HISTOPATHOLOGICAL DYNAMIC ASPECTS IN THE RAT CORNEA IRRADIATED WITH REPEATED DOSES OF UVB

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Summary

The cornea occupies the front of the fibrous tunic of the eye and resembles a diverging lens. The acute clinical effect of UVB on the eye is photokeratitis, which is a transient painful condition that can be called sunburn of the eye. The experimental study was performed in the Department of Physiology from the University of Medicine and Pharmacy "Iulius Hațieganu" Cluj-Napoca. Histological processing was done in the laboratory of Histology department of the Faculty of Veterinary Medicine, Cluj-Napoca. Animals used in this study were 10 Wistar rats (females) that were about 2 months. Rats were divided into five groups as follows: Group I, nonirradiated (control group); Group II, irradiated with 0.36 J/cm² (one dose); Group III, irradiated with 0.36 J/cm² (two doses at 24 hours interval); Group IV, irradiated with 0.36 J/cm² (three doses at 24 hours interval); Group V, irradiated with 0.36 J/cm² (four doses at 24 hours interval). At 24 hours after the last exposure, the animals were sacrificed and eyes enucleated, harvested and immersed in 10% buffered formalin. Serial sections of 5 μm were applied and stained with Masson’s trichrome method (panoptic Goldner version). Our results confirm that exposure to multiple doses of UV irradiation induces different changes than acute ones. After a single exposure of the cornea to UVB, the epithelium displayed severe acute and focal injuries. Repeated corneal exposures cause epithelial lesions that reveal a chronic tendency, reflected by the keratinisation tendency of the corneal epithelium, hypertrophy and metaplasia of the anterior part of corneal epithelium, hypertrophy and densification of the stroma, and metaplasia of the posterior corneal epithelium.

Key words: UVB radiation, corneal lesions, histopathological findings.

Introduction

The cornea occupies the front of the fibrous tunic of the eye and resembles a diverging lens whose transparency is given by some structural and physiological peculiarities: a highly ordered non-keratinized surface epithelium, an avascular and poorly cellularized stroma composed of thin collagen fibrils (which are orderly set in lamellae) and the endothelium.

It appears as a structural and physiological change of the sclera, and when chronic lesion tends to occur it resembles the sclera (histological and by ophthalmoscopy). If there is a rapid deterioration in any component of the cornea, it will undergo healing by ulceration. If the change is gradually installed, then the most likely response is adaptive corneal metaplasia (and the cornea resembles the skin). Corneal lesions may occur under the action of physical or chemical trauma, microbial agents, increased intraocular pressure, and rarely in innate metabolism errors (Grant Maxie et al., 2007). Some physical agents that may

114
traumatize the cornea include the UVB radiation that appears to be the most important. In general, the effect of UVB radiation depends on the wavelength and the amount of radiation absorbed by tissues. Continuous decline of the ozone layer is an aggravating factor for the action of ultraviolet radiation on the eye (Kabuyama et al., 2002). Accordingly, it is known that stratospheric oxygen and ozone molecules absorb 97-99% of high-frequency ultraviolet light of the sun (Tucker, 2007). Chronic exposure to the solar UVR can lead to some acute and chronic effects on the eye (Mahmoud et al., 2010). Acute clinical effect of UVB on the eye is photokeratitis, which is a transient painful condition that can be called sunburn of the eye (Gallagher and Lee, 2006). Chronic exposure with multiple doses of UV can cause severe eye disorders such as: cataract, squamous cell carcinoma, melanoma and some other corneal entities known as pterygium and pinguecula (Berwick, 2000).

Material and method
The experimental study was performed in the Department of Physiology from the University of Medicine and Pharmacy "Iulius Hațieganu" Cluj-Napoca, with the approval of the ethics committee and in accordance with Best Practices. Histological processing was done in the laboratory of Histology department of the Faculty of Veterinary Medicine, Cluj-Napoca. Animals used in this study were 10 Wistar rats (females) that were about 2 months and an average weight of 120g. Utilized rats received granulated standard diet (ad libitum) and ad libitum water. The environmental conditions were: 21-23 C, humidity 65%, and light alternation of 12 hours dark/night periods. Rats were divided into five groups (two animals in each group) as follows:
- Group I, nonirradiated (control group);
- Group II, irradiated with 0.36 J/cm² (one dose);
- Group III, irradiated with 0.36 J/cm² (two doses at 24 hours interval);
- Group IV, irradiated with 0.36 J/cm² (three doses at 24 hours interval);
- Group V, irradiated with 0.36 J/cm² (four doses at 24 hours interval).
Irradiation was performed after anesthesia with Ketamine 10% and Xylazine 2% (im, 0.1 ml/100g body weight from a mixture of two parts Ketamine and a part of Xylazine). The utilized lamp for UVB irradiation was UVB WALDMANN model UV 236B therapy system, which possesses a large spectrum and wavelength ranging from 280 to 360 nm.
At 24 hours after the last exposure, the animals were sacrificed and eyes enucleated and harvested (4 eyes per group). After the creation of lateral gaps (in every eye ball) in order to facilitate penetration of fixation solution, eyes were immersed in 10% buffered formalin. After two days the lens were extracted and remained into formalin for some other three days. Subsequently, the pieces were dehydrated with alcohol, cleared with n-Butanol and paraffin embedded. Serial sections of 5 µm were applied (approximately 200 for each piece) and stained with Masson’s trichrome method (panoptic Goldner version). Histological examination was done using the BX41 Olympus microscope.

Results and discussion
The cornea of rats in the control group I show uniform thickness and a normal structure (Fig. 1).

The irradiated cornea of rats from group II (a single irradiation at 0.36 J/cm²) has been irregularly thickened (Fig. 2), especially due to corneal edema present all through the stroma. The anterior epithelium is affected in a restricted area, where the severe necrosis affects mostly epithelial cells in the external half (Fig. 3). Manifested lesions have an acute character.

Cornea of rats exposed twice to 0.36 J/cm² UVB (i.e. group III) was
considerably thickened (particularly in outlying areas) compared to the former group (Fig. 4). The corneal epithelium did not show features of acute necrosis. The manifested lesions had a chronic tendency by presenting keratinisation in specific areas (Fig. 5).

In rats exposed to three doses of 0.36 J/cm$^2$ UVB the cornea is even thicker (Fig. 6) whereas the anterior corneal epithelium is extremely rough. In some corneal areas the epithelium is apparently thickened (Fig. 7) while in others slightly atrophied and with the tendency to keratinisation.

Rats exposed to four doses of 0.36 J/cm$^2$ UVB show marked thickening of the cornea (Fig. 8) and anterior epithelium (which is uneven in both thickness and structure). Therefore, areas with atrophied epithelium that has an obvious keratinisation trend (Fig. 9) alternate with others with thickened and hypertrophied to metaplastic epithelium. In these zones the corresponding endothelium shows focal metaplasia from simple squamous to stratified squamous epithelium (Fig. 10).

Information in the literature indicates that acute UVB irradiation induce significant changes in ocular structures (Mahmoud et al., 2010), whereas the chronic exposure leads to lower intensity changes that are gradually installed. The former phenomenon is generally called photoadaptation or tolerance. Mechanisms involved are not fully understood and usually imply early keratinisation, hyperplasia of the conjunctiva, epithelial metaplasia, DNA reparation etc. (Hamzavi, 2006). According to some authors, only 70-80% of UVB radiation that reaches the cornea is absorbed by it and its components. Application of multiple doses determines adaptive and healing changes that occur between irradiation exposures (Voke et al., 1999).

Our results confirm that exposure to multiple doses of UV irradiation induces different changes than acute ones. Thus, histological findings have suggested an overall increase in corneal thickness. Overall assessment of corneas from rats in all groups highlights that the UV aggression determines defensive actions for the local protection. The body tries to form more resistant structures following the action of radiation that (according to the number of UV exposures) may involve the anterior corneal epithelium and stroma (after one, two or three UVB exposures), and posterior epithelium following four UVB exposures. Changes that occur subsequent to repeated UVB irradiation are: keratinisation tendency of the corneal epithelium, hypertrophy and metaplasia of the anterior part of corneal epithelium, hypertrophy and densification of the stroma, and metaplasia of the posterior corneal epithelium. All these changes are adaptational mechanisms that increase the corneal strength, but unfortunately they are not physiological because they affect the functionality of the cornea. Our histopathological results are similar to those reported by some other researchers that stipulate after UVB irradiation the cornea becomes turgid, reduces its transparency, increase its level of hydration. As a result, the light absorption of the injured cornea is affected quite a bit (Cejka et al., 2007) according to the irradiation frequency and utilized dose (Cejka et al., 2011). Moreover, eye exposure to repetitive doses of UVB cause cellular death and decline the cellular density in the corneal epithelium (Johar et al., 2003) as an adaptation. In other words, photoadaptation is the body's response to UVB aggression, which involves major changes in the corneal components. The previous changes will have consequences in the functionality of the corneal components.

The changes in the anterior epithelium of the cornea after repeated exposures to 0.36 J/cm$^2$ UVB were directly related to the number of administrations. Thus, after a single exposure of the cornea to UVB, the epithelium displayed severe acute and focal injuries. Repeated corneal exposures cause
epithelial lesions that reveal a chronic tendency, reflected by the keratinisation processes, metaplasia and hypertrophy as adaptive processes. Unfortunately, all the previous lesions affect the functionality of the corneal epithelium, which should possess a highly ordered structure (without keratinisation) in order to be transparent (Grant Maxie et al., 2007).

In our study, the injuries in the corneal stroma were dependent on the number of UVR exposures. The major corneal changes were represented by the collagen proliferation (i.e. fibroplasia) that increases along with the number of exposures to UVB. As a consequence, the corneal stroma was significantly hypertrophied as an adaptive response to aggression exerted by UVB radiation. This thickening of the cornea increased its resistance to UVB radiation, but affected the functionality (transparency) of the cornea (which is allowed by a highly ordered structure). Accordingly, the transparency of the cornea is provided by a poorly cellularized stroma and thin collagen fibrils arranged in highly ordered lamellae that are separated by a distance of 620-640 angstroms. The former morphological details are critical for the uninterrupted passage of the light all through the cornea (Grant Maxie et al., 2007).

Chronic injuries of the cornea extended up to the corneal endothelium (following four exposures to 0.36 J/cm² UVB), which were represented by focal metaplasia (from simple squamous to stratified squamous epithelium). The increase of endothelial cell layers provided additional resistance, but impedes the corneal transparency and optimal fluid exchanges between aqueous humor and corneal stroma.

Conclusions

In conclusion, repeated exposures to UVB radiation trigger the development of particular events in the corneal components. They are gradually installed according to the number of UVB exposures, all the changes being included in the phenomenon of photoadaptation or tolerance. Although the photoadaptation increases the resistance of cornea to the action of UVB radiation, this adjustment is made in the detriment of its functionality.
Fig. 4. Cornea from group III exposed twice at 0.36 J/cm² UVB.

Fig. 5. Cornea from group III exposed twice at 0.36 J/cm² UVB; detail.

Fig. 6. Cornea from group IV exposed three times at 0.36 J/cm² UVB. (Goldner's Trichrome)

Fig. 7. Cornea from group IV exposed three times at 0.36 J/cm² UVB; detail. (Goldner's Trichrome)

Fig. 8. Cornea from group IV exposed times at 0.36 J/cm² UVB. (Goldner's Trichrome)

Fig. 9. Cornea from group IV exposed times at 0.36 J/cm² UVB; detail. (Goldner's Trichrome)
Fig. 10. Cornea from group IV exposed times at 0.36 J/cm² UVB; detail. (Goldner's Trichrome)

References
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