The Effect of Varying Levels of Progesterone with Fixed Level of Estrogen on Concentrations of Serum Leptin in Ovariectomized Rats

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ABSTRACT

Sexual hormones especially estrogen have most effects on the fat tissues on the leptin's production. Changes of concentrations of progesterone in sexual cycles & hormonotrapies make changes in the exodic processes of estrogen's target systems. Aim of this study is the measurement of these effects on the changes of plasma leptin's levels. For the correct & complete analysis results we recorded other factors like insulin – Glc – HDL – LDL – TG & cholesterol. This research was done on the 56 female rats that have been divided into 7 groups of 8 rats. Then we did ovariectomy them 10 days before the starting test for the control of hormone levels. Hormone levels have controlled so that groups received constant estrogen with the different progesterone concentrations that we could measure the amounts of the effects of both hormones to each others.

Key words: Estrogen – Progesterone – Leptin – Insulin – Ovariectomy – Rats.

Introduction

Estrogen and progesterone are the main hormones affecting the homeostasis of an organism, especially female animals. Prescribing substitutive hormones for ovariectomized mice or menopausal women compensates for the lack of leptin and takes it back to the time before ovariectomy or menopause.

According to the effect of progesterone on the amount of leptin produced by fat cells in response to estrogen, it is necessary to estimate the effects of varying amounts of progesterone. This helps us to determine the appropriate proportion of prescribed estrogen to progesterone that leads to either decrease or increase in the production of leptin. The significance of this study is to develop a method for determining the limits of appropriate prescription amounts of estrogen and progesterone and also offering an appropriate proportion of the hormones – since the appropriate range minimizes the side effects of either higher or lower amount of the hormones regarding the production of leptin.

Materials and Methods

The subjects of this study were 56wistar rats purchased from Lab Animals Center of Medical University of Tabriz. They have been kept and examined for about 2 months. They were kept in the light and the dark for 12 hours respectively. The temperature was 24 centigrade. The rats which were kept in boxes of five were free to have food. The rats were accidentally assigned to 7 groups, members of which were kept in 2 separate boxes with the same conditions. The rats were kept in their place (Drug Applied Research Center) for 2 weeks in order to become adapted to their new conditions. The rats of 6 groups ovariectomized in order to have same hormonal conditions. They were anesthetized by a combination of Ketamine-Xylazine. They were ovariectomized according to the study schedule with regard to rest period and the inception of the treatment. The day of ovariectomy was considered as day 0 and a 10- day period was spent for healing and stabilization. In this study, according to the prescribed amounts in physiological amounts which were reported in the literature and physiological goals of this study, 50 \( \mu \text{g/kg} \) daily prescription was done in the form of hypodermic injection in combination with sesame oil, the pharmacological amount of which was produced according to previous studies. 100 \( \mu \text{g/kg} \) of progesterone were considered.
as the base amount. The low-concentration group (group 4) received \( \frac{n}{2} \), the moderate-concentration groups (groups 5 and 6) received \( n \) and \( \frac{3}{2} n \), and high concentration group (group 7) had \( 2n \).

**Grouping And Treatment:**

G1 group was not ovariectomized and received only a medical solution called Sham Operated. Group 2, which was considered as a control group, was ovariectomized. G3 group was ovariectomized and received 50 µg/kg estrogen to demonstrate the independent effects of estrogen. Groups G4, G5, G6 and G7 were ovariectomized and received 50 mg/kg, 150 mg/kg, 100 mg/kg, and 200 mg/kg of progesterone respectively with 50 mg/kg of estrogen to reflect the effects of the varying doses of hormones and varying hormonal prescriptions on the level of produced serum leptin. The injections of hormones were begun 10 days after ovariectomy. The following table shows the treatment schedule.

<table>
<thead>
<tr>
<th>Group</th>
<th>Name</th>
<th>Ovariectomized</th>
<th>Dose of Estrogen</th>
<th>Dose of progesterone</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Sham operated</td>
<td>-</td>
<td>µg/kg/day0</td>
<td>mg/kg/day0</td>
<td>8</td>
</tr>
<tr>
<td>G2</td>
<td>Ov</td>
<td>+</td>
<td>µg/kg/day0</td>
<td>mg/kg/day0</td>
<td>8</td>
</tr>
<tr>
<td>G3</td>
<td>Ov + es</td>
<td>+</td>
<td>µg/kg/day50</td>
<td>mg/kg/day0</td>
<td>8</td>
</tr>
<tr>
<td>G4</td>
<td>Ov + Es + P1</td>
<td>+</td>
<td>µg/kg/day50</td>
<td>( \frac{5}{7} ) mg/kg/day</td>
<td>8</td>
</tr>
<tr>
<td>G5</td>
<td>Ov + Es + P2</td>
<td>+</td>
<td>µg/kg/day50</td>
<td>5 mg/kg/day</td>
<td>8</td>
</tr>
<tr>
<td>G6</td>
<td>Ov + Es + P3</td>
<td>+</td>
<td>µg/kg/day50</td>
<td>7/5 mg/kg/day</td>
<td>8</td>
</tr>
<tr>
<td>G7</td>
<td>Ov + Es + P4</td>
<td>+</td>
<td>µg/kg/day50</td>
<td>mg/kg/day10</td>
<td>8</td>
</tr>
</tbody>
</table>

The applied combinations include 17-β estradiol and progesterone which were provided by Abu Raihan Pharmaceutical Company and produced in Drug Applied Research Center of Tabriz after calculating the precise amounts and required concentrations. According to the conditions of this study sesame oil was considered as the selected appropriate solvent which was purchased from Barij Essans Company. The hormonal combinations were planned in a manner that each group had their single injection.

**Results And Discussions**

The specimens of blood from retro-orbital were gathered on day 21 and 31 according to the study schedule. Specimens are taken while the rats were anesthetized by ether. The serum was separated by centrifuging the specimens in 1800 r/s at 24 centigrade for 12 minutes and was examines by -specific ELISA kits purchased from Bio Vendor (rat leptin ELISA/ Bio Vendor. RD-1872s3). The obtained amounts are as follows:

<table>
<thead>
<tr>
<th>Plasma leptin ng/ml</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/38 ± 2/46</td>
<td>Sham Operated 1 G</td>
</tr>
<tr>
<td>0/90 ± 0/96</td>
<td>ov 2 G</td>
</tr>
<tr>
<td>3/73 ± 6/37</td>
<td>ov + Es 3 G</td>
</tr>
<tr>
<td>2/49 ± 5/66</td>
<td>ov + Es + P1 4 G</td>
</tr>
<tr>
<td>1/95 ± 2/43</td>
<td>ov + Es + P1 5 G</td>
</tr>
<tr>
<td>1/30 ± 7/5</td>
<td>ov + Es + P3 6 G</td>
</tr>
<tr>
<td>1/11 ± 0/90</td>
<td>ov + Es + P4 7 G</td>
</tr>
</tbody>
</table>

**Findings:**

Estrogen makes the leptin & insulin plasmic levels up & when we add progesterone occurs reduced concentration - dependent processes. Balance of these changes in some methods makes the vicinity levels of ovariectomized groups or sham operated group.

**Comparison And Conclusion:**

Because of the nature of the grouping in this study, three sets of comparison are possible. The first comparison can be drawn between sham operated group and other groups to highlight the difference between manipulated and natural physiological conditions of rats. The comparison of the serum levels of different groups shows that the ovariectomy in control group caused a significant decrease in the level of leptin in comparison with that of sham operated group. Group 3's results showed a two-time increase in serum leptin level by having estrogen in comparison with that of control group. Group 4 which received progesterone experienced a significant leptin level decrease in comparison with group 3. Groups 5, 6, and 7 experienced a gradual decrease of progesterone level in hormone proportions similar to that of control group.

The related data are presented in the following graph which shows the results of the analysis of the role of ovariectomy, estrogen and progesterone on the level of serum leptin.

On the other hand, the comparison among OV groups and other groups shows that there is a significant difference between this group and group 3 which received estrogen only and group 4 which received estrogen and lowest level of concentration of progesterone. With the increase of the level of progesterone, the levels of leptin in the groups are decreasing and approximate to that of control group so that there are no differences among the levels of leptin in these groups.

The third comparison was drawn to highlight the possible differences between the groups which received the combination of progesterone and estrogen and the one which had estrogen only. The results demonstrate that the increase of the level of progesterone causes a significant difference among these groups. The occurred differences are negatively correlated with the amount of progesterone.

These groups experience the decrease of the level of leptin with the increase of the level of progesterone. That is the level of leptin approximates to that of ovariectomized group so that there are no significant differences among them. The decrease of the leptin level may reach half of or even less than the level of sham operated group.

Estron, estradiol, and other estrogens and progesterone can enter the cell freely and be connected to cytoplasm receptors. Two separate receptors of estrogen have 500 and 600 amino acids each. These receptors are coded by different genes on different chromosomes which are called ErB and Era. Ligand connection area of ErB and Era are similar in 55 percent of the sequence of amino acids. Despite their tendencies to combine with estradiol are the same, their tendencies to combine with estron and a number of synthesized agonists and antagonists vary significantly.

The tissue distributions of these two ligands are to some extent overlapping. The effects of B-17 estradiol on gene copying in human and rodents are done by ErB and Era which both have the same intense tendency to be connected to estradiol.

The results of the study on the rats which lack ErB and Era show that these two receptors possibly have different biological functions so that they induce different intra-cell messages and metabolic responses. In each cell the type and number of receptors on the target tissue determine the sensitivity to the hormone. According to several studies done on the physiological functions estrogen and progesterone, the key point is the way these two hormones affect each other.

According to the studies, these two hormones not only do affect the type of responses but also influence the path of response production and its mechanisms. That is, one of the major roles of estradiol is to increase the production of ER and PR. For example, B-17 estradiol stimulates the genes which are related to progesterone receptor. In this study estradiol affects the growth of follicle and the generation of endometrium which pave the way for subsequent action of progesterone. It was slightly observed that Era induces PR in womb and glandular epithelial cells.

With regard to the previous studies the influencing element on the stored fat and metabolism of lipids in an organism is related to steroid hormones. In females estrogen causes fats to be gathered around hips, breasts, and hypodermal tissues and forms the females' body after maturation so that women have as twice much fat as men because of the outperformance of estrogen over progesterone. The main question of this study is the quality of interaction among different concentrations of progesterone and the fixed level of estrogen. That is if the increase of the sensitivity of the estrogen receptors of fat cells (which is induced by progesterone) lead to the higher production of lipid. Or in the second case, does the increasing effect of the receptor which is produced by progesterone on...
the estrogen receptors in fat tissue lead to the decrease of the level of leptin?

The obtained results are in line with the second case; that is, the increase of the level of the progesterone leads to gradual decrease of estrogen effects. In this study the amount of produced leptin was considered as the index of the level of the production of fat cells. Thus, its amounts were carefully examined in and compared among different groups.

As it is demonstrated in the results related to leptin, ovariectomy leads to the decrease of leptin level. The injection of estrogen leads to the increase in leptin level especially compared with that of control or sham operated group. In line with previous researches, this study shows that estrogen causes the increase of the amount of fat and the level of leptin in serum. With the adding of progesterone to treatments, the level of leptin decreased, so that, its amount approximated to that of control or sham operated group.

As the level of added progesterone reaches the natural level (groups 5 and 6), the level of the leptin of serum decreases so that it reaches to that of sham operated groups. There is no significant difference among these groups - leptin levels. However, there were significant differences among these groups and estrogen only group. The level of leptin in the groups which received progesterone had an indirect relationship with the level of progesterone.

By comparing these results with those of the previous studies and with regard to the fact that progesterone decreases the number of estrogen receptors in some tissues and increases the sensitivity of receptors in the other ones, it can be concluded that the decreasing effect of progesterone is dominant. That is as the level of progesterone increases, it forces the receptors of estrogen I fat cells to produce less leptin.

With regard to the appropriate grouping for treatments, multi-dimensional comparisons were possible so that control group, estrogen only group, and sham operated group can be independently examined with the ones received progesterone. For example the findings of this study demonstrated that the natural level of progesterone leads to the stable level of leptin in serum, similar to that of sham operated group. However, adding progesterone (1.5 to 2 times) increases the level of leptin. Moreover, decreasing the level of progesterone to the half of its natural level increases the level of leptin in serum. So in special cases aiming at higher or lower level of leptin the variation of the level of progesterone in the permissible range can be helpful.

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References


