EFFECTS OF ANTI-PROGESTERONE ON PROGESTERONE AND OESTROGEN RECEPTORS PRESENT IN STROMAL CELLS OF RAT UTERUS

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ABSTRACT

Objective: To investigates the effect of mifepristone (anti-progesterone) on stromal cells of the uterus of rats. **Study Design:** Laboratory based randomized controlled trials

Method: Sixty adult female rats were divided randomly into two groups, comprising of 30 animals in each group. In-group A 1ml of normal saline was given orally daily for three months while in group B mifepristone was given orally in a dose of 1mg/kg body weight daily for three months. All the animals were sacrificed next day after the last oral dose. 2ml blood was taken directly from the heart for measurement of progesterone levels. Sections were stained with hematoxylin and eosin for light microscopic study. Immunohistochemical staining procedure was done for demonstration of progesterone receptors

Results: The stromal cells were flattened and irregular in outline present around the glands. Some appeared fusiform or spindle shaped. In the experimental group the stromal cells were tightly packed. There was an increase in the number of infiltration of granulocytes and eosinophils in the stroma.

Progesterone antagonist application lowered the plasma concentration of progesterone.

The number of progesterone receptors in all uterine compartments of the experimental group were decreased and found statistically significant.

Conclusion: In conclusion, mifepristone affects stromal, glandular and epithelial morphology in the rat uterus.

Keywords: Mifepristone, receptors, oestrogen, progesterone, stromal cells

INTRODUCTION

Oestrogen effects predominate during the follicular phase of the estrous cycle, whereas progesterone dominates during the proliferative phase in non-pregnant uterus[1]. Oestrogen & progesterone have specific intracellular receptors members of the nuclear receptor super family of transcription factors[2].

Estraneprogestins are the precursors of Mifepristone, {17(,hydroxyl 11-(-(4-dimethylaminophenyl)-17(-(prop-1-ynyl)-estra-4, 9-dien-3-one,}[3]. Mifepristone binds strongly to the progesterone and glucocorticoid receptors, and to a lesser extent to the androgen receptor, thus mediating its effects at the receptor level[4].

This study was conducted to investigate the effect of mifepristone (anti-progesterone) on stromal cells of the uterus of rat.

MATERIAL AND METHODS

These laboratory based randomized controlled trials were conducted at the department of

Anatomy, Army Medical College Rawalpindi from Jan 2007 to March 2007. Institutional Ethical Committee of Army Medical College Rawalpindi approved all the procedures. Sixty healthy adult female Sprague Dawley rats weighing 200-300g were procured from the National Institute of Health Sciences Islamabad. The animals were randomly divided into two groups having 30 rats in each.

Group A (Control)

Thirty female rats were given 1ml of normal saline orally daily for three months.

Group B (Experimental)

Thirty female rats were given the drug (Mifepristone) orally in a dose of 1mg/kg body weight daily for three months.

All the animals were sacrificed next day after the last oral dose. 2ml blood was taken directly from the heart for measurement of progesterone level. About ½cm piece of tissue was taken from the middle of the right uterine horn. Approximately five microns thick sections were cut and stained with hematoxylin and eosin for light microscopic study. Immunohistochemical staining procedure was done for demonstration of progesterone receptors.

Microscopic observations

Qualitative Parameters

Morphology of the distribution of stroma was noted.

Statistical analysis

Data had been analyzed using SPSS version 15. Descriptive statistics were used to describe the data. Quantitative variables were compared through independent samples' t-test while qualitative variables were compared through chi-square test between cases and controls. P-value < 0.05 was considered as significant.

RESULT

Total 60 animals were included in the study, 30 in each group. In the control group the tubular sections showed three distinct layers (inner, middle and outer) of endometrium. The luminal side of inner layer was lined by single regular row of cylindrical cells.

The stroma consisted of stromal cells, network of collagen fibers stained with eosin, intermingled with amorphous ground substance, and several blood vessels (Fig 1). The stromal cells were flattened and irregular in outline present around the glands. Some appeared fusiform or spindle shaped. They had ovoid nuclei with acidophilic cytoplasm. The eosinophils were recognized by their specific pink stained granules. The lymphocytes with deep staining indented nucleus were also present.

While in experimental group at low magnification, inner layer was folded giving it an overall ruffled appearance as a result lumen was much reduced as compared to normal group. Epithelium was low columnar.

The superficial part of inner layer consisted of few glands and abundant stroma and a deeper layer had many glands and relatively less stroma. The stromal cells were tightly packed, having basophilic cytoplasm. The infiltration of the stroma with the eosinophils was observed. (Fig.2). Glands varied in size, some of them were dilated and cystic.

The mean progesterone level in experimental group was 2.8 + 0.09 ng/ml, which was lower than that of the control group i.e. 5.5 ± 0.8. The difference was found statistically significant when compared with control group (p =0.001).

The mild, moderate and marked progesterone receptors were counted in the luminal epithelial cells, glandular epithelial cells, stromal cells and myometrial cells. The number of progesterone receptors in all the compartments of the uterus was reduced in the experimental group as compared with the control group and found statistically significant (Fig.3) (Table-l).

DISCUSSION

The direct effect of the hormone is usually responsible for all the changes seen in the luminal epithelium in response to antiprogesterone[5].

The changes in the uterine wall are likely due to changes in the epithelial – stromal cell interactions and growth factor actions that are required for uterine development[6]. Mifepristone suppresses endometrial cell proliferation and thus, causes reduction in overall endometrial thickness[7]. Cyclical endometrial proliferation, differentiation and secretion during the human menstrual cycle are strictly controlled by oestrogen and progesterone. A balance is required between oestrogen and progesterone production to ensure normal endometrial growth and proliferation[8]. The absence of progesterone removes the 'progesterone brake' leading to persistent estrogenicity and constant endometrial proliferation. Although the ratio of stroma to glands remains normal. The endometrium can become disordered and vascular abnormalities such as dilated capillaries may become apparent[9]. T he effects of unopposed estrogen may lead to endometrial hyperplasia and possible malignancy, a fact to be kept in mind with long-term use of PR antagonists. In rats receiving long-term PR antagonist treatment estrogenic stimulation of the endometrium has been recorded[10]. The nonhuman primate endometrium, however, demonstrates endometrial atrophy and evidence of antiestrogenic. High doses of mifepristone (25mg/d and 50mg/d) cause variable effects, such as atypical cystic changes in eutopic endometrium[11]. Serum progesterone levels declined in experimental groups after mifepristone administration. No statistically significant changes in the progesterone levels were observed in the 2-day following the administration of 200mg mifepristone[12]. However, with 600mg of mifepristone administered progesterone levels increase on day 1 and then decrease significantly[13]. 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. The effects may be variable depending on the duration of pregnancy[14]. The highest density of stromal cells nuclei was observed in all the uteri of the experimental group. The stromal cells were tightly packed with scanty basophilic cytoplasm. The infiltration of the stroma with the eosinophils was observed. The increased level of leukocytes may not be a direct effect of antiprogestin. Mifepristone treatment may up-regulate potential chemokines that cause leukocyte traffic and interleukin-8 and an increase in MCP-1 and influence leukocyte influx in human decidua[15]. Dependent stromal compaction could be identified as one of the key morphological features of the antiprogestrogenic action of mifepristone. It is well established that the proliferation of endometrial stroma is progesterone - dependent in rat. Considering that both the oestrogen and progesterone receptor proteins are oestrogen-dependent, in the present study any change in the concentration or localization of endometrial ER and PR was determined to evaluate whether the antiprogestin-induced anti-oestrogenic effects are reflected in these receptors[16]. 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. An increase in the number of endometrial ER and PR has been observed after chronic administration of antiprogestin[17]. 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. Endometrial receptivity is strongly related to the down regulation of the progesterone receptor.

CONCLUSION
In the experimental group the stromal cells were tightly packed. There was an increase in the

number of infiltration of granulocytes and eosinophils in the stroma. Progesterone antagonist application lowered the plasma concentration of progesterone.

The number of progesterone receptors in all uterine compartments of the experimental group were decreased and found statistically significant. In conclusion, mifepristone affects stromal, glandular and epithelial morphology in the rat uterus.

ACKNOWLEDGEMENTS

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Table-l: Comparison of Number of Progesterone Receptors in Uterine Tissues between Control and Experimental Groups

Uterine tissues	Control (n = 30)	Experimental (n = 30)	P-value
Luminal Epithelium			
Mild	435 (9.6%)	35 (11 %)	
Moderate	2060 (45.3%)	118 (37.1%)	0.017*
Marked	2050 (45.1%)	165 (51.9%)	
Glandular Epithelium			
Mild	1721 (23.5%)	45 (25.1%)	
Moderate	2700 (36.8%)	104 (58.1%)	< 0.001*
Marked	2910 (39.7%)	30 (16.8%)	
Stromal Cells			
Mild	1120 (22.5%)	26 (14%)	
Moderate	1601 (32.2%)	114 (61.6%)	< 0.001*
Marked	2250 (45.3%)	45 (24.3%)	
Myometrial Cells			
Mild	1621 (26.8%)	17 (5.3%)	
Moderate	3040 (50.3%)	134 (42.1%)	< 0.001*
Marked	1380 (22.8%)	167 (52.5%)	

Values are expressed as Frequency (%)
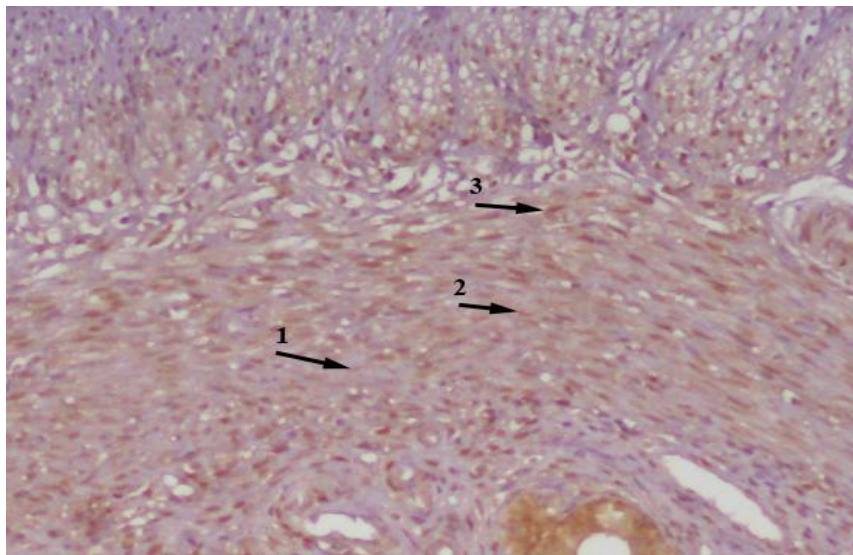
NS = Insignificant

* = Significant.

Figure 1: Photomicrograph of a cross section from uterine horn of animal no 12 of control group showing stroma consisted of stromal cells (SC), network of collagen fibers (CF) stained with eosin, intermingled with amorphous ground substance (GS). H&E stain.Bar = 50μm
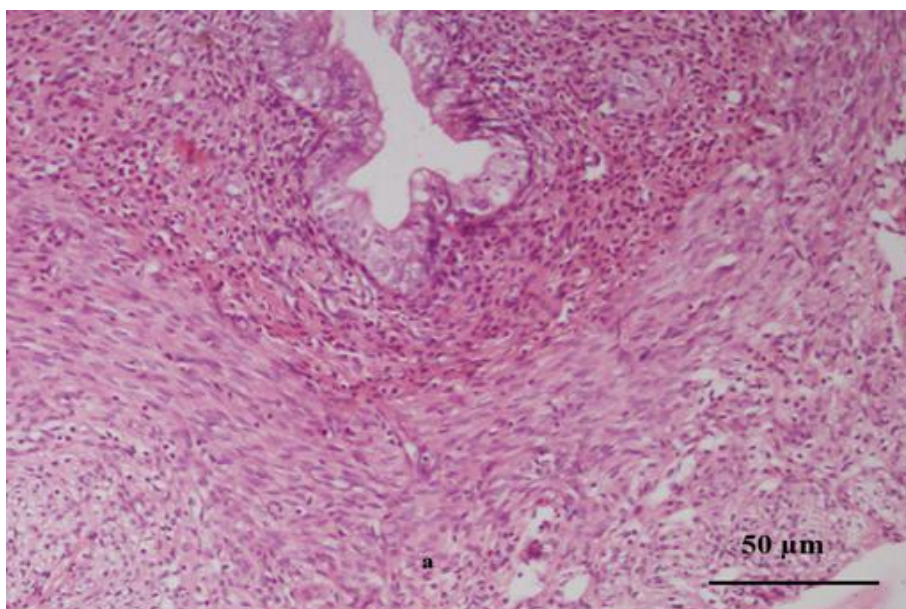

Figure 2: Photomicrograph of a cross section from uterine horn of animal no 27 of experimental group a, showing increased amount of connective tissue, increased number of infiltrating cells in the stroma. H&E stain. Bar =50μm
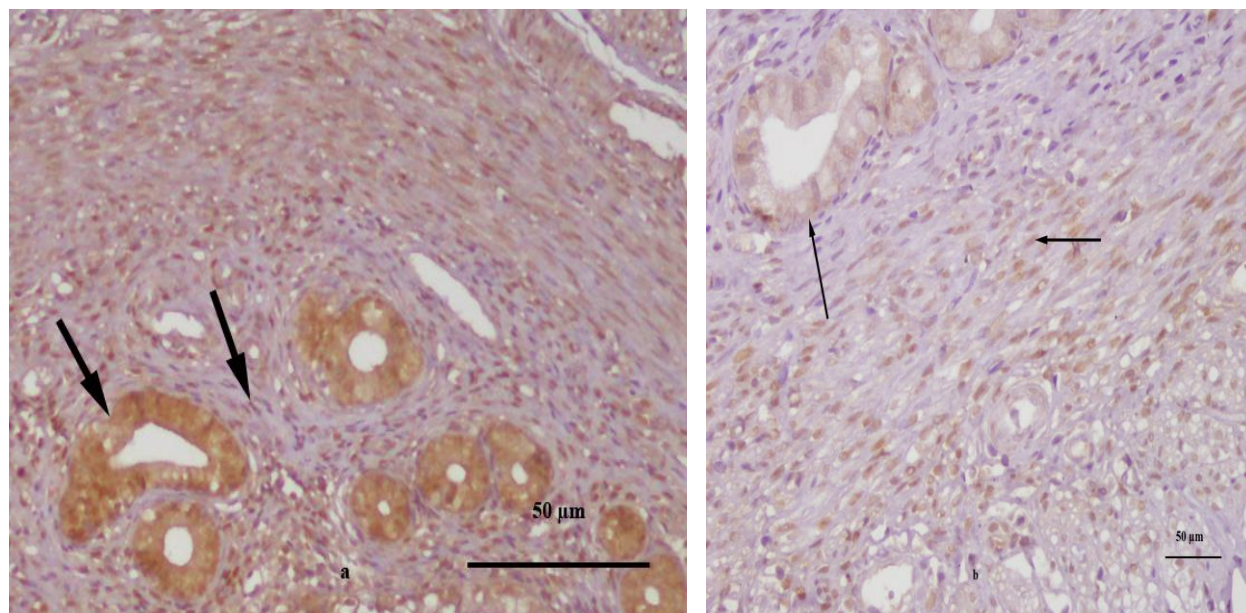
Figure3: Photomicrograph of a cross section of uterine horn from animal no 9 of control group a, showing moderately stained PR with immunostaining in luminal epithelium and stroma cells and mild stained PR with immunostaining in luminal epithelium and stroma cells of animal no 12 of experimental group b. Bar =50µm