HISTOMORPHOLOGY OF THE CORNEAL EPITHELIUM OF ANASTROZOLE TREATED RABBITS

Aamna Khalil, Khadija Qamar, Shadab Ahmed Butt

Army Medical College, National University of Sciences and Technology (NUST) Islamabad,

ABSTRACT

Objective: To evaluate the effects of prolonged use of anastrozole as an endocrine treatment of breast cancer on the corneal epithelium in an animal model.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, six months from Jun 2012 to Nov 2012.

Material and Methods: Twenty adult female NewZealand white rabbits were taken. Ten rabbits were placed in control group taking normal diet and 10 were given anastrozole orally in the normal dose of 1 mg/day (0.02 mg/kg/day). After the completion of the study, corneas were removed and grossly examined. The specimen were fixed and slides prepared for histomorphological examination. The epithelium in each slide was examined for any deposits, edema or increase in stratification and the height of the epithelium was measured for each eye. The results were compared between the groups for statistical significance.

Results: The epithelium had normal shape with no areas of any deposits, edema or ulceration. The mean epithelial height in the control group was 21.25 ± 4.29 µm and 21.00 ± 4.28 µm in the right corneas and left corneas, respectively. The mean epithelial height taken from the experimental group was 20.50 ± 4.97 µm and 21.00 ± 4.28 µm in right sided and left sided corneas, respectively. The *p* value was calculated to be 0.722 and 1.00 for the right and left corneas, respectively and no statistical significance was found in between the two groups.

Conclusion: Long term administration of anastrozole has no effect on the histological morphology of the corneal epithelium.

Keywords*:* Anastrozole, Dry eye syndrome, Epithelial microcysts, Keratitis, Tamoxifen, Vortex Keratopathy.

INTRODUCTION

The cornea is the anterior window of the eye. The human cornea is transparent and consists of five layers in transverse section. These are the corneal epithelium, Bowman's layer, corneal stroma, the Descemet's membrane and the corneal endothelium[1]. The rabbit cornea does not have the Bowman's layer. The corneal epithelium, with the underlying basement membrane, rests directly on the corneal stroma[2]. The corneal epithelium consists of approximately five layers of stratified squamous non-keratinized cells in both the species. The deepest layer consists of cylindrical cells[3]. The overall thickness of the rabbit cornea is less than that in human eyes (30-40 µm)[4].

The corneal surface must be extremely smooth and must have a high degree of transparency to allow the formation of a sharp image on the retina. Irregularity of the surface is extremely common after corneal disease and, many times, is the sole cause for reduction of vision on a large scale. The corneal epithelium is the first layer that the light has to pass through after crossing the tear film. The smooth epithelium itself can acquire uneven thickness after infectious keratitis, corneal edema, or dry eye. Previous stromal ulceration also causes the surface to lose its regular curvature. Surface irregularity and light scattering by the epithelium is often much more destructive to visual acuity than stromal edema or scarring[5]. With the rapid development of new drugs, ocular toxicities of systemic medication are becoming an important

Correspondence: Dr Aamna Khalil, PG Trainee NUST, Anatomy Department, Army Medical College, Rawalpindi.
Email: aamnakhalil@amcollege.nust.edu.pk
Received: 30 May 2013; Accepted: 2013

aspect of clinical practice. The adverse effects on the eyes range from dry eye syndrome, keratitis, keratopathies and cataract to blinding complications of retinopathy and optic neuropathy[6]. Systemic medications reach the cornea through aqueous humor, limbal vasculature and the tear film and the changes produced are often the result of the underlying chemical structure and properties of these drugs.

Anastrozole is a third generation non-steroidal aromatase inhibitor which significantly lowers serum estradiol concentrations. Aromatase inhibitors are rapidly replacing tamoxifen in postmenopausal patients with estrogen-receptor-positive cancers, who make up the majority of cases in patients with breast cancer[7]. Orally administered anastrozole is well absorbed into the systemic circulation and eliminated, primarily via hepatic metabolism[8]. Third generation aromatase inhibitors are very well tolerated and have short term adverse effects of which common ones are hot flashes, musculoskeletal pain, vaginal dryness and headache[9]. These drugs are superior to the previously used estrogen receptor modulators like tamoxifen as these newer drugs cause less endometrial hyperplasia[10] and venous thromboembolism[11]. In cornea tamoxifen use, similar to chloroquine, leads to vortex keratopathies and epithelial deposits[12,13]. Exemestane, also an aromatase inhibitor, has been linked to the formation of intraepithelial cysts in the corneal epithelium in a case report[14]. Though, anastrozole administration has been associated with dry eye syndrome in a clinical retrospective study carried out by Turaka et al[15], not many experimental studies have been reported to evaluate the effects on corneal epithelium.

The purpose of this study was to assess the effect of anastrozole use on the corneal epithelial height and to observe for any epithelial deposits or intraepithelial cysts.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was conducted in the Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad. The permission was taken from ethical committee on animal experiments of the Army Medical College, Rawalpindi. Twenty healthy adult non-pregnant female New Zealand white rabbits weighing 1.2 – 2 kg were used for the experiment. They were kept at room temperature in separate cages and were given normal animal house diet.

The animals were divided into two groups using random numbers table. Group A was the control group comprising of 10 animals. Group B included 10 rabbits that received anastrozole orally at a dose of 0.02 mg/kg/day. After six months, the animals were anaesthetized by giving chloroform inhalation in a glass jar. The eyeballs were dissected away from the surrounding structures in the anaesthetized rabbits. The corneas were removed by circumscribed incision along the limbal region and separated from the sclera and iris. They were fixed in 10% neutral formalin by placing in separate labeled containers for 48 hours. A transverse section of about 0.5 cm width was taken from the centre of each cornea. It was placed in duly labeled tissue cassettes and passed through a series of increasing concentrations of

Table-1: The comparison of corneal epithelial height in control and experimental groups.

Parameter	Control group (n=10)	Experimental group (n=10)	*p*-value
Epithelial height (µm) in right corneas (Mean ±S.D)	21.25 ± 4.29	20.50 ± 4.97	0.722
Epithelial height (µm) in left corneas (Mean ±S.D)	21.00 ± 4.28	21.00 ± 4.28	1.000

n- number of rabbits, S.D: Standard Deviation

alcohol from 70% to 100%, cleared in xylene and embedded in paraffin wax as labeled blocks.

Tissue were cut into 5 microns thick sections and slides were prepared by staining with haematoxylin and eosin (H and E) for the histomorphological examination. The slides were studied at 4 X, 10 X and 40 X objectives for the general appearance and architecture of the tissue. The corneas were qualitatively analyzed for the presence of any epithelial deposits. Ocular micrometer was calibrated with stage micrometer and used for the measurement of epithelial height with 40 X objective lens. The height of corneal epithelium was recorded in all the animals for the quantitative measurement and statistical comparison in between the groups.

Statistical Analysis

Data was analyzed by using SPSS version 17.0. Descriptive statistics were used to describe the results. Independent sample t tests was used to assess the significance of the results between the control and experimental groups. Results were considered significant at $p < 0.05$.

RESULTS

On gross examination all the corneas were found to be transparent with a smooth outer surface. Histological examination of the slides revealed a thick corneal stroma covered by epithelium but lacking the intervening Bowman's layer. The deeper surface of the cornea was lined by the Descement's membrane and a single layer of endothelial cells (Figure-1). The corneal epithelium was stratified squamous non-keratinized with five to six layers of cells in both groups. The corneas were examined thoroughly for any epithelial deposits or epithelial detachment from the underlying stroma. All the animals in the control as well as the experimental group showed clear corneal epithelium with no detectable deposits or discernible edema. The *p* value, thus calculated, was 1.00 showing no significant change in the experimental group. There were no areas of desquamation or ulceration visible in any slides. No foci of ulceration or scarring were present in the corneas.

The epithelial layer had a smooth contour in all the slides with no unevenness in any area. On higher magnification (40 X) the epithelial height was measured by ocular micrometer in three

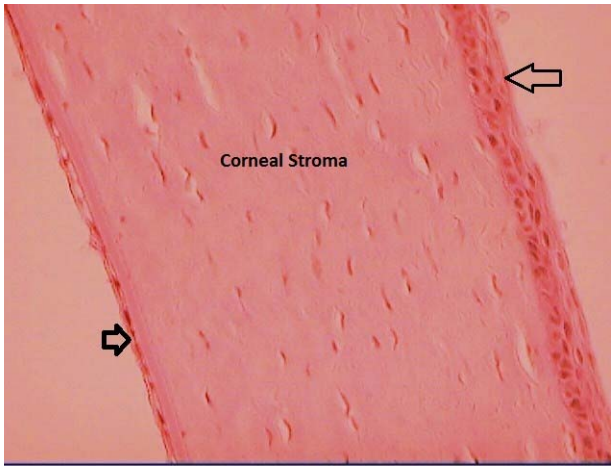

Figure-1: Photomicrograph of section of the cornea of the right eye of fifth animal of the control group showing corneal epithelium (arrow), stroma and endothelium (small arrow) (H and E, 100X).

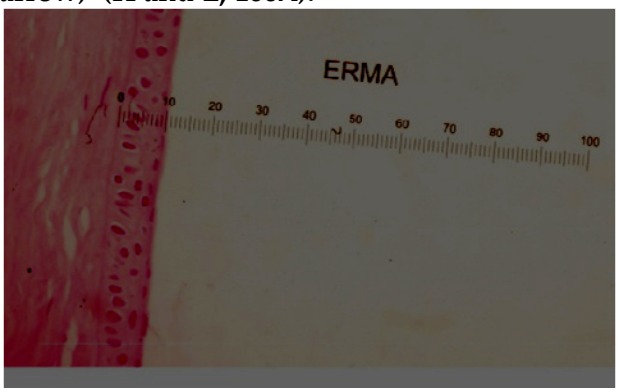

Figure-2: Photomicrograph of measurement of the height of the corneal epithelium in the right eye of the 7th animal of the experimental group (H and E, 400X)

areas of same specimen and the average taken as the final measurement (Fig-2). The mean of epithelial height in the control group was 21.25 ± 4.29 µm in the readings taken from the corneas of right eyes and 21.00 ± 4.28 µm in the corneas of the left eyes (Table-1). The epithelial height taken from the experimental group was 20.50 ± 4.97 µm for the right corneas and 21.00 ± 4.28 µm for the

left corneas (Table-1). The *p* value was calculated to be 0.722 and 1.00 and no statistical significance could be found in epithelial heights between the control and experimental groups.

DISCUSSION

The ocular anatomy of the rabbit is quite similar to that in humans and it continues to be one of the most commonly used animal models for ophthalmic research[4].

A large number of drugs produce cytotoxic effects on the corneal epithelium. Epithelial microcysts with degeneration of basal epithelial cells are associated with the use of cytarabine[16]. Epithelial deposits are reported with flecainide use[17].

Long term tamoxifen use has been demonstrated to cause ocular side effects. They were first documented by Kaiser- Kupfer and Lippman in 1978[18]. Further studies were done over the next few decades and conclusive evidence was found that the use of Tamoxifen is related with the development of keratopathy and retinopathy[19]. In the cornea the major changes seen by many authors included vortex keratopathies and corneal epithelial deposits[12,13]. Drug-induced lipidosis and vortex keratopathy is produced by amphiphilic medications such as amiodarone, chloroquine, suramin, clofazimine, etc[16]. Tamoxifen, like several other ampiphilic cationic drugs, may interfere with intralysosomal catabolism of polar lipids. The drug has been reported to cause prominent lipidosis-like alterations in most tissues when administered in high oral doses[20]. Even at a dose of 8.3 µmol/l, it has been reported to cause moderate phospholipidosis with the characteristic changes of abnormal lysosomes containing electron-dense deposits and membranous structures arranged in whorled arrays (myelin figures) present in the cells[21]. The generalized lipidosis induced by cationic amphiphilic drugs may also be associated with lipidotic alterations in the cornea. This was experimentally demonstrated in a study carried out by Drenckhahn et al[22]. In this study, rats were orally administered chlorpheniramine, iprindole and tamoxifen. All the animals showed clear lipidosis-like alterations in corneal cells.

There is also an increased risk of post sub-capsular cataract with tamoxifen use[23] but the underlying mechanism for such changes is quite different than that for the corneal changes and is related to the ability of the drug to block chloride channels in the lens leading to lens opacification and cataract formation[24]. It is evident that, although a drug is designed to act on the tissues in a specific mechanism to alleviate disease process, it can cause effects by many other undiscovered methods.

The aromatase inhibitors are the new development in the endocrine treatment of breast cancer. Exemestane is a selective steroidal aromatase inhibitor taken orally in such patients to lower the circulating estrogen levels. In a case report, administration of Exemestane caused the formation of intraepithelial cysts in the corneal epithelium[14]. Anastrozole selectively targets the aromatase enzyme in tissues and lowers the serum estrogen levels. In postmenopausal women with breast cancer, it is now the first line of endocrine treatment preferred over the previously used Tamoxifen[7]. In our study, the drug had no statistically significant effects on the cellular architecture or stratification of the corneal epithelium. Though, anastrozole administration has been associated with dry eye syndrome in a clinical retrospective study carried out by Turaka et al[15], not many experimental studies have been reported to evaluate its effects on corneal epithelium.

Side effects of aromatase inhibitors resemble those of tamoxifen to some extent but little is known about the effects of aromatase inhibitors on visual system[9].

CONCLUSION

The study was carried to evaluate the effects of anastrozole on the corneal epithelium. The use of aromatase inhibitors as chemopreventive agent, is increasing among postmenopausal women with breast cancer and it is imperative to assess their effects on the vision. There were no

statistically significant effects seen on the histomorphology of the corneal epithelium after six months of drug administration.

REFERENCES

1. Ross MH and Pawlina W. Histology. A Text and Atlas. Lippincott, Williams and Wilkins. 6th Edition. 2011.
2. Werner L, Chew J and Mamalis N. Experimental evaluation of ophthalmic devices and solutions using rabbit models. Vet Ophthalmol 2006; 9(5): 281-291.
3. Davis FA. The anatomy and histolgy of the eye and orbit of the rabbit. Trans. Am. Ophthalmol. Soc. 1929; 27:400-441.
4. Gwon, A. (2008) The rabbit in cataract/IOL surgery. In: Tsonis, P.A. (ed.) Animal models in eye research. Elsevier, pp. 184-204.
5. Dohlman CH. The function of the corneal epithelium in health and disease. Invest. Ophthalmol. Vis. Sci. 1971; 10(6):383-407.
6. Blomquist PH. Ocular complications of systemic medications. Am J Med Sci, 2011; 1: 62-69.
7. Smith IE , Dowsett M. Aromatase inhibitors in breast cancer. NEJM 2003; 348:2431-2442.
8. www.arimidex.net. Accessed on 26 October 2012.
9. Eisner A and Luoh SW. Breast cancer medications and vision: Effects of treatments for early-stage disease. Curr Eye Res 2011; 36(10): 867-85.
10. 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; 16:70-74.
11. ATAC Trialists' Group. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomized trial. Lancet 2002; 359:2131-2139. [Erratum, Lancet 2002; 360:1520.]
12. Noureddin BN, Seoud M, Bashshur Z, Salem Z, Shamseddin A , Khalil A. Ocular toxicity in low-dose tamoxifen: A prospective study. Eye, 1999; 13(6):729-33.
13. Muftuoglu O, Ucakhan OO and Kanpolat A. Clinical and in vivo confocal microscopy findings in patients receiving tamoxifen citrate. Eye Contact Lens 2006; 32:228-232.
14. Papathanassiou M, Nikita E, Theodossiadis P, Theodossiadis GP, Vergados I. Exemestane-induced corneal epithelial changes. Cutan Ocul Toxicol, 2010; 29(3):209-211.
15. Turaka K, Nottage JM, Hammersmith KM, Nagra PK, Rapuano CJ. Dry eye syndrome in aromatase inhibitor users. Clin Experiment Ophthalmol 2013; 41(3):239 -43.
16. Hollander DA and Aldave AJ. Drug-induced corneal complications. Curr Opin Ophthalmol 2004; 15:541-48.
17. Ikaheimo K, Kettunen R and Mantyjarvi M. Adverse ocular effects of flecainide. Acta Ophthalmol Scand 2001; 79(2): 175–76.
18. Kaiser-Kupfer MI and Lippman ME. Tamoxifen retinopathy. Cancer Treat Rep 1978; 62(3):315-20.
19. Dulley P and Senior J. Tamoxifen: a review of drug development for use in breast cancer and ocular adverse reactions. Optometry in Practice 2003; 4:117-27
20. Lullmann H and Lullmann-Rauch R. Tamoxifen-induced generalized lipidosis in rats subchronically treated with high doses. Toxicol and Appl Pharm 1981; 61(1):138-46.
21. Sawada H, Takami K and Asahi S. A taxicogenomic approach to drug-induced phospholipidosis: Analysis of its induction, mechanism and establishment of a novel in vitro screening system. Toxicol Sci, 2005; 83:282-292.
22. Drenckhahn D, Jacobi B and Lüllmann-Rauch R. Corneal lipidosis in rats treated with amphiphilic cationic drugs. Arzneimittel-Forschung 1983; 33(6):827-831.
23. Gorin MB, Day R, Constantino JP, Fisher B, Redmond CK, Wickerham L, et al. Long-term tamoxifen citrate use and potential ocular toxicity. Am J Opthalmol. 1998; 125:493-501.
24. Zhang JJ, Jacob TJC, Valverde MA, Hardy SP, Mintenig GM, Sepulveda FV, et al. Tamoxifen blocks chloride channels. A possible mechanism for cataract formation. J Clin Invest 1994; 94:1690-97.