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UVR-B induced cataract development in C57 mice

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Abstract

The evolution of the morphological appearance and intensity of light scattering in C57 mice lenses after exposure to ultraviolet radiation type B (UVR-B) was investigated. A total of 80, 6-week-old female C57BL/6 mice were divided into four groups ($n=20$). One eye in each animal was exposed in vivo to UVR-B in the 300 nm wavelength region (UVR-B-300 nm) to a dose of 5 kJ m^{-2} for 15 min. The radiation output had λ_{max} at 302 nm with 5 nm [FWHM]. The animals were consecutively sacrificed at 1, 2, 4 and 8 days after the exposure. Macroscopic lens changes were documented using grid- and dark field illumination photography. Light scattering in the exposed and contralateral not exposed lens was measured quantitatively. Morphological lens changes were documented using grid- and dark field illumination photography. In vivo exposure to UVR-B-300 nm induced subcapsular cataract in all exposed lenses and occasionally cortical and nuclear cataract at all investigated time points. Exposed lenses scattered light significantly higher on all investigated days compared to contralateral non-exposed lenses. A transient increase of light scattering peaking at day 2 in exposed as well as in contralateral not exposed lenses was identified. Light scattering of the lenses varies with latency time after exposure. A dose of 5 kJ m^{-2} UVR-B-300 nm induces light scattering in C57 mice lenses. The increase has a transient peak at 2 days after exposure. The variation of light scattering among days 1, 2, 4, and 8 indicates a dynamic change of scattering characteristics in the mouse lens following unilateral in vivo exposure to 5 kJ m^{-2} UVR-B-300 nm.

Keywords: cataract; UVR-B; light scattering; C57BL/6 mouse

1. Introduction

In the present study, the effect of in vivo exposure of the eye to ultraviolet radiation (UVR) with the spectral band B (UVR-B) on the evolution of cataract and light scattering in C57 mice lenses was investigated.

The cataractogenetic effect of UVR was established already at the end of the last century (Widmark, 1901). Several experimental and epidemiological investigations have since then shown a correlation between cataract development and exposure to UVR (Pitts et al., 1977; Jose and Pitts, 1985; Söderberg, 1988; Taylor, 1989; Cruickshanks et al., 1992; West et al., 1998). These epidemiological studies have consistently demonstrated an association between exposure to UVR and cortical cataract. The recent case-control study by Neale et al. (2003) from Australia presents evidence that also supports an association between exposure to the sun and nuclear cataract, especially when abundant exposure occurs in young age. Cataract has been acknowledged by the WHO as the leading cause of blindness in the world (Dawson and Schwab, 1981; Leske and Sperduto, 1983; Memoranda WHO, 1991; Seidman-Ripley and Huang, 1993). Approximately half of the world's 40–45 million blind people are blind from cataracts (Brian and Taylor, 2001). Cataract surgery is the most common surgical intervention in the developed part of the world (Hartmann and Anders, 1997). Cataract thus has a major impact on the world economy and existing health care insurance systems. It is therefore urging to define scientifically based preventive measures.

The effect of genetic modulation on the sensitivity of the lens to UVR-B exposure is currently unknown. The C57 mouse is a suitable model to study significance of genetic modulation of phenotype since mouse chromosomes

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represent the most thoroughly studied of all non-human genomes. Although chromosomal linkage is observed among several mammals, the human and the laboratory mouse genomes are by far the most extensively characterized and compared (Carver and Stubbs, 1997).

The current project aimed at providing basic information about in vivo UVR-B induced cataract development in the C57 mouse. This information is crucial to make it possible to study the effects of mutations, and lack of genes in knockout mice, on UVR-B sensitivity in the future.

2. Material and methods

C57 mice were unilaterally exposed to UVR-B. The intensity of forward light scattering in the lens was measured at various post-exposure intervals.

2.1. Experimental animal

The experimental animal was the 6-week-old female C57BL/6 mouse. The animals were kept and treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The use of animals for this experiment was approved by Stockholm's Norra Djurförsöksetiska nämnd, protocol: N227/03.

2.2. Experimental devices

UVR-B in the 300 nm wavelength region (UVR-B-300 nm) was generated with a high-pressure mercury lamp. The emerging radiation was collimated, passed through a water filter and a double monochromator ($\lambda_{max} = 303$ nm with 5 nm [FWHM]), and finally projected in a narrow beam on the cornea of the exposed eye (Söderberg, 1990a,b; Michael et al., 1996). The diameter of the field on the cornea was 6 mm. The contralateral eye was shielded with a black paper shield during exposure. The intensity of UVR was measured with a thermopile calibrated to a National Institute of Standard (NIST) traceable source.

The intensity of forward light scattering was measured with a light dissemination meter (Söderberg et al., 1990). This instrument uses the principal of dark field illumination. The illuminating light transilluminates a transparent object (e.g. a mouse lens) at 45° against the horizontal plane. At this angle, the light cannot enter the objective aperture. If the object scatters light in the forward direction, a defined fraction of light reaches the objective and is measured by a photodiode.

The scattering standard was a lipid emulsion of Diazepam (Stesolid Novum, Dumex-Alphapharma, Denmark). Light scattering was therefore expressed in transformed equivalent Diazepam concentration [tEDC] (Söderberg et al., 1990).

2.3. Experimental procedure

One eye of each mouse was exposed in vivo to UVR.

All animals were checked with a slit lamp prior to UVR exposure to exclude congenital cataract. The animal was unilaterally exposed for 15 min to 5 kJ m^{-2} UVR-B. The unexposed eye was kept as a control. Ten minutes preceding the exposure, the animal was anaesthetized with a mixture of 40 mg kg^{-1} ketamine and 5 kg^{-1} xylazine injected intramuscularly. Five minutes after the injection, 1% tropicamide was instilled in both eyes to induce mydriasis.

The animal was kept for a predetermined latency period from the start of the exposure to UVR. After the animal was sacrificed, the eyes were enucleated and the lenses were extracted. The tension in the eyes appeared normal at the time of dissection as observed by manual indentation of the globe. Remnants of the ciliary body were removed from the lens equator under a microscope, keeping the lens in BSS. Then, the intensity of forward light scattering was quantitatively measured three times for each lens. Thereafter, the macroscopic appearance of the lens was documented in incident illumination against a grid and in light- and dark field illumination photography.

2.4. Experimental design

The experimental design is given in Fig. 1.

Altogether, 80 animals were randomly divided into four equally sized latency groups (1, 2, 4 or 8 days). One eye in each animal was exposed to UVR-B-300 nm. Forward light scattering was measured three times for each lens.

2.5. Statistical parameters

The significance levels were set to 0·05 and the confidence coefficients to 0·95 considering the sample size.

3. Results

Seven out of the 160 examined lenses were damaged during the dissection. These lenses were together with the corresponding contralateral lenses excluded from the data analysis. In the latency group four, one mouse suffered from congenital eye defects and was additionally excluded from

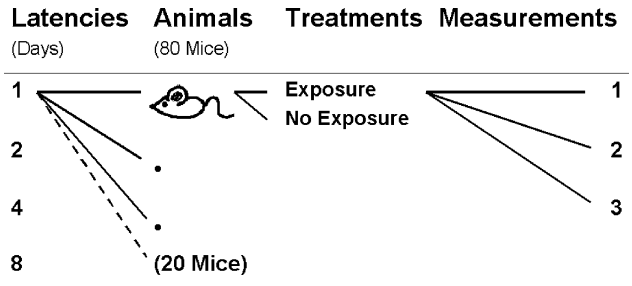

Fig. 1. Experimental design.

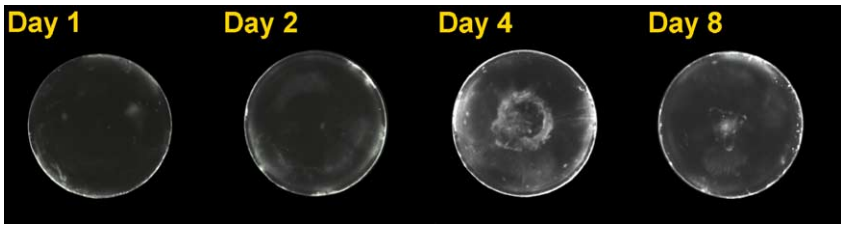

Fig. 2. Anterior subcapsular cataract in mouse lens 1–8 days after in vivo exposure to 5 kJ m^{-2} UVR-B-300 nm.

the data analysis. To allow a balanced design for the analysis of variance, the group sizes of all groups were adjusted to the group size of $n=17$ (equals group size of latency group four) by random exclusion.

3.1. Macroscopic appearance

Exposed lenses developed anterior subcapsular cataract (Fig. 2).

The opacity started as an annular ring of opaque granules close to the equator at day 1. The annulus gradually decreased in diameter during days 2–4 and ended up as a triangular shaped opacity in the region of the sutures at the anterior pole. The posterior surface of the exposed lenses was clear.

In addition to the anterior subcapsular cataract, cortical and nuclear cataract (Fig. 3) was identified in 19/80 exposed lenses.

The lenses of the non-exposed eyes were in majority clear with a smooth surface as seen with the naked eye (Fig. 4). However, in 10 animals, nuclear and or cortical cataract occurred bilaterally.

In 3/20 mice there was bilateral nuclear cataract 48 hr after exposure.

The eye bulbs of the animals with a bilateral response showed signs of ocular inflammation in both eyes such as iris adhesions, iris bombé and neovascularization.

3.2. Light scattering

When the evolution of light scattering was plotted for the exposed and the contra lateral lenses individually, it was found that the light scattering peaked 2 days after exposure in both lenses (Fig. 5).

The highest absolute light scattering values were found 2 days after the lenses were exposed to UVR. In some animals the light scattering also increased in the non-exposed lenses but never reached the level of the exposed lenses.

The difference of light scattering between exposed and contralateral not exposed lenses increased slightly from the first day to the second day after exposure but then decreased (Fig. 6).

The measurements of light scattering in the lenses were analyzed with a mixed model analysis of variance (Appendix). Exposed lenses scatter differently from contralateral not exposed lenses as indicated by the mean square for treatment (Table 1).

There is a variation of light scattering among latencies as revealed by the estimated mean square for latencies (Table 1). The difference of light scattering between exposed and unilateral not exposed lenses does not vary among latencies (Table 1).

The analyses of variance (Table 1) also allowed estimation of the variance for animals: σ_A^2 (0·00308 [$tEDC^2$]) and for measurements: σ_ε^2 (0·00001 [$tEDC^2$]).

4. Discussion

The present study was designed to elucidate the evolution of UVR-B induced cataract in C57 mice lenses. This knowledge is essential for future design of studies of the significance of genetic modulation on sensitivity of the lens to UVR-B.

The relationship between cataract and UVR exposure has been well established epidemiologically and experimentally in vitro in human and in animal models, especially rabbits, rats and guinea pigs (Pitts et al., 1983; Merriam et al., 2000; Malik et al., 1995). These in vivo studies are irreplaceable because it cannot be ruled out that the surrounding tissues of the lens are also involved in the formation of cataract.

It should be pointed out that the established animal models, including the mouse, have their limitations in studies of damage caused by optical radiation. Some species are nocturnal in contrast to humans. Further, animal eyes often differ anatomically, e.g. the mouse as well as the rat cornea is thinner than the human cornea (Dillon et al., 1999). Thus a higher sensitivity of the lens in these animals is expected due to higher transmittance of UVR-B through the cornea. Despite these differences, the C57 mouse model

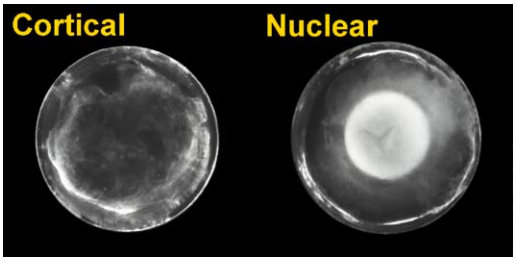

Fig. 3. Cortical and nuclear cataract in mouse lens after 5 kJ m^{-2} UVR-B-300 nm.

Fig. 4. Contralateral not exposed lenses.

offers important advantages compared to other rodents previously studied. Mice are available in large numbers at uniform size and to a reasonable cost. More important though, the C57 strain offers the possibility to study the effect of genetic modulation on altered sensitivity to oxidative stress, using knock out and transgenic mice. The results of Forker et al. (1997) support the hypothesis that environmental oxidative stress, like exposure to UVR-B, enhances the severity of genetically predetermined eye disease. The mouse and the human genomes are well mapped and extensively compared. The mouse model may therefore be an important tool for increased understanding of UVR-B induced cataract in humans.

The currently employed dose of UVR, 5 kJ m^{-2}, was chosen for this experiment because a preliminary experiment indicated that this dose is close to threshold level for formation of cataract in mice lenses. Further, this dose is equivalent to the dose found to induce permanent lenticular damage in the rabbit lens at 300 nm (Pitts et al., 1983).

4.1. Macroscopic appearance

The evolution of the subcapsular opacities organized in an annulus that shrank between days 1 and 8 (Fig. 2) probably reflects the central migration of newly formed anterior lens fiber ends. This indicates that the proliferation of lens epithelium in the germinative zone and subsequent differentiation of the newly formed cells into lens fibers may anatomically correspond to the light scattering induced.

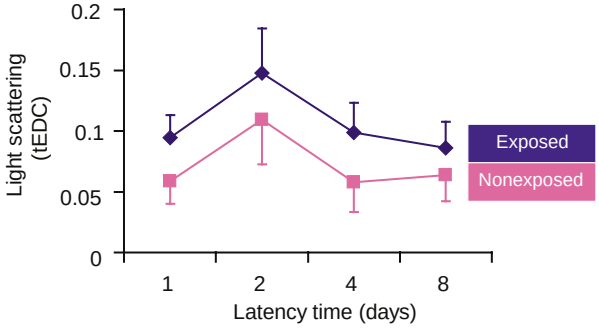

Fig. 5. Average light scattering of C57 mice lenses after in vivo exposure to 5 kJ m^{-2} UVR-B-300 nm. Bar is 95% confidence interval for mean: day 1: $n=18$, day 2: $n=19$, day 4: $n=17$, day 8: $n=18$.

The finding that cortical and nuclear cataract was found in addition to subcapsular cataract (Fig. 3) may indicate that there is a vast variation in sensitivity to oxidative stress in the lens. This is also reflected in the large error bars for light scattering in Figs. 5 and 6 and in the estimated variance for animals but not for the estimated variance for measurements.

4.2. Light scattering

The finding that there is a variation of light scattering among latencies (Table 1) indicates that there is a dynamic change of light scattering in the lenses, between 1 and 8 days after unilateral exposure to 5 kJ m^{-2} UVR-B-300 nm.

The fact that the present study demonstrated that a dose of 5 kJ m^{-2} UVR-B-300 nm induces significant light scattering difference between exposed and contralateral lenses (Table 1) demonstrates that the C57 mouse lens in similarity to lenses from many other species (Pitts et al., 1977; Merriam et al., 2000) is sensitive to UVR-B-300 nm.

The plot of the evolution of the difference of light scattering between exposed and its contralateral lens indicates that there is a transient peak light of scattering around 2 days after exposure (Fig. 6).

Kufoy et al. (1989) described the development of acute lens opacities immediately after anesthesia in rats. However, these opacities were transient and disappeared completely within 315 min after application of anesthesia,

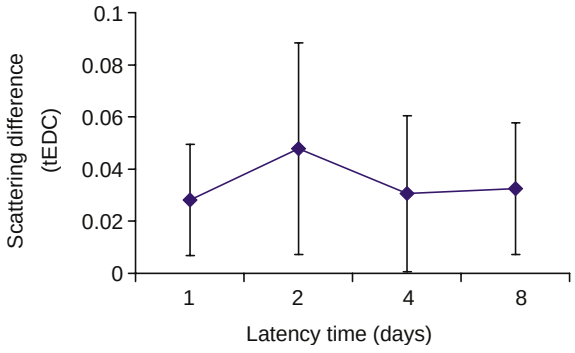

Fig. 6. Difference of light scattering between a lens exposed in vivo to 5 kJ m^{-2} UVR-B-300 nm and it's contralateral not exposed lens. Bar is 95% confidence interval for mean: day 1: $n=18$, day 2: $n=19$, day 4: $n=17$, day 8: $n=18$.

Table 1
Analysis of variance of light scattering in lenses from mice exposed unilaterally in vivo to 5 kJ m^{-2} UVR-B

Source of variation	Degrees of freedom	Estimated mean square ([tEDC.100])^2	Expected mean square	Test statistics F	Significance limit
Latencies	3	0.0707503	$\sigma_\varepsilon^2 + cn\sigma_B^2 + bcn\kappa_\alpha^2$	3.83*	2.76
Treatments	1	0.0951370	$\sigma_\varepsilon^2 + abn\kappa_\alpha^2$	15.28*	3.87
Animals	64	0.0184672	$\sigma_\varepsilon^2 + cn\sigma_B^2$	2061.06*	
Interaction latencies-treatments	3	0.0026115	$\sigma_\varepsilon^2 + n\sigma_{B\gamma}^2 + bn\kappa_{\alpha\gamma}^2$	0.42	1.41
Interaction animals-treatments	64	0.0062251	$\sigma_\varepsilon^2 + n\sigma_{B\gamma}^2$	694.76*	
Measurement error	272	0.0000009	σ_ε^2		

σ^2, the expected variance for the indexed source; κ^2, a factor for the systematical variation for the indexed source; α, latencies; B, animals; γ, treatments; ε, measurements; a, number of latencies ($a=4$), b, number of animals ($b=17$); c, number of treatments ($c=2$), n, number of measurements ($n=3$). Significance: *$p=0{\cdot}05$.

thus long before the presently observed increase of light scattering 2 days after exposure.

The finding that light scattering increased in the contralateral non-exposed lens (Fig. 5) after exposure to UVR-B-300 nm is consistent with reports by Pitts et al. (1983); Söderberg et al. (1990). Both groups found an increase in light scattering in non-exposed contralateral lenses in in vivo experiments with rabbits and rats. Kufoy et al. (1989) report that the widely used anesthetic combination of xylazine and ketamine has the potential to cause dry eye syndrome and secondary uveitis in rats 8–15 days after anesthesia. However, in the present experiment the time between anesthesia and measurement was shorter with the inflammatory signs already observed 1 day after application of anesthetics and exposure to UVR-B. In addition, the dose of xylazine and ketamine, respectively, in our study was ½ and 4/5 of the dose used by Kufoy et al. The fact that the bilateral inflammatory effect was only observed in a few animals although all were bilaterally dilated with tropicamide makes it unlikely that tropicamide caused this effect. Furthermore, tropicamide is a clinically widely used drug and to our knowledge there is no report of an associated inflammatory reaction in the anterior chamber. Therefore, our finding that eyes in mice that suffered from a bilateral cortical and or nuclear cataract showed signs of inflammation in both eyes rather indicates that there may be a systemic response involved in the formation of cataract that causes a sympathetic reaction also in the unexposed contralateral eye.

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Appendix. Analysis of variance for forward light scattering

The data were analyzed with an analysis of variance according to the following model:

$$X_{ijkl} = \mu + \alpha_i + B_{j(i)} + \gamma_k + (\alpha\gamma)_{ik} + \varepsilon_{l(ijk)}$$

An individual light scattering observation, X_{ijkl}, equals the sum of the expected total mean, μ, a term for the systematical variation between latencies, α_i ($i=1,\ldots4$), a term for the systematical variation between animals, $B_{j(i)}$ ($j=1,\ldots17$), a term for the systematical variation between treatments, γ_k ($k=1,2$), a term for the interaction between latencies and treatment $(\alpha\gamma)_{ik}$, and a term for the measurement error, $\varepsilon_{l(ijk)}$ ($m=1,\ldots3$).

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