Effects of UVB-induced damage on corneal tissues in surgical menopause female rats by bilaterally ovariectomy

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Abstract: Background aims: Menopause is associated with increased production of inflammatory cytokines, such as TNF-α and TGF-β. The elevated cytokine levels likely contribute to the increased incidence of inflammatory diseases after menopause, such as osteoporosis, and neurodegenerative cardiovascular diseases and visual disease. To determine the role of 17β-estradiol and involvement of its receptors in the prevention of light-induced corneal disorders, we performed oxidative UVB-induced corneal injury model following surgically ovariectomized (OVX) female rats. Effects of UVB-induced damage on corneal tissues in OVX were compared to that in no OVX female animals. **Methods:** Fifteen female Sprague-Dawley rats were randomly divided into three groups. Corneal oxidative injury was induced by exposure to UVB irradiation at 560 μW/cm^2 for five days, followed surgical menopause induced by bilaterally OVX. The experimental animals without surgically OVX were used as surgical treatment controls and animals without UVB irradiation as blank controls. Leukocyte accumulation and central corneal epithelial thickness after injury were examined by histopathology analysis, respectively. **Results:** UVB irradiation caused substantial damage to the corneas, including thinning of corneal epithelial layer, induction of neovasculazation. After surgical menopause by bilaterally ovariectomy, corneal disorders were significantly enhanced compared with UVB and blank control groups. Studies showed that increased central corneal epithelial disorders and corneal neovasculazation after ovariectomy in vivo in terms of change in corneal epithelial thickness and vessel length (P<0.05). **Conclusions:** The studies demonstrated that surgical menopause intensity effects on UVB radiation-induced corneal oxidative disorders in female rats, including decreased central corneal epithelial thickness and increased corneal neovasculazation. [Shiu-Jau Chen, Ching-Ju Lee, Yang-Cheng Wen, Tzer-Bin Lin, Hsien-Yu Peng, Hsiang-Jui Liu, Cheng-Yu Tsai, Hung-Yu Li, Kuang-Wen Tseng. **Effects of UVB-induced damage on corneal tissues in surgical menopause female rats by bilaterally ovariectomy** [*Life Sci J.* 2014;11(5):428-433] (ISSN:1097-8135). http://www.lifesciencesite.com. 60

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1. Introduction

Women experience instabilities in sex steroids hormonal changes throughout their life span in association with puberty, ovarian cycles, pregnancy, and aged menopause. Corneal thickness and curvature variations are indicated to be influenced by steroid hormonal levels during ovarian cycles [1]. Female corneal tissues attained minimal thickness just before ovulation, maximal thickness at the beginning or end of the menstrual cycle, and increased corneal thickness during the phase pregnancy [2-3].

In addition to physical changes such as variations in thickness or curvature of corneal tissue, studies demonstrate that elderly women exhibit a more significant degree of alterations to ocular tissues compared with elderly men [4-6]. An increased prevalence of higher morbidity ocular disorders in postmenopausal women in particular has been revealed in epidemiologic researches [7-8]. Disorders such as age-related macular degeneration (AMD) idiopathic full-thickness macular hole, cataract, dry eye, and glaucoma have been decisively related to gender and age difference. Women are at a greater hazard for developing some ocular disorders and leading cause of blindness, which are very common during menopause. Furthermore, an elderly female population under estrogen replacement therapy has shown a reduced prevalence of nuclear cataract, dry eye and glaucoma, indicating some

beneficial effects of the steroid hormone estrogens on ocular physiology [9-12].

The female sex hormone estrogens are long known for their thoughtful effects on both male and female reproductive systems. Estrogens regulate growth, differentiation, and function of diverse tissues traditionally connected with various tissues. Two types of intracellular ERs, ER-α and ER-β, are encoded by genes on chromosomes 6q25 and 14q23 [13]. ER-α and ER-β are found in some organs at parallel levels, sometimes in different cell types within the same organ. Whereas in others, one or the other subtype are expressed predominates. ER-α is predominantly expressed in urogenital tract, the bone, breast, gastrointestinal tract, white adipose tissue, and ocular tissues. ER-β is predominantly expressed in the urogenital tract, gastrointestinal tract, bone marrow, vascular endothelium, lung, and certain regions of the neural tissues [14]. The analysis of amino acid sequence, numbers in percentages show the protein sequence similarity between human ER-α and ER-β. Nuclear ERs acts as a transcription factor that modulates gene expression by directly binding to DNA at specific estrogen response elements (EREs). Otherwise, rapid nongenomic effects also follow ER-ligand interactions [15]. Recently, an intracellular estrogen-binding site located on the endoplasmic reticulum has been described as a G protein-coupled receptor, for example, GPR30 (G-protein coupled receptor 30), mediating intracellular calcium mobilization [16]. The rapid effects activated including calcium-calmodulin-dependent kinases or other signal transduction pathways of the mitogen-activated protein kinase family and phosphoinositide 3-kinase [14, 16-17].

Female gonadal hormone estrogens have been demonstrated as affecting induction of ocular disorder in epidemiologic investigations. However, 17β-estradiol and involvement of its related-receptors in the prevention of light-induced corneal disorders has yet to elucidate. We investigated the impact of estrogen in the prevention of UVB-induced corneal disorders in surgical menopause female rats by bilaterally ovariectomy. Corneal damage was graded according to corneal epithelial thickness and corneal neovascularization length.

2. Material and Methods

Experimental animals

Female Sprague-Dawley rats (n = 15; 210-275g) were obtained from the National Laboratory Animal Center (Taipei, Taiwan, ROC). Animals were quarantined and allowed to acclimate for one week before beginning experimentation. The animal room temperature was maintained at 25±2 °C with a relative humidity of 55±5%. Air handling units in the animal rooms will be set to provide approximately 12 fresh air changes per hour. Animals will house 3-4 per cage under standard laboratory conditions with a 12 hours light/dark cycle. Care and treatment of animals were in accordance with standard laboratory animal protocols approved by the Animal Care Committee (Mackay Medical College). Food and water were available ad libitum. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee, and the animals were cared for in accordance with the institutional ethical guidelines. Animals were ovariectomized bilaterally via two small lumbar incisions under anesthesia with ethrane (Abbott, Abbott Park, IL, USA), and were tested 20-30 days after surgery. On experimental days, rats were anesthetized with intraperitoneal urethane (1.2 g/kg, i.p.).

UVB irradiation animal model

Fifteen rats were randomly split into three groups, including Group Ⅰ : blank control, Group II: UVB (exposure to UVB irradiation without treatment), and Group III: UVB/ OVX (exposure to UVB irradiation following surgically ovariectomized (OVX)). To induce retinal damage in vivo, eyes of the animals in Groups II and III were exposed to UVB irradiation using the method as the previous study [18] with slight modification. The energy output of UVB was measured with Ultrospec 3000 UV-visible spectrophotometer (Pharmacia Biotech) from 260 nm to 800 nm. The wavelength of the light source peaked at 308 (range, 280-315 nm). After anesthesia, eyes were exposed to 550 μW/cm^2 of UVB light (UVGL-58; UVP Inc., San Gabriel, CA) for four minutes in a darkroom. The entire UVB irradiation course will be completed in a consecutive five-day period.

Corneal tissues isolation

Following corneal euthanasia, tissue was immediately collected from both eyes of the control and experimental animals by careful dissection to avoid contamination. After removal, the corneas were washed in phosphate-buffered saline (PBS) and immersed overnight in 4% paraformaldehyde. After which they were dehydrated in increasing concentrations of ethanol and xylene, and embedded in paraffin. Sections were cut and deparaffinized with xylene and decreasing concentrations of ethanol. All samples in an experiment were subsequently processed at the same time to avoid potential variation.

Histopathological analysis of corneal epithelial thickness

Sagittal sections of 7μm were cut and stained with hematoxlin (Sigma-Aldrich). After 5 more rinses in PBS, samples were mounted and viewed on a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany). The corneal section with the longest corneal length were collected and measured for the corneal morphology and the corneal epithelium thickness of the central area. Images were measured at 10 representative locations for corneal epithelial thickness.

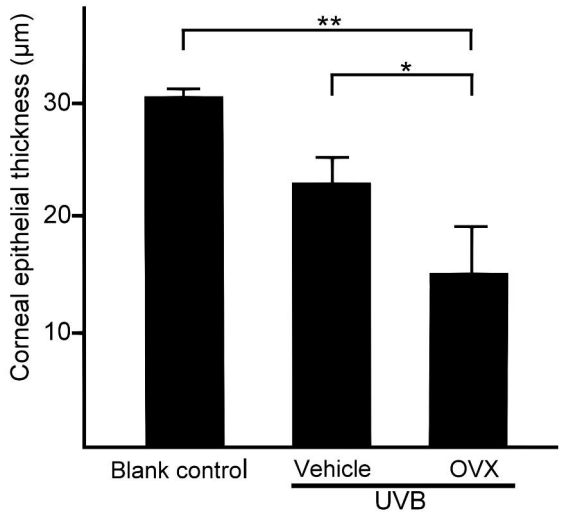

Fig. 1: The effect of surgical menopause on UVB induced corneal disorders
Epithelia were significantly thinner in eyes exposure to UVB irradiation following surgically ovariectomized (OVX) compared to blank control and exposure to UVB irradiation without surgically ovariectomized. * indicates $p<0.05$, ** indicates $p<0.01$.

Corneal angiogenesis

For histograms of relative corneal neovascularization, the method was modified from the previous study [19]. Experimental animals were anesthetized, and the maximum vessel length of the neovascularization zone, extending from the base of the limbal vascular plexus toward the central cornea. The contiguous circumferential zone of neovascularization was measured as clock hours with a 360° reticule.

Statistical analysis

To analyze the differences between groups the Mann-Whitney U test analysis with two tailed probability was used and a p value of <0.05 was considered significant. Results are presented as means ± SDs (standard deviations). For each experiment, surgery was performed on all animals in a standardized fashion, and animals were randomized to the different treatment and control groups.

3. Results

3.1 UVB-induced corneal damage

Light microscopic examination of the corneas of UVB irradiation rats revealed stromal edema and minimal inflammatory response, the latter comprising some polymorphonuclear neutrophil-like cells and lymphocytes, which was not observed in the non-irradiated blank controls (results not shown). The thinning of the corneal epithelial layer was also detected in the eyes of animals with UV exposure. Histopathologically, the corneal epithelial layers with UVB exposure were significantly thinner than those of the non-irradiated blank control group. The epithelial cells generally exhibited wrinkles in the corneal surface were often observed in the UVB group.

3.2 Increase of UVB-induced corneal epithelial disorders in surgical menopause experimental animals

We next wanted to test whether surgical menopause by bilaterally ovariectomy is critical for increase of UVB-induced corneal epithelial disorders. We examined the morphological properties of blank control, exposure to UVB irradiation, and exposure to UVB irradiation following surgically ovariectomized (Figure 1). The mean of blank control corneal epithelium thickness in non-irradiated eyes was 31.6±0.8μm. However, the epithelial cells generally exhibited wrinkles in the corneal surface were often observed in the UVB irradiation with or without surgical menopause groups. The corneal epithelial thickness in eyes without bilaterally ovariectomized was 23.1±2.2 μm after UVB irradiation. The mean corneal epithelial thickness in eyes with bilaterally ovariectomy was 16.2±3.3μm after UVB-exposure. Irradiated corneal epithelial tissues were significantly thinner in eyes treated with surgical menopause compared with blank control and irradiation without surgical menopause groups.

3.3 Advanced UVB-induced corneal angiogenesis in surgical menopause experimental animals

To evaluate the effects of surgical menopause in vivo, corneas of ovariectomized bilaterally animals were irradiated with UVB to monitor the development of neovascularization (Figure 2). Animals without UVB irradiation represented blank control. UVB exposure lets induced corneal angiogenesis after 5 days. At the end of experiment (day 5), the average vessel length in corneas treated with UVB following surgically ovariectomized (1.21 ±0.11mm) was more increased than that of corneas treated with UVB (0.83±0.16mm, $p<0.05$) .In other

words, UVB irradiation following surgically ovariectomized resulted in significantly increased corneal neovascularization compared to that blank control and UVB irradiation without surgical menopause groups.

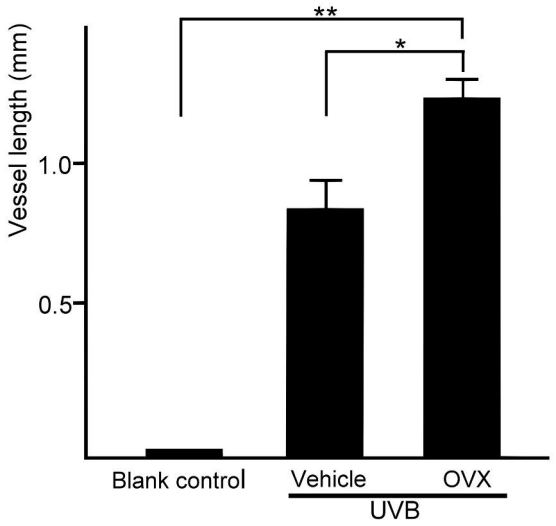

Fig. 2: The effect of surgical menopause on UVB induced corneal angiogenesis

Corneal neovascularization response in corneas was expressed as length of central extension from the limbus. The average vessel length in corneas was significantly increased in eyes exposure to UVB irradiation following surgically ovariectomized (OVX) compared to blank control ($p<0.01$) and exposure to UVB irradiation without surgically ovariectomized. * indicates $p<0.05$, ** indicates $p<0.01$.

4. Discussion

Light-induced ocular discords have been demonstrated by the results of human and animal studies [20-21]. The absorption properties of the cornea contribute to the protection of the retina against the hazards of UV light exposure. The cornea absorbs about 90% of UVB radiation and is most sensitive to UVB damage. Epithelial tissues of the corneal surface generally exhibited wrinkles were often observed in the UVB irradiation groups in the study (results not shown).

Corneal epithelial tissues originate from surface ectoderm, as do epidermis, conjunctival epithelia, and vaginal epithelium. It is also well recognized that corneal epithelial thickness like vagina is sensitive to variable levels of estrogens, not only during the ovarian cycle, but also during other phases of a woman's life [22-23]. It is well known that UV irradiation leads to inflammatory reactions in the corneal and skin epithelia. In this study, we also demonstrated that UVB-induced corneal disorders. In addition, this present study first to demonstrate the increase of UVB-induced corneal disorders in surgical menopause experimental animals. Increase of corneal epithelial disorders and advanced UVB-induced corneal angiogenesis were observed in animals after exposure to UVB irradiation following surgical menopause by bilaterally ovariectomy in our study.

Several epidemiologic observations suggest a potential participation of ERs in the homeostasis of the eye. However, the mechanisms involved remain unclear. Interesting, gender-based differences in the occurrences of many important ocular conditions raise the possibility that ERs may have direct effects on the eye. Disorders such as keratoconjunctivitis sicca, AMD, cataract, and idiopathic full-thickness macular hole, have been conclusively associated with sexual and age categories in a number of epidemiologic studies [7-8]. These investigations demonstrated an increased prevalence of high-morbidity eye diseases, especially in the aged and postmenopausal individuals.It also demonstrated that the number of ERs-positive cells in ovariectomized animal was significantly deceased compared to shammed group [24]. Taken together, it will be appropriate to suggest that the decrease of ER expression following ovariectomy enhanced UVB-induced corneal disorder, although precise mechanism remains need to be address.

Cytoprotective effects of ERs have been widely studied in various experimental models both in vivo and in vitro. ER-α- and ER-β-selective agonist have been reported to promote the survival of mesencephalic cells following exposure to glutamate, superoxide anion, hydrogen peroxide, β-amyloid protein, sodium azide, kainate or N-methyl-d-aspartate [25]. Moreover, it was demonstrated that cells in the animal model of ERα knockout mice were more vulnerable to dopamine depletion than wild type mice [26]. Menopause is associated with increased production of inflammatory cytokines, such as TGF-β, NF-κB, interleukins and TNF-α. The elevated cytokine levels likely contribute to the increased incidence of inflammatory diseases after menopause, such as osteoporosis, and neurodegenerative cardiovascular diseases and visual disease [27-30]. Estrogens inhibit the release cytokines from multiple cell types, suggesting that pro-inflammatory genes are major targets for ERs. It has been demonstrated that ERs exert their anti-inflammatory activity by repressing the expression of multiple NF-κB-driven cytokine genes [31, 32]. Studies recommended that ERs repress genes by blocking the binding of NF-κB to the promoter. Our study indicated that surgical menopause enhanced UVB-induced epithelial disorders and

neovacuolization in corneal tissues.

5. Conclusion

The normal process of aging in women is accompanied by depression in hormonal levels due to the inception of menopause. The results in the article reveal that surgical menopause intensity effects on UVB radiation-induced corneal oxidative disorders in female rats. Corneal changes including decreased central corneal epithelial thickness and increased corneal neovasculazation after exposure to UVB irradiation following surgically ovariectomized. These results reveal the potential for female sex hormones and related receptors in the treatment of diseases involving oxidative stress as an essential component for pathogenesis and progression in corneal tissues.

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