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LABORATORY INVESTIGATION

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Dose-response function for lens forward light scattering after in vivo exposure to ultraviolet radiation

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Abstract ● Background: It is known that different types of radiation, as well as aging and metabolic disorders, can cause cataract. Several epidemiological investigations show a correlation between cataract development and the dose of ultraviolet radiation (UVR) received. It is well established experimentally that exposure of animal eyes to UVR induces cataract. The purpose of the present study was to determine the dose-response function for UVR-induced opacities in the rat lens after in vivo exposure. ● Methods: Sprague-Dawley rats received 0.1, 0.4, 1.3, 3, 5, 8 or 14 kJ/m^2 UVR (λ_{MAX}=300 nm, $\lambda_{0.5}$=10 nm) unilaterally for 15 min. At 1 week after exposure both lenses were removed, photographs were taken and the intensity of forward-scattered light was measured. ● Results: One week after UVR exposure, opacities occurred on the lens surface, as observed with a microscope. With increased UVR dose the opacities became more intense and occurred also in the equatorial area of the lens, but not in the nucleus. The intensity of forward light scattering increased with increased UVR dose between 3 and 14 kJ/m^2. No significant change in intensity of forward light scattering was observed for lower UVR doses. ● Conclusion: The intensity of forward light scattering in the rat lens increases exponentially with increased UVR dose between 0.1 and 14 kJ/m^2.

Introduction

In the present study, the dose-response function for forward light scattering in the rat lens after short-term in vivo exposure to ultraviolet radiation (UVR) was examined.

We used the intensity of forward-scattered light to objectively and quantitatively describe lens opacities. A light beam entering the eye is refracted and then projected onto the retina. All light that is scattered away from this beam in the forward direction reaches the retina at other points than the main beam and disturbs visual acuity. The intensity of the light scattered forward by the lens is measured in the present experiment.

Epidemiological studies [5, 23] and experimental exposure of animals to UVR [1, 4, 7, 8, 12, 22] show a relationship between the UVR exposure and induced lens opacities. Most epidemiological studies relate UVB radiation (280 to 315 nm wavelength) to cortical cataracts [5, 23, 24].

In vitro [1, 7, 22] and in vivo [4, 8, 12] animal studies show that UVB radiation induces cataract more efficiently than UVA radiation.

In the present experiment, radiation peaking in the center of the UVB radiation band at 300 nm was used. For this waveband, Pitts et al. [12] found a threshold for permanent lenticular damage at 5 kJ/m^2 for the rabbit. The dose of 5 kJ/m^2 is also known as close-to-threshold dose for rats, whereas 20 kJ/m^2 destroys the lens totally [10].

The annual terrestrial dose of UVB radiation is about 1.5 MJ/m^2 in central Europe and up to 4.5 MJ/m^2 in central Africa [15]. In contrast to the narrow-band radiation used in the experiment, the solar UVB radiation is broad band and the intensity rapidly increases towards longer

wavelengths. Therefore, only the spectral values at a particular wavelengths should be compared. The spectral irradiance at 300 nm as applied for the lowest UVR dose in the present experiment (0.1 kJ/m^2) is 10 mW/(m^2 · nm) in the corneal plane. The same spectral irradiance is measured at the earth surface with the sun in the zenith and a clear sky [25].

Earlier experiments have shown that forward light scattering develops over the course of 1 week and then remains constant up to 32 weeks after exposure [10, 18]. Söderberg and Löfgren found that after a dose of between 3 and 300 kJ/m^2 the intensity of forward light scattering is induced proportional to the logarithm of the dose [19].

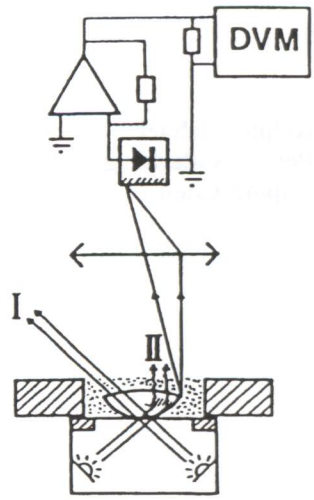

Fig. 1 Schematic drawing of the Light Dissemination Meter. Examined lens under dark-field illumination *I* Ideally clear lens, *II* opacified lens, *DVM* digital volt meter. (Reprinted with permission from [20])

Methods

The Sprague-Dawley rat was the experimental animal. One eye of each rat was exposed in vivo to UVR. Intensity of forward light scattering caused by the lens was measured after in vitro isolation of the lens.

Experimental devices

The radiation from a high-pressure mercury lamp (HBO 200 W, Osram, Germany) was collimated, passed through a water filter and then an interference filter ($\lambda_{MAX.}$=300 nm, half band width 10 nm) and finally projected on the cornea of the exposed eye (Söderberg) [10, 17]. The spectrum of the radiation was published earlier [10].

The intensity of forward light scattering was measured with a Light Dissemination Meter [20]. This instrument uses the principle of dark-field illumination. The illuminating light transilluminates a transparent object 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 the light reaches the objective and is measured by a photodiode (Fig. 1).

The scattering standard was a lipid emulsion of diazepam (Diazemuls, KabiVitrum, Sweden) and the unit was therefore expressed as transformed equivalent Diazemuls concentration ($_t$EDC) [20].

Experimental procedure

Female Spraque-Dawley rats were unilaterally exposed at the age of 6 weeks. Ten minutes preceding the 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, tropicamide was instilled in the exposed eye. After another 5 min, the animal eye was exposed to UVR for 15 min. Exposure was unilateral and the beam covered only the cornea and the eye lids of the exposed eye.

Altogether, 80 rats were divided into 8 dose groups. The exposure was done in two batches, a low-dose batch and a high-dose batch. The low-dose batch was exposed first and then the high-dose batch was added to provide a wider dose interval for the estimation of the dose-response function. Rats of the low-dose batch were exposed to 0.1, 0.4, 1.3 or 5 kJ/m^2 UVR, and rats of the high-dose batch were exposed to 3, 5, 8 or 14 kJ/m^2 UVR. The dose of 5 kJ/m^2 was used in both batches to ensure that the results of exposure were reproducible. Each animal was kept for 1 week from the start of the exposure to UVR. It was then killed with an overdose of pentobarbital sodium (Pentobarbitone; 200 mg/kg, intraperitoneally), followed by cervical dislocation. Thereafter, the eyes were enucleated and the lens was extracted and placed in balanced salt solution (BSS). Remnants of the ciliary body were removed from the lens equator under a microscope, keeping the lens in BSS. Photographs were taken with 6 times magnification for each lens against a dark background and a white grid. Finally, intensity of forward light scattering was measured three times for each lens in BSS. The animals were kept and treated according to the ARVO *Statement for the Use of Animals in Ophthalmic and Vison Research.*

The intensity of forward light scattering was fitted against UVR dose with stepwise multiple regression considering polynomials of increasing order (Appendix). The confidence interval for the regression curve was calculated. Confidence coefficients were set at 0.95.

Results

One week after exposure to UVR, lens opacities were visible with the naked eye in lenses exposed to 3 kJ/m^2 or higher doses (Fig. 2). Exposed lenses had haze on their anterior and posterior surface that became more intense with increased dose (Fig. 2c, d). After exposure to 14 kJ/m^2, the surface became opaque, strong equatorial

Table 1 Intensity of forward light scattering (95% confidence interval for the mean) in exposed and non-exposed lenses and for the difference between exposed and non-exposed lenses

UVR dose group (kJ/m^2)	Forward light scattering ($_t$EDC)			Sample size
	Exposed lens	Non-exposed lens	Difference	
0.1	0.15±0.01	0.15±0.01	0.00±0.02	9
0.4	0.16±0.02	0.17±0.03	0.00±0.03	9
1.3	0.16±0.02	0.18±0.02	−0.01±0.02	9
3	0.20±0.03	0.14±0.01	0.06±0.03	10
5	0.24±0.03	0.14±0.01	0.09±0.03	19
8	0.32±0.06	0.14±0.03	0.18±0.06	10
14	0.62±0.21	0.14±0.01	0.49±0.21	9

opacities were visible and the ciliary body was often attached to the lens (Fig. 2e).

The data from the two groups exposed to 5 kJ/m^2 were pooled because a comparison with *t*-test showed no significant difference between the groups. Five lenses were destoyed during enucleation and handling, so five pairs of lenses had to be excluded from the data analyses (Table 1).

There was no significant difference in forward light scattering between exposed and non-exposed lenses 1 week after exposure to a UVR dose of 0.1, 0.4 and 1.3 kJ/m^2 since the confidence intervals for the mean difference in forward light scattering between exposed and non-exposed lenses included zero for these doses (Fig. 3). After exposure to 3, 5, 8 and 14 kJ/m^2 the forward light scattering of exposed lenses differed significantly from that of non-exposed lenses. The mean difference in forward light scattering increased with increasing UVR dose. Concurrently, the interindividual variability increased with increasing dose (Fig. 3).

The result of the multiple stepwise regression with a 95% confidence interval is presented in Fig. 3. The analytical process for calculation of the dose-response function is given in the Appendix.

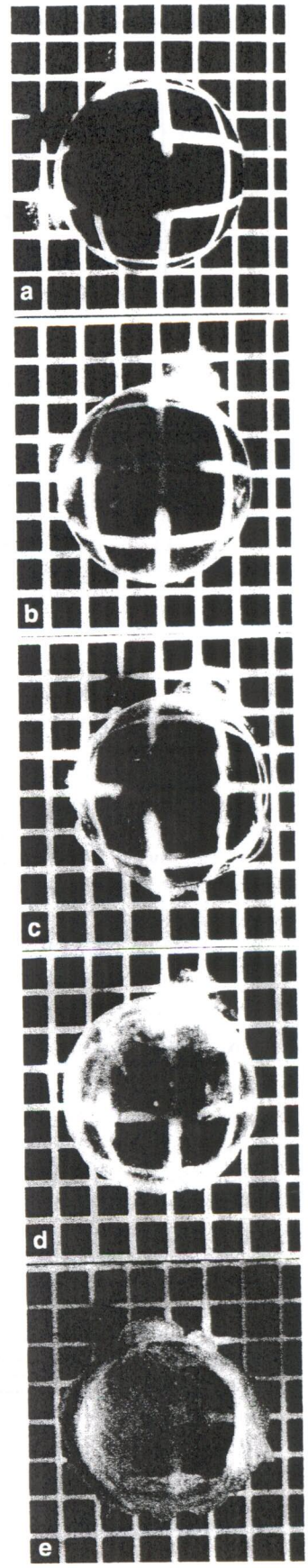

Fig. 2a–e Photographs of isolated rat lenses 1 week after exposure to UVR against a black background with a white grid. The distance between the white wires is 0.75 mm. Measured intensity of forward light scattering is given in parentheses.
a Non-exposed (0.126 ₍EDC);
b 3 kJ/m^2 (0.185 ₍EDC);
c 5 kJ/m^2 (0.311 ₍EDC);
d 8 kJ/m^2 (0.347 ₍EDC);
e 14 kJ/m^2 (0.703 ₍EDC)

Discussion

Qualitative results were obtained from photographs taken of the entire extracted lens kept in BSS. Opacities occurred on the lens surface 1 week after UVR exposure. With increased dose the opacities become more intense and occurred also in the equatorial area of the lens, but not in the nucleus. It was not possible to distinguish the exact location of the damage. The opacities could be located in the lens capsule, the epithelium and in the outer cortical lens fibers. The fact that both, the anterior and posterior lens surface appeared opaque suggest that not only the lens epithelium is damaged, but also the outer cortical fibers. The lens capsule might be less involved in the damage, because it transmits a large part of the UVR at 300 nm [21].

The increase of forward light scattering as function of UVR dose found in the present experiment (Fig. 3) probably represents the first part of a sigmoid function, which starts with an exponential increase, has a turning point and then flattens asymptotically. This idea is supported by the earlier findings of Söderberg and Löfgren [19]. They found a logarithmic dependence of forward light scattering for higher UVR doses (up to 300 kJ/m^2), which probably represents the second part of the sigmoid function. These findings concerning the dose-response function will serve as a basis for threshold dose estimations for UVR.

Ultraviolet radiation at 300 nm with a half band width of 10 nm was used for exposure. The transmission of the

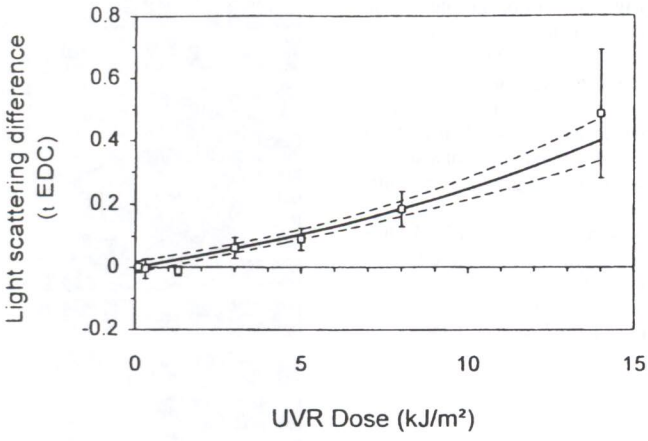

Fig. 3 Light scattering difference after a certain UVR dose. The bars represent 95% confidence intervals for the mean difference in intensity of forward light scattering between the lenses of the exposed and non-exposed eyes 1 week after a UVR dose of either 0.1, 0.4, 1.3, 3, 5, 8 or 14 kJ/m^2 [n=9, except for 3 and 8 kJ/m^2 (n=10) and 5 kJ/m^2 (n=19)]. The *solid line* is the best fit obtained with stepwise multiple regression considering polynomials of increasing order, and the *dashed lines* represent its 95% confidence interval

cornea at this wavelength is low, but not zero. In vitro measurements of corneal transmission vary from <2% [6] to 20% [14] for the rat, 9% [3] for the human and 24% [2] for the rabbit. The small fraction of the UVR that reaches the lens is strongly absorbed by the lens [2, 3] and probably damages the lens directly.

For the lowest UVR dose which induced significant forward light scattering (3 kJ/m^2), the spectral dose at 300 nm was $H_{300\,nm}$=270 J/(m^2 · nm). An outdoor worker with the sun in the zenith would receive this spectral dose during 75 h;

$$t = \frac{H_{300\,nm}}{E_{300\,nm} \cdot R_{ocular/ambient}} \quad (1)$$

Here, t is the exposure time, $H_{300\,nm}$ is the spectral dose at 300 nm as applied in the experiment and $E_{300\,nm}$=10 mW/(m^2 · nm) is the ambient terrestrial spectral irradiance at 300 nm with the sun in the zenith and a clear sky as measured by Wester on the Canary Islands [25]. $R_{ocular/ambient}$=0.1 is the ocular-to-ambient exposure ratio. This ratio gives the relationship between the irradiance on the earth surface and the ocular surface. Rosenthal et al. [13] found the ocular-to-ambient exposure ratio to be between 0.02 and 0.17, depending on clothes, working conditions and time of the year.

There are a few reported cases in which people who received significant UVR doses from artificial sources subsequently developed cataract which could be correlated to this exposure. Lerman [9] reported three patients who received 0.3–0.4 W/m^2 UVR (λ=300–400 nm) for about 200 h over an 18-month period related to their occupation. He found posterior subcapsulr and zonular cortical opacities in one or both eyes. Müller-Breitenkamp et al. [11] reported anterior and posterior subcapsular opacities in both eyes of a 65-year-old patient 30 years after unintended exposure to UVR. The patient received 0.7–2 kJ/m^2 per day UVR-B and -C during the winter season over 15 years. The time interval of the exposure prior to the examination was confirmed by measuring the distance between the lens capsule and the cortical opacities [11].

In the present experiment, all animals exposed to 14 kJ/m^2 had an inflammation of the conjunctiva and the cornea. The inflammation was less intense after 8 and 5 kJ/m^2 and occurred in 75% and 50% of the exposed eyes, respectively. After exposure to a lower dose no external inflammation of exposed eyes was observed. The majority of animals exposed to 14 to 8 kJ/m^2 and some exposed to 5 kJ/m^2 developed a corneal epithelial defect 1 week after exposure. This occurred only occasionally with lower doses.

To summarize, 1 week after UVR exposure, opacities occur superficially in the lens. With increased UVR dose the opacities become more intense and also occur in the equatorial area of the lens, but not in the nucleus. There is no significant change in intensity of forward light scattering after exposure to UVR doses between 0.1 and 1.3 kJ/m^2. The intensity of forward light scattering increases with increased UVR dose between 3 and 14 kJ/m^2. The intensity of forward light scattering in the rat lens increases exponentially with increased UVR dose between 0.1 and 14 kJ/m^2.

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Appendix

The forward light scattering data had incresing variability in the y-coordinate with increasing magnitude of the x-values. Because normality and homoscedasticity of the data is needed for regression, the data were transformed [27]:

$$y' = \ln(y+1) \quad (2)$$

Because of expressed heteroscedasticity this transformation was performed three times:

$$y''' = \ln\{\ln[\ln(y+1)+1]+1\} \quad (3)$$

Then, a stepwise multiple regression considering polynomials of increasing order was performed to find the best fitting, least complex polynomial function [26]. According to this method a linear regression of the triple logarithmic-transformed forward light scattering was found to describe the dose-response function:

$$y = \mathrm{EXP}\{\mathrm{EXP}[\mathrm{EXP}(m \cdot x) - 1] - 1\} - 1 \quad (4)$$

Here, y is the difference in forward light scattering in the lens between the exposed and the non-exposed eye (in $_t$EDC), x is the UVR dose (in kJ/m^2) and m=0.018418 is the slope of the linear regression line. The confidence interval $CI_{y'''}(x)$ for the regression line is calculated according to Eq. 5.

$$CI_{y'''}(x) = [m \cdot x \pm t_{n-2;0.95} \cdot SE_{y'''}(x)] \quad (5)$$

Here, n is the sample size, $t_{n-2;0.95}$ the Student's t distribution (with the two-sided significance level set at 0.95) and $SE_{y'''}(x)$ the standard error of y'''. The standard error $SE_{y'''}(x)$ depends on x [16]:

$$SE_{y'''}(x) = s_{y/x} \cdot \sqrt{\frac{1}{n} + \frac{(x-\bar{x})^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2}} \quad (6)$$

Here, $s_{y/x}$=0.05047 is the square root of the estimated residual variance of the regression line, n=75 is the number of observations, $\bar{x}$=4.672 is the mean of the UVR doses x and x_i is the UVR dose of exposure i in the sample (i=1..n). The sum over $(x_i-\bar{x})^2$=1494.05 in the current experiment. Finally, the confidence interval is transformed back to the original values of forward light scattering with the inverse of Eq. 3.

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