Original Article

The Effect of Mifepristone Antiprogestrogen on the Number of Progesterone Receptors in the rat Uterus

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Abstract

Objective: To investigates the effect of mifepristone on the number of progesterone receptors in the rat uterus**. Study Design:** Laboratory based randomized controlled trials. **Place & Duration of the study:** Department of Anatomy, Army Medical College Rawalpindi from Jan 2007-March 2007. **Method:** Sixty adult female rats were divided randomly into two groups, comprising of 30 animals in each group. In control 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. Immunohistochemical staining procedure was done for demonstration of progesterone receptors **Results:** In the control group Total number of PR stained receptors in all the compartments of the uterus were 40400. In the experimental group the number of progesterone receptors in all uterine compartments of the experimental group was 986 Significantly lower level of progesterone while higher levels of estrogen level were noted in the experimental group as compared to control group. **Conclusion:** Long term mifepristone administration suppresses the endometrial proliferation The number of progesterone receptors in all uterine compartments of the experimental group were decreased and found statistically significant It also lowered the plasma concentration of progesterone. While the plasma concentration of the estrogen was raised. **Key Words**: Mifepristone, receptors, estrogen, progesterone, endometrium.

INTRODUCTION

The effects of Estrogen & Progesterone are mediated through interaction with specific intracellular receptors that are members of the nuclear receptor super family of transcription factors. Binding of the steroids to their cognate receptors induces conformational changes in receptor structure leading to receptor demierization, posttranslational modification, and binding to specific enhancer DNA elements in the promoters of specific genes and recruitment of co regulator proteins that interact with general transcriptional machinery to elaborate hormone triggered changes in promoter activity[1.] Mifepristone is a progesterone receptor antagonist used as an abortifacient in the first months of pregnancy, and in smaller doses as an emergency contraceptive. Mifepristone acts at the receptor level, binding strongly to the progesterone and glucocorticoid receptors, and to a lesser extent to the androgen receptor. Mifepristone is potent, antiprogesterone and antiglucocorticoid and a weak antiandrogen.. The metabolites of mifepristone also bind to the progesterone receptor[2]. 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[3]. 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[4].

MATERIAL & 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)

Thirty female rats were given one ml of normal saline orally daily for three months.

GROUP-B (EXPERIMENTAL)

Thirty female rats 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. Five microns thick sections were cut and Immunohistochemical staining procedure was done for demonstration of progesterone receptors.

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Number of mild, moderate, and marked brown stained progesterone receptors in the luminal epithelium, glandular epithelium, stromal cells and myometrial cells were counted per cross section and their mean was calculated (fig1).

RESULTS

Total sixty animals were included in the study, 30 in each group. In control group Progesterone receptors (PR) were evaluated as mild, moderate and marked stained in different parts of the uterine sections. Mild, moderate and marked stained progesterone receptors were counted in the luminal epithelial cells, glandular epithelial cells, stromal cells and myometrial cells. Total number of stained receptors in all the compartments of the uterus was 22948.

Total number of PR in the luminal epithelium was 4550 (Mild stained 435, moderate stained 2060 and marked stained 2050). (Fig.2a)

Total number of PR in the glandular epithelium was 7331 (Mild stained 1721 moderate stained 2700 and marked stained 2910).

Total numbers of PR in the stromal cells were 4971 (Mild stained 1120, moderate stained 1601 and marked stained PR was 2250). (Fig.2a).

Total number of PR in the myometrial cells of the uterus was 6101 (Mild stained 1621, moderate stained 3040 and marked stained 1440). (Appendix XIII).

In this group luminal epithelium and myometrial cells showed more number of marked staining PR as compared to the mild and moderate stained PR.

In the glandular epithelium and stromal cells moderate staining PR were more in number as compared to the mild and marked stained PR.

In experimental group total number of PR in all the compartments of uterus was 991which was very less as compared with the control group .The difference was statistically significant (Table I). Total number of PR in the luminal epithelium was 309(Mild stained 35, moderate stained 118 and marked stained 156). The number of progesterone receptors in the luminal epithelium of the uterus was reduced in the experimental group as compared with the control group (Fig.2 b). (Bar chart I).

Total number of PR receptors in the glandular epithelium were179.(Mild stained 45, moderate stained 104 marked stained 30). The numbers of progesterone receptors in the glandular epithelium of the uterus were reduced in the experimental group as compared with the control group (Bar chart II).

Total number of PR in the stromal cells was 185. (Mild stained 26, moderate stained 114 and marked staining 45). The number of progesterone receptors in stomal cells of the uterus were reduced in the experimental group as compared with the control group (Fig.2b). (Bar chart III).

Total number of PR in the myometrial cells of the uterus was 318.(Mild stained 17, moderate stained 134 and marked staining 167) (Fig.3b). The number of progesterone receptors in myometrial cells of the uterus was reduced in the experimental group as compared with the control group (Bar chart IV).

Luminal epithelium and myometrial cells showed more number of marked staining PR as compared to the mild and moderate staining PR. In the glandular epithelium and stromal cells moderate staining nuclei PR were more as compared to the mild and marked staining PR nuclei. 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 2.8 + 0.09 ng/ml which was lower than that of the control group .The difference was found significant statistically when compared with control group (p =0.001) (Table. II).

Table-1

Mean number of progesterone receptors in uterine tissues of control and experimental groups

Progesterone Receptors	Control		Experimental		Statistical Significance of the difference (p-value)
	No. of Animals	Mean ± SE	No. of Animals	Mean ± SE	
Luminal epithelium	30	151.5 ± 6.9	30	10.6 ± 1.3	<0.001
Glandular epithelium	30	224.4 ± 12	30	5.70 ± 1.0	<0.001
Stromal cells	30	168.7 ± 9.5	30	6.5 ± 1.9	<0.001
Myometrial cells	30	199.7 ± 17	30	10.6 ± 2.9	<0.001

Table-2

Comparison of study variables between control and experimental groups

Parameters	Control (n=30)	Experimental (n=30)	p-value
Progesterone ng/ml	5.5 ± 0.8	2.8 ± 0.09	0.001*
Estrogen pg/ml	41.7 ± 0.66	83.6 ± 1.2	0.001*

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

Figure-1

Photomicrograph of a cross section from uterine horn of animal of control group showing mild (1). Moderate(2),and marked (3) stained nuclei.

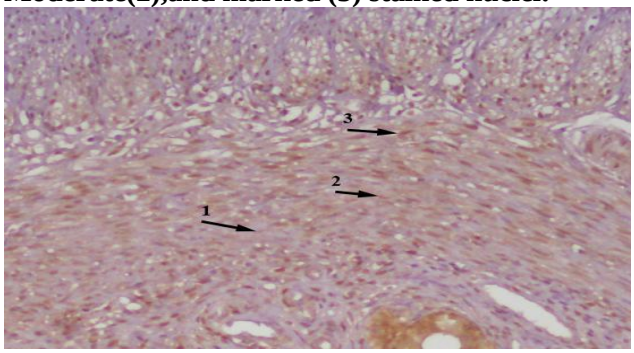


Figure-2

Photomicrograph of a cross section of uterine horn from animal no 9 of control group a, showing moderately stained PR with immunostaining in luminal epithelium and stroma cells and mild stained PR with immunostaining in luminal epithelium and stroma cells of animal no 12 of experimental group b. Bar =50µm.

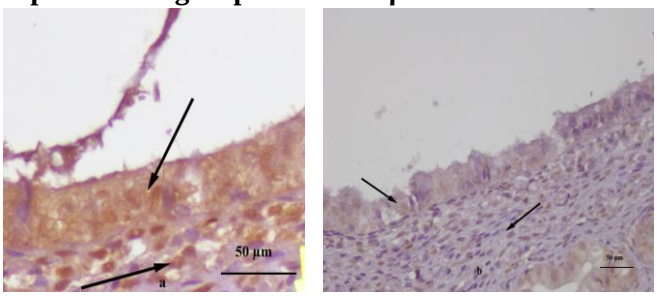


Figure-3

Photomicrograph of a cross section of uterine horn from animal no 8 of control group a, showing moderately stained PR with immunostaining in myometrium and mild stained PR with immunostaining in myometrium of animal no 11 of experimental group b. Bar =50µm

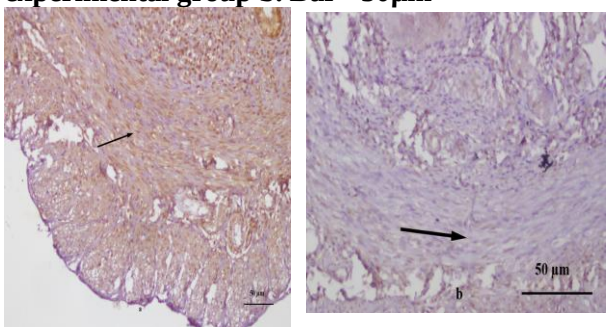


Figure-4

Statistical significance of number of progesterone receptors in luminal epithelium of control and experimental groups

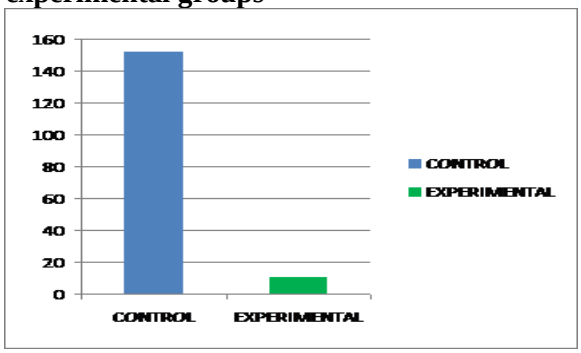


Figure-5

Statistical significance of number of progesterone receptors in glandular epithelium of control and experimental groups

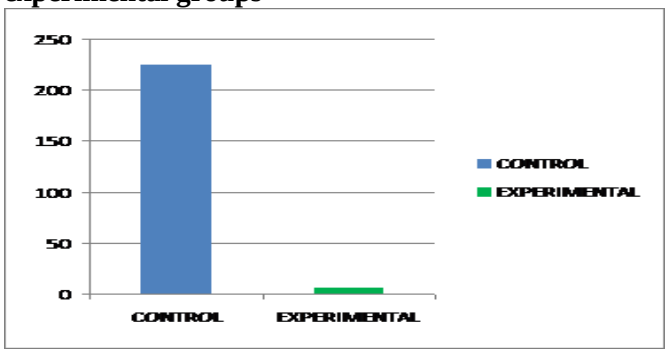
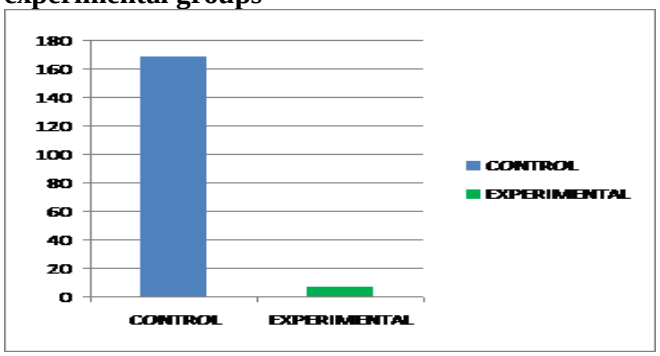


Figure-6

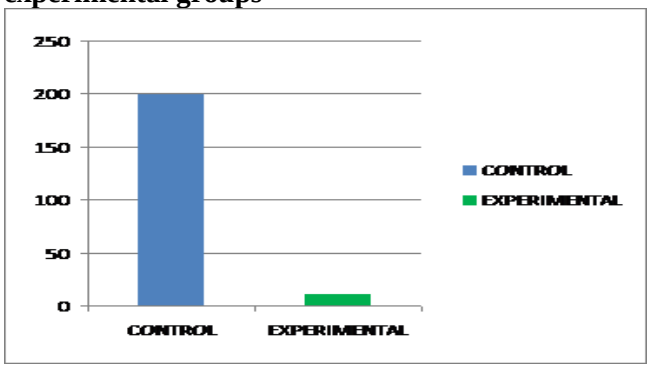
Statistical significance of number of progesterone receptors in stromal cells of control and experimental groups



Statistical significance of number of progesterone receptors in stromal cells of control and experimental groups

Figure-7

Statistical significance of number of progesterone receptors in myometrial cells of control and experimental groups



DISCUSSION

In this study of 3 months we have studied the long term effects of mifepristone treatment on rat endometrium. In the present study an attempt was made to determine whether the antiprogestin-induced anti-estrogenic effects are reflected as change in the concentration or localization of endometrial PR, as these receptor proteins are estrogen-dependent. Total number of PR in all the compartments of uterus was 991which was very less as compared with the control group .The difference was statistically significant The number of progesterone receptors in the luminal epithelium, glandular epithelium, stromal cells and in the myometrial cells of the uterus was reduced in the experimental group as compared with the control group.

Luminal epithelium and myometrial cells showed more number of marked staining PR as compared to the mild and moderate staining PR. In the glandular epithelium and stromal cells moderate staining nuclei PR were more as compared to the mild and marked staining PR nuclei .

The most obvious change in steroid receptor distribution in the uterus was the reduction in staining intensity PR in the glandular epithelium and stromal cells. There is a report demonstrating elevation in endometrial ER and PR after chronic administration of antiprogestin[5]. Progesterone receptor concentration could be expected to be highly associated with successful implantation since many of the relevant local factors such as cytokines and growth factors are progesterone-regulated. Down regulation of the progesterone receptor has been shown to be highly associated with development of endometrial receptivity[6] .RU 486 appears to act by high affinity binding to the progesterone receptor, trapping it into an inactive DNA-receptor complex [7]. The data on the inhibitory action by RU 486 therefore strongly suggests that the proliferative effects of progesterone are mediated via its receptor[8]. In the experimental group luminal epithelium and myometrial cells showed more number of marked staining PR as compared to the mild and moderate staining receptors. In the glandular epithelium and stromal cells moderate staining nuclei PR were more moderate in staining as compare to the mild and marked staining PR nuclei. A previous study showed that progesterone dependant down-regulation of PR in endometrial glandular cells was inhibited by mifepristone[9]. Numbers of

progesterone receptors were more markedly reduced as in all the compartments of the uterine tissues when compared with the control and experimental groups. Mifepristone action in the endometrium is mediated by the PR with higher affinity of mifepristone for the PR compared with ER[10]. The high efficacy of Mifepristone administered at a dose of 1mg/kg could also be demonstrated in glands and luminal epithelium where progesterone –induced vacuolization was blocked completely[11]. Endometrial stromal cells mediate many of the effects of steroids through the steroid-induced secretion of various growth factors[12]. Retention of a significant number of ER and PR receptors in the stromal cells at all times would be advantageous because these cells would be able to synthesize and release growth factors with greater rapidity in response to changes in plasma concentrations of estrogens and progesterone[13] However, reports on variation in the expression of PR in human endometrium were expressed in most glands and stroma. The scores for PR immunostaining in stromal cells were slightly higher than the ones in glandular cells[14]. Anti-progestins block estradiol action on glandular proliferation and suppress the overall growth of the primate endometrium. The mechanism involved in this endometrial anti-proliferative effect is not known, but the receptors may play a role[15]. Epithelial PR can directly mediate antiproliferative effects of mifepristone, at least in vitro and, because stromal PR is also increased, an enhanced sensitivity of the stroma to hormones could block any progesterone-dependent, stromal–epithelial interactions involved in glandular mitosis. Therefore the suggested mechanism of action probably has a cell specific character[16]. 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) [17]. Our data suggest that a disturbance of normal stromal function may explain why antiprogesterone treatment ultimately leads to endometrial atrophy. If we presume that stromal compaction is due to a lack of interstitial fluid possibly caused by decreased vasopermeability. Mifepristone treatment, induced reduction in the production of VEG+/VPF would be in agreement with the observed high stromal cell density[18]. Thus several mechanisms of action of antiprogestines might contribute to their potential usefulness as contraceptive agents preventing implantation. In stroma Mifepristone increases cellular density and in glands antagonizes the action of progesterone and blocks secretory transformation. Both actions appear to be medicated by the PR[19]. Serum progesterone levels declined in experimental groups after mifepristone administration. Level of estrogen hormone was elevated in the experimental group as compared with the control group. Previous reports concerning the effect of mifepristone on progesterone secretion have not been entirely congruent[20]. In a study using 200 mg of mifepristone, no statistically significant changes in the progesterone levels were observed in the 2-day follow-up[21]. In another study, with 600 mg mifepristone, progesterone levels increased on day 1 and then decreased significantly[22]. The paradoxical effects (Mifepristone both raises and lowers progesterone levels) have also been explained by the hypothesis that mifepristone can act either by preventing the progesterone effect or in a way that is similar to that of progesterone, which always stimulates its own secretion by autoregulation[23].

CONCLUSION

It is concluded that in long term mifepristone affected the endometrial proliferation and induced histomorphological changes in the uteri of the experimental rats. Progesterone antagonist application lowered the plasma concentration of progesterone. While the plasma concentration of the estrogen was raised. The number of progesterone receptors in all uterine compartments of the experimental group were decreased and found statistically significant. The disturbance of normal stromal function explains that antiprogestin treatment ultimately leads to endometrial atrophy. This contributes to their potential usefulness as contraceptive agents preventing implantation:

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