EFFECTS OF 13- CIS-RETINOIC ACID ON THE TAMOXIFEN INDUCED UTERINE HISTOLOGICAL CHANGES IN THE RABBIT

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ABSTRACT

Objective: To study the effects of 13-cis-retinoic acid on the tamoxifen induced uterine histological changes in the rabbit.

Study Design: Experimental – randomized controlled trial.

Place and Duration of study: The study was conducted for 4 months at the department of Anatomy, Army Medical College and National Institute of Health in 2007.

Material and Methods: The animals were randomly divided into three groups, a control group A, and two experimental groups B and C, consisting of thirty rabbits each. The experimental groups were treated with tamoxifen only and tamoxifen plus retinoic acid, respectively. The animals were sacrificed after three months. The uteri were then processed for paraffin embedding. Sections were then assessed for the luminal epithelial height, endometrial area and percentage of mitotic figures.

Results: The results obtained were suggestive of uterine proliferation by tamoxifen. The adjuvant administration of 13-cis-retinoic acid produced a statistically significant ($p = 0.002$) inhibitory effect on the tamoxifen induced increase in the area of endometrium, whereas no significant suppressive effect of this drug has been observed on the other parameters when compared with Group B.

Conclusion: 13-cis Retinoic acid has not shown a significant role in the reversal of tamoxifen induced changes in the uterine tissue after a short term administration of three months.

Keywords: 13-cis Retinoic, Tamoxifen, Rabbits, Uterus.

INTRODUCTION

Carcinogenesis is a multistep process, resulting from mutagenic damage to growth-regulating genes and their products, that ultimately leads to development of invasive or metastatic cancers. Neoplasms of the breast and the reproductive system are one of the leading causes of death and morbidity among women worldwide. Exposure to exogenous or endogenous estrogens has been identified as the main event of carcinogenesis in these hormone responsive tissues. Tamoxifen(TAM), a selective estrogen receptor modulator (SERM), was developed as an anti-estrogen for the treatment of breast cancer. It exhibits antiestrogenic activity in the breast and estrogen-like actions on the endometrium. Endometrial malignancies are frequently preceded by a stage of hyperplasia that may progress to carcinoma in situ. The concept of cancer chemoprevention involves interventions at the earliest stages of carcinogenesis i.e. epithelial hyperplasia and hypertrophy. Cancer chemoprevention is the use of specific chemical compounds to prevent, inhibit or reverse carcinogenesis.

13-cis-retinoic acid is a retinoid which has been found to be effective in preventing epithelial carcinogenesis in animal studies on rats and also preventing the malignant conversion of chemically induced benign tumors. Pre-malignant conditions like oral leukoplakia, cervical dysplasia and xeroderma pigmentosum have been treated in chemopreventive trials with 13-cis-RA(Retinoic Acid). However the drug has not been studied in relation to its effects on the pre malignant and malignant conditions of the uterus. Our aim was to study the effect of 13-cis-retinoic acid on the uterine histological changes induced by tamoxifen in the rabbit.

MATERIAL AND METHODS

The study design was experimental–randomized controlled trial. It was conducted over a period of four months at the department of Anatomy, Army Medical College Rawalpindi in 2007. Ninety adult female New Zealand

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White rabbits were procured from the National Institute of Health Islamabad. Their approximate age was six months to one year and their weight ranged from 1.2 – 2 kg. All rabbits received the normal animal house diet. They were kept in cages, at the room temperature of 18ºC - 26ºC for 3 months. Only healthy, active and non-pregnant animals were included in this study, whereas unhealthy, inactive animals and those with any physical deformity were excluded.

The animals were randomly divided into three groups A, B and C. Each group comprised of thirty female rabbits. Group A was the control, animals in Group B were given tamoxifen, orally at a dose of 5mg/kg/day for 3 months and Group C received tamoxifen orally at a dose of 5mg/kg/day for three months along with 13-cis-retinoic acid (Isotretinoin), orally at a dose of 1mg/kg/day for three months. Strength of one tablet of tamoxifen (Nolvadex - ICI) is 10 mg, so 30 tablets were dissolved in 30 ml of distilled water (10mg/ml). A capsule of Isotretinoin (Roaccutane - Roche) is 20 mg, so one capsule was dissolved in 20 ml of soya bean oil (1mg/ml). The required dose of both drugs was given to the respective rabbits through oral gavage.

The animals of all the three groups were sacrificed after three months of the experimental period. The genital tract was carefully dissected and removed through a midline incision on the skin of the abdomen. A 0.5 cm section from the mid portion of each of the uterine horns was excised and put in 10% neutral buffered formalin. The sections were processed and stained with Haematoxylin and Eosin (H&E) for the routine histological study.

The images of the endometrium were captured in Nikon digital camera from the eyepiece with the ocular micrometer, the scale of which was to be used as a reference of known distance for the calculations afterwards. Each image was opened in morphometric computer software "Image J", by National Institute of Health USA, for calculating area of the endometrium. The height of epithelium was measured under high power field 40 x objective. The height was taken from the basement membrane up to the upper limit of the cell facing the lumen. It was taken from three regions of each section and their mean was calculated. The glandular epithelium was observed for the mitotic figures. The number of glandular epithelial cells along with the mitotic figures was counted in ten consecutive high power fields (HPFs) and the percentage was calculated.

The data was analyzed using SPSS version 13.Descriptive statistics were used to describe the data.. All the study variables amongst the groups were compared through ANOVA followed up by Post –hoc Tukey test.

RESULTS

The animals remained active and healthy throughout the study period. In group A the transverse section of the uterine horn showed a star shape lumen, (Fig-1), encircled by three distinct layers. The endometrium was lined by simple columnar epithelium, where the long axis was at right angle to the basal lamina. The cells had an eosinophilic cytoplasm with a smooth, oval, central basophilic nucleus. Their nuclei, all tend to be aligned at the same level (Fig2). These cells showed little variation in size and shape and formed a column of cells towards the adluminal surface of the horns.

The stroma was highly cellular, the most abundant cells were endometrial stromal cells with plump vesicular nucleus (Fig-2). The endometrial glands on transverse sections were round to oval in shape, and embedded in abundant stroma without any crowding. They were lined by simple columnar epithelium. They had eosinophilic cytoplasm and oval, basophilic nuclei, which had smooth outlines (Fig-3). There were occasional mitoses in the glandular epithelium.

In group B the lumina of the uterine horns were slit like and mostly obliterated by the mucosal infoldings (Fig-4) as there was an apparent increase in the thickness of the endometrium. The glands were dilated and varied in their shapes and sizes but there was no crowding. The lining epithelium of the glands was simple columnar and there were frequent mitoses (Fig-5).

Table -1: Comparison of histological parameters between the groups

Parameter	Group A (n=30)	Group B (n=30)	Group C (n=30)	ANOVA p value
Epithelial Height Luminal (µm)	10.360 ± 0.255	10.972 ± 0.183	10.417 ± 0.290	0.160
Area of the Endometrium (μm^2)	1816125.733 ± 57892.712	2927806.767 ± 139764.635	2399751.867 ± 82444.6027	< 0.001
Percentage of Mitotic Figures	0.064 ± 0.012	0.111 ± 0.009	0.108 ± 0.005	< 0.001

Values were expressed as mean ±SE

Table -2: Post-hoc comparison of histological parameters between the groups

Group Comparisons	Epithelial Height Luminal	Area of the Endometrium	Percentage of Mitotic Figures
Group A vs Group B	0.192	< 0.001	0.001
Group A vs Group C	0.986	< 0.001	0.003
Group B vs Group C	0.255	0.001	0.968

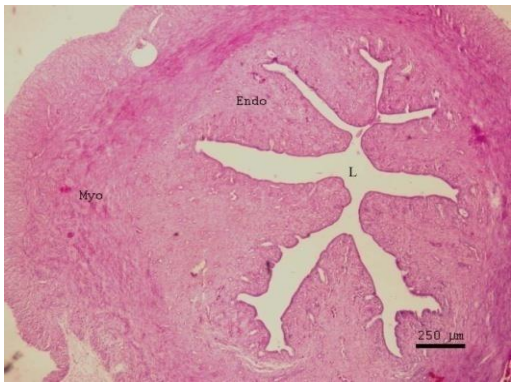

Fig-1: Transverse section of uterine horn in control group, showing star shaped lumen. Myo-Myometrium, Endo- Endometrium, L-Lumen. H&E stain, photomicrograph.

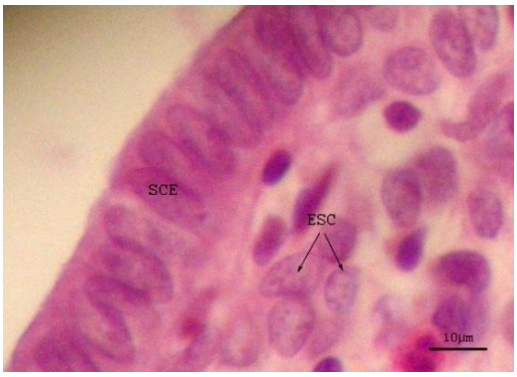

Fig-2: SCE-The simple columnar epithelium, lining the lumen of the uterine horn. ESC-Endometrial Stromal Cells. H&E stain, photomicroghraph.

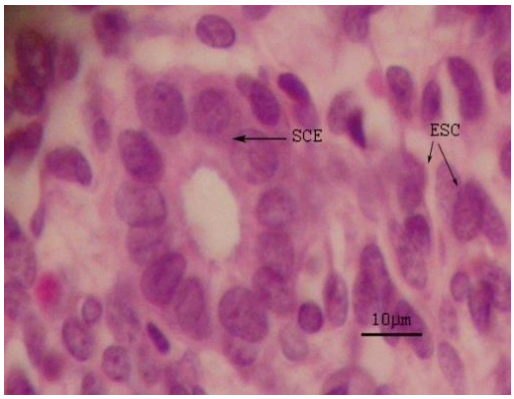

Fig-3: SCE-The simple columnar epithelium, lining the glands. ESC-Endometrial Stromal Cells. H&E stain, photomicroghraph.

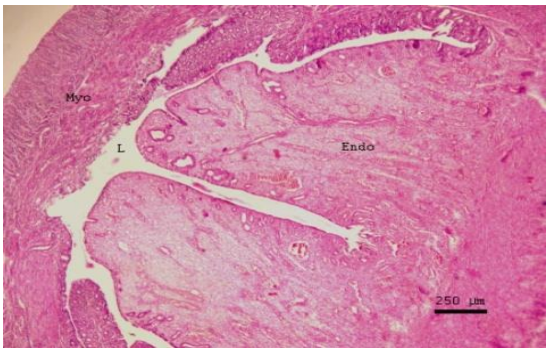

Fig-4: The slit like lumen of uterine horn in Exper group B. Myo-Myometrium, Endo-Endometrium, L-H&E stain, photomicroghraph.

The histological features in group C were similar to Group B. The lumina of the horns were obliterated and slit like. The glands in this group were dilated and varied in sizes and shape. The glandular epithelium had apparently less mitotic figures.

The mean ± SE of microscopic observations and their statistical significance has been summarized in Table1- and Table-2.

DISCUSSION

Our study shows a proliferative effect on the endometrial component of the uteri. There was an increase in the area of endometrium as compared to controls ($p< 0.001$) in both the experimental groups. Most of the histological changes observed in the endometrium after tamoxifen treatment (100 µg orally) represents typical estrogenic-like uterotrophic effects (Sourla et al. (1997). These changes are also seen after estradiol treatment in laboratory animals. The study by Carthew et al. (1999) has shown that there is an increase in DNA synthesis in the endometrial stromal and myometrial compartments of the rat uteri. They have used BrdU (5-Bromo-2´deoxyuridine) labeling index in the different tissue compartments of the uterus, and also examined the immunohistochemical expression of nuclear estrogen receptor alpha (nERalpha), and nuclear progesterone receptor (nPR). Tamoxifen (1 mg/kg) caused a smaller increase of BrdU labeling index in the endometrial stroma and myometrium.

The endometrial area in 13-cRA treated group of our study reduced as compared to tamoxifen treated group only, and this reduction was significant. This drug has been found to exert proliferative effect in a few cell lines and paradoxically also blocked differentiation in chondrocytes, osteocytes and adipocytes. The existence of many types of RA nuclear receptors and their regulation in a tissue specific manner has been postulated as likely reason for such observations. The divergent effects of RA may also be explained by different levels of cellular retinol binding protein affecting the transfer of RA across the plasma membranes .

The height of luminal epithelium was measured in our study by micrometry. There was only a slight increase in the epithelial height in group B and even less so in Group C. This was not found to be significant on statistical analysis. Epithelial height has been measured by Stygar et al. (2003) in their study

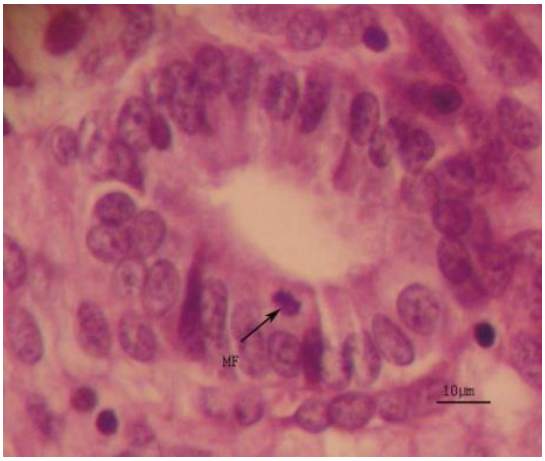

Fig-5: Mitotic figures as seen in the glandular epithelium of Experimental group B. MF-Mitotic figure. H&E stain, photomicroghraph.

conducted in Sweden to see the effects of SERM treatment on growth and proliferation in the rat uterus. Estradiol and TAM (500 µg tamoxifen IM injections once daily for two days) treatment showed strong stimulation of luminal epithelial height, with active proliferation in glandular epithelium, luminal epithelium as well as in the stroma. It has been documented in the study conducted by Cora et al. (2007) that the luminal epithelial cell height is a hallmark of estrogen action in rodents. Significant dose dependent increases in the epithelial height were detected at 30 µg/kg TAM. In the study of Goss et al. (2009) it has also been demonstrated that the administration of tamoxifen alone and in combination with steroidal aromatase inhibitors leads to an increase in the epithelial lining height of ovariectomized rats.

Luminal height has also been observed to be increased by Sourla et al. (1997)11 in the uteri of intact mice treated with tamoxifen for six months. The comparatively less increase in the height of epithelium in retinoic acid treated group is also supported by the observations mentioned in the study by Tickle et al (1989) . They have studied the short term effect of retinoic acid on the height of epithelium. With increasing concentrations of retinoic acid there is a progressive decrease in the epithelial height of the apical wing bud ridge in chick embryos.

Many biomarkers and intermediate endpoints of carcinogenesis have been evaluated as markers of proliferation by the use

of appropriate animal models in various studies. In addition to area of endometrium the proliferative index included in our study was the percentage of mitotic figures. The tamoxifen treated group showed an increase in percentage as compared to the controls. There was a negligible effect of 13-cRA on the suppression of mitosis as compared to Group B, which was not statistically significant.

CONCLUSION

13-cis retinoic acid significantly reduced the proliferative effect of tamoxifen on the area of the endometrium only. The luminal epithelial height were not suppressed in a period of three months.

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