Effect of Antiprogestrogen on Myometrial Thickness and Progesterone/Estrogen Receptors in the Rat Myometrium

Khadija Qamar* , Liaqat Ali Minhas**, Zarmina Saga**
*Department of Anatomy, Army Medical College, Rawalpindi; 
**Department of Anatomy, Rawalpindi Medical College, Rawalpindi

Abstract

Background: To study the histomorphological effects of Mifepristone treatment on rat uterus.

Methods: In this experimental study, sixty adult female rats were divided randomly into two groups, comprising of 30 animals in each group. In group A one ml of normal saline was given orally daily for three months while in group B mifepristone was given orally in a dose of 1 mg/kg body weight daily for three months. All the animals were sacrificed next day after the last oral dose. Two ml blood was taken directly from the heart for measurement of estrogen and progesterone levels. About ½ cm piece of tissue was taken from the middle of the right uterine horn. Sections were cut and immunohistochemical staining procedure was done for demonstration of progesterone and estrogen receptors

Results: In the experimental group the number of progesterone and estrogen receptors in the uterine myometrium of the experimental group were decreased and found statistically significant. Significantly lower level of progesterone while higher levels of estrogen level were noted in the experimental group as compared to control group. In the experimental group thickness of the myometrium was reduced

Conclusion: Long term mifepristone (Antiprogestrogen) administration suppresses the endometrial proliferation. It decreases the number of progesterone and estrogen receptors. It lowers the serum levels of progesterone, while estrogen levels elevates it.

Keywords: Mifepristone, estrogen, progesterone, myometrium.

Introduction

Estrogen and Progesterone responsive tissues are initially determined by the tissue distribution of receptor proteins whose restricted spatiotemporal expression identifies tissues targeted for hormonal response. However, tissues that express receptors for estrogen and progesterone exhibit physiologically diverse responses to the same steroidal ligand. Functional diversity arises from the existence of two structurally related but nonidentical receptors of each hormone and by the ability of a single receptor subtype elicits diverse transcriptional response to a specific ligand. Steroid receptors including those for estrogen and progesterone have a modular protein structure consisting of distinct functional domains capable of binding steroidal ligand, dimerization of ligand receptors, interaction with hormone responsive DNA elements, and interaction with coregulator proteins required for bridging receptors to the transcriptional apparatus.

Mifepristone acts at the receptor level, binding strongly to the progesterone and glucocorticoid receptors, and to a lesser extent to the androgen receptor. It is relatively five times greater than progesterone, three times greater than dexamethasone and four times less than testosterone, respectively. Mifepristone, like progesterone, enters target cells and reaches its receptors; however, it interacts differently from progesterone and may produce different conformational changes in the receptor. By occupying the progesterone receptor in the nucleus, progesterone modifies the receptor’s shape, enabling it to bind to chromatin, and this binding leads to gene transcription and protein synthesis.

Material and Methods

These laboratory based randomized controlled trials were conducted at the department of Anatomy, Army Medical College Rawalpindi from Jan 2007-
March 2007. Sixty healthy adult female Sprague Dawley rats weighing 200-300 g were procured from the National Institute of Health Sciences Islamabad. The animals were randomly divided into two groups of 30 each. Group A (Control) comprised thirty female rats and were given one ml of normal saline orally daily for three months. Group B (Experimental) comprised thirty female rats and were given the drug (Mifepristone) orally in a dose of 1 mg/kg body weight daily for three months. All the animals were sacrificed next day after the last oral dose. Two ml blood was taken directly from the heart for measurement of estrogen and progesterone levels. Uterine horns along with a portion of vagina was removed, trimmed and placed into 10% Formalin for 24 hours. About ½ cm piece of tissue was taken from the middle of the right uterine horn. It was passed through ascending series of alcohol from 70% to 100%, cleared in xylene, infiltrated and embedded in paraffin wax at 58°C. Sections were stained with hematoxylin and eosin for light microscopic study. Immunohistochemical staining procedure was done for demonstration of estrogen and progesterone receptors.

Microscopic observations:
On microscopic examination thickness of the myometrium was taken by measuring the maximum thickness of the myometrium between two points on uterine wall one on the side merging with the endometrium and other up to the inner limit of the serosa. On immuno histochemistry number of mild, moderate, and marked brown stained progesterone and estrogen receptors in the myometrial cells were counted per cross section and their mean was calculated.

**Results**

Total sixty animals were included in the study, 30 in each group. In control group the Middle layer (Myometrium) consisted of elongated spindle shaped cells with pointed ends. Nuclei were elongated and centrally located. These were smooth muscle cells, composed of interlacing bundles of long slender fibers arranged in ill defined layers of thick inner circular and outer longitudinal layers. The longitudinal muscle layer was closely applied to the circular layer with only a minimal amount of connective tissue having network of tubular structures lined with single layer of flattened cells representing the blood vessels(Fig 1). The considerable numbers of mitotic figures were observed in the muscle layers. The mean thickness of myometrium was355.3±16.1 µm. (Table I).

<table>
<thead>
<tr>
<th>Table 1: Number of Progesterone Receptors in Uterine Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone Receptors</td>
</tr>
<tr>
<td>Myometrial cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Number of Estrogen Receptors in Uterine Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen Receptors</td>
</tr>
<tr>
<td>Myometrial cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Comparison between Control and Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Thickness of myometrium (µm)</td>
</tr>
<tr>
<td>Progesterone ng/ml</td>
</tr>
<tr>
<td>Estrogen pg/ml</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; NS = Insignificant; *= Significant

Total number of Progesterone receptors (PR) in the myometrial cells of the uterus was 6101 (Mild stained 1621, moderate stained 3040 and marked stained 1440) (Table I). In the control group myometrial cells showed more number of marked staining PR as compared to the mild and moderate stained PR. Mild, moderate and marked estrogen receptors were also counted in the myometrial cells. Total number of ER in the myometrial cells of the uterus was 5771 (Mild stained 1110, moderate stained 2980 and marked stained 1681) (Table 2).

In experimental group middle layer (myometrium) as compared with controls was decreased in thickness. Their spindle shaped cells were loosely arranged in ill defined layers of inner circular and outer longitudinal layers. The amount of connective tissue intervening between these layers was increased. Increased number of infiltration of granulocytes was observed. Eosinophils were more in number (Fig 2). The outer most layers were composed of loose connective tissue with large number of dilated vessels as it was in the control group. The mean thickness of myometrium (148.6±9.6 µm) was less than that of control group. The difference was highly
significant statistically ($p = 0.001$) when compared with the control group (Table 3).

![Image](image1.png)

**Figure 1:** Uterine horn of animal of control group showing connective tissue having network of tubular structures lined with single layer of flattened cells representing the blood vessels (arrows).

![Image](image2.png)

**Figure 2:** Uterine horn of animal of control group showing stroma consisted of stromal cells (SC), network of collagen fibers (CF) stained with eosin, intermingled with amorphous ground substance.

![Image](image3.png)

**Figure 3:** Uterine horn from animal of control group showing moderately stained PR with immunostaining in myometrium and mild stained PR with immunostaining in myometrium of animal.

Total number of PR in the myometrial cells of the uterus was 318. (Mild stained 17, moderate stained 134 and marked staining 167) (Fig 3). The number of progesterone receptors in myometrial cells of the uterus was reduced in the experimental group as compared with the control group (Fig 4). Myometrial cells showed more number of marked staining PR as compared to the mild and moderate staining PR.

The mild, moderate and marked estrogen receptors (ER) were also counted in the myometrial cells. Total number of ER in the myometrial cells of the uterus was 3806. (Mild stained 550, moderate stained 1610 and marked stained 2220). The numbers of estrogen receptors in myometrial cells of the uterus were reduced in the experimental group as compared with the control group (Fig 5). Myometrial cells showed more number of marked staining ER as compared to the mild and moderate staining estrogen receptors.

![Image](image4.png)

**Fig 4:** Progesterone receptors in myometrium of control and experimental groups.

![Image](image5.png)

**Fig 5:** Progesterone receptors in myometrial cells of control and experimental group.

![Image](image6.png)

**Fig 6:** Estrogen receptors in myometrium of control and experimental groups.

The mean serum estrogen level was (83.6±1.2 pg/ml), which was higher than that of the control group. The difference was found significant statistically when compared with the control group. The mean progesterone level was lower in experimental group than that of the control group. The difference was found significant statistically when compared with control group ($p = 0.001$) (Table 3).

**Discussion**

In the present study, the long term effects of Mifepristone treatment on rat myometrium were studied. The experimental group displayed the decrease in myometrial thickness, as compared to control group. Progesterone induced proliferative activity was profoundly reduced in experimental group. Mifepristone antagonizes the estrogen-induced proliferative response in uterine stromal and myometrial cells. Cell proliferation in the normal uterus corresponded with high serum levels of steroid hormones during the estrous cycle, and apoptosis occurred in the rat uterus in all cell types following sharp, cyclical declines in serum hormone levels. The responsiveness of uterine mesenchymal cells decreases...
in both proliferative and apoptotic rates observed in myometrial and stromal cells of mifepristone treated animals. 6

In the present study an attempt was made to determine whether the antiprogesterin-induced anti-estrogenic effects are reflected as change in the concentration or localization of endometrial ER and PR, as both of these receptor proteins are estrogen-dependent. 7 Total number of PR in all the compartments of uterus was less as compared with the control group. The difference was statistically significant. The number of progesterone receptors in the myometrial cells of the uterus was reduced in the experimental group as compared with the control group. Progesterone receptors (PR) mediate multiple aspects of female reproduction and are important targets for reagents that can modulate progesterone-dependent events. Many such reagents have been developed, and they range from full PR antagonists (PAs) to compounds with mixed agonist/antagonist actions, currently known as selective progesterone receptor modulators (SPRMs). 8 Myometrial cells showed more number of marked staining ER as compared to the mild and moderate staining estrogen receptors. This reflects significant change in the levels of endometrial ER in the treated groups as compared with the control group. Estrogen receptor is associated with shifts in uterine weight, proliferation and morphogenesis. Uterine ER levels were decreased by administration of progesterone to estrogen-treated rats. The progesterone-mediated decrease in ER protein has been shown in breast cancer cells to result from decreased cellular ER mRNA levels, likely to reflect decreased transcription of the ER gene, since the effect was seen rapidly without shortening of the ER mRNA half-life. 9

The observation of the presence of ER at relatively high concentrations in smooth muscle cells of the myometrium of control group has led to the conclusion of a direct action of this steroid on smooth muscle cells. This suggests that populations of ER and PR in the endometrial stroma and myometrium are more stable than those in the epithelium. As the expression of estrogen receptors in our cases was associated with high plasma oestradiol concentrations, it is possible that the expression of these receptors can be oestradiol independent. Antiprogesterin administration also inhibits estrogen-dependent endometrial cell proliferation and growth. 10

**Conclusion**

The antiproliferative effects of antiprogesterone therapy formulates basis for their clinical utilization.

**References**