ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Histological findings in the Wistar rat cornea following UVB irradiation

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Abstract

The acute clinical effect of UVR on the eye is photokeratitis, which is an inflammatory state that might be regarded as the sunburn of the eye. In this study, we used a rat model to assess the histological injuries induced in the intact rat cornea following its exposure to UVB radiation. A total of 15 two-months-old female Wistar rats were purchased from the Animal Facility of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The rats were fed *ad libitum* and kept under standard conditions with a 12 hours light/dark cycle. The rats were randomly divided into five groups, including control group (no UVB exposure), group II (a single exposure to a dose of 45 mJ UVB/cm^2 for 47 seconds), group III (a single exposure to 90 mJ UVB/cm^2 for one minute and 57 seconds), group IV (a single exposure to 180 mJ UVB/cm^2 for three minutes and 57 seconds), and group V (a single exposure to 360 mJ UVB/cm^2 for five minutes and 26 seconds). After 24 hours of recovery, the rats were sacrificed by cervical dislocation. The rat eyes were extracted, harvested and fixed in 10% buffered formalin. The eye samples were then processed through paraffin technique for further histological examination. We found that, following the UVB exposure, the cornea showed significant inflammatory responses (infiltration of polymorphonuclear leukocytes), hemorrhage and gross damages such as superficial and deep ulcerous keratitis and epithelial exfoliation. The severity of these findings was associated with the increase of UVB radiation intensity and exposure period.

Keywords: eye, cornea, UVB irradiation, Wistar rat.

Introduction

Ultraviolet light (UVL) is an electromagnetic radiation with a shorter wavelength than that of visible light, but longer than X-rays (range from 400 nm to 10 nm). The UVL is used for the treatment of some skin conditions (*e.g.*, psoriasis and vitiligo) and in food processes to destroy unwanted microorganisms and to pasteurize fruit juices [1]. Nearly all people are conscious of the effects of UV radiation (UVR) through the painful condition of sunburn, but the UVR has many other effects, both beneficial and damaging, on human health [2]. Extended exposure to solar UVR may result in some effects on the skin, eye and immune system [3].

Among ultraviolet radiation, type B (UVB), particularly UVB having 300 to 320 nm wavelengths, has gained interest, since it is mostly absorbed by corneal stroma and lens [4, 5]. An augmented risk of ultraviolet radiation on the eye has been associated to the decrease in stratospheric ozone [6]. Stratospheric oxygen and ozone molecules absorb 97–99% of the sun's high frequency ultraviolet light [7].

There are numerous papers studying UVR effects on the ocular lenses. The lens ability to protect against UVR, and its ability to repair damages induced by UVR, is of crucial importance to avoid cataract development. The influence of UVR-induced damage and repair processes on the lens metabolites are not fully understood [8]. Ultraviolet radiation (UVR) damages the lens through several mechanisms, leading to formation of protein cross-linking, damage to the membrane transport system, and changes in cellular DNA. These alterations have a major impact on the metabolic pathways in the lens [8]. The production of reactive oxygen species (ROS) is a well-documented path for UVR damage [9, 10]. Increased levels of oxidants perturb the fine balance between production of free radicals and their eradication by the oxidant scavengers. This might lead to further alterations of the biological processes in the lens. As shown in various reports, major changes in enzyme activities and metabolic concentrations have been detected after UVR exposure [8, 11, 12].

The acute clinical effect of UVR on the eye is photokeratitis, which is an inflammatory state that might be regarded as the sunburn of the eye. The chronic effects following the eye exposure to UVR are photokeratitis, climatic droplet keratopathy, cataract, pinguecula, and pterygium formation [13–15].

In this study, we used a rat model to assess the histological injuries induced in the intact rat cornea

following its exposure to UVB radiation. The number of published accounts of microscopic lesions induced by UVB-irradiation of the cornea is relatively low.

Materials and Methods

A total of 15 two-months-old female Wistar rats were purchased from the Animal Facility of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The rats weighted about 120 g on arrival and were fed *ad libitum* and kept under standard conditions with a 12 hours light/dark cycle. The rats were acclimatized to the laboratory for one week before the experiments. The experiments were reviewed and approved by the Ethical Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

The 15 rats were randomly split into five groups, including blank control group (no UVB exposure), group II (a single exposure to a dose of 45 mJ UVB/cm^2 for 47 seconds), group III (a single exposure to 90 mJ UVB/cm^2 for one minute and 57 seconds), group IV (a single exposure to 180 mJ UVB/cm^2 for three minutes and 57 seconds), and group V (a single exposure to 360 mJ UVB/cm^2 for five minutes and 26 seconds). Each group contained three rats. In order to expose the corneas to UVB irradiation, the rats were anesthetized with 10% Ketamine and 2% Xylazine. The corneal UVB-irradiation was realized using a lamp (UVB Waldmann, model UV 236 B therapy system, Waldmann, Germany). The wavelength range of UVB light was 280–360 nm. The distance interposed between the rat cornea and UVB lamp was about 10 cm.

After a single UVB exposure of rats' cornea, the animals were allowed to recover (for about 24 hours) and then sacrificed by cervical dislocation. The rat eyes were extracted, harvested and fixed in 10% buffered formalin for 48 hours (each eyeball was punctured laterally for a better fixation and crystalline extraction). The eye samples were then embedded in paraffin, cut at 5-µm thickness, and mounted on glass slides. Goldner's trichrome stain was performed for histological examinations. The eye sample harvesting and histological processing was realized in the Department of Histology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca.

Results

A relatively uniform corneal thickness was noticed in the control group. There were no microscopic changes in the structure of the cornea in this group (Figure 1).

The eyes from group II generally exhibited mild damages in the corneal structure, as represented by the modest stromal edema.

The histological assessment of the cornea showed significant difference between the eyes from group III and those from the control group and group II. The rat eyes from group III generally exhibited serious corneal damages as represented by the irregular hypertrophy of the cornea in all its length (Figure 2). Ruptures in the central corneal surface, leading to ulceration, were often observed. The Bowman's membrane was still present and continuous covering the stroma in the ulcerative corneal regions (Figure 2). Both superficial (*i.e.*, epithelial) and stromal corneal edema were noticed. Several large clefts were noticed throughout the whole corneal stroma suggesting edema. Mild epithelial spongiosis, intercellular edema and intraepithelial vesicles were encountered in the peripheral corneal epithelium. Furthermore, the corneas frequently contained discreet polymorphonuclear leukocytes in all the stroma. More abundant neutrophils were noticed underneath the ulcerative regions (junctional leukocytic infiltrate).

The granulocytic stromal infiltrate of the cornea was scarce in the peripheral regions (close to sclerocorneal area). Besides, the sclerocorneal regions of the cornea had a marked focal hypertrophy suggested by large stromal clefts displacing the collagen fibers.

Significant histological changes were observed in cornea of the rats from the IVth group. Severe irregular thickness in all the corneal length occurred because of some large spaces (*i.e.*, edema induced clefts) separating and displacing the stromal collagenous fibers (Figure 3).

Similarly to the previous group, the cornea suffered epithelial ulceration in its central region. Critical epithelial changes were detected in the remaining corneal epithelium. Severe intercellular edema led to the compression atrophy of some epithelial cells (suggested by hyperchromatic nuclear pyknosis and cellular shrinkage). Additionally, superficial epithelial layers suffered bullous vacuolation. The corneal Bowman's membrane was intact and continuous (including in the ulcerative corneal regions), and leukocytic infiltrate followed a similar pattern to the one from the previous group (Figure 4). In contrast, in group III, there was a more severe corneal edema and the inner part of the stroma was less affected.

Quantitatively, major differences were found between group V and the previous groups. Thickness of the cornea increased considerably (especially in its central region) about three times in contrast to the control group. The centrally placed corneal epithelium was entirely necrotized having the tendency of desquamating as large masses. Besides, the subjacent Bowmans's membrane suffered focal necrosis because of epithelial necrosis extension in the corneal stroma (Figure 5). The epithelial necrosis did not occur to the periphery of the cornea (to the sclerocorneal junction).

In this group, the inflammatory infiltrate was diffuse and abundant in comparison to groups III and IV. The inflammation extended not only to epithelial–stromal junction, but also all through the corneal stroma depth. Polymorphonuclear leucocytes and cellular debris were noticed in the necrotized epithelium of the cornea (Figure 6). Excluding the polymorphonuclear leukocytes, some multinucleated giant cells were identified among the inflammatory cells from the eye stroma.

Diffuse and severe stromal infiltration with polymorphonuclear leukocytes (including mast cells and macrophages) and extensive edema were associated with liquefaction of the corneal stroma (keratomalacia)

(Figure 7). However, numerous stromal microvessels, hemorrhage and fibroplasia complete the histological findings noticed in this group. The microvessels' number and diameter, and keratomalacia diminish at the sclerocorneal limbus (Figure 8).

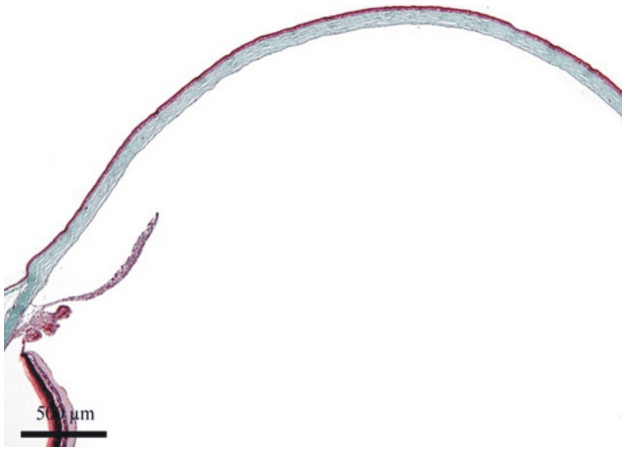

Figure 1 – ***Normal aspect of the rat cornea from the control group. Goldner's trichrome staining.***

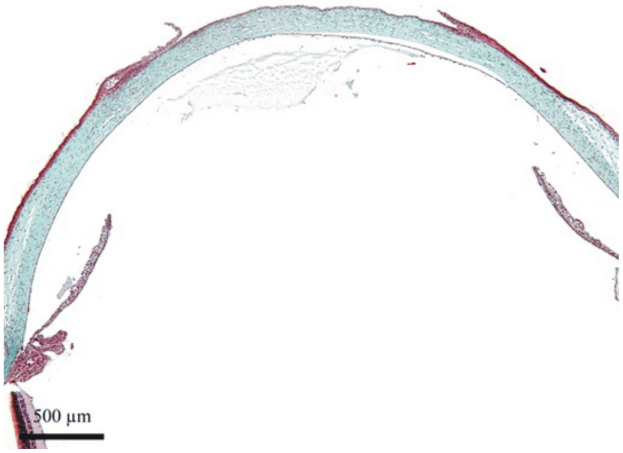

Figure 2 – ***Irregular hypertrophy of the rat cornea, central epithelial ulceration and stromal edema (experimental group III). Goldner's trichrome staining.***

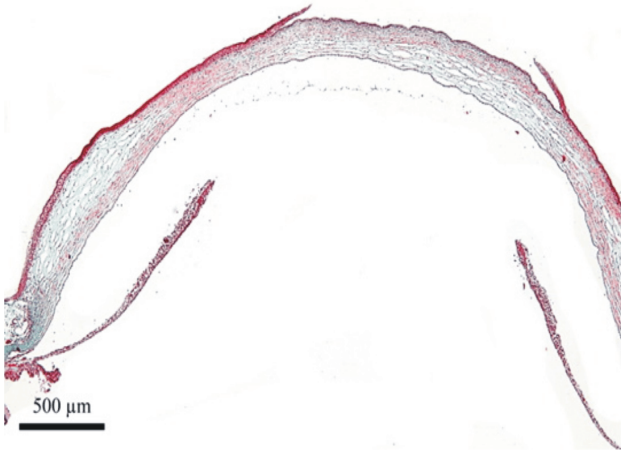

Figure 3 – ***Marked diffuse irregular hypertrophy of the rat cornea, central epithelial ulceration and severe stromal edema represented by large clefts displacing the stromal collagenous fibers (experimental group IV). Goldner's trichrome staining.***

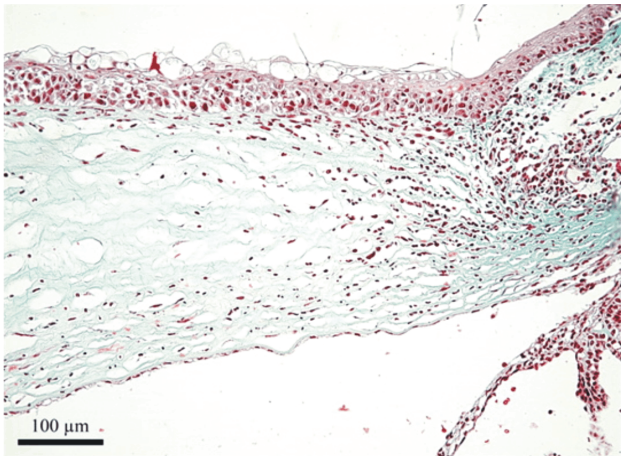

Figure 4 – ***Severe intercellular edema and hypertrophy of the corneal epithelium, bullous vacuolation of the superficial epithelial layers, leukocytic infiltrate and edema in the corneal stroma (experimental group IV). Goldner's trichrome staining.***

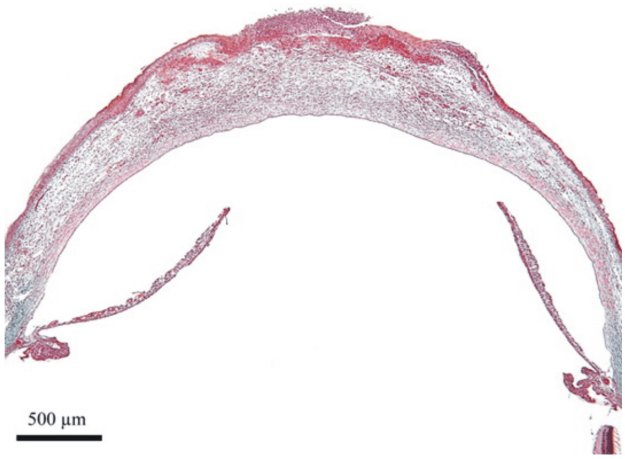

Figure 5 – ***Marked diffuse irregular hypertrophy of the rat cornea, central epithelial necrosis, diffuse inflammatory infiltrate, hemorrhage and severe edema in the stroma (experimental group V). Goldner's trichrome staining.***

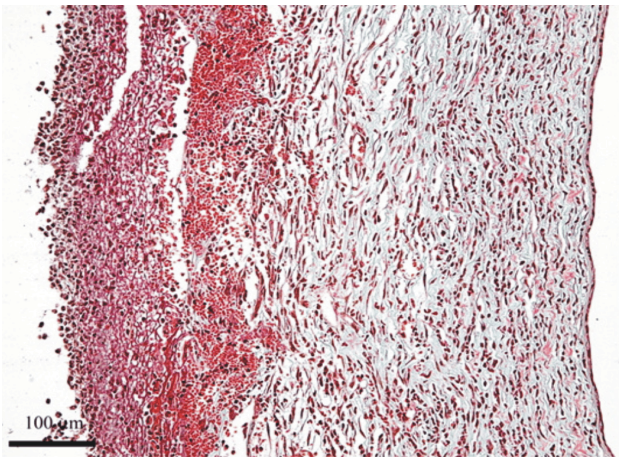

Figure 6 – ***Polymorphonuclear leucocytes and cellular debris in the necrotized epithelium of the cornea, hemorrhage and associated fibroplasia in the stroma (experimental group V). Goldner's trichrome staining.***

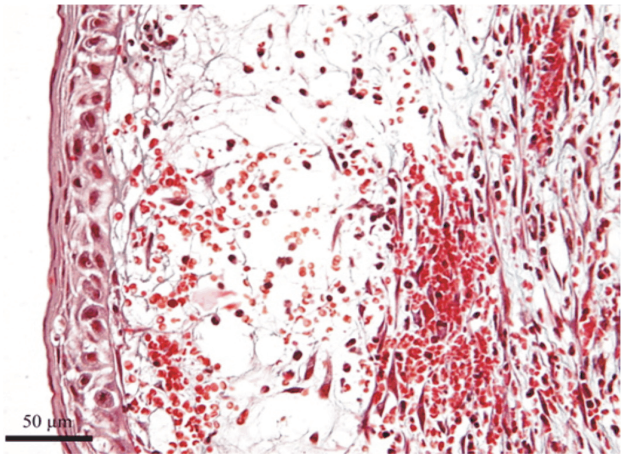

Figure 7 – ***Extensive edema associated with keratomalacia, numerous stromal microvessels and hemorrhage (experimental group V). Goldner's trichrome staining.***

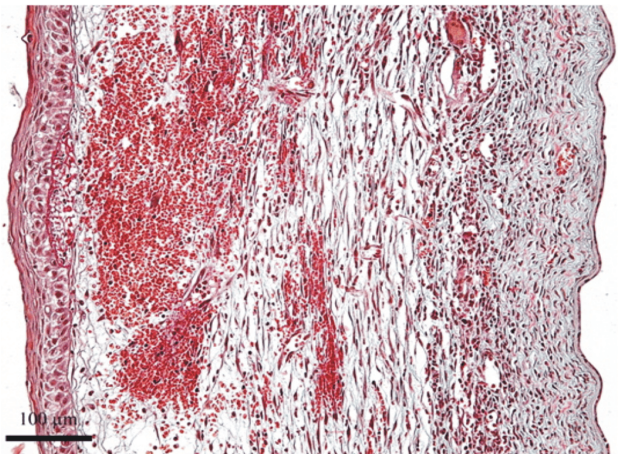

Figure 8 – ***Invasive hemorrhage and numerous stromal microvessels and associated fibroplasia (experimental group V). Goldner's trichrome staining.***

Discussion

The eye is dependent on visible light energy and can be damaged by it and the contiguous ultraviolet and infrared wavelengths. Illness of the eye in which sunlight has been involved has been named ophthalmohelioses, and these conditions create a major problem to the eye health in many communities. The ophthalmohelioses have a remarkable impact on patients' quality of life and have considerable implications on the cost of health care [16]. The understanding of the intracellular mechanisms involved has conferred various insights into how treatments have been developed (for the management of ocular surface squamous neoplasia). The theory of peripheral light focusing has also offered a path in the prevention of these diseases. This has resulted in improved sunglass design and the additional progress of UV-blocking contact lenses [17, 18].

In contrast to some other reports approaching the involvement of ROS on corneal tissue following exposure to UVB, this paper describes histological details induced by UVB radiation. The cornea is directly exposed to UV light and most of the information rests on investigations using animals and short-time exposures at high intensities. This step is necessary if we are to make significant progress toward better understanding of the practical impact of solar radiation on the eye. Epidemiologic data suggests that corneal injury from UVR is a threat when prolonged exposure takes place in regions containing much ultraviolet (UV) or in exceedingly reflective environments [19, 20].

In the present study, some histological details regarding effects of UVB radiation on rat cornea have been provided. We found that higher doses and longer durations of UVB exposure induced severe lesions in rats' cornea in groups II–IV in comparison to the unexposed group (group I).

Rats from group II exposed to a dose of 45 mJ UVB/cm^2 for 47 seconds (sacrificed at 24 hours after UVB irradiation) showed mild corneal lesions (*i.e.*, discrete stromal edema displacing the collagen fibers). In group III, the corneal lesions (following exposure to 90 mJ UVB/cm^2 for one minute and 57 seconds and sacrificed at 24 hours after UVB irradiation) are more severe in both the epithelium and stroma. The thickness of the cornea increased following the stromal and epithelial edema. Epithelial necrosis and desquamation showing a denuded Bowman's membrane (superficial ulcerative keratitis) were also noticed. The surface epithelium (still present at the sclerocorneal limbus) showed intercellular edema and intracellular spongiosis, and associated suprabasilar and intraepithelial bullous keratopathy. The stroma presented large clefts and moderate junctional infiltrate with granulocytes (*i.e.*, neutrophils).

Exposure of the rats' cornea to 180 mJ UVB/cm^2 (group IV) for three minutes and 57 seconds (sacrificed at 24 hours after UVB irradiation) generated significantly more injuries. The corneal stroma was irregularly thickened due to the edema. In its central region, the corneal epithelium was necrotized and desquamated leading to corneal ulceration. Similarly to the previous group, moderate to severe intercellular edema and intraepithelial bullous keratopathy occurred. A focal granulocytic infiltrate has been noticed in the stroma adjacent to sclerocorneal limbus. Finally, in the rats' cornea from group V (exposure to 360 mJ UVB/cm^2 for five minutes and 26 seconds) the damages involved all the corneal layers. Apart from the lesions already presented (in the groups III and IV), some others were observed, such as: (a) deep ulcerative keratitis represented by the extension of the necrosis in the corneal stroma (superficial epithelial coagulation necrosis associated with stromal necrosis and discontinuous Bowman's membrane); (b) hyperemia and severe hemorrhages underneath the necrotized corneal epithelium; (c) diffuse infiltration of leukocytes (neutrophils, macrophages, and few mast cells) in the corneal stroma associated with keratomalacia (progressively liquefaction of the corneal stroma); (d) the presence among inflammatory cells of giant multinucleated cells; (e) numerous stromal microvessels in all the width of the cornea due to increased neoangiogenesis.

The injuries caused by UV irradiation to cornea are named photokeratitis (also known as ultraviolet keratitis). Photokeratitis is characterized by exfoliation of the corneal epithelium, diminished visual perception, inflammation,

edema, eye redness, and burning-like pain from the ocular surface [21, 22]. Moreover, the damages induced by photokeratitis might not be limited only to the corneal epithelium (as we also noticed in our study). UV irradiation can go deeper through the epithelial layer and provoke inflammatory responses that involve the full corneal thickness [23–26].

Similar findings following UVB exposure of the rats' cornea have been noticed by Chen BY *et al.* (2011) [27]. The mice were anaesthetized with their ocular surfaces exposed to UVB light (0.72 J/cm^2/daily). Histologically, the corneal epithelial layers with UVB exposure were considerably thinner than those of the control group are. The epithelial cells usually exhibited more condensed nuclei, suggesting occurrence of cell death. Ruptures in the corneal surface were frequently noticed in the UVB group. Besides, the corneas with UVB exposure frequently contained invasive polymorphonuclear leukocytes in the stroma and in the aqueous humor. Some polymorphonuclear leukocytes appeared to fasten to the endothelial layer, suggesting a potential threat of attack to the corneal endothelial cells [27].

Some other histological and ultrastructural details have been reported by Mahmoud BL *et al.* (2010) in the rats' cornea (exposed to a single UVB irradiation at a dose of 1.2 J/cm^2) [3]. Cornea of UV irradiated rats revealed epithelial thinning with cellular degeneration, exfoliation and metaplasia. Bowman's membrane was focally discontinuous or absent. Stroma presented intermittently arranged widely separated collagen fibers, degenerated few keratocytes with neovascularization, interstitial hemorrhage and cellular infiltration. Ultrastructurally, epithelial cells exhibited nuclear membrane irregularities, chromatin condensation, mitochondrial degeneration and loss of rER [3].

In our experiment, the UVB irradiation caused obvious corneal damages starting with a minimum UVB-irradiation of 90 mJ UVB/cm^2 for one minute and 57 seconds (a single UVB exposure of the rat cornea). The histological changes increased along with irradiation intensity and UVB exposure of the cornea. The most severe lesions were observed following eye exposure to 360 mJ UVB/cm^2 for five minutes and 26 seconds. Previous reports had indicated that UVB can induce gross lesions following corneal exposure to some large doses (*e.g.*, 0.72 J/cm^2 to 1.2 J/cm^2) [3, 27]. This study investigated the effects of lower doses of UVB (45 mJ to 360 mJ UVB/cm^2) on the rat cornea. Our findings proved that even lower UVB doses induce histological changes in the rat cornea (despite the fact that gross changes were not always observed).

The cellular and molecular mechanisms underlying photokeratitis have been widely studied in the latest years [28, 29]. Evolution of the disease engages a range of proinflammatory molecules such as interleukins, cytokines, matrix metalloproteinases (MMPs) and nuclear factor-κB (NF-κB) [30–33]. Among them, NF-κB activation stimulated by UVB has been usually accounted [27, 28, 31]. NF-κB (if translocated into the nucleus) will make the transcription of many down-stream genes possible, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Both of them are the key mediators in the recruitment of inflammatory cells [27, 34–36].

Conclusions

A rat model of UVB-induced photokeratitis was established and significant histological findings in the rats' cornea were noticed following UVB exposure. Five groups were used, exposed to different intensities of UVB radiation (45 mJ UVB/cm^2 to 360 mJ UVB/cm^2, once). We found that, following the UVB exposure the cornea showed significant inflammatory responses (*i.e.*, infiltration of polymorphonuclear leukocytes), hemorrhage and gross damages such as superficial and deep ulcerous keratitis and epithelial exfoliation. The severity of these findings was associated with the increase of UVB radiation intensity and exposure period.

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Received: December 20th, 2012

Accepted: April 10th, 2013