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Changes of Corneal Optical Properties after UVB Irradiation Investigated Spectrophotometrically

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Summary

Ozone depletion leads to an increase in UV rays of solar radiation reaching the surface of the Earth which is harmful to biological systems. Of the eye, the cornea is directly open to increased amount of UV rays of which mainly UVB rays are capable to induce reactive oxygen species damaging the cells. Previous studies showed that the irradiation of the cornea with UVB rays leads to morphological as well as metabolic disturbances of the cornea. Also, corneal hydration and corneal light absorption are increased after UVB rays. These changes were observed after five days of repeated irradiation of the cornea with UVB rays. The aim of the present paper was to examine how early the changes of corneal hydration and light absorption occur after UVB irradiation. The rabbit corneas were irradiated with UVB rays for one, two, three or four days. Corneal light absorption was examined spectrophotometrically and corneal hydration measured by pachymeter (as corneal thickness). Results show that changes of corneal hydration and light absorption appear early after UVB irradiation and increase along with the number of irradiations. In conclusion, irradiation of the rabbit cornea with UVB rays leads to harmful changes of its optical properties.

Key words

Corneal hydration • Corneal light absorption • Absorption coefficients • UVB rays

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Introduction

With the depletion of the ozone layer, a danger to biological systems arises from the increased penetration of UV rays of longer (UVA) as well as shorter (UVB) wavelength, known to induce reactive oxygen species generation (superoxide and hydroxyl radicals, hydrogen peroxide, singlet oxygen) (Messeley and Mackie 1997, Young 1997, Wenk *et al*. 2001). These toxic oxygen products are dangerous to biological systems, causing cellular damage by reacting with lipids, proteins and DNA (Kehrer 1993, Wei *et al*. 2009).

The eye and particularly the cornea are directly exposed to UV rays. The cornea absorbs and detoxifies the majority of UVB rays (approximately 80 %), and thus it acts as a UVB filter. These processes are ensured by corneal tissue components and fluids containing important low molecular weight antioxidants (Ringvold 1997, 1998, Brubaker *et al*. 2000, Ringvold *et al*. 2000, Bilgihan *et al*. 2001, 2003) as well as high molecular

weight antioxidants (Atalla *et al*. 1988, Behndig *et al*. 1988, Abedinia *et al*. 1990, Downes *et al*. 1992, Uma *et al.* 1996, Pappa *et al.* 2001, Piatigorski 2001). Of the anterior eye segment tissue components, the corneal layers (particularly the epithelium) play the key role in the protection of the inner eye against the effect of UVB rays (Mitchell and Cenedella 1995, Kolozsvari *et al.* 2002, Podskochy 2004).

Irradiation of the cornea with UVB rays leads to profound morphological disturbances of the cornea (Koliopoulos *et al*. 1979, Haaskjold *et al*. 1993, Podskochy *et al*. 2000, Čejková *et al*. 2001, 2004, Rogers *et al*. 2004), release of pro-inflammatory cytokines (Kennedy *et al*. 1997) as well as decrease in corneal antioxidants: superoxide dismutase and glutathione peroxidase (Čejková *et al*. 2000, Lodovici *et al*. 2003), aldehyde dehydrogenase (Downes *et al*. 1992, Manzer *et al*. 2003), and ascorbic acid (Tessem *et al*. 2005). In contrast, reactive oxygen and nitrogen species are generated in increased amounts (Čejková *et al*. 2001, 2005).

Tessem *et al.* (2005) found that five days of repeated irradiation of the rabbit cornea with UVB rays (daily dose 1.6 J/cm^2) evoked significant changes in metabolites in the cornea. Using similar mode of irradiation and the daily dose 1.01 J/cm^2, Čejka *et al.* (2007) described that the irradiated cornea with increased hydration absorbed much more light (particularly in the UVB region) than the normal cornea. The aim of this investigation was to examine at which time interval of irradiation with UVB rays the changes of corneal hydration and light absorption start to be increased. Therefore, the rabbit corneas were irradiated with UVB rays once a day (daily dose 1.01 J/cm^2) for one, two, three or four days, and corneal light absorption (together with the corneal thickness) was investigated.

Methods

UVB irradiation of rabbit corneas

Adult New Zealand white rabbits (2.5-3.0 kg) were used in our experiments. The investigation was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were anesthetized by an i.m. injection of Rometar (Xylazinum hydrochloricum, Spofa, Prague, CR, 2 %, 0.2 ml/kg b.w.) and Narkamon (Ketaminum hydrochloricum, Spofa, 5 %, 1 ml/kg b.w.). The rabbits were divided into four groups (each group consisted of six rabbits). The open eyes of the rabbits (both eyes of each rabbit under general anesthesia) were irradiated with a UVB lamp (Bioblock Scientific, Illkirch Cedex, France; 312 nm wavelength, 6W) from a distance of 0.03 m for 5 min, once a day (the dose per day was 1.01 J/cm^2). Only the corneas were irradiated; the rest of the eye surface was protected from UV rays. The first group of rabbits was irradiated once, the second group two times, the third group three times and the fourth group of animals four times. (After every irradiation of animals and awaking from anesthesia, analgesia was performed: Ketonal, ketoprofenum, Ljubljana, Slovenia, 1.0 mg/kg, i.m. injection). A UV lamp stand – with an exactly determined distance between the lamp and the eyes – was employed for irradiation. The plane of the lamp was parallel to the tangential plane of the eye (at a right angle to the optical axis of the eye). The intensity of total dose of irradiation was measured with Cole-Parmer radiometer equipped with UVB probe (instruments manufactured by Cole-Palmer Inc., Vernon Hills, Illinois, USA). The total dose of irradiation was also checked using these devices. Although the used source of UVB emits only UVB rays with a peak at 312 nm (according to the irradiation spectrum given by the manufacturer), a UVC sensor (Cole-Parmer Inc.) was employed to detect any potential emission of UVC rays. After finishing the last irradiation (day 1, 2, 3 or 4), the animals were left without further irradiation for 24 h, then they were anesthetized by an i.m. injection of Rometar (Xylazinum hydrochloricum, 2 %, Spofa, 0.2 ml/kg b.w.) and Narkamon (Ketaminum hydrochloricum, 5 %, Spofa, 1 ml/kg b.w.) and the central thickness of the corneas in each experimental group was measured using an ultrasonic pachymeter SP-100. Subsequently, the animals were sacrificed under thiopental anesthesia (Thiopental, Spofa). Immediately after the death of the animals, the corneas were used for spectrophotometrical analyses. Normal non-irradiated corneas also analyzed spectrophotometrically served as controls.

The central corneal thickness of anesthetized rabbits in each experimental group was measured (using an Ultrasonic Pachymeter SP-100, Tomey Corporation, Nagoya, Japan) before the irradiation procedures and, for every irradiation interval, one day after the end of irradiation before sacrificing the animals.

Spectrophotometry of the whole corneas

Immediately after the sacrificing the animals the corneas were spectrophotometrically examined. The

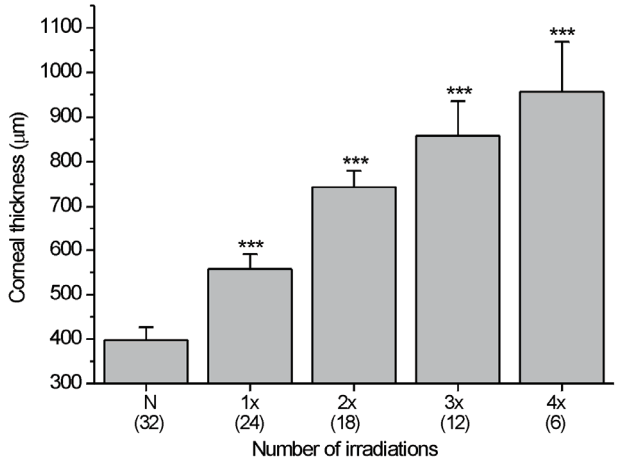

Fig. 1. Thickness of normal corneas and corneas irradiated with UVB rays (daily dose 1.01 J/cm^2) once daily for one, two, three or four days. The corneal thickness was measured by an ultrasonic pachymeter at the beginning of each experiment, before each irradiation, and before sacrificing the animals (the total number of measurements is indicated under each column). *** significantly different ($p<0.001$) when compared to normal corneas.

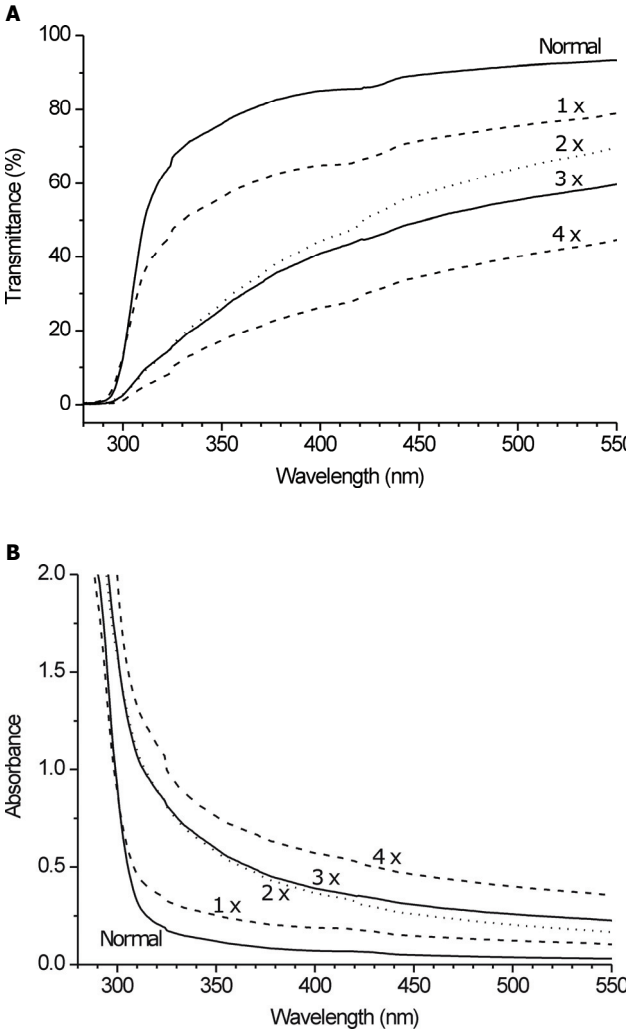

Fig. 2. Results of the spectrophotometry of the corneal centers, expressed either as the spectrum of transmittance T = T (λ) (**A**), or absorbance A = A (λ) (**B**) of normal corneas and corneas irradiated with UVB rays (1.01 J/cm^2) once daily for one (1x), two (2x), three (3x) or four (4x) days. The spectra are averaged from 16 traces for normal corneas and 6 traces for each group of irradiated ones. Significant differences of T and A, respectively, in selected wavelengths (312 nm, 320 nm, 550 nm) between normal corneas and corneas irradiated with UVB rays (1x, 2x, 3x, 4x) by one-way ANOVA with Dunnett´s *post-hoc* test were found with the exception of A in 312 nm. (Note that for wavelengths shorter than about 300 nm, the spectra show instrumental stray light error rather than the corneal optical properties).

corneas were excised from the sclera. From the center of each cornea a circle of 6 mm diameter was cut out using a special circular knife. Each of these circles was placed into a 6 mm diameter hole in a stainless steel sheet insert, covered on both sides with quartz glass, and the whole assembly was placed between two halves of an insert (made of acryl glass) designed to fit inside a standard quartz cuvette. The insert also contained a 6.0 mm hole that coincided with the measuring light beam of the spectrophotometer, and the instrumental light entered the measured piece of cornea from the epithelial side, i.e. from the same direction as light entered the cornea in situ.

The stainless steel sheets were manufactured in advance in the growing row of thicknesses (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3 mm). For the spectrophotometrical measurement, the insert with the same thickness as the thickness of the normal or irradiated cornea or the nearest higher sheet insert thickness was used.

Before the spectrophotometrical measurements, the corneas (with the whole assembly described above) were submersed into 340 mOsm/kg PBS in the measuring cuvette. Reference spectrophotometrical measurements (with metal sheets for normal and irradiated corneas) were conducted with the same assembly bathed in the same solution (only without the samples). The absorbance readings were made over a range of wavelengths 300-650 nm using a HELIOS b 84021 v4.55 scanning spectrophotometer (Spectronic Unicam, Cambridge, UK) with a wavelength resolution (step size) of 1 nm. The obtained data were expressed either as the spectrum of transmittance T = T (λ) or absorbance A = A (λ).

$$A = -\log T$$

For the absorbance the following formula is valid: $A = \alpha . d$, where α [mm^{-1}] is the coefficient of absorption, and d [mm] is the thickness of the light absorbing sample layer.

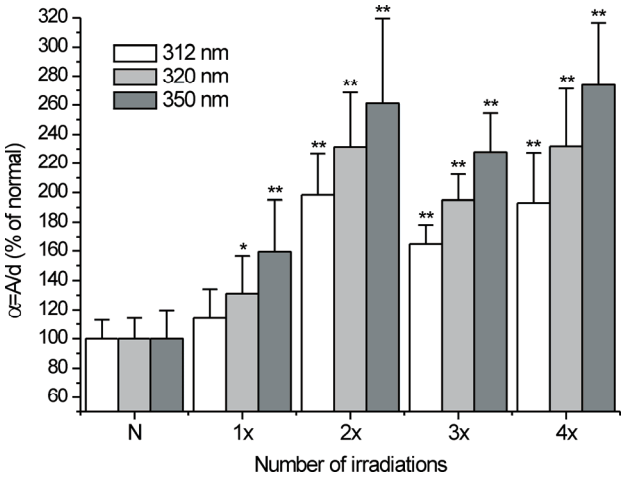

Fig. 3. Corneal optical properties of normal and UVB irradiated corneas shown as the extinction coefficient α=A/d. Corneal thickness was taken one day after the end of individual irradiations, before sacrificing the animals for spectrophotometrical examinations. The data were converted to % of the mean of normal values for each selected wavelength. n=16 for normal corneas, and n=6 for each group of irradiated ones. Statistically significant differences between the irradiated values and the corresponding normal values are marked with * ($p<0.05$) or ** ($p<0.01$).

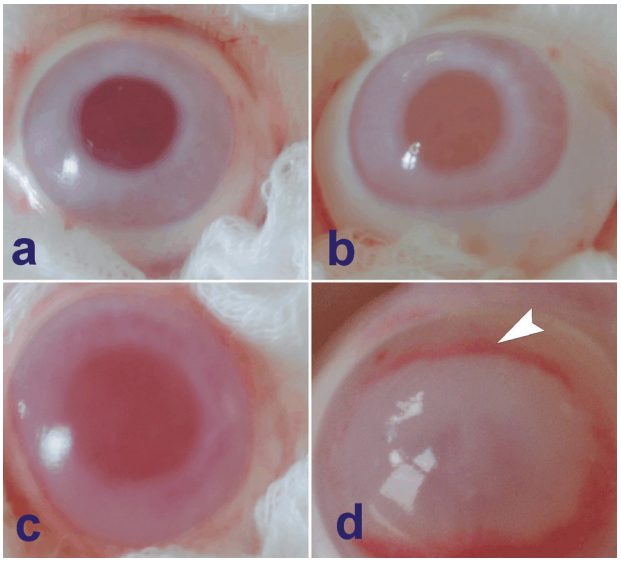

Fig. 4. Macroscopic picture of the normal rabbit cornea and rabbit corneas irradiated with UVB rays. Photographic images were done one day after the end of individual irradiations, immediately after sacrificing the animals. **a** – normal cornea; **b** – cornea irradiated once. No changes of corneal transparency can be seen; **c** – cornea irradiated two times. The corneal transparency is slightly changed (cornea becomes opalescent); **d** – cornea irradiated four times. The changes of corneal transparency are highly pronounced. Corneal neovascularization appears at the limbus (arrow).

Statistics

The data are generally presented as mean ± S.D. The Kruskal-Wallis test (non-parametric ANOVA) with the Dunn´s test was used for statistical evaluation of corneal thickness measurements, while for the absorption coefficient data, ANOVA followed by the Dunnett´s test were employed. Both tests were calculated using InStat ver. 3.06 (GraphPad Software, Inc., San Diego, CA).

Results

The corneas irradiated once did not show any macroscopical changes. From day two to day four of repeated irradiation, the corneas became opalescent (they gradually turned grayish). When the cornea was irradiated four times, corneal vascularization appeared at the limbus (Fig. 4). After one UVB dose, the corneal thickness increased (increased hydration), and continued to increase with every further irradiation (Fig. 1). The increase in corneal hydration was at the beginning linear but later (from day three to day four of irradiation) the increase of corneal hydration became slower. Simultaneously, the transparency of the corneas measured as tissue transmittance also decreased with every irradiation (Fig. 2A). When the spectrophotometrical data for selected wavelengths were converted to the absorption coefficients (α = A/d), it was evident that the observed changes were not uniform throughout the measured spectral range and that also they depended on the number of irradiations (Fig. 3). Changes in absorption coefficient α against normals (in selected wavelengths) (Fig. 3) were significant with the exception of the wavelength 312 nm after one irradiation. In this case the increase of absorbance A (decrease of transmittance, respectively) (Fig. 2A,B) was caused mainly by the increase in corneal hydration (significant against normal, Fig. 1). In addition, there was a qualitative difference in corneal absorption between single and repeated irradiations, manifesting as a flattening of the transmittance traces with the maximum changes centered around 320 nm (Figs 2A and 3).

Discussion

Our results show that the light absorption in the rabbit cornea irradiated with UVB rays increases soon after UVB irradiation, in parallel with an increase in corneal hydration (measured as central corneal thickness). Later (from day two to day four), with repeated irradiation, some other qualitative changes in the composition of the corneal tissue also appear that contribute to further changes in corneal transparency.

The broad increase in corneal light absorption seen even after one UVB irradiation can be a direct

consequence of increased corneal hydration. Corneal transparency is highly dependent on normal hydration of the cornea (Meek *et al*. 2003b). Light scattering appears along with corneal swelling. This scattering has been ascribed to the disruption caused by the arrangement of collagen fibres. Changes in the refractive index of the extracellular matrix make only a small contribution to the increase in light scattering when the cornea swells (Meek *et al*. 2003b). In the hydrated corneal stroma, fluid is not uniformly distributed. This is more pronounced in the posterior lamellae due to known differences in glycosaminoglycans between the anterior and posterior stroma (Meek *et al*. 2003a).

In our previous studies (Čejková *et al*. 2001, 2005, Čejka *et al*. 2007) as well as in papers of other authors (Podskochy 2004, Rogers *et al*. 2004) the damage of the corneal epithelium and a considerable reduction of epithelial thickness after UVB irradiation were reported. Podskochy *et al*. (2000) described that apoptosis appeared to be a mechanism of corneal cell death after UVB rays. According to Kennedy *et al.* (1997) acute UV irradiation exposure results in the induction of cornea-derived proinflammatory cytokines. Moreover, the irradiation of the cornea with UVB rays leads to the decrease of antioxidants (Downes *et al*. 1993, Čejková *et al*. 2000, Lodovici *et al*. 2003, Manzer *et al*. 2003, Tessem *et al*. 2005), whereas the generation of reactive oxygen and nitrogen species is significantly increased (Čejková *et al*. 2001, 2005).

Tessem *et al.* (2005) found that the repeated irradiation of the cornea with UVB rays for five days (daily dose 1.6 J/cm^2) caused significant disturbances of corneal metabolites. Čejka *et al*. (2007) found that repeated irradiation with UVB rays for five days (daily dose 1.01 J/cm^2) evoked greatly increased corneal light absorption throughout the whole measurable spectral range (particularly in the UVB region). In this paper it was reported that the cornea absorbed more light already after one daily dose of UVB rays (1.01 J/cm^2). Examination of the absorption coefficient α at various wavelengths suggests that the increase in corneal light absorption is initially mainly due to an increase in corneal hydration and later – with further irradiation – also due to changes in corneal composition that further increase corneal light absorption (decrease corneal transparency) at certain wavelengths. These latter changes will be the subject of our next study.

Zigman (1995) described a dose of 0.105 J/cm^2 of UVB rays during 1 hour exposure of the human cornea to sunlight. When we compare our daily UVB dose of 1.01 J/cm^2 with the dose calculated for solar UVB radiation reaching the human cornea, our dose is equivalent to 10 hours exposure time in sunlight (the approximate summer daily exposure). Our results show that this dose evoked a significant increase in corneal hydration and light absorption.

In conclusion, the repeated irradiation of the cornea with UVB rays leads to the profound changes of its optical properties.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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