Histological changes induced by tamoxifen versus tamoxifen plus 13-cis-retinoic acid on rabbit uterine glands

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Abstract
Objective: To study the effects of tamoxifen versus tamoxifen plus 13-cis-retinoic acid on the histology of uterine glands in rabbits.
Methods: The experimental, randomised, controlled trial was conducted at the Army Medical College, Rawalpindi, from March 2009 to June 2009 and comprised rabbits acquired from the National Institute of Health, Islamabad. The animals were randomly divided into three equal groups: group A had controls, group B was treated with tamoxifen, and group C with tamoxifen plus retinoic acid. The uterine weight and cross-sectional diameter of uterine horns were measured after sacrifice. The uteri were processed for paraffin embedding. The sections were then assessed for stratification of glandular epithelium, changes in the glandular shape and glandular epithelial height. SPSS 13 was used for statistical analysis.
Results: Tamoxifen administration resulted in variation of glandular shape and increase in glandular epithelial height in group B as compared to control group, p < 0.001 and 0.005 respectively. The adjuvant administration of 13-cis-retinoic acid showed a suppressive effect only on glandular epithelial height, when compared with Group B (p=0.01).
Conclusion: The 13-cis-retinoic acid has no significant inhibitory effect on uterine glandular proliferation induced by tamoxifen after a short-term administration of three months.
Keywords: 13-cis Retinoic acid, Tamoxifen, Rabbits, Uterus.

Introduction
Endometrial hyperplasia is characterised by a proliferation of endometrial glands that may progress to or coexist with endometrial carcinoma.1 Endometrial hyperplasia virtually always results from chronic oestrogen stimulation and has been related to the exogenous exposure to oestrogens. Tamoxifen is a selective oestrogen receptor modulator (SERM) which was developed for the treatment of breast carcinoma. It exhibits anti-oestrogenic activity in the breast and oestrogen-like actions on the endometrium.2 The drug is associated with an increased risk of endometrial cancer.

Cancer chemoprevention is the use of specific chemical compounds to prevent, inhibit or reverse carcinogenesis.3,4 This involves interventions at the earliest stages of carcinogenesis i.e. epithelial hyperplasia and hypertrophy. It has been observed in animal studies that 13-cis-retinoic acid (13cRA) inhibits the process of epithelial carcinogenesis and also prevents the progression of chemically-induced benign into malignant tumours.5 13cRA has been used in drug trials as a chemopreventive agent in the treatment of pre-malignant conditions like cervical dysplasia, leukoplakia and xeroderma pigmentosum.6,8 However, there is dearth of literature on studies involving the effects of this drug on the uterine conditions, either pre-malignant or malignant. The current study was planned to evaluate the effect of 13cRA on uterine glandular changes induced by tamoxifen in rabbits.

Material and Methods
The experimental, randomised, controlled trial was conducted in the Department of Anatomy at the Army Medical College, Rawalpindi, from March 2009 to June 2009 and comprised adult female New Zealand White rabbits acquired from the National Institute of Health (NIH), Islamabad. The age of the animals was 6-12 months and their weight ranged from 1.5-2kg. All rabbits received the normal animal house diet. They were kept at room temperature in separate cages. Only healthy, active and non-pregnant animals were included.

The animals were randomly selected and divided into three groups: group A had controls, while group B was given tamoxifen orally at a dose of 5mg/kg/day, and group C received tamoxifen orally at a dose of 5mg/kg/day along with 13cRA (Isotretinoin), orally at a dose of 1mg/kg/day. Strength of one tablet of tamoxifen
Nolvadex - ICI) was 10mg, so 30 tablets were dissolved in 30ml of distilled water (10mg/ml). A capsule of Isotretinoin (Roaccutane - Roche) was 20mg, so one capsule was dissolved in 20ml of soya bean oil (1mg/ml). The required dose of both drugs was given to the respective rabbits through oral gavage.

All animals were sacrificed at the end of the three-month study period.

The body-weight of all the animals was recorded at the start of the study as well as before the sacrifice. The genital tract was carefully dissected and uteri were removed and trimmed. Luminal fluid was expressed out and the uteri were blotted dry. The uteri were then weighed using a digital precision balance. The mid portion of each of the uterine horns was excised and put in 10% neutral buffered formalin. After fixation, the diameter of the horns was measured with the scale. A 0.5cm section of tissue was cut from each specimen of the uterine horns. It was processed and embedded in paraffin wax at a temperature of 58ºC. The processed samples were labelled for the respective animal. The sections measuring 7µm were cut and then mounted on labelled clear glass slides. The sections were stained with Haematoxylin and eosin (H&E). The slides were observed under the required objectives with ocular micrometre placed in the eyepiece. The ocular had been calibrated with the stage micrometre.

Glandular epithelial height was measured on transverse sections of glands. Height was measured from basement membrane till the apex of cell facing the glandular lumen. Three glands were selected from three different regions of the uterine horn in 40X objective magnification and average was calculated.

Five to six serial sections were prepared for each specimen of control and experimental groups. These were observed under high power field for any apparent stratification of the glandular epithelium and it was recorded as present or absent. Any change from the usual rounded to oval shape of glands, like cystic dilatations, epithelial budding and infoldings, in transverse section of glands, were noted in 40X objective magnification and recorded as present or absent.

Data was analysed using SPSS 13. Mean and standard deviations (SD) were calculated for all numerical data. All quantitative variables among the groups were assessed with Kruskal Wallis Test and followed up by post-hoc test. Chi-squared test was applied to analyse the stratification of glandular epithelium and variations in the shape of glands followed by post-hoc test for comparison between two groups.

Table-1: Quantitative Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Mean ± SD</th>
<th>Group B Mean ± SD</th>
<th>Group C Mean ± SD</th>
<th>Kruskal Wallis Test p value</th>
<th>Group comparisons p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of uterus (g)</td>
<td>1.091 ± 0.388</td>
<td>3.510 ± 0.670</td>
<td>3.373 ± 0.534</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cross-sectional diameter of uterine horn (cm)</td>
<td>0.403 ± 0.099</td>
<td>0.697 ± 0.159</td>
<td>0.680 ± 0.130</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Glandular Epithelial Height (µm)</td>
<td>10.139 ± 1.112</td>
<td>10.917 ± 0.987</td>
<td>10.244 ± 0.809</td>
<td>0.007</td>
<td>0.005</td>
</tr>
</tbody>
</table>

SD: Standard Deviation.

Table-2: Qualitative Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Present</th>
<th>Group A Absent</th>
<th>Group B Present</th>
<th>Group B Absent</th>
<th>Group C Present</th>
<th>Group C Absent</th>
<th>Chi-square Test p value</th>
<th>Group comparisons p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratification of Glandular Epithelium</td>
<td>Nil</td>
<td>30</td>
<td>2</td>
<td>28</td>
<td>1</td>
<td>29</td>
<td>0.355</td>
<td>0.154 0.317 0.557</td>
</tr>
<tr>
<td>Variations in Shape of Glands</td>
<td>Nil</td>
<td>30</td>
<td>26</td>
<td>4</td>
<td>26</td>
<td>4</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 &lt; 0.001 1.000</td>
</tr>
</tbody>
</table>
epithelium. The cells had eosinophilic cytoplasm and oval, basophilic nuclei, which had smooth outlines (Figure-2).

Histological features were almost similar in both treatment groups. The lumina of the horns were obliterated and slit-like. Glandular epithelial height was increased in both the experimental groups, but more in group B (p=0.011). The glands in both these groups were dilated and varied in sizes and shape (Figure-3), changes like epithelial outpouchings and infoldings were also seen in a few cases (Figure-4). The lining epithelium of the glands was simple columnar, but initial stages of stratification were apparent in 2(6.67%) animals in group B and in only 1(3.33%) in group C. The nuclei of the lining epithelial cells were pleomorphic and varied from small condensed to large oval in size. The variation in the shape of glands was observed in 26(86.67%) animals in group C (Table-2). The stroma of experimental groups showed stromal cells with vacuolation and pyknotic nuclei, red cell infiltration and epithelial cell lining damage.

Discussion
In our study the weight of uteri increased after treatment with tamoxifen in the experimental group B compared to the controls (p<0.001). Dose-dependent increases in uterine weight were observed in a study following three
consecutive daily oral treatments of tamoxifen (TAM) in mice at 72 hours. To optimise the anti-oestrogen therapy in breast cancer patients, end organ changes were studied in rats. Uterine weight was increased by tamoxifen within a range of clinically relevant dose. Effects of tamoxifen on the uterus, vagina and breast in ovariectomised cynomolgus monkeys have been mentioned. Growth hormone has been suggested as a cause of increase in the weight of uteri exposed to SERM.

No significant change in our study was seen in the 13cRA treated group compared to the group which received tamoxifen alone (p=0.209), suggesting that there was no effect of 13cRA on the increase in the weight of uterus mediated by the tamoxifen.

There was an increase in cross-sectional diameter of the uterine horns in both the experimental groups compared to the controls (p<0.001). Similar but not identical findings have also been reported in studies where the luminal circumference of uteri was increased under the effects of tamoxifen. Tamoxifen has shown varied uterine effects (uterine atrophy, increased uterine weight and epithelial cell hypertrophy). However, studies on the early effects of tamoxifen on the uterus after only three months of therapy are limited. No significant inhibitory effects of 13cRA was seen on the diameter of the uterus in our study.

The height of glandular epithelium was measured in our study by micrometry. There was an increase in the epithelial height in group B and less so in group C (p<0.011). Effects of tamoxifen have been studied by a study where glandular epithelial cell height increased compared to the controls (p<0.05) in ovari intact rats. In another study, 6-month treatment of intact female mice with tamoxifen resulted in a moderate hypertrophy of glandular epithelium.

Only 2(6.67%) animals in group B and 1(3.33%) in group C were found to have stratification of glandular epithelium which was not significant compared to the controls. Stratification of the glandular epithelium was observed in tamoxifen-treated ovari intact mice. However, isotretinoin has not been studied in this context along with tamoxifen. In addition, the uterotrophic effects in groups B and C included pleomorphism in the nuclei of the lining glandular epithelial cells and these varied from small condensed to large oval in size.

The uterotrophic effects in both the experimental groups showed a significant change in the size and shape of glands compared to controls (86.67%) (p<0.001). But use of isotretinoin did not reverse these changes in group C. Along with these changes in the glands, the stroma of experimental groups also showed cells with vacuolation and pyknotic nuclei, red cell infiltration and epithelial cell lining damage (Figure-4). Tamoxifen has been observed to produce cystic endometrial hyperplasia in adult cynomolgus macaques. In an evaluation of endometrial histopathologic findings from 700 patients treated with TAM for breast cancer, pathologic changes were found in 39.86% cases. In a Canadian study the researchers compared the effects of TAM and EM-800 on the uterus, vagina and mammary gland in mice. This resulted in histological changes characterised by an increased number, crowding and dilatation of the endometrial glands. Hyperplastic changes were seen to be reversed by retinoids in one study. The reversal of proliferative effect with retinoids has been documented in studies with pharmacologically-induced hyperplasia of the rat prostate tissue. These observations, however, do not correlate with the effects of retinoic acid seen in our study. This may be because of difference in the target tissue examined. This can also be related to in vitro experimental design of this study. Moreover, the difference in the target receptors for 13cRA and other isomers of retinoic acid can also explain the variability in observation.

**Conclusion**

13cRA did not significantly inhibit the proliferative effect of TAM on the weight of rabbits, the uterine weight, cross-sectional diameter of uterine horns and glandular histomorphology over three months. However, exclusive 13cRA administration may be studied for achieving the desired results in future trials.

**References**