertilization is relevant to the biotechnical world with the intent of giving couples the option to have a child. In the case of three-parent I.V.F, these ARTs can be beneficial to the healthcare of individuals.

Conclusion

As a result, the use of three-parent I.V.F has the potential to improve the health of future children. Other situations with three-parent families on reflection include homosexual couples with children, step-parents, and even adoptive families are already seen. The bond shared by genetics is one that can never be broken. But, what about the children who grew up not knowing who their parents were? However, they do have the emotional bond shared with their current parents. That emotional bond is something that can be worth so much more than sharing genetics. There are nontraditional two parent families with better bonds than the average traditional family. Mothers carrying mitochondrial diseases are faced with the option of adoption or risk having a child with critical health problems. The first solution would be to just utilize the method of spindle transfer. It would reduce the number of embryos created which will only lead to destruction. A second solution for families considering this reproductive technique, would be to commit the family to follow-ups to regulate and monitor any unintended consequences. So far, studies have been in place using mice, fruit flies, macaques, copepods, and humans. Mitochondrial replacement I.V.F treated human embryos have survived up to the blastocyst stage; four macaques, disease-free, have been born and were found healthy at three years old. If we are considering the health and social related benefits of living worry free from debilitating diseases, and can still maintain a genetic bond to the parents' children, this ART would be ethical to use.

Acknowledgements

I would love to acknowledge my father who took care of me and made sure I got all the work I needed to done. Without him, I wouldn't be in the situation I am now. I'd love to make him proud and impress him with the things I do.

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Kisspeptin-54: A Novel, Safe Method of Stimulating Ovulation

Ivanna Quiceno

Mater Dei Catholic High School

Objective

When stimulating ovulation in IVF treatments, the widely used hormone human chorionic gonadotropin, hCG, has been shown to cause ovarian hyperstimulation syndrome (OHSS) in some patients. This poster will show that the hormone kisspeptin-54, recently isolated from the peptide kisspeptin, is a better, safer choice of hormone to use in comparison to hCG when administered.

Abstract

Infertility cases are treated through different treatments, such as in vitro fertilization (IVF). Before the woman achieves oocyte pickup however, she might have to take the hormone, human chorionic gonadotropin (hCG), to stimulate egg maturation. hCG can be harmful to the ovaries, so the hormone kisspeptin-54 also used to stimulate ovulation may be safer for women undergoing IVF. Kisspeptin is normally present during a healthy pregnancy and stimulates egg development and the release of female sex hormones in the human body. In a recent study, patients were given a single injection of kisspeptin-54 to induce a luteinizing hormone surge and egg maturation. Of the 53 women's eggs in the study conducted by the Imperial College London, 51 developed into embryos. These results were accomplished using kisspeptin because in some women, hCG directly targets the ovaries without any regulation causing them to be over stimulated, which can potentially be life threatening. Ovarian hyperstimulation syndrome (OHSS), where the ovaries become painful and swollen, can lead to kidney failure and more. Therefore using a different effective hormone would be safer to trigger egg maturation during the IVF process. Kisspeptin-54 does not directly target the ovaries as hCG does, making it less likely to cause OHSS. At the end of the same study, 12 of the women became pregnant without observable negative side effects. In conclusion, the hormone kisspeptin-54 may prove to be safer for woman undergoing IVF. It will also be helpful in the field of oncofertility because it is a less harmful hormone than hCG while stimulating ovulation.

Materials and Methods

In this study, there were 53 eligible female patients age 18 to 34 years old who received a single IVF treatment cycle. They were first given an antagonist to prevent premature ovulation and then they were administered a single subcutaneous injection of the hormone, kisspeptin-54 after three ovarian follicles of 18 mm diameter were visible visible on an ultrasound. (This hormone was purified and synthesized, put into vials, and stored at -20°C and reconstituted in 0.9% saline prior to its injection into the females). The kisspeptin-54 was administered to induce a luteinizing hormone surge and egg maturation, and the females in the study were given different dosages. In the first phase of the study, nine women were given the three lowest doses of kisspeptin-54 (0.4, 0.8, and 1.6 nmol/kg). In the second phase of the study, the next nine women were given three higher doses (3.2, 6.4, and 12.8 nmol/kg). Before these kisspeptin injections however, injections of FSH were stopped 12 hours prior in order to minimize the effect that the GnRH antagonist had on the kisspeptin-54. Then, 36 hours after the kisspeptin injection, the eggs were extracted transvaginally, assessed for maturation, and then finally fertilized by sperm injection which led to the creation of one or two embryos.

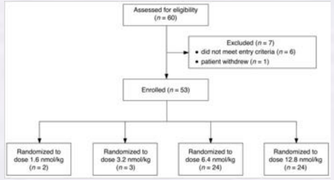

Figure 1. The amount of patients in the study that were administered kisspeptin-54 to induce egg maturation during IVF therapy[1]

Results

This study proved that kisspeptin-54 would be a viable substitute for the hormone hCG in order to stimulate ovulation in women undergoing IVF treatment. After the single injection of kisspeptin-54, egg maturation was observed in every patient. While it was found to be difficult to collect the eggs in the patients with the 3.2 nmol/kg dose of kisspeptin-54, there were still good egg maturation statistics in the patients, with 75-85% mature recovered eggs in all of the kisspeptin-54 doses. During the study there was also found to be surges in reproductive hormonal secretions in the 12 hours following the kisspeptin injection. Kisspeptin peaked an hour after injection and then fell to pre-injection levels after 12 hours. LH levels peaked 4-6 hours following, FSH and estradiol showed some patterns of peaking, and progesterone levels continuously rose for 12 hours. However, these different peaking levels did not affect the pregnancy rates as fertilization occurred in 92% of patients. The rate of embryo transfer was 92%, and the high-quality embryo transfer occurred in 58% of patients. This led to 12 women achieving clinical pregnancies with a pregnancy rate of 23%. Ten women gave birth to healthy babies while 2 women had miscarriages for reasons not associated with kisspeptin-54. The responses seemed to be dose related with better results as the doses were increased. Overall, kisspeptin-54 was well received in all 53 of the patients and did not cause OHSS.

	Kisspeptin-54 dose (nmol/kg)			
	1.6 ($n = 2$)[a]	3.2 ($n = 3$)[a]	6.4 ($n = 24$)[a]	12.8 ($n = 24$)[a]
2PN fertilized oocytes	2, 0	5, 1.2	5.1 (2.9)	6.8 (3.8)
Cleaved embryos at day 3	2, 0	5, 1.2	5.0 (2.9)	6.3 (3.4)
Embryos at day 3 graded as 633 or above	0, 0	5, 1.1	3.8 (2.7)	4.5 (2.7)
Patients with day 5 transfer (n)	0	1	18	17
Embryos at day 5[b]		4	5.2 (2.0)	6.9 (2.3)
High-quality embryos at day 5[c]		3	1.9 (1.4)	2.4 (1.7)
High-quality embryos (> 3A/B) transferred at day 5[c]		1	1.3 (0.8)	1.5 (0.7)

[a]List of raw values for individual subjects, unless otherwise stated. [b]Mean (SD), unless otherwise stated. [c]Calculated using only patients with day 5 transfer.

Table 1. The egg maturation in patients after receiving different doses of kisspeptin-54[1]

Conclusions

Studies have now proven that kisspeptin-54 is a reliable hormone to trigger egg maturation in IVF patients resulting in pregnancy rates comparable to those when using hCG. Since the hormone kisspeptin is found naturally in the human body as a peptide hormone that triggers ovulation, it was not difficult to transition into the use of kisspeptin-54. After all, kisspeptin is found in large amounts with elevated levels lasting the whole nine months during a normal human pregnancy. Therefore, this is more of a natural hormone and was found to have no side effects. This hormone as opposed to hCG which is the commonly used hormone to trigger egg maturation. However, the problem with hCG is that it stimulates the ovaries by binding directly to the LH receptor without a regulator. This causes an overstimulation of the ovaries which can lead to OHSS. As a result, GnRH agonists have been used to stimulate ovulation as they are able to stimulate the GnRH receptor on the pituitary gland to release LH. However, while GnRH has been found to have lower rates of OHSS, it also has been associated with lower pregnancy rates when compared to hCG. Kisspeptin-54 generates a LH surge by activating the hypothalamus and when used as a trigger for egg maturation, results in peak levels of serum LH. This ideal fertilization situation created by kisspeptin led to a blastocyst formation rate of 49.4%. In conclusion, kisspeptin-54 is able to induce egg maturation in women undergoing IVF therapy just as well as the other two hormones without any risk of OHSS and with a high pregnancy rate.

	Kisspeptin-54 dose (nmol/kg)			
	1.6	3.2	6.4	12.8
Patients treated	2	3	24	24
At least 1 egg collected	2	3	23	24
At least 1 M2 egg	2	3	22	24
At least 1 fertilized egg	1	3	22	23
Embryo transfer	1	3	22	23
High-quality embryo transfer	0	1	15	15
Biochemical pregnancy at 12 days	1	1	11	8
Clinical pregnancy at 6 weeks	1	0	7	4

Number of successes are shown. Doses of kisspeptin-54 are in nmol/kg.

Table 2. The response to the treatment of hormone kisspeptin-54, and the progression of pregnancy following IVF treatment[1]

Relevant Applications to Biotechnology

Kisspeptin-54 will be able to help the IVF treatments run more smoothly and without as many complications. This is because kisspeptin does not cause OHSS and has a high pregnancy rate, which will contribute to the effectiveness of the whole IVF process. In the past, complications such as OHSS have caused the patients to have to stop treatments, thus prolonging their fertility process. However, with kisspeptin this problem is practically eliminated as there is no possibility of contracting OHSS.

Acknowledgments

I would like to thank Dr. Ericka for always being so full of energy and making me excited to learn, and for being the great big sister and role model. I would also like to thank Dr. Saunders for helping me with my abstract and poster and Ms. Winter and Miss Nina for helping all the sessions run smoothly making them very enjoyable. I would like to thank my family for always pushing me to do my best in everything that I do. Last, but not least, I would like to thank my OSA sisters for being an amazingly smart and beautiful group of girls to spend my summer with.

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The Comparison of the Possible Advantages of IVM over Traditional IVF in Women with Polycystic Ovarian Syndrome (PCOS)

UC San Diego Health Sciences

Joanna Rodriguez

Kearny International Business

the Oncofertility™ Consortium Saturday Academy

Objective

Polycystic Ovarian Syndrome (PCOS) is an endocrine disorder which is present in about 5-10% of females of reproductive age.[2] It is a hormonal imbalance, and causes the ovaries to make more androgens (male hormones) than normal, and the development and release of eggs during ovulation can be affected; there can also be a high risk of Ovarian Hyperstimulation Syndrome (OHSS). The objective of this poster is to compare In-vitro fertilization to In vitro maturation, and compare the outcomes of each fertility treatment for women with PCOS resulting in a full term pregnancy and healthy child.

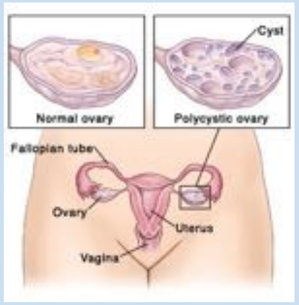

***Figure 1:** A comparison of a normal ovary to a polycystic ovary.

Abstract

One out of eight couples have difficulty conceiving or sustaining a pregnancy, and one-third of infertility is in account of the female. IVF is a common fertility treatment women undergo hoping to become pregnant; IVM is another fertility treatment, but not much data has been gathered about the success of the treatment. IVF and IVM have the same goal: treat infertile women into becoming pregnant. IVF patients′ eggs are retrieved at a mature state; meaning that prior this stage, they have to constantly inject themselves hormones which, with PCOS patients, may lead to OHSS; IVM patients′, eggs are retrieved at an immature stage, the patient then doesn't have high risks of developing OHSS. A number of studies show the benefits of women undergoing IVM treatments in comparison to IVF; becoming pregnant under the circumstances may not be as hard as maintaining a full term pregnancy with a live baby. Full concrete studies haven′t yet been found, although there are many more treatments to help women with PCOS. In addition, IVM has a potential application in women desiring fertility preservation after cancer treatment without exposing them to high levels of estrogen in similar cycles. One can then infer that IVM has a higher outcome for potential Oncofertility advancement and innovation for improved patient care and un-met medical need.

Methods and Materials

A study conducted from January 2005 to December 2009 compared IVM and IVF with patients with PCOS; patient′ s were grouped by age, closest date of treatment, and number of attempt.[4] IVM was the initial offer and IVF was the alternative treatment throughout this stage.[4] IVM patients were to reach about 6mm of endometrial thickness or a leading follicle of 10mm to 12mm, when one of both conditions were met, 10,000 IU hCG; 8 hours later oocyte retrieval was scheduled beginning with the largest follicle.[5] In a second study 104 women with PCOS underwent their first IVF treatment between 2002 and 2009, their study measured cumulative live birth in regards to IVF; the studies conducted on the IVF results of the group resulted in live birth.[3] One group was made up of 104 women with a history of PCOS, and fertility treatment was conventional.[3]

A third similar study also searched for a "superiority" between IVM and IVF in women with PCOS; it was a study conducted between January 2009 and December 2011.[5] The patients who underwent IVF, had an embryo transfer done between days 2-3 of induced bleeding.[5] Patients who underwent IVM, however, had a performed protocol of unstimulated cycle- minimal gonadotropin. When endometrial thickness exceeded 6mm/ follicle had a diameter of 10-14mm, hCG was administered to the patient 38 hours before oocyte retrieval; oocytes were then matured in a medium to mature.[5]

Table 1.
Comparison of IVM to IVF cycles in PCOS patients.

	IVM (n = 108)	IVF (n = 108)	p
Age	32.9 ± 4.1	33.5 ± 4.7	NS
No. of oocytes collected*	17.09 ± 13.6	19.4 ± 9.5	NS
Total No. of MII (matured) oocytes*	10.5 ± 6.5	15.3 ± 8.8	<0.0001
Day 0 MII oocytes*	2.2 ± 2.9	15.3 ± 8.8	<0.0001
Day 1 MII oocytes*	5.2 ± 3.6	–	–
Day 2 MII oocytes*	3.0 ± 3.7	–	–
Fertilization rate (%, ± SD)	70.2 ± 20.2	68.7 ± 22.1	NS
Cleavage rate (%, ± SD)	92.4 ± 13.0	95.2 ± 11.7	0.03
No. of embryo transferred*	3.4 ± 0.8	2.8 ± 1.0	<0.0001
Implantation rate (%)	60 (16.1)	64 (21.6)	0.07
Pregnancy rate (%)	52 (48.1)	48 (44.4)	NS
Clinical pregnancy rate (%)	48 (44.4)	45 (41.6)	NS
Twin pregnancy rate (%)	8 (15.3)	17 (35.4)	0.02
Triplets or quadruplets rate (%)	2 (3.8)	1 (2.0)	NS
Clinical miscarriage rate	19(17.5)	18(16.6)	NS
Delivery rate (%)	29 (26.8)	27(25.0)	NS

* Mean ± SD.

***Figure 2:** Table 1 shows the pregnancy rate, delivery rate, and clinical miscarriage between IVM and IVF.[4]

Table 2.
IVM embryologic and cycle outcome data 2005–2009.

	2005	2006	2007	2008	2009	p**
Number of cycles	39	77	60	66	68	–
Age*	32 ± 2	33.1 ± 2.2	32.9 ± 0.6	33.2 ± 0.2	32.2 ± 3.7	NS
Eggs collected*	19.4 ± 4.5	16.6 ± 0.4	19.8 ± 14.2	18.6 ± 8.9	16.8 ± 9.4	NS
Matured eggs*	13.3 ± 3.7	10.8 ± 1.5	13.8 ± 10	11.7 ± 4.7	10.0 ± 4.8	NS
M2 at collection day*	1.2 ± 0.8	1.3 ± 0.9	2.3 ± 1.6	1.8 ± 1.2	2.1 ± 1.9	0.003
M2 at day1*	7.1 ± 0.1	5.5 ± 3.1	7.2 ± 5.1	4.2 ± 0.9	5.1 ± 3.5	0.021
M2 at day 2*	4.8 ± 2.7	4 ± 0.7	4.2 ± 5.5	1.9 ± 0.6	2.7 ± 2.8	0.007
Fertilization rate (% ± SD)	49.9 ± 2.6	70.3 ± 4.6	71.5 ± 12.5	67.2 ± 9.0	69.9 ± 24.6	0.001
Cleavage rate (% ± SD)	89 ± 7.7	88.4 ± 1.0	91 ± 0.6	92.4 ± 5.3	93.2 ± 13.2	NS
Embryos transferred	3.6 ± 0.9	3.6 ± 0.9	3.6 ± 0.9	3.5 ± 0.3	3.4 ± 0.6	NS
Implantation rate (%)	11/142 (7.7)	27/281 (9.6)	25/214 (11.6)	26/231 (11.2)	36/199 (18)	0.005
Chemical pregnancy rate (%)	15/39 (38.4)	27/77 (35)	28/59 (47.4)	26/65 (40)	36/61 (59)	0.04
Live birth rate (%)	6/39 (15.3)	12/77 (15.5)	15/59 (25.4)	14/65 (21.5)	23/61(37.7)	0.01

NA; not available; NS; not significant.

* Mean ± SD.

** When comparing 2005 and 2009 only.

***Figure 3:** Table 2 shows the live birth rate for IVM throughout the study period.[4]

Results

In the first study, the pregnancy rate between both fertility treatment results were comparable.[4] In figure 2, the pregnancy rate for PCOS patients is higher for IVM in comparison to IVF patients; a twin pregnancy rate is higher for IVF, and for triplets or quadruplets the number is higher by 1% for IVM.[4] Although IVM has a higher chance of miscarriage, it also has a higher rate of delivery rate than that of IVF.[4] In figure 3, the live birth rate for PCOS patients undergoing IVM increases throughout the years of the study.[4] In the second study, researchers concluded and backed up the first study's information about PCOS patients having a higher miscarriage rate under IVF.[3] In the third study, the data collected included age, infertility diagnosis, and hormonal level as well as antral follicle counts.[5] A 1,52 cycles of IVM and IVF were overall evaluated throughout the study.[5] The mean number of oocytes retrieved were significantly lower in the IVF group in comparison to IVM.[5] Results obtained in this study indicated that IVM is a simpler procedure as well as safe for women with PCOS; IVM can avoid long-term and short-term complications.[5]

Conclusion

The conducted studies have debated the issue of the fertility treatments, IVM and IVF, for women with PCOS. Studies have researched the outcomes of IVM and IVF, live births, and miscarriages. PCOS patients wishing to undergo a fertility treatment have a better option with IVM. For one, the risks of developing OHSS is much less likely with IVM in comparison to IVF. [4 5] Without the use of hormonal injections, like the ones used in IVF, there is a much more positive alternative for PCOS patients.[4] IVM has had lower success rate compared to IVF ten years prior to the first study first conducted in 2005; however, after the end of the study in 2009, studies demonstrated improving outcomes from IVM.[4] The third study concluded that the fertilization rate and quality of embryos were higher in the IVF by more than 10%; the implantation rate was higher in the IVM group (22% vs 10%).[5] In vitro maturation is a better medical fertility treatment for patients with PCOS due to its lower risks of developing OHSS.[5]

Applications to Biotechnology

In vitro maturation has promising outcomes as an alternative to conventional IVF treatment for women with PCOS.[3] Many of these future outcomes are possible because of many innovative technology which can be used to better the outcomes of patients. Women with PCOS will have a lot more of positive outcomes in the future due to improvements of dealing with PCOS and their fertility treatments. Greater expectancy of the improvements are due to biotechnology and researchers behind it. In vitro maturation is a better medical fertility treatment for patients with PCOS due to its lower risks of developing OHSS, its live birth rates, and the low cost of the treatment; research projects are said to be aiming in closing the gap between IVM and IVF.[5] IVM offers an important place in women with oocyst preservation in Oncofertility in the future.

Acknowledgements

I would first like to thank Dr. Senegar-Mitchell, Mrs. Winter, and Nina Caudill for being an incredible help throughout the academy. I would also like to thank all the doctors who were very welcoming and made us feel apart of the team, and the CHOC team in Orange County for having us and giving us so much. My OSA sisters for being so helpful and friendly throughout the Oncofertility Science Academy, we learned incredibly amazing information. Lastly, I would like to thank my parents for giving me continuous encouragement to do the best I can, and to not give up, even when everything seems so dark.

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Correlation Between the Drug Accutane and the Affects on the Pituitary levels and Fetal Development

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NiNa Tabrizi

Objective

Accutane has been routinely prescribed to men and women for the treatment of acne since 1979. Side effects, associated with its use, manifested as malformations in children whose mothers were prescribed the medication while pregnant. The number of miscarriages also increased. The objective of this research is to understand the adverse effects of the drug Accutane, also known as Isotretinoin and to investigate how it alters pituitary and gonadal hormone secretion and how it produces malformations in humans.

Abstract

Accutane, also known as Isotretinion, is a drug licensed to treat severe acne cysts since 1979. It is taken orally over 20 weeks and there was a correlation with the women exposed to the drug in their first trimester and their children who were born with major birth defects. Within a year and a half, a correlation between taking the drug and congenital malformations appeared. This syndrome consists of a set of malformations in the central nervous system, head and face, and heart. The LH and FSH, GH (growth hormone), testosterone, and prolactin lowered.[4] The FDA reported infants born with internal and external abnormalities and malformations. These abnormalities have occurred in 42 percent of infants who's mothers took Accutane. [5]

Materials and Methods

There were many studies on this subject performed on both pregnant and non pregnant women, and men. One study focused on how Isotretinoin influences pituitary hormone levels in acne patients. A correlation between taking Accutane and the effects on the pituitary levels in both males and females was also discovered. The LS and FSH in males was lowered. In men and women the GH, LH, prolactin, and FSH was lowered. In regards to estradiol, progesterone, and 17- hydroprogesterone, their was no change. [5] Besides this research, there is some contrary to it stating that pituitary, adrenal, and gonadal hormones are not changed. There was a 4 week study where there were no marked changes in any of these hormones, although there was a decrease in precursor androgens, androstenedione, and testosterone in 6/9 patients. The study also showed that there was no elevated LH/FSH in a patient with polycystic ovarian syndrome.[6] Although the information is contrary to the first study, there is more evidence supporting that specific pituitary hormones are lowered. In 14 pregnancies where the mother was prescribed Accutane, but no pregnancy tests were taken beforehand, resulted in four live infants with no birth defects, one live infant with multiple defects, four spontaneous abortions, and five induced abortions. [2] Other women on Accutane were asked to enroll in the Boston University Accutane Study (BUAS). Over the study period from 1989 to October 1999, 500,000 women enrolled and 958 of the women became pregnant. 834 of these pregnancies ended in termination, either elective, spontaneous, or due to ectopic pregnancy, 110 resulted in live births, 14 had unknown outcomes, and of the 60 with medical records, 8 had congenital abnormalities.[1] Another study was done on hamsters regarding the abnormalities that occur from taking Isotretinoin. In their research they used a scanning electron microscope (SEM) to check the embryonic and fetal hamsters craniofacial structures at 4, 8, 12, 24, 48, and 72 hours after administering the drug. Using SEM they could observe embryonic and fetal craniofacial structures as well as embryos recovered 4 hours after treatment. Craniofacial damage was obvious within 8-12 hours showing a collapse of the forebrain.[3] The forebrain is the largest part of the brain, mostly consisting of the cerebrum, which is needed for perception, memory, and higher thought process. There was also a low number of incidences consisting of cleft lip, a facial malformation. These results showed that Isotretinoin in hamster embryos was unsafe. This information converts to humans, because animal trials are always taken before the FDA approves them for humans.

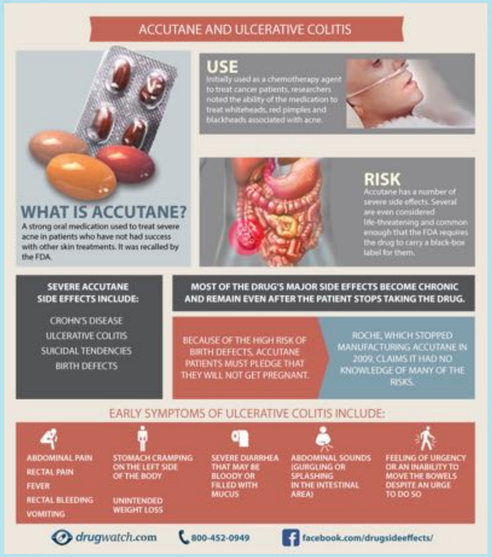

Figure 1 (Left): Information about the drug Accutane, provided by Drugwatch.com

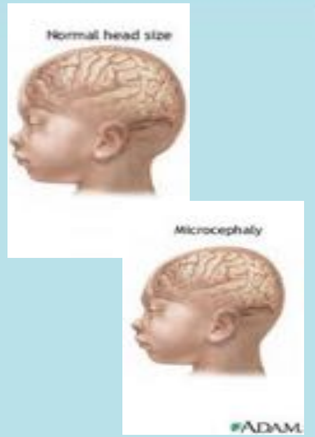

Figure 2: Top is a normal baby head. Bottom is a baby born with microcephaly, which is one of the deformities that can occur when a mother takes Accutane.

Figure 3: This chart demonstrates the difference in pituitary hormones of both men and women. [4]

	Pretreatment Mean ± SD or Median (IR)	Posttreatment Mean ± SD or Median (IR)	*p*-value
Estradiol (pg/ml)	84.0 (75.0)	67.0 (95.5)	0.615
FSH (mIU/ml)	6.0 ± 3.6	5.9 ± 2.5	0.874
LH (mIU/ml)	8.1 (11.6)	6.7 (6.0)	< 0.02
Prolactin (ng/ml)	13.3 ± 5.7	11.6 ± 4.4	< 0.02
Progesterone (ng/ml)	1.9 (3.8)	1.5 (2.6)	0.791
17-hydroxyprogesterone (ng/ml)	2.3 (2.1)	2.3 (2.2)	0.191
Total testosterone (ng/ml)	0.7 (0.4)	0.5 (0.4)	< 0.005
DHEAS (µg/dl)	217.6 ± 104.9	205.6 ± 80.0	0.354
Free testosterone (pg/ml)	2.9 (2.0)	2.5 (1.1)	0.338
SHBG (µmol/l)	40.6 ± 19.8	51.7 ± 34.1	0.055
Cortisol (µg/dl)	13.1 ± 5.2	11.0 ± 5.8	< 0.005
ACTH (pg/ml)	28.9 ± 17.0	24.9 ± 13.6	< 0.05

Results and Interpretations

The overall results of this information shows that the pituitary hormones are lowered and there are children with malformations. In a study using a scanning electron microscopes (SEM) to see how Isotretinoin affects fetal hamster craniofacial structures, scientists discovered that after administering 50 mg/kg of Isotretinoin to hamsters that within 8-12 hours there was hypoplasia of the maxillary and mandibular processes of the first branchial arch, a rudimentary second arch, and apparent collapse of the forebrain.[3] In another children born with Isotretinoin syndrome often demonstrated serious external ear abnormalities, including microtia (small ear), anotia (no ear), or stenosis of the external ear canal; micrognathia (small jaw); a flat depressed nasal bridge, and ocular hypertelorism (widely spaced eyes).[1] Alongside that there are internal malformities. The results proved that taking Accutane while pregnant results in birth defects including brain, heart, and face deformities. The FDA has rated this drug as X drug because of the dangers it has shown in pregnant women, but has not discussed the changes in pituitary and gonadal hormones. This research shows that the LH, FSH, testosterone, prolactin and GH all decreased. The levels of estradiol, progesterone, 17-hydroprogesterone, and FSH did not change.[5] Although there is some information contradicting this, there is more evidence supporting it.

Conclusions

From these results it can be concluded that the drug Isotretinoin, commonly known as Accutane, does influence hormones in the body and malformations of humans. This research shows that the LH, FSH, testosterone, prolactin and GH all decreased. [4] This was proven by recording results from pregnant and non-pregnant women, and men who were prescribed Accutane. All this information was used by the FDA to rate the drug X, further meaning that it should not be taken by pregnant women, because of its effects on the embryo that result in internal and external abnormalities such as cleft palate, missing ears, facial dysmorphism, and central nervous system malformations.[2] It was also discovered that the change of miscarriage also rises when taken during pregnancy. All these incidences were later known as fetal Isotretinoin syndrome.[1]

Relevant Applications to Biotechnology

This information is important in helping understand what Accutane does to women when it is taken during pregnancy. The effects it has on children is drastic and irreversible. It helps doctors make accurate health care decisions for their patients and at the same time they are avoiding life threatening malformations in their patients embryos. This research further allows scientists know how Accutane affects hormones in both males and females who receive the drug orally over 20 weeks. This will allow healthcare professionals explain the changes their patients are feeling. This has made it necessary for scientists to make acne medications made with benzoyl peroxide which can be used to treat acne during pregnancy.

Acknowledgments

I would like to first thank Ms. Winter, without her dedication to this program it would not continue to thrive. I would also like to thank Dr. Ericka, Dr. Saunders, Dr. Chang, and the other educators for their help with my scientific endeavors. Without them I would not have had this amazing opportunity to learn and experience what being a research scientist really is . I would like to thank my OSA sisters for being supportive and helping me through out this adventure and lastly to my parents for getting me out the door with food. I've learned so much this summer and have had an unforgettable time.

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The Possible Risk of Epithelial Ovarian Cancer Due to Increased Levels of Interleukin-6 in the Ascitic Fluid in OHSS Patients

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Scripps Ranch High School

Objective

Infertile women seeking IVF treatment may have an increased risk of ovarian cancer. Basic research shows that patients, who suffer from OHSS, a severe, but fortunately rare, side effect of IVF treatment where fluid from the ovaries escapes into the abdominal cavity, might be at risk of epithelial ovarian cancer (EOC). This may be caused by the high amount of proteins present in ascites which are fluid cavities found in the peritoneal regions of chronic OHSS patients. One protein in particular, Interleukin 6 (IL-6), might have a major role as it is present in both OHSS and EOC. The objective of this poster is to demonstrate the correlation between the excessive amounts of IL-6 resulting from OHSS and the risk of EOC by presenting and comparing results from several studies.

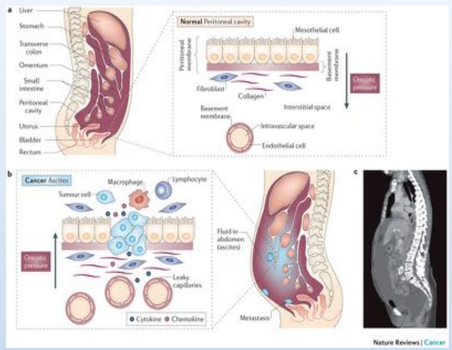

Figure 1. The diagram above shows the difference in the biology of a normal peritoneal cavity and a peritoneal cavity with ovarian cancer. As seen in (b.), as malignant ascites form, there is more leakage of fluid. High protein concentration, which includes cytokines such as IL-6, can aid in changing the peritoneal membrane, reducing the oncotic pressure difference and allowing more fluid to enter the peritoneal cavity. Retrieved from http://www.nature.com/nrc/journal/v13/n4/fig_tab/nrc3432_F1.html

Abstract

Ovarian hyperstimulation syndrome (OHSS) is a consequence of IVF treatment in which a patient suffers from fluid escaping her ovaries. A common symptom of severe OHSS is the formation of ascites which contain proteins such as IL-6. During OHSS, high amounts of IL-6 can be produced by vascular endothelial growth factor (VEGF), which may contribute to angiogenesis of epithelial ovarian cancer (EOC) tumor cells. The objective of this poster is to demonstrate the correlation between the excessive amounts of IL-6 resulting from OHSS and the risk of EOC by comparing results from several studies. A study has found that the IL-6 receptor found in ascites can lead to signaling pathways which can lead to EOC angiogenesis. By using enzyme-linked immunosorbent assay, a study has shown a 37.5-fold increase in the amount of IL-6 in the ascitic fluid of EOC patients. Several studies tested the amount of IL-6 present in blood serum and ascites of EOC patients. One such study found that tumor size increased as levels of IL-6 present rose in ascites of EOC patients. From this research, it can be concluded that there may be a correlation between the amount of ascites due to the presence of IL-6 formed during OHSS and the risk of subsequent EOC prompting the need for further research. If there is a risk of EOC, then it is necessary for patients to know prior to starting IVF treatment. Research for preventing the formation of ascites during IVF treatment should be conducted as well.

Methods and Materials

There have been several studies focused on the significance of the amount of IL-6 present and the subsequent growth of epithelial ovarian cancer tumors. One of these studies measured the levels of IL-6 in 39 recently diagnosed EOC patients by using enzyme-linked immunosorbent assay (ELISA) tests. Another study used several assays such as an intravasation assay to determine whether IL-6 signaling pathways can lead to the progression of malignant tumors. Several other studies have been done to determine the difference between the levels of IL-6 in ascitic fluid and serum fluid. One of these studies collected blood samples from patients with epithelial ovarian cancer to determine levels of IL-6 by using ELISA tests as well. Another compared serum fluid and ascitic fluid retrieved from 70 recently diagnosed but untreated EOC patients before they entered their first surgery. The IL-6 levels were measured with a B9 bioassay.

Results

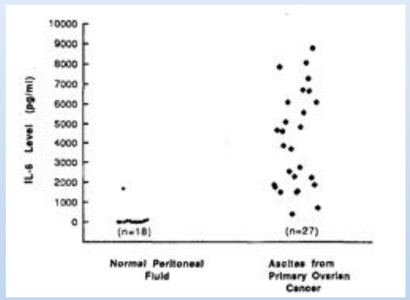

Figure 2. This scatter plot compares the levels of IL-6 levels in normal peritoneal fluid and in ascites from primary ovarian cancer. There is a significant increase. (Carson, L. F., *et al.* 2006.)

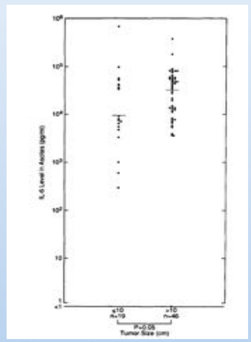

Figure 3. This scatter plot compares the levels of IL-6 with tumor size. (Federici, M. G., *et al.* 1994.)

Overall, the studies seemed to agree that IL-6 is present in excess in the ascites of EOC patients. One study concluded that IL-6 levels increased in EOC patients since the levels of IL-6 present in the ascites ranged from 408 to 8908 pg/ml. In control serum, however, the median range was 0 pg/ml. Another study has shown an average increase of 37.5-fold in the amount of IL-6 in the ascitic fluid of ovarian cancer patients. Also, a study discovered that tumor size increased as the levels of IL-6 rose (Fig. 3). Likewise, the volume of ascites was found to be increased as more IL-6 was present. Interestingly, increased levels of IL-6 were found in the ascitic fluid, and not in the blood serum. This can prove to be detrimental as one study has concluded that the IL-6 receptor, sIL-6α, found in ascites can lead to the production of IL-6 signaling pathways. Furthermore, another study found that the more sIL-6Rα present in ascites, the more IL-6 trans-signaling occurred on endothelial cells. The receptor for IL-6, sIL-6Rα, showed signs of preventing apoptosis, or cell death, ensuring the survival of endothelial ovarian cancer cells. In another study, EOC patients with median IL-6 levels greater than 2662 pg/ml showed shorter progression-free survival, meaning the cancer began to metastasize sooner. Data suggests that there is a correlation between increasing amounts of IL-6 signaling pathways and the progression of malignant tumors.

Discussion

From this basic research, it can be concluded that there could be a correlation between the amount of ascites due to the presence of IL-6 formed during OHSS and the subsequent risk of EOC prompting the need for further research. If there is a risk of EOC, then it is necessary for patients to know prior to starting IVF treatment as it is the duty of medical practitioners to ensure the safety of their patients. Furthermore, research for preventing the formation of ascites during IVF treatment should be conducted as well since IVF treatment has been a great asset in fertility treatment for many years.

Application to Biotechnology

As the live birth rate for IVF increases, more women are choosing to undergo IVF treatment. Some of these women develop OHSS and as a result, ascites may form in their peritoneal cavities. If there is a risk of EOC due to the ascites formed during OHSS, then the safety of the patient is undermined. More research should be done to ensure that ascites don't form during IVF treatment.

Acknowledgments

I would like to thank everyone involved with the Oncofertility Science Academy for taking the time to realize the importance of young women in science and for giving me an opportunity to learn. This academy has changed the way I look at the world. A special thanks to Dr. Senegar-Mitchell for being a wonderful mentor and teaching me how important it is to embrace who I am as a person. I would also like to thank Dr. Saunders, Mrs. Winter, Nina Caudill and my OSA sisters for their constant support and guidance.

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The Correlation between Etanercept and Infertility Linked to Endometriosis

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San Marcos High School

Objective

Endometriosis, of unknown origin and unpredictable course, mainly strikes in the reproductive years. It occurs when the tissue typically lining the endometrium grows elsewhere in the body. The only symptom for many of the 10-15% women who have endometriosis is infertility. Though endometriosis patients may be fertile, 24-50% of infertile women may have endometriosis. With endometriosis related infertility, there is a 2-4.5% conception per month, compared to 15-20% among normal couples. It is not known how Stage I or II of endometriosis impede fertility, since reproductive physiology is typically normal. Subtle changes including overproduction of tumor necrosis factor-a (TNF-a), an inflammatory cytokine, are associated with embryotoxicity and other effects which may cause infertility.[2 5] A possible treatment option for endometriosis associated infertility may be TNF-a inhibitor, including etanercept. Etanercept (Enbrel) is a soluble TNF-a receptor and is already used for certain inflammatory disorders. The objective of this study is to evaluate the correlation between the administration of etanercept and decreased infertility with mild and moderate endometriosis through TNF-a inhibition.

Abstract

TNF-a, an inflammatory cytokine, is consistently linked to endometriosis, a common cause of infertility. Current treatments for endometriosis may have deleterious effects, including early menopause. Etanercept, a soluble TNF-a receptor and potential treatment, correlates with reductions of endometriotic red lesions in baboons[1]. The objective of this study is to evaluate if etanercept correlates with decreased rates of endometriosis (stages I and II) associated infertility through TNF-a inhibition in animals. In two studies [8 3] of pregnant rats treated with etanercept, each group was given a substance known to boost TNF-a production, either lipopolysaccharide or shiga toxin type 2, to measure fetal death and preterm delivery rates, respectively. In another study [4] lesions surgically implanted in rats were measured for size, surface area, and nitrogen oxide levels during treatment with etanercept. The results, respectively, were reductions in rat fetal deaths and decreased rat preterm deliveries. Also, nitrogen oxide levels as well as the size and numbers of lesions were reduced. Etanercept reduces TNF-a's fetal apoptotic effects. It reduces levels of nitrogen oxide, high levels of which are linked to infertility. Etanercept's effects on infertility have not been tested enough in humans, including correlations with gametes, ovulation, embryotoxicity, and endometriosis. Etanercept and endometriosis associated infertility may be adversely correlated in animals. More testing is required before establishing etanercept as an infertility treatment for mild or moderate endometriosis in humans.

Methods and Materials

In two studies, rats pregnant for 14.5-15 days (about 3 per group) were given etanercept (10 mg/kg) 6 hours before administration of LPS (100 µg/kg in saline),[8] or injection with 0.5 ml culture supernatant from recombinant E. coli at a dose equal to 0.7 ng Stx2 or 50 pg LPS/gram, respectively.[3] 3 days later, the dams in the first group were killed and measured for the number of viable fetuses compared to dead and resorbed fetuses. The second group was observed daily until all dams had established delivery time and fetal status, which were recorded. TNF-a concentrations in both groups were measured by ELISA. 41 other rats were surgically given endometriosis.[4] 21 days later, implants were surgically checked for viability through inspection and surface area measurements. When divided into groups, some of the rats were given etanercept at 2.016 mg/kg twice every three days. In the third laparotomy, they were killed and implants were measured for surface area. They were then removed and measured for nitrate and nitrite levels by Griess reaction. All the studies involved animals that were otherwise physiologically normal in reproductive structures.

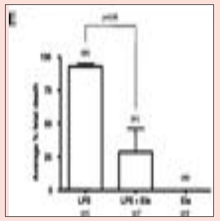

Figure 1:
Etanercept reduced fetal deaths due to LPS by 63% (Renaud, et. al., 2011).

Figure 2:
Surface area and nitrogen oxide levels decreased with treatment with etanercept more than the control (Cayci, et. al., 2011)

Measurement of each Group	Control	Etanercept
Average Surface Areas (mm^2) At the Beginning of Treatment	16	16
Average Surface Areas (mm^2) At the End of Treatment	9	0
Average Surface Area Change (%)	-43.75	100
Average Nitrate/Nitrite Levels (µmol/L) At the End of Treatment	80.5	19.8

Results

Etanercept reduced fetal death rates due to LPS by 63%[8] and prevented preterm delivery of dead fetuses by 30%.[3] In 2 hours, LPS increased TNF-a levels from 0 ng/ml to 25 ng/ml. Also, Stx2 more than doubled TNF-a concentration, which was 110 in rats with Stx2, compared to 50 in control rats. Mean surface area (mm^2) of the endometriotic implants was 9 in the control group with endometriosis, compared to 0 in the group with endometriosis given etanercept.[4] Average nitrogen oxide levels (µmol/L) were 19.8 in the etanercept group, compared to 80.5 in the control and 91.1 in the group without endometriosis. Therefore, Stx2 and LPS were linked to high levels of TNF-a as well as decreased rates of fetal survival. Increased levels of TNF-a have also been shown to induce apoptosis in fetal cells.[7] This occurs when TNF-a binds to TNFRI and activates the capase apoptotic pathway, probably causing the inability to carry a pregnancy full term. Etanercept also increased fetal survival rates and reduced number of preterm deliveries in the presence of abnormally high TNF-a levels. This correlation indicates that etanercept can relieve infertility in animals that occurs with pregnancy loss and early still born deliveries due to high levels of TNF-a and inflammation. However, these are just two aspects of infertility associated with endometriosis. Reduction of lesions indicates that the risk of progression of endometriosis leading to altered physiology is lessened. Additionally, a study found that increased levels of nitrogen oxides in endometriotic peritoneal fluid correlated with decreased rates of fertilization. This could perhaps imply damaged or altered gamete functioning.[6] Inhibition of TNF-a, a probable factor in this infertility, has been proven to reduce pregnancy losses due to TNF-a and the hostility of the reproductive environment with less lesions and nitrogen oxide levels. Even though etanercept reduces nitrogen oxide levels and a possible cause for infertility, whether etanercept can improve nitrogen oxide infertility due to endometriosis remains to be studied.

Conclusions

There is an opposing correlation of etanercept and reduced infertility related to endometriosis through excessive TNF-a in animals . At the same time, only a few factors regarding etanercept's effect on endometriosis related infertility have been studied. With further funding, support, and acknowledgement, further trials could determine such correlation. There should be further studies on animals and then humans directly relating infertility during endometriosis and etanercept. After studying etanercept's direct effects on the impact of gametes, ovulation, embryotoxicity, and endometriosis on infertility, one can be closer to determining etanercept's correlations with endometriosis induced infertility. This could result in the emergence of a treatment in etanercept for endometriosis, that, unlike hormonal therapy or some surgeries, does not suppress ovulation, induce premature ovulatory function, and can relieve infertility.

Applications to Biotechnology

Etanercept is a TNF-a inhibitor that is genetically engineered from Chinese hamster ovary cell lines. It is the combination of soluble TNFR2 receptor and the Fc region of human immunoglobulin 1 (IGg1). Recombinant proteins, including etanercept, enable the inhibition or expression of certain desired genes with a minimized chance of rejection as a nonself protein. Also, the use of recombinant E. coli in a cell culture in this study allowed for maximal expression of the toxin. This induced an inflammatory response through TNF-a production. The ELISA method also allowed for efficient determination for the presence of TNF-a. That way, it was ensured one was measuring TNF-a's effect on fetal death rates. Future use of recombinant protein can enable efficient ways to produce etanercept and produce more of the proteins to be tested in terms of effects on endometriosis associated infertility. For instance, TNF-a could be produced at a fast rate to measure how efficient etanercept can block TNF-a to preserve gametes and embryos. Additionally, ELISA can ensure the integrity of such tests by making sure TNF-a and other related causes of endometriosis related infertility the independent variables dealing with the correlation.

Acknowledgements

I would like to thank Dr. Senegar-Mitchell, Mrs. Winter, all of the OSA lecturers, and my OSA sisters for their support, knowledge, and time. I would also like to acknowledge my family who has helped me every step of my way. Finally, I would like to thank the authors who provided information from which to base my findings.

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Comparing the Effects of Monoclonal Antibody 3C23K and Hormone MIS on Human Ovarian Cancer Cells Grafted in Mice

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Objective

Ovarian cancer is a fatal gynecological cancer mostly due to the fact that there are not effective treatment methods. Chemotherapy and radiation kill any rapidly dividing cells which mean that they would even kill normal cells that need to rapidly divide. This causes side effects like hair loss and blood problems. One major side effect is infertility, and preserving fertility might not be an option for many cancer patients. To avoid these side effects, monoclonal antibodies and hormones can be used to target cancer cells by binding to cancer cell surface proteins. Both 3C23K (mAb) and hormone MIS(Müllerian Inhibiting Substance) target MISRII to inhibit ovarian tumors. The purpose of this poster is to show which drug will most effectively bind to MISRII to inhibit the tumor growth in ovarian cancer.

Abstract

Ovarian cancer is one of the most deadly gynecological cancers and the seventh cause of death in women worldwide. Radiation, although known to eradicate cancer, can cause infertility in patients, so alternative methods need to be found. This research will show which drug, 3C23K or MIS, would more effectively lead to a faster decrease in tumor volume thus serving as a better alternative method for patient therapy. Monoclonal antibodies (mAb) interact with very specific targets, thus preventing any unwanted side effects like infertility. 3C23K (mAb) binds to the cancer cell surface receptor, MISRII, and triggers the engagement of immune effector cells which then attack and kill the cells. MISRII is expressed in most ovarian cancers including epithelial ovarian cancer (90% of ovarian cancer) as confirmed by Immunohistochemistry studies. Human ovarian cancer tumors were xenografted into mice, and tumor volumes of experimental mice injected with 3C23K were compared with those of control groups. Müllerian Inhibiting Substance (MIS), a hormone known to cause the regression of the Müllerian ducts in the male embryos, and even the regression of the surface epithelium of the ovaries (this function allows inhibition of ovarian tumor), can also inhibit ovarian cancer by targeting MISRII. Immunosuppressed mice were implanted with OVCAR8 or IGROV1 human ovarian cancer cells beneath the renal capsules. Mice were injected daily with recombinant human MIS, and these mice showed a larger decrease in tumor volume than did the control mice. Both drugs inhibited tumor growth in mice proving to be alternative methods to radiation.

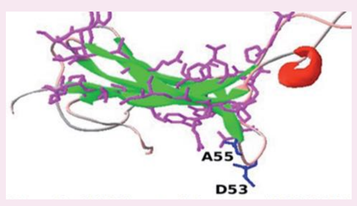

Figure 1. 3D model of MISRII extracellular region. Blue represents 3C23K epitope. [3]

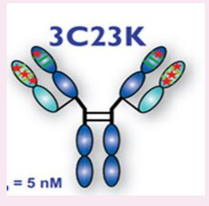

Figure 2. Molecular structure of mAb 3C23K. [3]

Methods and Materials

Comparisons between the drugs will be made by looking at two different studies, each highlighting one specific drug. Both drugs were tested for their inhibitory effects on ovarian cancer through immunosuppressed xenografted mice. Study on MIS: Selected female nude mice injected with MIS had whole body irradiation, which serves to destroy or suppress the recipient's immune system, preventing immunologic rejection of transplanted tissue, 24 hours before OVCAR 8 tumor grafting.[1] On the day of OVCAR 8 tumor grafting, each animal was given 10 micrograms (µg) of purified MIS in 100 µl of Phosphate Buffer Saline (PBS) by intraperitoneal or intramuscular injections. [1] The MIS injected was rhMIS (recombinant MIS) secreted from Chinese Hamster Ovarian cells. [1] The controls were mice injected with just the buffer. [1] One experiment had mice injected with purified MIS for two weeks (10 µg/day) and then harvested the OVCAR 8 tumor a week later, while in another experiment mice were injected(10 µg/day) for 3 weeks and then harvested the OVCAR 8 tumor the next day. [1] Study on 3C23K by LFB Biotechnologies in France: Ovarian tumor models established on Swiss nude (immunosuppressed) female mice: Granulosa tumor model COV434-MISRII (COV434-WT transfected with MISRII cDNA), and Asc1A5 cell line derived from patient ascites (pockets of fluid). [3] [6] The treatment schedule for the mice was 3C23K mAb intraperitoneal injections (10 mg / kg/ injection) on established tumor once a week for 4 weeks; this administration procedure was known as "Q7D4". [6] Combination therapy was also experimented; 3C23K(10 mg/kg/injection) plus paclitaxel(15mg/kg/injection)and 3C23K(10 mg/kg/injection) plus carboplatin(15mg/kg/injection); they were administered by the "Q7D4" procedure. [6] The controls in this study were injected with only PBS. [3] [6]

Results and Interpretation

The following is the results for the 3C23K study. When the COV434-MISRII and the Asc1A5 tumor models were treated with 3C23K, there was more anti-tumor activity then was in the controls (Fig 3). [3] A Kruskal-Wallis test confirmed that tumor volumes of the tumor models were significantly different from the controls. This test calculated the P-values which when greater than or equal to 0.05, there is no statistically significant difference between the mean of the control and experimental groups, but when P values are less than 0.05 this indicates a significant difference. [6] Most of the P values ranged from .01 to .04 thus proving that the null hypothesis, which was that this drug does not inhibit cancer, wrong.[6] Both models exhibited efficacy of 3C23K because there was no weight loss in the mice suggesting that there were not many side effects. [3] [6] The inhibition of tumor growth (T / C) was calculated using this formula: T / C = (average of the experimental TV /average of TV in the control group) x 100. [6] According to the National Cancer Institute, the following are the conditions used to determine antitumor activity of a drug/product: The drug is considered ineffective when the T / C >42%, has an antitumor effect when 10 %< T/C<42%, and is certainly effective when the T / C < 10%. [6] In this case (Figure 4), the T/C, when 3C23K was administered on the COV434-MISRII and Asc1A5 tumors, was between 42% and 10%, so 3C23K does have an antitumor effect, but it is not completely effective. [6] The combination therapy showed greater anti-tumor effects and proved to be more effective(T/C< 10%) then just antibody or anti-cancer agent alone. [6] These results of combination therapy could be taken into consideration once 3C23K becomes an option as a treatment method, and then could be used as different methods of administration for this antibody therapy.

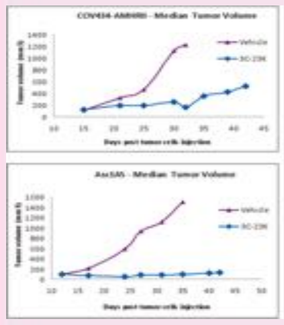

Figure 3. Graphs of Median tumor volume of the two tumor models- experimental(3C23K) and control tumors.[3]

Days	19	26	34	37	41	45	48	51	55
Volume (mean) median									
Vehicle	93	88	358	615	765	1163	1664		
3C23K 10mg/kg q7d x4	89	107	93	139	102	217	407	277	623	712
paclitaxel 15mg/kg q7d x4	90	224	270	350	415	950	1350		
3C23K 10mg/kg + paclitaxel 15mg/kg q7d x4	91	71	94	90	44	134	169	174	252	365	324
T/C ratio									
Vehicle - 3C23K 10mg/kg q7d x4	95	57	26	23	21	27	29		
Vehicle - paclitaxel 15mg/kg q7d x4	97	21	20	65	93	83	63		
Vehicle - 3C23K 10mg/kg q7d x4 + paclitaxel 15mg/kg q7d x4	98	38	28	18	8	11	12		
3C23K 10mg/kg q7d x4 - paclitaxel 15mg/kg q7d x4 + paclitaxel 15mg/kg q7d x4	96	47	36	36	30	32	36		
3C23K 10mg/kg q7d x4 - 3C23K 10mg/kg q7d x4 + paclitaxel 15mg/kg q7d x4	100	87	101	70	97	42	40	30	41	51
paclitaxel 15mg/kg q7d x4 - 3C23K 10mg/kg + paclitaxel 15mg/kg q7d x4	101	31	35	27	11	14	14		

Figure 4. Median tumor volume and T/C ratios of controls, 3C23K and combination therapy(paclitaxel).[6]

The following is the results of the MIS study. During tumor harvest, the final tumor size was measured in each nude mouse, the graft size ratios (GSR) of experimental (MIS treated) and control animals were calculated(Fig. 5). [1] In the first experiment where experimental mice were injected with MIS for two weeks, the control mice population(n=4) had OVCAR 8 tumors that reached an average graft size ratio of 4.439 ± 1.064 after three weeks of growth, while the experimental mice(n=4), which were measured at three weeks, had an average GSR of 1.065 ± 0.69 ($P < 0.019$). [1] In the second experiment where experimental mice were injected with MIS for 3 weeks, the OVCAR 8 tumors implanted in control mice(n = 8) had an average GSR of 9.16 ± 0.72, while the experimental mice had an average GSR of 3.71 ± 0.471 ($P < 0.001$). [1] The lower the GSR the more effective the drug; in this situation the graft size ratio was lower for mice with tumors treated with MIS. The P values of the experimental groups are less than .02 thus proving that the null hypothesis, which was that this drug does not inhibit tumor growth, wrong and proving the alternative hypothesis, that the drug does inhibit tumor growth, right.

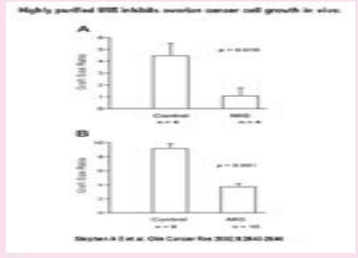

Figure 5. Graft size ratio of tumors; A is experiment one while B is experiment two.[1]

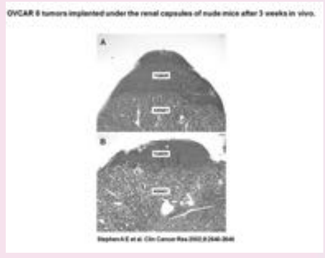

Figure 6. Shows decrease in OVCAR 8 tumor, implanted on kidney, after MIS treatment.

Discussion

When looking at the numerical and statistical data of the studies, one can see that MIS and 3C23K both effectively target MISRII to inhibit tumor growth. Based on time though, MIS seems to inhibit tumor growth faster. However to determine the efficacy of the drug, the advantages and disadvantages must also be acknowledged. There are general side effects associated with monoclonal antibodies like 3C23K which are nausea, fever, low blood pressure, vomiting, and rashes. Clinical trials need to be performed in order to determine the effects of the antibody on humans and to see if there are any severe side effects. Clinical trials need to be performed for MIS too in order to find the correct dosage in order to avoid possible side effects like regression of major female reproductive organs. If human clinical trials demonstrate that these drugs do not have many side effects and are fast treatment methods for ovarian cancer, then there is a chance that both can be used. As of right now, there is not enough evidence to prove that one drug is better than the other.

Applications to Biotechnology

Without biotechnology, the development of these drugs would not have been possible. The monoclonal antibodies were produced through the fusion of myeloma cells with the B cells (hybridomas) from a mouse that had been injected with the desired antigen, and then a hybridoma that produced the specific antibody was chosen and a culture was made to extract the antibodies from. Tissue grafting has improved drug testing which has made drugs more readily available for human use. Several tests like ELISA were helpful in determining certain variables throughout the experiments. The ability to recombine the genetic data of these drugs has allowed for more effective treatment, and maybe advances in recombinant DNA procedures will lead to improvements in cancer treatment. Once there is a complete understanding of the molecular aspects of cancer and these anti-cancer drugs, this type of biological therapy may more effectively treat cancer.

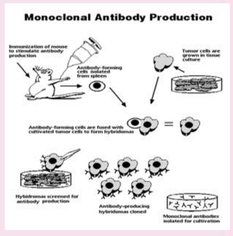

Figure 7. The process of monoclonal antibody production. Retrieved from http://www.bio.davidson.edu/courses/MolBio/MolStudents/01rakarnik/mab.html

Acknowledgements

Without the help of Dr. Ericka, Ms. Winter, Dr. Saunders, Dr. Chang and several other educators, I would not have received this opportunity and the ability to learn and do so much. I would like to thank my parents for supporting me through this scientific journey. Through this journey, I was able to learn more than just about science, I learned how to be a better person from all the different educators, doctors, and speakers. Thank you so much for this opportunity where I got to learn so much about such an amazing scientific field, and got to see what it is like to be a doctor or researcher. Thank you for the lab coat, it really made me feel like a scientist!

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The Therapeutic Potential of Focal Adhesion Kinase Inhibitors in Epithelial Ovarian Cancer

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Introduction

Ovarian Cancer is the deadliest gynecological disorder in the US, with a five-year survival rate of only 45%. [6] Epithelial Ovarian Cancer (EOC), the most dangerous form of ovarian cancer, constitutes about 90% of all ovarian cancer cases. Taxane-based chemotherapy agents such as paclitaxel, which interfere with tumor cell mitosis, have long been used as adequate first-line EOC therapy. Even so, EOC recurs in 80% of cases due to rapidly acquired resistance to these traditional cytotoxic agents, and most patients succumb to recurrent disease. [2] Thus, novel therapeutic approaches are needed to more effectively combat EOC. A new therapeutic target has recently been examined: a nonreceptor tyrosine kinase involved in many cytoplasmic signal pathways essential to tumor progression, Focal Adhesion Kinase (FAK). The objective of this research poster is to evaluate if FAK inhibition is a viable method for suppressing EOC tumor progression.

Abstract

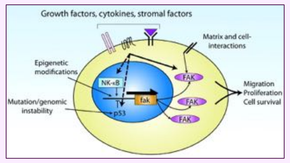

Figure 1. FAK plays an essential role in regulating cell migration, proliferation, and survival via important cytoplasmic signaling pathways. Overexpression may promote tumor cell invasion. *Adapted from diagram by Parsons JT, Slack-davis J, Tilghman R, Roberts WG. (2008)* [5]

Focal Adhesion Kinase is a key cytoplasmic component of integrin-mediated signal pathways that regulate vital cell functions including adhesion and survival. [5] However, overexpression of FAK, most likely due to gene amplification, has been shown also to promote tumor progression and invasion. [1] Recent studies in mice have tested novel small-molecule FAK inhibitors capable of competitively inhibiting FAK phosphorylation in EOC cells, and consequently diminishing FAK activity. One preclinical study tested the potency of FAK inhibitor VS-6063 against 2 taxane-resistant EOC cell lines xenografted into female mice models. 4 different treatment groups were administered to the mice: a vehicle control, VS-6063, paclitaxel, and a combination regimen involving both VS-6063 and paclitaxel. Although paclitaxel alone did not show any considerable effect in reducing tumor burden, VS-6063 alone significantly reduced proliferation and angiogenesis while increasing apoptosis. This resulted in substantial decreases in tumor weight (42.6-67.1% reduction compared to the control). However, combination therapy proved the most effective, reducing tumor weight by about 87.2-91.6%. [3] These results confirm the viability of FAK inhibitors in treating EOC, especially when used in conjunction with taxane-based chemotherapy agents. In addition, they possibly give insight into the therapeutic mechanisms of FAK inhibitor action. The study promotes the further development of this novel therapeutic approach and encourages future testing in human clinical trials.

Methodology

A study conducted at the Anderson Cancer Center of the University of Texas tested the effects of FAK inhibitor VS-6063 on mice afflicted with human EOC xenografts. Female athymic nude mice aged 8-12 weeks were given intraperitoneal (i.p.) injections of either HeyA8-MDR or SKOV3-TR taxane-resistant EOC cells, and randomly divided into 4 groups of 10 mice for each cell line. 3-4 weeks after tumor cell injection, 3 groups from each cell line were administered 1 of 3 different treatment variables: twice daily 25 mg/kg VS-6063 administered orally, weekly paclitaxel through i.p. injection (2 mg/kg for SKOV3-TR cell line mice and 2.5 mg/kg for HeyA8-MDR cell line mice), or a combination regimen involving both VS-6063 and paclitaxel each in their respective dose scheduling. The fourth group was used as a control and administered a vehicle solution both twice a day orally and weekly intraperitoneally. Mouse models were sacrificed on day 28 for HeyA8-MDR models, and day 35 for SKOV3-TR models, or earlier if they seemed moribund. Tumor weight and number of nodules were averaged and recorded, and HeyA8-MDR tumors were evaluated for angiogenesis, apoptosis, and proliferation using immunohistochemistry (IHC) to examine potential mechanisms of FAK inhibitor function.

Freshly cut frozen samples were evaluated for angiogenesis using anti-CD31 antibody IHC staining, and data was quantified as micro-vessel density (MVD, the average number of vessels in 10 random fields of view under x200 magnification). Formalin-fixed and paraffin-embedded samples were evaluated for apoptosis with anti-cleaved-caspase-3 antibody staining, and for proliferation with anti-Ki67 antibody staining, and the data was quantified as the percentage of cells displaying positive expression. [3]

Results

The results confirmed FAK inhibitor potential in reducing EOC tumor burden, despite taxane resistance. As expected, paclitaxel therapy alone was ineffective in reducing tumor burden in either cell line model. However, VS-6063 therapy alone was very successful, reducing tumor weight by 42.6% (P=.041) in the HeyA8-MDR model and 67.1% (P<.001) in the SKOV3-TR model as compared to paclitaxel therapy alone. Even so, combination paclitaxel + VS-6063 therapy was by far the most effective, reducing tumor weight by 87.2% (P=.02) in the HeyA8-MDR model and 91.6% (P=.04) in the SKOV3-TR model. The number of tumor nodules decreased in parallel to tumor weight.

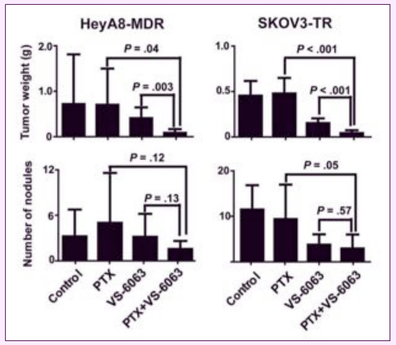

Figure 2. Comparing the effects of paclitaxel (PTX), VS-6063, and paclitaxel + VS-6063 on mouse models afflicted with HeyA8-MDR and SKOV3-TR cell line tumors. *Adapted from charts by Kang Y, Hu W, Ivan C, et al. (2013)* [3]

(Figure 2)[3] In both cell line models, angiogenesis and proliferation decreased with VS-6063 treatment, with most significant results shown by combination therapy (Figure 3, A and B). Apoptosis also increased in a similar pattern (Figure 3, C). These results indicate that some of the success mechanisms of FAK inhibitors in reducing tumor burden come from the suppression of FAK-related pathways that control angiogenesis, proliferation, and survival. [3] It is interesting to note that in both cell line models, the additive effect of combination FAK inhibitor + taxane-agent therapy proved even more superior to the effect of FAK inhibitors alone. This outcome suggests that FAK inhibitors succeed not only by countering angiogenesis and proliferation while inducing apoptosis, but also by resensitizing taxane-resistant cells to taxane therapy. Other studies have been conducted into FAK's role in nuclear signal pathways that regulate chemoresistance, and some correlation has been noted between downregulated FAK expression and declined chemoresistance. However, further study is still necessary to confirm this relationship. In any case, these outcomes prove that FAK inhibitors are effectual in reducing tumor burden in mice models, and potentially have therapeutic efficacy for treating EOC in humans.

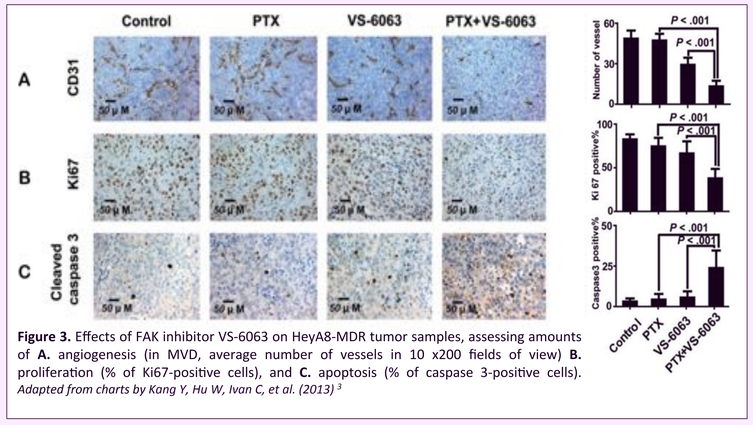

Figure 3. Effects of FAK inhibitor VS-6063 on HeyA8-MDR tumor samples, assessing amounts of **A.** angiogenesis (in MVD, average number of vessels in 10 x200 fields of view) **B.** proliferation (% of Ki67-positive cells), and **C.** apoptosis (% of caspase 3-positive cells). *Adapted from charts by Kang Y, Hu W, Ivan C, et al. (2013)* [3]

Discussion

Epithelial Ovarian Cancer has been and still is a challenging obstacle to the medical and patient community. However, the results from this study support a novel medical innovation that has therapeutic potency in reducing EOC tumor progression by reducing tumor cell proliferation and angiogenesis and inducing apoptosis. Evidence also points to FAK inhibitors' ability to resensitize taxane-resistant tumor cells to paclitaxel, and likely other taxane drugs as well. Because resistance of tumor cells to first-line chemotherapy threatens dreaded recurrence for not only EOC patients but also other cancer patients, this versatile drug will become so essential to future success in general cancer treatment if developed and utilized. Therefore, it is just so necessary that further research be done to better understand the mechanisms by which FAK mediates chemoresistance in cancer cells. There currently is a clinical trial being conducted by biopharmaceutical company Verastem for VS-6063 in advanced EOC patients, testing FAK inhibitor ability to target cancer stem cells, major sources of tumor recurrence and regeneration that develop chemoresistance very quickly. [3] However, it is still only in Phase 1/1B. Additional trials must be promoted to spread awareness about this novel therapy to patients and public.

Applications to Biotechnology

Evaluation of angiogenesis, proliferation, and apoptosis in EOC tumor samples was made possible by reliable IHC biotechnology. IHC exploits the antigen-specific properties of antibodies to mark certain target antigens present in a sample of biological tissue for easy visualization. Ki67 is present only in cells undergoing division, so it serves as an excellent target antigen for marking and measuring proliferation. Similarly, cleaved caspase 3 is a target marker of apoptotic activity, and CD31 is a target marker for angiogenic activity. IHC technology is valuable in many similar kinds of experiments.

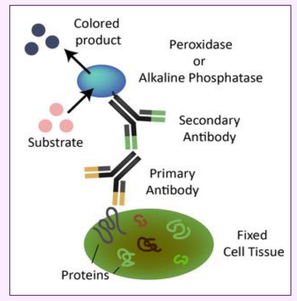

Figure 4. Indirect IHC works via a series of antibodies that are used to mark specific target proteins within a tissue sample. *Retrieved from: http://www.rockland-inc.com/ihc-products.aspx*

Acknowledgements

I would like to sincerely thank Dr. Ericka for her endearing moral support and Ms. Winter for planning the academy. A special thanks to Dr. Saunders, Dr. Chang, and all the other professionals from whom we learned myriads during the academy. My gratitude towards Dr. Schlaepfer for sparking my interest in the topic of this poster. And finally, to all my OSA sisters who made this summer truly unforgettable.

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