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Effects of Estradiol and Tamoxifen on Leukocyte Distribution in Response to Restraint Stress in Mice (Mus musculus)

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Effects of Estradiol and Tamoxifen on Leukocyte Distribution in Response to Restraint Stress in Mice (Mus musculus)

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Natural Sciences at Carroll College, Helena, Montana

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April 5, 2004
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Acknowledgments

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Abstract

Selective estrogen receptor modulators (SERMs), drugs that either mimic or block the action of estrogen but do not have its side effects, have been under intense research since the early 20th century because of their distinct biological functions as antagonists and agonists in cells through their unique molecular conformations. Previous, unpublished research has found a correlation between a reduction in the leukocytic stress response in mice and injection with a natural form of estrogen, estradiol. The present study tested the effects of a SERM, tamoxifen, on the leukocyte distribution in mice after subjection to restraint stress. No difference was found in the leukocyte distribution between stressed mice that were treated with tamoxifen and those that were injected with the vehicle. Further, in opposition to previous work, there was no difference found in the leukocyte distribution in mice injected with estradiol, which exhibited an augmented leukocytic stress response, compared to the mice that were injected with the vehicle.
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Introduction

The relationship among the nervous, endocrine, and immune systems is a complex, but fascinating one. Consequently, the connection between these systems is constantly being studied and is well documented. Studies have shown that glucocorticoids, released in response to stress, may inhibit the function of lymphocytes and macrophages and the production of proinflammatory cytokines, involved in the immune response (Rohleder and others, 2001; Stefanski and Engler, 1998). Glucocorticoids also cause a change in the distribution of leukocytes when animals are subjected to restraint stress (Kelley, 1985).

Interestingly, it has been shown that estrogen not only stimulates the secretion of glucocorticoids, but induces a shift in these cytokines as well (Rohleder and others, 2001). Estrogen receptors have been found on various immune cells and have been shown to induce varying reactions according to the dosage of estrogen present. Estrogen acts by inhibiting proinflammatory cytokine production at higher concentrations and stimulating their production at lower concentrations.

A previous, unpublished study showed that a pharmacological dose of estradiol, a form of estrogen, decreased the change in leukocyte distribution in mice that were subjected to restraint stress (Smart, 2001). This study mimicked the previous one, but tested a synthetic estrogen in addition to estradiol to see if the same effect could be observed. In this study, I administered pharmacological dosages of tamoxifen, a synthetic estrogen receptor modulator (SERM), to mice and observed their leukocyte distribution in response to restraint stress. SERMs, drugs that either mimic or block the action of estrogen, but do not have its side effects, have been under intense research since
the early 20th century because of their distinct biological functions as antagonists and agonists through molecular confirmation (Paige and others, 1999). In this study, I hypothesize that tamoxifen will have the same effect as estradiol on the change in leukocyte distribution caused by restraint stress.
Background

Stress

Stress is a generalized, nonspecific response of the body to any factor that disrupts its ability to maintain homeostasis. The following are the five known types of stressors: physical, chemical, physiologic, psychological or emotional, and social. In this study, I implemented an external physical stressor: restraint. A stressor can be perceived by receptors throughout the body or by areas of the brain. The hypothalamus receives all of this input and responds to stress depending on how the brain perceives it. If the stress is viewed as avoidable, the brain stimulates the sympathetic nervous system, which stimulates the adrenal medulla to release catecholamines (epinephrine/norepinephrine). On the other hand, if the brain views the stress as unavoidable, it will try to adapt to the stress by secreting CRH, which eventually is involved in cortisol secretion.

Cortisol is a glucocorticoid that is produced in the zona fasciculata and zona reticularis in the cortex of the adrenal gland. The secretion of cortisol is regulated by the hypothalamus-pituitary-adrenal cortex axis. Stress positively acts upon the hypothalamus to secrete corticotropin-releasing hormone (CRH), which in turn stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) into the bloodstream. This hormone binds to cells in the adrenal cortex and stimulates them to secrete cortisol. Its actions are to increase blood glucose levels by stimulating gluconeogenesis, inhibit glucose uptake, increase blood amino acids by stimulating protein degradation, and increase blood fatty acids by stimulating lipolysis. These effects are seen to provide the
brain with more nourishment and the body with the building blocks that would be needed if injury occurred.

Furthermore, these higher levels of cortisol secretion have been found to impact the immune system. Glucocorticoid receptors have been found on leukocytes, and it has been shown that higher concentrations of glucocorticoids suppress the functions of the immune system (Paavonen, 1987).

Leukocytes

White blood cells (WBCs) are the principal component of the immune system and function by destroying foreign substances such as bacteria and viruses. Leukocytes are produced from undifferentiated pluripotent stem cells in the red bone marrow. There are five main types of WBCs, which are further divided into two main subgroups—the polymorphonuclear and mononuclear leukocytes. Within the polymorphonuclear leukocyte group reside the neutrophils, eosinophils, and basophils while within the mononuclear reside the monocytes and lymphocytes. In this study, I focused mainly on the distribution of the lymphocytes and neutrophils.

Lymphocytes originally derive from precursor cells in bone marrow, but most are produced from pre-existing lymphocytes found in lymphoid tissue. Two types of lymphocytes are known: B cells and T cells. B cells are responsible for antibody production once activated by a foreign antigen. After activation, B cells produce B memory cells, which are quick to react if the identical antigen is encountered during a later time. There are two types of T cells: T helper cells (CD4) and T cytotoxic cells (CD8). Cytotoxic T cells directly destroy cells that have been invaded by pathogens such
as viruses, bacteria, protozoa, or fungi by releasing toxic cytokines. T helper cells “help” the B cells’ production of antibodies and the recruitment of other T cells by releasing cytokines. Glucocorticoids have been shown to suppress the major types of these cytokines produced by helper cells, interferon-gamma and interleukin-2, thus suppressing the inflammatory effect (Rohleder and others, 2001). Neutrophils are derived from the red bone marrow. They respond to an inflammatory reaction by chemotaxis and mainly function by phagocytosis. As a general response to invasion, phagocytosis includes engulfing foreign material and digesting it within a lysosome.

Estrogen

Estrogen is a female sex steroid that is cholesterol-based and is produced in the ovaries and, in small amounts, in the adrenal cortex. Estrogen is responsible for the development of the breasts, ovaries, vagina, and uterus and regulation of the reproductive cycle. The female reproductive cycle can be broken into two stages: the follicular stage and the luteal stage.

The follicular stage begins with the rising level of FSH (follicle stimulating hormone) and LH (leutenizing hormone), which are released from the adenohypophysis after stimulation by the hypothalamic hormone GnRH (gonadotrophic releasing hormone). FSH stimulates the proliferation of the granulosa cells and LH the thecal cells. These two types of cells, which make up the follicle, are responsible for estrogen production and secretion. Granulosa cells are also responsible for the secretion of inhibin, which acts by negative feedback to block the secretion of FSH by the adenohypophysis. Rising estrogen levels act through negative feedback by inhibiting the
hypothalamus and adenohypophysis, especially inhibiting FSH production. Furthermore, estrogen primes the endometrium, in that it initiates its growth and activates and sensitizes the endometrium’s receptors for progesterone, the hormone most responsible for endometrial growth.

A surge in LH is characteristic of ovulation. The oocyte finishes meiosis I and is released from the ovary. The follicle cells remaining in the ovary transform into a corpus luteum, which begins releasing both progesterone and estrogen. This increase in progesterone not only inhibits the release of FSH and LH, but also enhances the growth of the endometrial layer.

In primates, if fertilization of the ovum does not occur, levels of estrogen and progesterone begin to decrease, which causes the sloughing of the endometrial layer and rising levels of LH and FSH. Thus, the entire cycle starts over again.

Regulating the female reproductive cycle is not the only function of estrogen. Estrogen receptors have been found on various immune cells, including neutrophils and monocytes. Estrogen has been found to inhibit cellular immunity by causing a shift in the cytokine balance. This shift has been shown to be dose dependent, with inhibition of proinflammatory cytokine production at higher concentrations and stimulation at lower concentrations (Rohleder and others, 2001). The inhibitory action of estrogen on proinflammatory cytokine production mimics that of the glucocorticoids. Furthermore, it was reported by Rohleder and others (2001) that in animals, estrogen stimulates the production of glucocorticoids, which if produced at a high level, would suppress the immune system.
SERMs

Selective estrogen receptor modulators (SERMs) are drugs that either mimic or block the action of estrogen but do not have its side effects (Shang and Brown, 2002). Estrogen’s mechanism of action is through nuclear estrogen receptors that control gene transcription. For example, two well studied SERMs, tamoxifen and raloxifene, are shown to bind to the nuclear estrogen receptor, which then changes shape, forms a pair with another receptor-hormone complex, and subsequently attaches to a docking site called an estrogen response element (ERE) on DNA. This binding, in turn, activates the formation of a transcription complex, which is made up of a myriad of proteins including coactivators around the original complex. It is this final complex that is responsible for gene transcription. It activates RNA polymerase, which is responsible for making messenger RNA, the template for protein synthesis. The newly synthesized proteins subsequently induce changes within the cell.

The action of SERMs can be viewed as estrogenic or antiestrogenic, in that it can either mimic the effects of naturally produced estrogen or block or oppose its effects. SERMs have been shown to block the action of estrogen by binding to the nuclear estrogen receptors and causing a shape change of the receptor in such a way as to inhibit the formation of the transcription complex, in particular the binding of coactivator proteins (Jordan, 1998). On the other hand, SERMs can mimic the action of estrogen by binding to the estrogen receptors and causing a nearly identical shape change that promotes the binding of coactivator proteins and the formation of the transcription complex. Also, it has been hypothesized that some coactivator proteins may be able to tolerate the abnormally shaped receptor (caused by SERM binding) and continue with
their formation of the transcription complex (Jordan, 1998). Finally, new evidence has shown that cells can produce alpha and/or beta estrogen receptors and SERMs affect each of these receptors differently. For example, upon binding to an alpha receptor, a SERM may act in an antiestrogenic fashion while, upon binding to a beta receptor it may act in an estrogenic manner or vice versa (Paige and others, 1999).

Tamoxifen, the SERM used in this study, is currently being used as an effective treatment for hormone-responsive breast cancer patients in that it acts in an antiestrogenic manner in the breast cells. However, it has been shown to display estrogenic activity in the uterus and has been linked to an increase in endometrial hyperplasia and cancer. A study done in 2002 (Shang and Brown) found that tamoxifen induced the recruitment of co-repressors to EREs in mammary cells, while stimulating the recruitment of co-activators in endometrial cells. Thus it is possible that it may have an antiestrogenic or estrogenic effect in the leukocytic response to stress in mice.
Materials and Methods

Animals

I used prepubescent female Swiss-Webster mice (*Mus musculus*) obtained from stock supplies at Simonsen Laboratories in Gilroy, California. The mice were placed on a 12-hour light, 12-hour dark cycle with a room temperature kept at 25°Celsius. The four-and-one-half to six weeks old mice had free access to tap water and food (Mazuri Rat Chow) during experimentation.

Drug Preparation

The dosages (Table 1) were based on previous work (Dyer and others, 1980; Hilakivi-Clarke, 1996) and were recalculated based on relative body weights of the animals. All hormones were administered in a sesame oil injection vehicle.

Table 1. Hormones and dosages administered.

<table>
<thead>
<tr>
<th>Hormone dosage</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no stress)</td>
<td>No injection</td>
</tr>
<tr>
<td>Control (stress)</td>
<td></td>
</tr>
<tr>
<td>Sesame oil/Vehicle</td>
<td>N/A</td>
</tr>
<tr>
<td>Estradiol Priming Dose</td>
<td>0.01mg/1mL</td>
</tr>
<tr>
<td>Experimental Estradiol Dose</td>
<td>0.2mg/1mL</td>
</tr>
<tr>
<td>Experimental Tamoxifen Dose</td>
<td>4.0mg/1mL</td>
</tr>
</tbody>
</table>

Injections

One group of controls was not given injections of the sesame vehicle while another was given injections. Experimental groups were all given the required priming
and final hormone dosages. To control movement, the mice were placed on a wire screen suspended about 10 centimeters above the lab bench and held by a partner while subcutaneous injections were given dorsally. After experimentation, it was found that the injections were accepted best if each mouse was injected with 0.03mL on the left and right sides of the body, with a total injection amount of 0.06mL.

Restraint Procedure

Half of the restraint cages were made from three-inch-long sections of one-inch-diameter opaque PVC pipe. Holes were drilled about every centimeter in the pipe to allow for air circulation in the cage. The ends of the cage were closed with rubber stoppers to prevent escape by the mice. The other half of the restraints were commercially made, clear, plastic restraints similar in size and structure to the handcrafted ones. All but the control mice were restrained for a two-hour period administered once during the experimental procedure (Smart, 2001).

Phlebotomy

For each experimental group, blood was drawn at approximately the same time each day to prevent uncontrolled variation. On days when stress was applied, blood was drawn immediately before and after the restraint was administered. Blood was taken from the capillary beds located in the tail. Each time blood was drawn, a small nick in the end of the tail was made, and a drop of blood was placed directly onto a microscope slide to perform a leukocyte differential count (Brosschot and others, 1992).
Wright Staining

I used the leukocyte differential count staining procedure of Neal and Kalbus (1983), which consisted of submersing slides for 30 seconds in the Wright’s stain, three minutes in phosphate buffer (pH 7.2), followed by a short rinse with phosphate buffer. The slides were then allowed to air dry.

Quantification of Data and Statistical Analysis

To quantify the effects of restraint and estradiol and tamoxifen on peripheral leukocyte distribution, I counted 100 cells on each blood slide twice, averaging the number of each kind of cell per count and then pooled the averages in each treatment group to produce group means and standard deviations.

I used a one-way analysis of variance (ANOVA) to test for differences between the leukocyte distributions in the stressed groups as well as the injected groups. In addition, I used a two-way multivariate analysis of variance (MANOVA) to test for the differences between the various experimental groups as well as the timed trials. I then used the Tukey HSD test of ratios as a post analysis to assess which groups were different. The PC program Statistica, version 5.1 (1997), was used for the statistical analysis.


Results

Effects of Stress

The effect of stress on the leukocyte distribution of the mice was compared. There was a significant change in the leukocyte distribution of mice that were stressed compared to those who were not stressed (Figures 1a and 1b, Table 2). The lymphocyte count of the stressed group decreased significantly compared to the non-stressed group, while the neutrophil count increased significantly.

Effect of Time on Effect of Estradiol

The effects of estradiol on the leukocyte distribution with the application of restraint at different times after injection were compared. It was found that there was no significant difference between the times of the administration of the restraint and leukocyte distribution (Figures 2a and 2b, Tables 3a, 3b, 4a, and 4b). As the 72-hour time period produced the most interference in the leukocytic stress response, this time was selected in the following study for the application of restraint.

Effects of Injection of Vehicle (Control), Estradiol, and Tamoxifen in Stressed Mice.

The third study compared the leukocyte distribution in response to stress in mice that were injected with the vehicle (control), estradiol, or tamoxifen. There was no significant change in the distribution of leukocytes of the mice injected with estradiol compared to the vehicle group (Figure 3a, 3b and Tables 5, 6). The vehicle mice exhibited the expected stress response in that their lymphocyte count went down and their neutrophil count went up (Figures 3a, 3b). The mice injected with estradiol showed an
augmented response to stress in that their lymphocytes decreased and their neutrophil count increased more than those of the vehicle group, although not significantly (Figure 3a, 3b). The distribution of leukocytes between the mice injected with tamoxifen and those injected with estradiol was not significantly different (Figures 3a, 3b and Tables 5, 6). The tamoxifen mice’s lymphocyte count decreased less than that of the vehicle group, and the neutrophil count increased less than that of the vehicle group, but again not significantly (Figures 3a, 3b and Tables 5, 6).
Figure 1a. Effects of Stress on Neutrophil Distribution in Stressed and Non-Stressed Mice. The stressed animals showed an increase in percentage of neutrophils. The change represents the average change in neutrophil percentage before and after the period in which stress was applied. Seventy animals were used in this study: 40 in the non-stressed groups and 30 in the stressed groups. (The error bars represent standard error.)
Figure 1b. Effects of Stress on Lymphocyte Distribution in Stressed and Non-Stressed Mice. The stressed animals showed a decrease in lymphocyte percent. The change represents the average change in lymphocyte percent before and after the period in which stress was applied. Seventy animals were used in this study: 40 in the non-stressed groups and 30 in the stressed groups. (The error bars represent standard error.)
Figure 2a. Effects of Estradiol and Stress on Neutrophil Distribution Over Time. A comparison of the effect of estrogen on neutrophil distribution when restraint was applied at different times post-injection. No statistical difference was found between the times or between the groups. Five animals were used in each group. (The error bars represent standard error.)
Figure 2b. Effects of Stress and Estradiol on Lymphocyte Distribution Over Time. A comparison of the effect of estrogen on lymphocyte distribution when restraint was applied at different times post-injection. No significant difference was found among the groups. Five animals were used in each group. (The error bars represent standard error.)
Figure 3a. Effects of Stress and Injection on Neutrophil Distribution. All stressed mice showed an increase in neutrophil distribution, with the mice injected with estradiol showing the largest increase. The change represents the average change in neutrophil distribution before and after the period in which stress was applied. Twenty animals were used in the no stress, no injection group, while the rest of the groups used ten per group. (The error bars represent standard error.)
Figure 3b. Effects of Stress and Injection on Lymphocyte Distribution. All stressed mice showed a decrease in lymphocyte distribution, with the mice injected with estradiol showing the largest decrease. The change represents the average change in lymphocyte distribution before and after the period in which stress was applied. Twenty animals were used in the no stress, no injection group, while the rest of the groups used ten per group. (The error bars represent standard error.)
Table 2. Univariate Analysis (ANOVA) of Leukocyte Changes in the Presence of Stress

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>P-value (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>&lt;0.000*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;0.000*</td>
</tr>
</tbody>
</table>

Table 3a. Multivariate Analysis (MANOVA) of Neutrophil Changes Among Timed Trials

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>P-value (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0.465</td>
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</table>

Table 3b. Tukey HSD Test of Ratios Between Neutrophil Counts Among Timed Trials

<table>
<thead>
<tr>
<th></th>
<th>0 Hours</th>
<th>24 Hours</th>
<th>36 Hours</th>
<th>48 Hours</th>
<th>60 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hours</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 Hours</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>48 Hours</td>
<td>0.979</td>
<td>0.999</td>
<td>0.958</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 Hours</td>
<td>0.953</td>
<td>0.997</td>
<td>0.982</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 Hours</td>
<td>1.000</td>
<td>0.996</td>
<td>0.423</td>
<td>0.934</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td>84 Hours</td>
<td>1.000</td>
<td>1.000</td>
<td>0.666</td>
<td>0.994</td>
<td>0.982</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table 4a. Multivariate Analysis (MANOVA) of Lymphocyte Changes Among Timed Trials

<table>
<thead>
<tr>
<th>Leukocyte</th>
<th>P-value (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>0.338</td>
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</table>
Table 4b. Tukey HSD Test of Ratios Between Lymphocyte Counts Among Timed Trials

<table>
<thead>
<tr>
<th></th>
<th>0 Hours</th>
<th>24 Hours</th>
<th>36 Hours</th>
<th>48 Hours</th>
<th>60 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hours</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 Hours</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>48 Hours</td>
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<td>0.998</td>
<td>0.942</td>
<td></td>
<td></td>
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<tr>
<td>60 Hours</td>
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<td>0.994</td>
<td>0.972</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 Hours</td>
<td>1.000</td>
<td>0.994</td>
<td>0.320</td>
<td>0.895</td>
<td>0.833</td>
<td></td>
</tr>
<tr>
<td>84 Hours</td>
<td>1.000</td>
<td>1.000</td>
<td>0.531</td>
<td>0.982</td>
<td>0.959</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table 5. Tukey HSD Test of Ratios Between Neutrophil Counts Among Test Groups

<table>
<thead>
<tr>
<th></th>
<th>No Stress</th>
<th>Stress Vehicle</th>
<th>Stress Tamoxifen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress Vehicle</td>
<td>&lt;0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress Tamoxifen</td>
<td>0.036*</td>
<td>0.803</td>
<td></td>
</tr>
<tr>
<td>Stress Estradiol</td>
<td>&lt;0.000*</td>
<td>0.452</td>
<td>0.091</td>
</tr>
</tbody>
</table>
Table 6. Tukey HSD Test of Ratios Between Lymphocyte Counts Among Test Groups

<table>
<thead>
<tr>
<th></th>
<th>No Stress</th>
<th>Stress</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Injection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress Vehicle</td>
<td>&lt;0.000*</td>
<td>0.040*</td>
<td>0.450</td>
</tr>
<tr>
<td>Stress Tamoxifen</td>
<td>0.843</td>
<td></td>
<td>0.106</td>
</tr>
</tbody>
</table>

*Denotes significant difference when comparing groups down a column and across a row (p<0.05).
Discussion

Like Smart’s (2001), this study once again found a significant difference in the leukocyte distribution between the stressed and non-stressed mice. However, contrary to the hypothesis, it was found that mice injected with tamoxifen displayed an opposite response in their leukocyte distribution after restraint-stress than those injected with estradiol. Further, this study showed a completely opposite response in the estradiol group compared to Smart’s (2001) study. Rather than an alleviated stress response, this study showed that estradiol augmented the leukocytic stress response, although not significantly. This finding can be correlated to recent literature, in that it has been shown that non-human, female animals have a greater glucocorticoid response to stress due to estrogen’s stimulation of glucocorticoid secretion (Rohleder and others, 2001; Da Silva 1994).

Much new biological data exists that may explain the difference in the leukocyte distribution between the groups of mice injected with estradiol versus those with tamoxifen. Due to the nature of SERMs, that of being synthetically made and subsequently having different amino acid sequences and quartenary structures than estradiol, each one may affect an estrogen receptor in a unique manner. As discussed in the background, they may have an estrogenic or anitestrogenic effect on the receptor and the subsequent intracellular response. Thus it is possible that tamoxifen had an antiestrogenic effect on the immune cells and their response to the glucocorticoids present in response to the restraint-stress.

The failure of my study to replicate Smart’s (2001) experiment may have been caused by injection error. Before injecting the mice with a final dose of their respective
hormone at a previously determined time, I injected the mice with a priming dose recommended by the literature. The tamoxifen mice were injected with a priming dose of estradiol and then a final dose of tamoxifen. However, the groups of estradiol mice were accidentally injected with a priming dose of tamoxifen rather than estradiol. This error may have interfered with the action of the subsequent dose of estrogen.

Problems also arose with the injections in that they often leaked out of the mice. After experimentation, it was found that approximately 0.01mL of the injection was leaking out of the mice. The injection procedure was subsequently altered in that rather than receiving 0.05mL of the injection to one side of the body, the mice received 0.03mL on each side of the body to prevent leakage and to compensate for the lost injection. As mentioned in the introduction, studies have shown that the leukocytic response is often dependent on the dosage of estrogen (Rohleder and others, 2001). Thus it is possible to assume that with the leakage and dosage increase the mice may not have been receiving the clinical dosages recommended to cause a response, and this discrepancy may have skewed the data.

It was also difficult to maintain constant laboratory conditions. For example, the laboratory temperature fluctuated immensely throughout the summer, and thus the laboratory setting was much cooler than that of the previous study. In addition, the laboratory was experiencing technical difficulties with its chemical hoods, and thus the alarms were frequently being activated. This disturbance, in turn, may have affected the stress levels of the mice and consequently their leukocyte distributions.

Inconsistencies in experimental protocol and the fluctuating lab environment may have compromised the validity of my results. Although the estrogen data did not
replicate previous unpublished work, my results were consistent with the recent finding that estrogen can stimulate glucocorticoid secretion. If laboratory conditions can be stabilized, it would be useful to repeat this work in order to resolve the action of estradiol and SERMs on leukocyte distribution in response to restraint stress.
Literature Cited


