

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

Shabnam Hamid,<sup>1</sup> Atika Khurshid,<sup>2</sup> Shadab Ahmed Butt<sup>3</sup>

### 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. (JPMA 65: 491; 2015)

### Introduction

Endometrial hyperplasia is characterised by a proliferation of endometrial glands that may progress to or coexist with endometrial carcinoma.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3,4</sup> 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.<sup>5</sup> 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.<sup>6-8</sup> 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 5 mg/kg/day along with 13cRA (Isotretinoin), orally at a dose of 1mg/kg/day. Strength of one tablet of tamoxifen

.....  
<sup>1,3</sup>Department of Anatomy, Army Medical College, Rawalpindi, <sup>2</sup>Department of Anatomy, Yusra Medical College, Islamabad.

**Correspondence:** Shabnam Hamid. Email: drshabnamhamid@gmail.com

(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 58°C. 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.

## Results

The 90 rabbits in the study were divided into 3 groups of 30(33.3%) each. Mean quantitative microscopic observations and their statistical significance were worked out, and after dissection, weight of the uteri was noted and the cross-sectional diameter of the mid portion of each uterine horn was measured (Table-1).

In group A, the transverse section of the uterine horn showed a stellate shaped lumen encircled by endometrium, myometrium and perimetrium. The endometrium was lined by simple columnar epithelium. The epithelium invaginated into the stroma, forming the simple tubular glands. The glands on transverse sections were rounded to oval and were invariable in shape (Figure-1), and embedded in abundant stroma without any crowding. They were lined by simple columnar

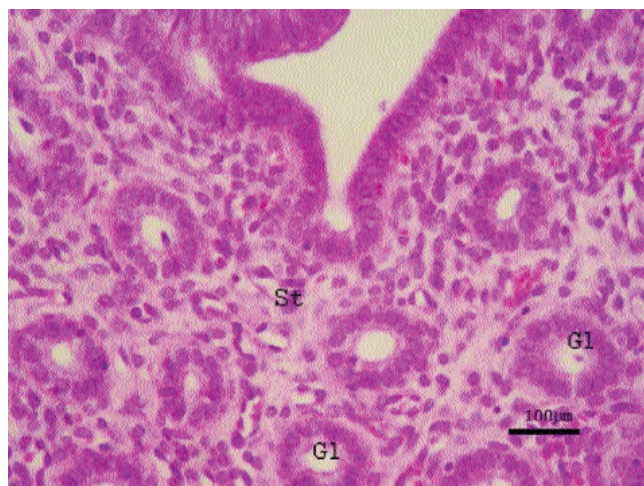
**Table-1:** Quantitative Parameters.

| Parameter                                     | Group A        | Group B        | Group C        | Kruskal Wallis Test<br>p value | Group comparisons p value |           |           |
|-----------------------------------------------|----------------|----------------|----------------|--------------------------------|---------------------------|-----------|-----------|
|                                               | Mean ± SD      | Mean ± SD      | Mean ± SD      |                                | Group A/B                 | Group A/C | Group B/C |
| Weight of uterus (g)                          | 1.091 ± 0.388  | 3.510 ± 0.670  | 3.373 ± 0.534  | < 0.001                        | < 0.001                   | < 0.001   | 0.209     |
| Cross-sectional diameter of uterine horn (cm) | 0.403 ± 0.099  | 0.697 ± 0.159  | 0.680 ± 0.130  | < 0.001                        | < 0.001                   | < 0.001   | 0.797     |
| Glandular Epithelial Height (µm)              | 10.139 ± 1.112 | 10.917 ± 0.987 | 10.244 ± 0.809 | 0.007                          | 0.005                     | 0.562     | 0.011     |

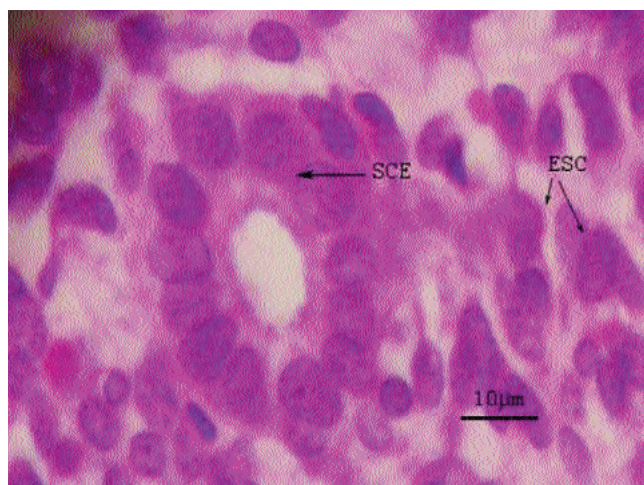
SD: Standard Deviation.

**Table-2:** Qualitative Parameters.

| Parameter                              | Group A |        | Group B |        | Group C |        | Chi-square Test<br>p value | Group comparisons p value |           |           |
|----------------------------------------|---------|--------|---------|--------|---------|--------|----------------------------|---------------------------|-----------|-----------|
|                                        | Present | Absent | Present | Absent | Present | Absent |                            | Group A/B                 | Group A/C | Group B/C |
| Stratification of Glandular Epithelium | Nil     | 30     | 2       | 28     | 1       | 29     | 0.355                      | 0.154                     | 0.317     | 0.557     |
| Variations in Shape of Glands          | Nil     | 30     | 26      | 4      | 26      | 4      | < 0.001                    | < 0.001                   | < 0.001   | 1.000     |



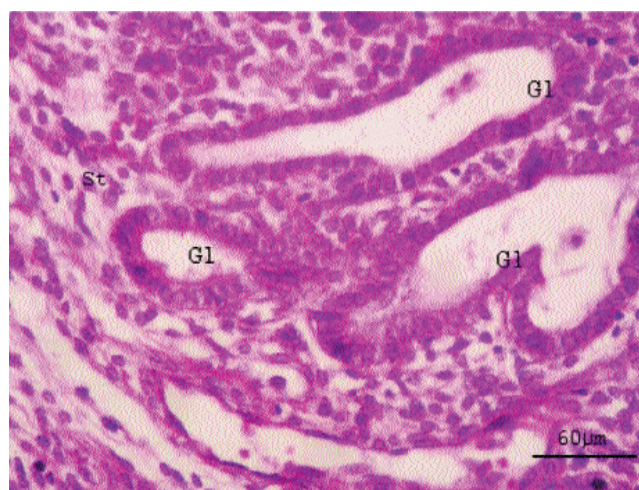
**Figure-1:** Endometrium of control group showing glands (Gl) in transverse section and stroma (Sr). Haematoxylin and eosin (H&E) stain, photomicrograph.



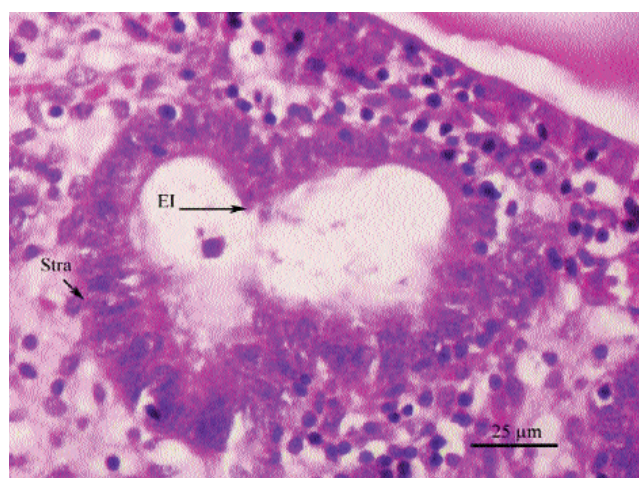
**Figure-2:** Simple columnar epithelium (SCE), lining the glands and Endometrial Stromal Cells (ESC). Haematoxylin and eosin (H&E) stain, photomicrograph.

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



**Figure-3:** Endometrium of experimental group showing dilated glands (Gl) in transverse section and stroma (Sr). Haematoxylin and eosin (H&E) stain, photomicrograph.



**Figure-4:** Endometrium of experimental group showing dilated glands with epithelial infoldings (EI) and stratification (Stra) of epithelium. Haematoxylin and eosin (H&E) stain, photomicrograph.

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.<sup>9</sup> To optimise the anti-oestrogen therapy in breast cancer patients, end organ changes were studied in rats.<sup>10</sup> 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.<sup>11</sup> Growth hormone has been suggested as a cause of increase in the weight of uteri exposed to SERM.<sup>12</sup>

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<sup>9</sup> 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).<sup>3</sup> However, studies on the early effects of tamoxifen on the uterus after only three months of therapy are limited.<sup>13</sup> 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<sup>14</sup> where glandular epithelial cell height increased compared to the controls ( $p<0.05$ ) in ovary intact rats. In another study,<sup>15</sup> 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 ovary intact mice.<sup>15</sup> 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.<sup>16</sup> In an evaluation of endometrial histopathologic findings from 700 patients treated with TAM for breast cancer, pathologic changes were found in 39.86% cases.<sup>17</sup> In a Canadian study<sup>15</sup> 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.<sup>18</sup> The reversal of proliferative effect with retinoids has been documented in studies with pharmacologically-induced hyperplasia of the rat prostate tissue.<sup>19</sup> 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

1. Kurman RJ, Norris HJ. Endometrial hyperplasia and related cellular changes. In: Kurman RJ. Blaustein's Pathology of the Female Genital Tract. 4th ed. New York: Springer, 1994; p.441.
2. Gradishar WJ. Tamoxifen - what next? *Oncologist* 2004; 9: 378-84
3. Kellof GJ, Crowell JA, Steele VE, Lubet RA, Boone CW, Malone WA, et al. Progress in cancer chemoprevention. *Ann NY Acad Sci* 1999; 889: 1-13.
4. Kellof GJ, Hawk ET, Karp JE, Crowell JA, Boone CW, Steele VE, et al. Progress in clinical chemoprevention. *Semin Oncol* 1997; 24: 241-52.
5. Bukhari SMH, Shahzad SQ, Naeem S, Qureshi GR, Naveed LA. Role of chemical carcinogens in epithelial and mesenchymal neoplasms with tumor initiation-promotion protocol and the effect of 13-cis retinoic acid in chemoprevention. *J Coll Physicians Surg Pak* 2002; 12: 302-6
6. Kraemer KH, DiGiovanna JJ, Moshell AN, Tarone RE, Peck GL. Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *N Engl J Med* 1988; 318: 1633-7.
7. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, et al. 13-cis- retinoic acid in the treatment of oral leukoplakia. *N Engl J Med* 1986; 315: 1501-5
8. Kavanagh JJ, Lippman SM, Paredes-Espinoza M, Erikson AO, Delgadillo-Madrueno F, Paredes-Casillas P, et al. The combination of 13-cis retinoic acid and interferon  $\alpha$ -2a with radiation therapy

- in squamous cell carcinoma of the cervix. *Int J Gynecol Cancer* 1996; 6: 439-44.
  9. Cora FJ, Lyle DB, Kurt JW, Agnes LF, Timothy RZ. Comparative temporal and dose-dependent morphological and transcriptional uterine effects elicited by tamoxifen and ethynylestradiol in immature, ovariectomized mice. *BMC Genomics* 2007; 8: 151.
  10. Goss PE, Qi S, Hu H. Comparing the effects of atamestane, toremifene and tamoxifen alone and in combination, on bone, serum lipids and uterus in ovariectomized rats. *J Steroid Biochem Mol Biol* 2009; 113: 233-40.
  11. Sikoski P, Register TC, Lees CJ, Lundeen S, Hutchison J, Brown KH, et al. Effects of two novel selective estrogen receptor modulators, raloxifene, tamoxifen, and ethinyl estradiol on the uterus, vagina and breast in ovariectomized cynomolgus monkeys (*Macaca fascicularis*). *Am J Obstet Gynecol* 2007; 196: 75.e1-7.
  12. Stygar D, Muravitskaya N, Eriksson B, Eriksson H, Sahlin L. Effects of SERM (selective estrogen receptor modulator) treatment on growth and proliferation in the rat uterus. *Reprod Biol Endocrinol* 2003; 1: 40.
  13. Morales L, Timmerman D, Neven P, Konstantinovic ML, Carbonez A, van Huffel S, et al. Third generation aromatase inhibitors may prevent endometrial growth and reverse tamoxifen-induced uterine changes in postmenopausal breast cancer patients. *Ann Oncol* 2005; 16: 70-4.
  14. Nephew KP, Osborne E, Lubet RA, Grubbs CJ, Khan SA. Effects of oral administration of tamoxifen, toremifene, dehydroepiandrosterone, and vorozole on uterine histomorphology in the rat. *Proc Soc Exp Biol Med* 2000; 223: 288-94.
  15. Sourla A, Luo S, Labrie C, Bélanger A, Labrie F. Morphological Changes Induced by 6-Month Treatment of Intact and Ovariectomized Mice with Tamoxifen and the Pure Antiestrogen EM-800. *Endocrinol* 1997; 138: 5605-17.
  16. Wang H, Isaksson E, von Schoultz B, Cline JM, Sahlin L. Effect of long-term treatment with steroid hormones or tamoxifen on the progesterone receptor and androgen receptor in the endometrium of ovariectomized cynomolgus macaques. *Reprod Biol Endocrinol* 2003; 1: 7.
  17. Deligdisch L, Kalir T, Cohen CJ, de Latour M, Le Bouedec G, Penault-Llorca F. Endometrial histopathology in 700 patients treated with tamoxifen for breast cancer. *Gynecol Oncol* 2000; 78: 181-6.
  18. Mater A, Muller-Salamin L, Lasnitzki I, Bollag W. Ultrastructural analysis of the retinoid-induced reversal of epithelial hyperplasia and metaplasia. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1980; 33: 199-212.
  19. Chopra DP, Wilkoff LJ. Inhibition and reversal by beta-retinoic acid of hyperplasia induced in cultured mouse prostate tissue by 3-methylcholanthrene or N-methyl-N'-nitro-N-nitrosoguanidine. *J Natl Cancer Inst* 1976; 56: 583-9.
-