Effects of Raloxifene on Ovarian Tissue of Mature Female Rats

Ebadi Zahra¹, Kargar Jahromi Hossein¹, Abedi Hassan Ali²*, Farzam Mohsen³ and Farzam Mohammad⁴

¹Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran.
²Research Center for Noncommunicable Diseases, Faculty of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.
³Department of Physiology, Veterinary School, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
⁴Department of Anatomy and Embryology, International Branch, Shiraz University, Shiraz, Iran.

doi: http://dx.doi.org/10.13005/bbra/1376

(Received: 15 April 2014; accepted: 10 May 2014)

Raloxifene as a selective non-steroidal regulator has profound estrogen-like effects on bone but anti-estrogen effects on endometrium and breast tissue. The aim of this study is to evaluate the effect of Intra-peritoneal injection of Raloxifene in different doses on ovarian tissue of mature female rats. Forty female Wistar rats weighing 180-200 g range were selected and divided into control, sham and experimental (1,2,3) groups. Raloxifene dissolved in distilled water with dosage of 30, 60 and 120 mg/kg BW were within four weeks injected to experimental groups 1, 2 and 3 respectively. Control group did not receive any drugs while sham group received distilled water. All the injections were conducted intraperitoneally in pathological studies. The parameters were the counts of initial, first, secondary, graph, uterus follicles and yellow body. The results of initial and uterus follicles counts in comparison with the control group show no significant difference (P<0.05). Secondary and graph follicles in experimental groups 2 and 3 showed no significant decrease compared to the control group (P<0.05) and number of initial follicles and yellow body in experimental group 3 showed a significant decrease compared to the control group (P<0.05). Results revealed that Raloxifene may cause a slight change in the number of ovarian follicles.

Key words: Raloxifene, ovary, female rats.

Evista, a drug with the generic name “Raloxifene hydrochloride”, is used for the treatment of osteoporosis. Raloxifene hydrochloride and its hydrochloride salt have recently been recognized as keoxifene which has been invented two decades ago¹. Raloxifene hydrochloride has the same structure as benzothiophene. Since this drug only affects female sex hormones, it is merely prescribed for women. It is also used in order to prevent vertebral fractures². Raloxifene as a non-steroidal selective estrogen regulator functions as estrogen imitator in some tissues and has non-estrogenic activities in other ones. In fact Raloxifene is an alternative means of estrogen treatment for those who are in risk of osteoporosis whereas it has an anti estrogenic property³. The general chemical formula of Raloxifene is C_{28}H_{27}NO_{4}S.HCl which corresponds to a net molecular weight of 510.05. Raloxifene belongs to a class of drugs known as SERMs (Selective Estrogen Response Modifiers)⁴.
Raloxifene has anti-estrogenic effects on breast cancer. This drug can decrease breast cancer hormones by preventing the secretion of these hormones. This drug can favorably change the biochemical signs of vascular disease by reducing LDL-cholesterol, fibrinogen and lipoprotein and also by increasing LDL2-cholesterol without increasing triglyceride. Scientists are investigating the question that whether these favorable chemical effects can cause protection against vascular disease. The main concern in using Raloxifene is the high risk of blood clotting in veins. Therefore blood clotting in veins prevents the use of Raloxifene.

In a study on rats in which each rat, before and during intercross, had received 5 mg/kg Raloxifene on a daily basis, no pregnancy was observed in the end. As a result the sexual cycle in female rats was disrupted and ovulation process was interrupted. Implantation of the embryo was also delayed and failed. The size of newborn rats became small by using of this drug during pregnancy. The reported growth effects include a decrease in the newborn weight, delay in skeleton growth, delay in growth, incomplete or abnormal formation of heart, edema and Hydrocephalus in children. In a study done by Patrick et al. (1997), on the effects of Raloxifene on ovarian cancer in postmenopausal women, it was shown that Raloxifene should not be used on women suffering from ovarian cancer. Furthermore, a treatment with Raloxifene in postmenopausal women does not cause any increase in ovary size.

MATERIALS AND METHODS

In this experimental study all code of conducts for research work with laboratory animals which is approved by the American organization called SPCA (Society for the Prevention of Cruelty Animals) in 2006 are obeyed. In this research, forty, mature female Wistar rat weighing 180± 20 g were used which was supplied by the center of breeding and keeping of laboratory animals in Islamic Azad University of Jahrom. They were kept in light condition of 12h light and 12h darkness. Standard compressed animal food (Pellet) was used in order to feed the rats. They were divided into 5 eight-member groups. The first group was control rats, second group sham rats which received distilled water, third group was the rats which received 30 mg/kg Raloxifene, forth group was the rats which were injected 60 mg/kg Raloxifene and fifth group was the rats which received 120 mg/kg Raloxifene. All injections were done intraperitoneally during 4 weeks with disposable insulin syringes. In this study the lethal dose of Raloxifene (LD_{50}) was determined 240 mg/kg. Therefore sub-lethal doses (30, 60, 120 mg/kg) were used. Raloxifene was supplied by Osveh Pharmacy Company. At the end of test period, all animals were anesthetized with ether and were weighted by AND (Japanese model digital scale) in 0.01 accuracy. Then the ovaries were removed by surgery and adipose tissues around them were removed carefully. Each animal’s right and left ovaries were separately weighted and until the preparation of tissue slices they were kept in glasses containing formalin fixation. Then tissues were sent to histology lab for the preparation of slides. The results were analyzed by SPSS software (version 20), ANOVA analysis test and DUNCAN statistical test. Average and standard deviation were estimated. P ≤ 0.05 was taken as statistical significant level.

RESULTS

Due to the body-weight results, there is no significant difference between experimental, sham and control groups (table 1).

According to the results obtained from measuring the weight of right ovary in all groups, experimental groups show no significant difference compared to control group (table 1).

According to the results obtained from measuring the weight of left ovary in all groups, no group shows a significant difference compared to control group (table 1).

Results obtained from counting the number of initial follicles related to ovaries between different groups show that the average of these follicles in non group shows meaningful difference than control groups (table 1).

Results obtained from counting the number of the initial follicles related to ovaries between experimental groups 1, 2 show no significant difference compared to control group, but the experimental group 3 shows a significant decrease compared to control group and experimental group 1 (table 1).
Results obtained from counting the number of secondary follicles related to ovaries between different groups show that none of the experimental groups had a meaningful change compared to the control group, but experimental group 3 shows a significant decrease compared to the experimental group 1 (table 1).

Results obtained from counting the number of the graph follicles related to ovaries between different groups show that there is no meaningful difference between experimental groups 1, 2, 3 compared to control group. But the experimental groups 2, 3 showed significant decrease compared to experimental group 1 (table 1).

Results obtained from counting the number of the yellow body related to ovaries between different groups show that there is no significant difference between experimental groups 1, 2 in compared to control group, but the experimental group 3 shows a significant decrease compared to experimental group 1 (table 1).

Results obtained from counting the number of the uterus follicles related to ovaries between different groups show that there is no significant difference between experimental groups compared to control group (table 1).

Table 1. Average comparison in different groups

<table>
<thead>
<tr>
<th>Experimental 3</th>
<th>Experimental 2</th>
<th>Experimental 1</th>
<th>Blank</th>
<th>Control</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.6±9.8a</td>
<td>15.510.2a</td>
<td>16.0111.1a</td>
<td>15.210.3a</td>
<td>15.810.6a</td>
<td>Weight of Body</td>
</tr>
<tr>
<td>0.0520.02a</td>
<td>0.0410.02a</td>
<td>0.0380.006a</td>
<td>0.0490.002a</td>
<td>0.0470.003a</td>
<td>Weight of Right Ovary</td>
</tr>
<tr>
<td>0.0450.008a</td>
<td>0.0380.009a</td>
<td>0.0330.003a</td>
<td>0.0440.002a</td>
<td>0.0480.002a</td>
<td>Weight of Left Ovary</td>
</tr>
<tr>
<td>1.70.35a</td>
<td>2.80.45a</td>
<td>3.20.8a</td>
<td>2.10.47a</td>
<td>2.60.24a</td>
<td>Initial follicles</td>
</tr>
<tr>
<td>6.51.7a</td>
<td>7.16ab</td>
<td>10.50.35b</td>
<td>9.51.5b</td>
<td>9.20.58b</td>
<td>First follicles</td>
</tr>
<tr>
<td>1.10.64a</td>
<td>2.50.48ab</td>
<td>3.20.86b</td>
<td>2.10.65ab</td>
<td>2.80.58ab</td>
<td>Secondary follicles</td>
</tr>
<tr>
<td>5.011.7a</td>
<td>5.81.6a</td>
<td>9.71.5b</td>
<td>6.80.79ab</td>
<td>6.40.87ab</td>
<td>Graph follicles</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Raloxifene is a drug which is discovered in the past two decades. Due to the selective nature of Raloxifene it is called the Selective Estrogen Response Modifiers (SERMs). Estrogenic and anti-estrogenic effects of Raloxifene in organization and regulation of nerve system in controlling reproduction performance is quite clear. In fact, there are paradoxical results about estrogenic or anti-estrogenic behaviors of Raloxifene on hypothalamus–hipofisis-gonad axis. According to table 1, there was no significant difference seen in the weights of ovaries in experimental groups 1, 2, 3 compared to the control group. TenaSempere, et al. (2004) after studying Raloxifene effects on female mice stated that this drug causes a decrease inovary weight9.

Pinla, et al (2002) also claimed that this drug causes a decrease inovary weight10 which does not match up with the results of this study. Previous studies expressed that this drug can cause fragmentation in action of hypothalamus–hipofisis-gonad axis and can cause a decrease in hipofisis volume. According to some researches carried out in the past, this drug causes a decrease in hipofise weight which can cause ovary weight loss. In this study, however, this result is not obtained though.

Results obtained from counting the number of the ovarian follicles showed that the number of initial, secondary and graph follicles did not have a significant difference in any of the experimental groups compared to the control group. But the first follicles in experimental group 3 showed a significant decrease compared to experimental group 1.

According to the research about effects of this drug on female mice which is carried out by Tena-Sempere, et al. (2004) and Gerald (2001) (9, 11) ovulation rate and level of progesterone was reduced. Gerald, on the other hand, stated that this drug causes swelling in follicles and hyperplasia in granulosa cells, a change in ovarian morphology and decrease in ovulation rate. In another study by
Pinela (2001) on “Raloxifene effects on operation of male mouse reproduction” expressed that this drug cause testis atrophy but it has less effects on reproduction system of male mouse. Furthermore, by injecting this drug to female mice, abundant horny cells in vaginal smear were found. Pinela also stated that this drug increase prolactin level. After maturation alarge amount of FSH and LH hormones are released from anterior hipofisis and cause secretion of LH (producer of yellow body) and FSH (follicle-stimulating hormone) from anterior hipofisis andthesese hormones cause secretion of ovarian hormones called estrogen and progesterone from ovary. Low concentration of estrogen also inhibits gonadotropins releasing. Increase in prolactin reduced the secretion of gonadotropinsby affecting the pulsatile release of GnRH. Therefore increase in prolactin causes FSH reduction. Reduction of follicle-stimulating hormone, FSH, can affect the growth and reduction of follicles process.

According to the table 1, no significant difference was seen in uterus follicles compared to control group.

Alisiya (2010) observed uterus follicles in ovarian tissue when he injected this drug to female mice.

Successful distinction of follicles depends on the presence of steroid and growth factors which stimulates follicle distinction and protectsthe cells against cellular death. The growth and distinction of ovarian follicles are done by proliferation and differentiation of granulosa cells. Estrogenic hormones in ovary have the main role in control of growth, evolution, homeostasis and planned death. In this study the amount of uterus follicles is not significant. Indeed by increasing the time of using of this drug, increasing in uterus follicles may happen.

According to table 1 experimental group 3 showed significant decrease compared to average number of yellow body.

In a research conducted by Gerald (2001), it is stated that this drug can cause anextremely decrease in yellow body as well as reducing progesterone. Another research claimed that the number of yellow body was reduced which matches up with the changes of yellow body in this study. Transformation of granulose cells and inner theca into yellow body is essentially dependent on the secretion of LH hormone from anterior hipofisis. Moreover, yellow body cells, influenced by LH hormone, replace their enzyme group and start to discharge progesterone and estrogen. Yellow body is the main source of progesterone. A decrease in Yellow body in this study can be caused by the reduction of the LH hormone.

REFERENCES


