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Maximum tolerable dose for avoidance of cataract after repeated exposure to ultraviolet radiation in rats

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Abstract

The purpose of the present study was to determine the impact of inter-exposure interval between repeated equivalent exposures of ultraviolet radiation (UVR) on threshold accumulated dose for cataract development. Female Sprague–Dawley rats were randomly divided into 5 inter-exposure interval groups with 20 rats in each group. The inter-exposure intervals were 6 h, 1, 3, 9 and 30 days respectively. Each inter-exposure interval group was divided into 5 dose-subgroups. Only one eye of each rat was exposed to ultraviolet radiation ($\lambda_{max} = 300$ nm). The total dose incident on the cornea, in each subgroup varied between $0 \sim 10$ kJ/m^2. One week after the second exposure, the rats were sacrificed and both lenses were extracted. The intensity of forward light scattering was measured and macroscopic morphology was documented. Maximum tolerable dose (MTD) for each inter-exposure interval was estimated based on the experimentally determined dose-response function. The difference of intensity of light scattering between exposed and contralateral non-exposed lens decreased as a function of inter-exposure interval between the two equivalent exposures. The accumulated $MTD_{2.3:16}$ was 5.3, 5.1, 5.4, 5.8, and 6.0 kJ/m^2 UVR-B for the 6 h, 1, 3, 9 and 30 day inter-exposure interval between the two exposures, respectively. The shorter the inter-exposure interval between two subsequent exposures, the more damage. The time constant for repair of lens damage after in vivo exposure to close to threshold dose was estimated to be eight days and the fraction of repairable damage to be 20%. The accumulated threshold dose for damage after two repeated equivalent exposures to UVR-B increases as a function of inter-exposure interval up to at least 30 days inter-exposure interval.

Keywords: ultraviolet radiation (UVR); cataract; rat; light scattering; dose-response function; maximum tolerable dose (MTD)

1. Introduction

The current study was undertaken to elucidate the effect of inter-exposure interval between repeated equivalent exposures of ultraviolet radiation (UVR) on threshold accumulated dose for cataract development.

UVR is electromagnetic radiation in the wavelength range between X-rays and visible light. UVR is separated into four wavebands; far UVR (1–100 nm), UVR-C (100–280 nm), UVR-B (280–315 nm) and UVR-A (315–400 nm). Natural radiation from the sun remains the most common source of UVR. The sunlight humans receive is filtered by the atmosphere and consists of UVR (UVR-A and UVR-B), visible light, and infrared radiation. UVR from the sun that reaches the surface of the earths is largely composed of UVR-A and a small component of UVR-B (Pitts and Kleinstein, 1993).

The spectral sensitivity of the lens for acute cataract development after a high dose of UVR has been elucidated with qualitative (Pitts et al., 1977) and quantitative (Merriam et al., 2000) evaluation of lens damage. Based on these studies, an action spectrum has been developed for acute cataract development after exposure to a high dose of UVR (Sliney et al., 2004). The action spectrum shows that UVR-B around 300 nm is the most toxic waveband for the lens (Merriam et al., 2000; Pitts et al., 1977). An action spectrum for *in vitro* UVR-induced cataract using

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whole porcine lenses was established (Oriowo et al., 2001). The threshold dose, estimated as ED_{50} was at 300 nm (UVR-B) 0.069 J/cm^2 and at 365 nm (UVR-A) 137.19 J/cm^2. Thus, the threshold dose for UVR-B was about 2000 times lower than that for UVR-A.

However, it may be premature to exclude UVR-A or even visible light in the etiology of human cortical cataract (Dillon et al., 1999). A recently published study showed that a 4–5 month exposure of guinea pigs to a biologically relevant level of UVR-A induces deleterious effects in the center of the lens (Giblin et al., 2002). A comparison of the effect of UVR-A and UVR-B in porcine lenses in vitro demonstrated that a high dose of UVR-A and a combination of a low dose of UVR-A and a low dose of UVR-B, respectively, impairs lens cell anatomy and the optical properties of the lens (Oriowo et al., 2002).

On the assumption of strict wave band additivity, the action spectrum allows estimation of the acute effect of any broadband source on the lens, e.g. the sun. No action spectrum is known for chronic exposures. The lack of an action spectrum for chronic exposure renders uncertain estimates of risk for cataract after chronic exposures to a broadband source (Sliney, 2002a).

Cataract related to UVR-B exposure is associated with photo oxidation of proteins, lipids and nucleic acids (Söderberg, 1990a). UVR initially causes DNA damage in the lens epithelium (Reddy et al., 1998b; Söderberg et al., 1986) and inactivates a number of metabolic enzymes (Anwar, 2001; Reddy and Bhat, 1998a). There is also a high concentration of anti-oxidative molecules and repair enzymes in the lens. These enzymes will repair proteins damaged by oxidation. Thioltransferase demonstrates a remarkable resistance to oxidation (Lou, 2003).

When acute cataract development was monitored after exposure to UVR-B in the 300 nm wavelength region, it was demonstrated that the sensitivity of the lens to UVR-B also depends on age (Dong et al., 2003, 2005a; Löfgren et al., 2003), exposure time, and the time interval between two repeated UVR-B exposures (Ayala et al., 2000; Michael et al., 1999).

UVR-induced cataract has a continuous dose-response relationship (Michael et al., 1996; Söderberg, 1990b). The in vivo threshold for UVR-induced cataract was established for rabbit (Pitts et al., 1977) and rat (Söderberg et al., 2003; Söderberg et al., 2002). The current safety limits for avoidance of UVR-induced cataract in humans (Sliney et al., 2004) are based on the experimental action spectrum (Merriam et al., 2000; Pitts et al., 1977), analysis of health risk (Sliney, 2002b) and clinical experience.

Maximum Tolerable Dose (MTD) (Dong et al., 2005a; Söderberg et al., 2002) is a threshold estimate considering the continuous dose-response function for UVR-induced cataract (Michael et al., 1996). After an exposure to $MTD_{2.3:16}$, there is a 16% probability that the exposed lens will express more forward light scattering than is found in less than 2.3% of a normal population, the tolerance limit (Dong et al., 2005a; Söderberg et al., 2002).

The purpose of the current study was to establish the dependence of $MTD_{2.3:16}$ on the interval between two equivalent exposures to UVR-300 nm.

2. Methods

2.1. Experimental animal

The albino Sprague–Dawley rat (M & B A/S, Denmark) was the experimental animal. The animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Ethical approval was obtained from the Northern Stockholm Animal Experiments Ethics Committee. The rats were fed with Standardkost rodent diet from BK-universal (Scanbur BK, Sweden). Four rats were housed to a cage, and the light/dark cycle was set to 12 h/12 h.

2.2. UVR-B source

The radiation from a 350 W high-pressure mercury lamp (Oriel 6286, LOT-oriel, Darmstadt, Germany) was collimated, passed through a water filter, and then a double monochromator set at 300 nm. The resulting spectral output was measured with a spectrometer (Ocean Optics PC 2000, Ocean Optics, Dunedin, Florida, USA). The output peaked at 300 nm and had a 9 nm full width at half maximum (Fig. 1).

Irradiance was measured with a thermopile (7104, Oriel Instruments, Stratford, Connecticut, USA) in the corneal plane. The thermopile had been calibrated to a NIST (National Institute of Standards and Technology) traceable source by the Swedish National Bureau of Standards.

2.3. Light scattering measurement

The intensity of forward light scattering was measured with a Light Dissemination Meter developed by Söderberg et al. (1990). The light scattered forward from the lens is collected by the optics of a camera equipped with a photodiode in the film plane.

The readings were calibrated with a standard lipid emulsion of the drug diazepam (Stesolid Novum, Alpharma AB, Stockholm, Sweden) and the unit of intensity of forward light

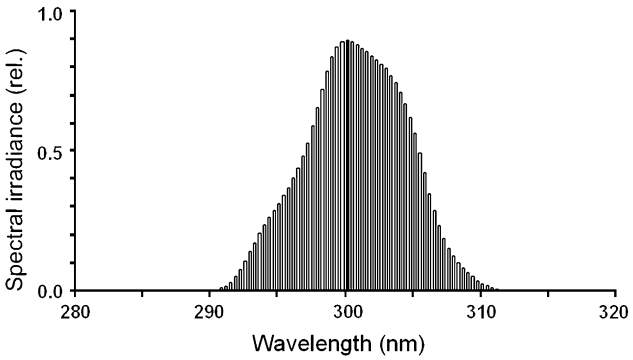

Fig. 1. Spectral distribution of the radiation.

scattering was expressed as transformed Equivalent Diazepam Concentration (tEDC) (Söderberg et al., 1990). The technique allows detection of less than 1% change in a light scattering standard.

2.4. Animal procedure

Ten minutes preceding the UVR-B exposure, the animal was anesthetized with a mixture of 95 mg/kg ketamine and 14 mg/kg xylazine, injected intraperitoneally. Five minutes after the injection, the mydriatic tropicamide was instilled in both eyes and both eyes were checked with a slit lamp microscope. After another five minutes, one eye of each animal was exposed to a narrow beam of UVR-B covering only the cornea and the eyelids with specific doses set on the corneal plane. The exposure time was 15 min. After 6 h, 1, 3, 9 or 30 days, the same dose of UVR-B was repeated with the same procedure.

One week after the second exposure, the rat was sacrificed by carbon dioxide asphyxiation. The eyes were enucleated and both lenses were extracted and placed in balanced salt solution (BSS, Alcon, USA). Remnants of the ciliary body were removed from the lens equator. The light scattering in both the exposed and the non-exposed lens was measured and dark-field photographs were taken of each lens.

2.5. Experimental design

A total of 101 female, 6-week old outbred Sprague–Dawley (SD) rats were included in the experiment. One rat died during the anesthesia. The rats were randomly divided into five interval-groups (6 h, 1, 3, 9 and 30 days) with 20 rats each (Fig. 2).

The rats in each inter-exposure interval group were further randomly allocated to one of five dose-subgroups.

The UVR doses were selected in order to optimize the regression by splitting the total dose interval squared into 4 similar equidistant doses. The square root of the equidistant doses was then used for the experiment (Eq. (1)).

$$H_{e:g} = \sqrt{(g-1)\frac{[E(\mathrm{MTD})]^2}{2}} \quad (1)$$

Here, $H_{e:g}$, is the subgroup dose, for the g:th subgroup ($g = 1 \ldots 5$) and E(MTD), is the expected MTD. The expected MTD was set to 3.5 kJ/m^2 based on a previous experiment (Söderberg et al., 2002). Thus, the subgroup doses were 0, 2, 3, 4, and 5 kJ/m^2, for each interval group. The total dose received after two repeated exposures were 0 (2×0), 4 (2×2), 6 (2×3), 8 (2×4), and 10 (2×5) kJ/m^2, respectively.

3. Results

3.1. Macroscopic appearance

Under dark-field illumination, more details of lens changes related to UVR exposure were observed than under incident illumination against a grid (Fig. 3).

In non-exposed lenses, it was possible to identify the anterior and posterior capsule and the equator under dark-field illumination (Fig. 3A). The grid under the lens was clear in incident illumination (Fig. 3B). After exposure to 2×2 kJ/m^2 UVR-B, 30 day inter-exposure interval, lenses showed vacuoles and opacities on the equator (Fig. 3C) and the anterior surface was rough and appeared corrugated in incident illumination against a grid (Fig. 3D).

Considering exposure to 2×4 kJ/m^2, the 9 and 30 day inter-exposure interval groups developed less opacities than the shorter inter-exposure interval groups (Fig. 4).

After 2×4 kJ/m^2 UVR-B exposure, all exposed lenses in all inter-exposure interval groups developed macroscopically visible opacities. Severe equatorial cataract developed in the 6 h, and 1 and 3 day inter-exposure interval groups. Mild subcapsular and equatorial opacities developed in the 9 and 30 day inter-exposure interval groups (Fig. 4).

3.2. Intensity of forward light scattering and MTD

The intensity of forward light scattering in all inter-exposure interval groups increased with increasing dose of UVR-B (Fig. 5).

The difference of intensity of forward light scattering between exposed and contralateral non-exposed lens decreased with increasing inter-exposure interval (Fig. 6).

The $\mathrm{MTD}_{2.3:16}$ was calculated according to Eq. (2) (Dong et al., 2005a)

$$\mathrm{MTD}_{2.3:16} = \sqrt{\frac{\sigma}{k}} \quad (2)$$

and was estimated to be 5.3, 5.1, 5.4, 5.8, and 6.0 kJ/m^2 total dose accumulated during two equivalent exposures for the 6 h,

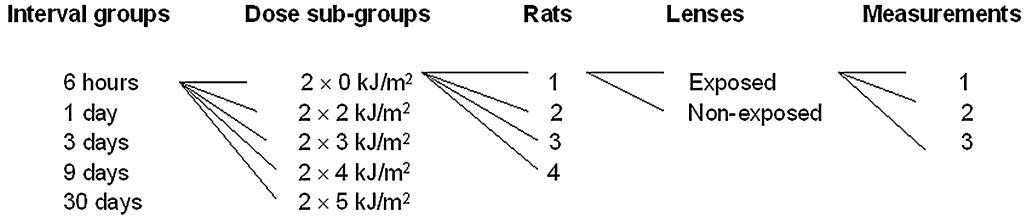

Fig. 2. Experimental design.

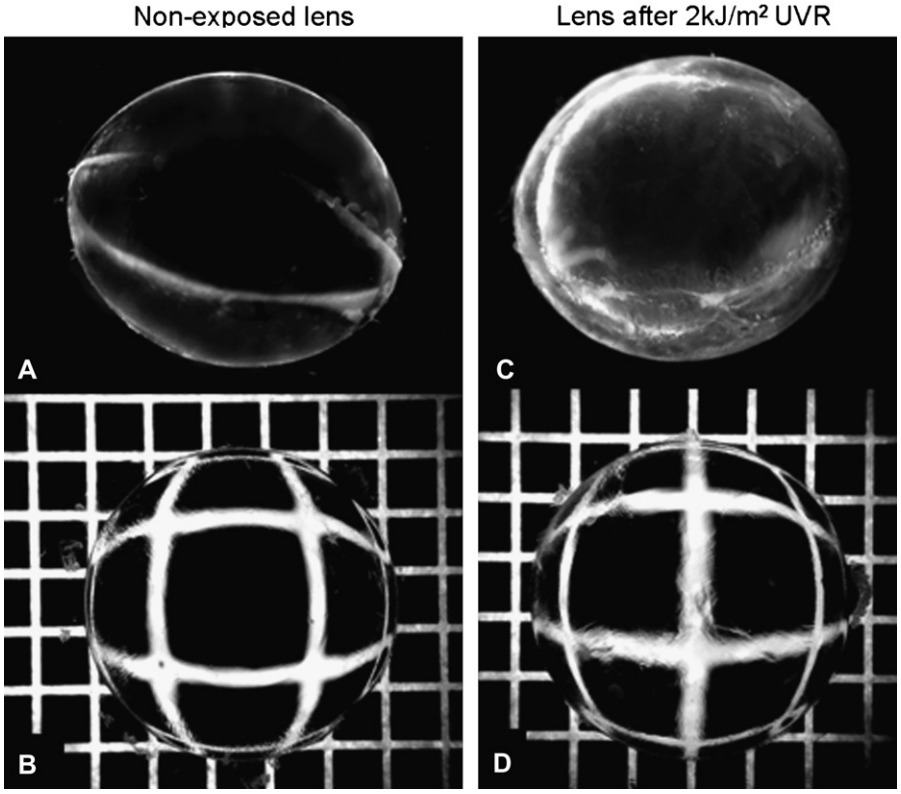

Fig. 3. Non-exposed (A and B) and exposed lens (C and D) after 2×2 kJ/m^2 UVR-B exposure with 30 days inter-exposure interval. Upper images: Dark-field illumination. Lower images: Incident illumination against a grid.

1, 3, 9 and 30 day-inter-exposure interval groups, respectively (Fig. 7).

These data indicate that the threshold dose increases with a declining increase as a function of inter-exposure interval. Fitting the data to first order kinetics (Eq. (3))

$$\mathrm{MTD}_{2.3:16} = 5.14 + 0.90\left(1 - \mathrm{e}^{-0.13*\mathrm{IntExpInt}}\right) \quad (3)$$

provides parameter estimates for the threshold dose accumulated during two single exposures, $\mathrm{MTD}_{2.3:16} = 5.14$ kJ/m^2, when the inter-exposure interval (IntExpInt) is 0. The rate constant for the increase (0.13) expresses the repair rate. The time constant, 0.13 days^{-1}, for repair at close to threshold dose, is 8 days.

In addition to the increased light scattering in the lens, dose-dependent corneal damage developed. After the higher doses (2×4 and 2×5 kJ/m^2), most rats developed corneal haze and edema at 1 to 3 days after the first UVR exposure. Nine and thirty days after the first 4 or 5 kJ/m^2 UVR exposure, the corneal edema and haze disappeared. After the high dose exposures, corneal opacity and neovascularization were occasionally observed 9 or 30 days post exposure (Fig. 8).

A slight hyphema and uveitis were also observed at one week after the second exposure in the eyes that had been exposed to high accumulated dose.

4. Discussion

In the present study, the dependence of the threshold dose, accumulated during two equivalent exposures, on the time interval between the exposures was elucidated.

4.1. Methodology

For adequate protection against toxic effects of UVR to the lens, it is necessary to understand the mechanisms involved in lens damage after exposure to UVR. Further, the exposure limits for avoidance of toxic effects must be known. In vitro systems may be used to study the mechanisms involved in damage.

Several human lens epithelial (HLE) cell lines (HLE B-3 and HLE B-4) are now available. However, the properties of HLE cells in culture change during growth. It was recently shown that the transcripts for α-crystalline decreased markedly at higher passages (Fleming et al., 1998; Wang et al., 2003). Further, in vivo the lens is subjected to humoral factors from surrounding tissues and interaction between cells at varying differentiation within the lens. Therefore, in vitro findings with a lens in organ culture (Zigler et al., 2003; Tumminia et al., 1994) or HLE cell lines should be cautiously interpreted to the in vivo situation. Relevant exposure limits thus can only be obtained with in vivo exposures.

In the current study, we decided to study the albino rat because we have previous experience of the toxic reaction in the

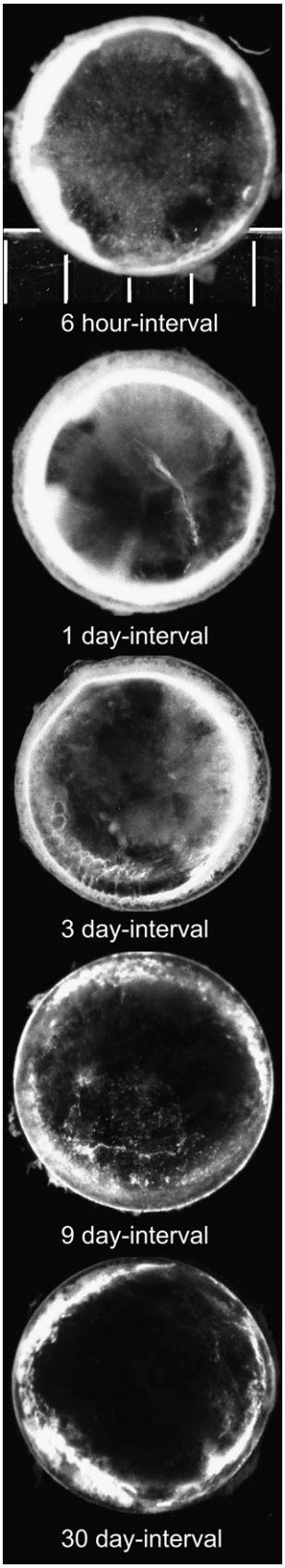

Fig. 4. Lenses after exposure to 2×4 kJ/m^2 UVR-B from different inter-exposure interval groups. The scale of the ruler is 1 mm.

albino rat after repeated in vivo exposure to UVR-B (Ayala et al., 2000). In order to provide a more general interpretation of the present findings, similar experiments should be repeated on other species. It has been shown that the pigmented rat is more resistant to UVR exposure than the albino rat (Löfgren et al., 2003). The morphological differences of the lens fibers between albino and pigmented rats may be one of the reasons. Under scanning electron microscopy, more ball-and socket junctions were found on the lateral surfaces of lens fibers in pigmented rats than in albino rats. Notable differences in shape and size of ball-and socket junctions were also observed. The role of ball-and socket junctions in calcium homeostasis in the lens may account for differences in cataractogenesis between pigmented and albino rats (Yamada et al., 2002).

We selected the dose range in the current experiment with the intention to be able to determine the threshold for damage. We found in a preliminary experiment that lenses were severely damaged after 2×8 (16 kJ/m^2) and 2×10 (20 kJ/m^2) with a 30 day inter-exposure interval. This is consistent with previous findings that severe lens damage developed after a single dose of 20 kJ/m^2 in vivo UVR exposure (Michael et al., 1996). We therefore limited the dose range in the current experiment to 2×5 kJ/m^2. The inter-exposure interval range for the current experiment was selected in order to be able to separate two equivalent exposures up to 30 days. It was assumed that after 30 days, there should be little biological memory of the first exposure.

UVR-induced cataract has a continuous dose-response function (Michael et al., 1998a). Therefore, the MTD strategy (Söderberg et al., 2002) was used for threshold dose estimation.

4.2. Implications of the results

The continuous dose response functions presently observed (Fig. 5) were consistent with the previous observation of a continuous dose response function for UVR-induced cataract in the albino rat (Michael et al., 1998a). Similarly, when plotting the intensity of forward light scattering induced for each inter-exposure interval, the continuous dose response relationship for UVR-induced cataract is observed (Fig. 6). The fact that the intensity of induced forward light scattering decreased with increasing inter-exposure interval for all doses (Fig. 6) indicates that repair mechanisms have been triggered.

There seem to be a slightly lower threshold for damage around 1 day inter-exposure interval than for other inter-exposure intervals (Fig. 7). This differs from our previous findings when studying toxicity of UVR (Ayala et al., 2000). In that study, we found that there was a maximum toxicity expressed when the inter-exposure interval was on the order of 72 h. A difference between the two studies is that in the current study, we measured the lowest dose that induced a measurable increase of forward light scattering. In the previous study (Ayala et al., 2000), we measured the toxic effect, as the intensity of forward light scattering induced by an approximately double threshold dose. In addition to methodological differences,

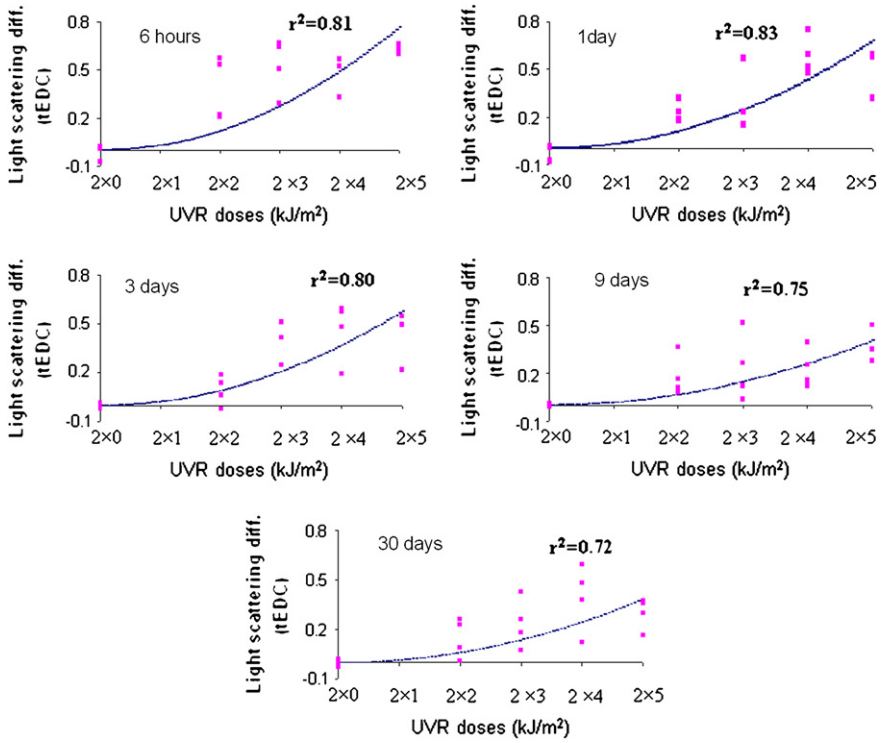

Fig. 5. Dose-response functions for the cumulated dose of two equivalent exposures to ultraviolet radiation with an inter-exposure interval between the exposures of 6 h, 1, 3, 9 or 30 days.

the UVR doses were different in two studies: UVR-induced lens epithelial cell apoptosis is dose-dependent. This may be one reason that we currently found the lowest threshold dose in the one day inter-exposure interval group. However, in the previous study the highest light scattering was in the 72 h inter-exposure interval group.

In the current study, repair after the first exposure modifies the threshold after the second exposure. Therefore, the threshold after the second exposure will be a function of the repair after the first exposure and the damage induced by the second exposure (Fig. 7). Then, the increase of threshold from 0 inter-exposure interval to infinite inter-exposure interval divided by the threshold at 0 inter-exposure interval expresses the fraction of repairable damage (Eq. (4)).

$$\mathrm{Rd} = \frac{0.90(1 - e^{-0.13 * \mathrm{IntExpInt}})}{5.14} \quad (4)$$

Here, the fraction of repairable damage, Rd, was estimated to 0.18.

The peak toxicity of UVR seen at an inter-exposure interval of approximately 72 h after well above threshold dose (Ayala et al., 2000) indicates that at well above threshold dose, the second dose damages the repair mechanism and that the repair mechanism is most sensitive after approximately 72 h. Alternatively, photosensitization such as reactive oxygen species induced by UVR may enhance the lens response to the second exposure well above threshold exposure.

There seem to be a slightly lower threshold for damage around one day inter-exposure interval (Fig. 7). This could be due to a temporarily increased sensitivity in the lens. That would be consistent with the previous finding that programmed cell death induced by a single 5 kJ/m^2 UVR exposure peaked at 24 h after exposure (Michael et al., 1998b). The replacement of dead cells would demand a higher rate of mitosis in the germinative zone, and secondly UVR exposure at this time may have a more damaging effect.

There could however be other UVR sensitive repair systems involved. There are high concentrations of anti-oxidative molecules and repair enzymes in the lens. The increased threshold doses with the increasing interval between two exposures (Fig. 7) indicate that there is a continuous repair even after 30 days.

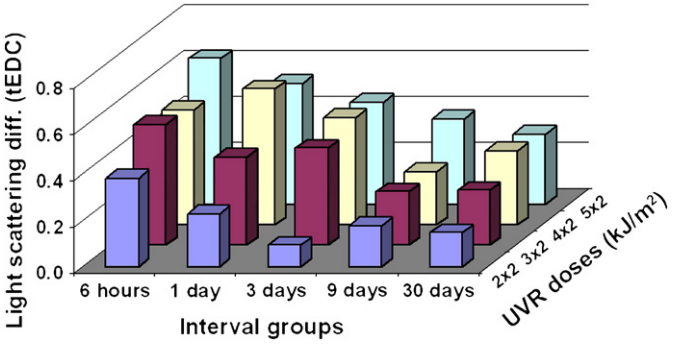

Fig. 6. Light scattering differences between exposed and contralateral non-exposed eye after two equivalent exposures. The light scattering was measured 7 days after the second UVR-B exposure for all inter-exposure intervals. *n* for each column = 4.

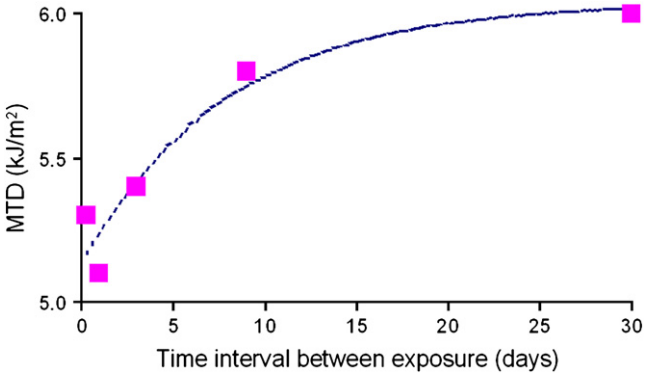

Fig. 7. $MTD_{2.3:16}$ for 6 h, 1, 3, 9 and 30 day inter-exposure interval of two equivalent exposures. MTD plotted is the sum of two repeated doses.

It has been shown that α-tocopherol (vitamin E) protects the rat lens against cataract from in vivo exposure to UVR-B (Ayala and Söderberg, 2004) directly as an antioxidant and/or indirectly by increasing glutathione (GSH) concentration (Ayala and Söderberg, 2004). Heat Shock Proteins (HSPs) may play a role as an antioxdant in lens. HSPs increase in abundance in cells subjected to thermal as well as oxidative stress (Ganea, 2001). Individual members of the extended HSPs are usually identified by reference to their molecular mass (eg, HSP 90, HAS 47). HSP-90 α expression peaks shortly before the beginning of lens apoptosis, suggesting a close relationship with lens apoptosis (Hooven et al., 2004). HSP 47 is expressed selectively within the endoplasmic reticulum of cells that synthesize and secrete type I and type III collagen (Williams, 2000). However, the relationship between HSP 47 and photochemical damage induced by UVR in lens epithelium needs to be elucidated. Unscheduled DNA synthesis have been observed after UVR exposure and is believed to be excision repair of DNA damage induced by UVR (Söderberg et al., 1986).

Michael et al. (2000) found that 56 days post-exposure, the epithelial layer appeared normal, and the extra cellular spaces had normalized. But abnormal fibers were found between the 60th and 100th growth shell below the capsule. The subtle damage to the lens fibers induced by UVR may accumulate during lifetime and contribute to the formation of age-related cataract. The balance between lens damage and repair play a critical role in cataract development.

MTD in the corneal plane *in vivo* can be used as an indicator of threshold dose in the lens *in vitro*. In the present study, the average MTD was approximately 5.5 kJ/m^2 in the corneal plane (Fig. 7). The corneal transmittance at 300 nm is 32% in the rat (Dillon et al., 1999) and the aqueous transmittance is 98% in the rat (Dong et al., 2005b). Then, the lenticular MTD in vitro is approximately 1.7 kJ/m^2 for the rat (5.5 $kJ/m^2 \times 0.32 \times 0.98 = 1.7\ kJ/m^2$). This lenticular threshold dose is similar to Oriowo et al.'s observation (Oriowo et al.,

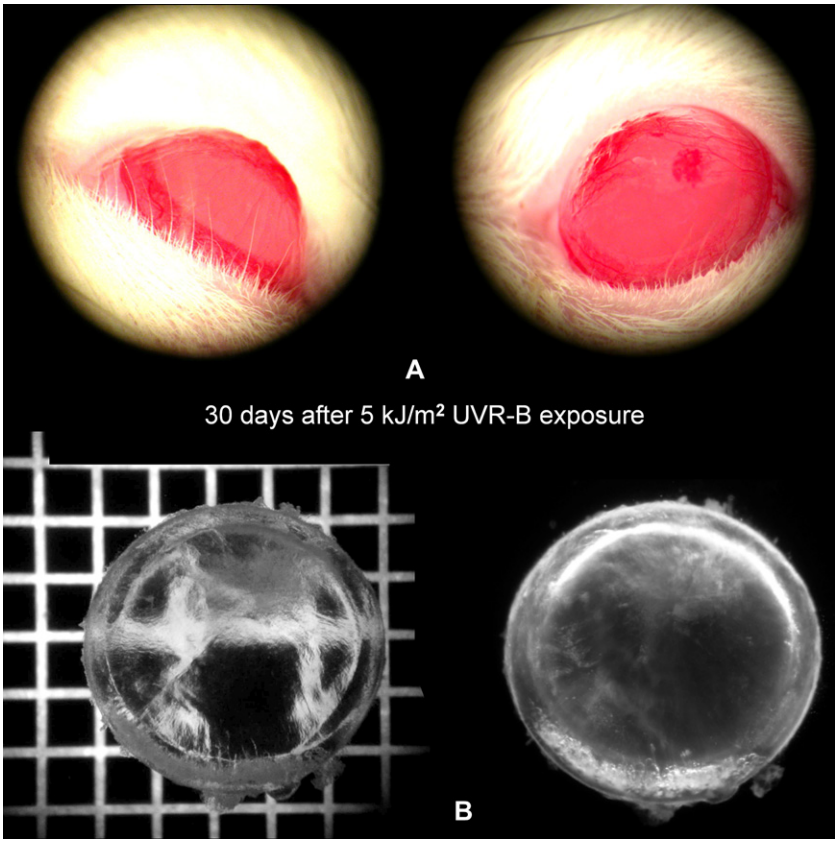

Fig. 8. Corneal and lens changes. A. Corneal neovascularization formed at 30 days after 5 kJ/m^2 UVR exposure. B. Cataract that developed seven days after the second 5 kJ/m^2 UVR exposure.

2001). They found that the threshold dose of UVR-B in vitro at 300 nm for permanent cataract was 1.4 kJ/m^2 for porcine lenses.

However, different experiments must be cautiously compared. Pitts et al. (1977) estimated threshold qualitatively based on a binary dose response model. It was however later demonstrated that the dose-response model for UVR induced cataract is continuous (Michael et al., 1998a). Oriowo et al. (2001) used a qualitative measure of cataract and a binary dose-response model. They, then applied the ED_{50} strategy for threshold dose estimation. Applying a continuous dose response model and a quantitative measure of cataract, it was demonstrated with the similar criterion for threshold, MTD (Söderberg et al., 2002) that the MTD varies among species such as mouse, rat, and guinea pig (Söderberg et al., 2006). It is also known that threshold varies with exposure time (Dong et al., 2005b), and animal age (Dong et al., 2003, 2005a).

Wahlman et al. (2003) found photo oxidative damage of hypericin in cultured bovine lenses after in vitro exposure to 0.2 J/cm^2 (0.2 J/cm^2 = 2 kJ/m^2) UVR-B. Hypericin absorbs UVR and binds to lens crystallins. The spectral distribution of the source used by Wahlman et al. (2003) was not given. Wahlman et al. (2003) do not provide information on how the detector was calibrated, and the spectral response of the detector is not given. Lacking this information, a direct comparison between the dose used by Wahlman et al. (2003) and the dose used in the current paper is difficult.

It may be argued that the corneal edema and haze observed 1 to 3 days after the first exposure to UVR may have hindered the second exposure to UVR. However, the fact that the highest UVR doses, for all inter-exposure intervals, evoked the most intense forward light scattering in the lens, at least indicates that the hindering was of little significance. Furthermore, comparing the groups that were exposed to the same dose of UVR, more severe corneal damage was observed in groups that received the second exposure at 6 h, one and three days after the first exposure, than in the groups that received the second exposure 9 or 30 days after the first exposure. Despite this, the intensity of forward light scattering was always higher for the short inter-exposure intervals. This finding was consistent with previous observations (Ayala et al., 2000; Michael et al., 1999).

The fact that we observed an inflammatory reaction in the anterior part of the eye one week after a high accumulated dose indicates that, in addition to a pure UVR effect on the lens, there may be an effect secondary to the inflammatory response in the eye. However, UVR-induced uveitis classically is not considered to cause cataract (Pitts and Kleinstein, 1993).

The current animal experimental data could not be directly extrapolated to humans. The human eye has a thicker cornea and a deeper anterior chamber than the rat. Therefore, the same corneal dose becomes a lower dose on the lens surface in the human eye than in the rat eye. Furthermore, a different content of antioxidant molecules such as glutathione (GSH), ascorbic acid (vit C), and α-tocopherol (vit E) may further modulate the response. However, the current safety limit for avoidance of UVR-induced cataract (Sliney et al., 2004) is based on the data from animal experiments (Pitts et al., 1977), epidemiological investigations and continuous evaluation of feedback information about the validity of current safety standards in field applications.

The current results demonstrate that the interval between two repeated exposures influences the lens threshold to UVR-B exposure. The amount of repair in the lens increases with time after the first exposure. To our knowledge, this is the first quantitative estimate of; the threshold dose for repeated UVR-B induced lens damage, the repair rate, and the fraction of repairable damage, in the lens after in vivo exposure to UVR. The current strategy for estimation of repairable damage provides a tool to study the impact of various antioxidative systems on the repair in the lens, in vivo.

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