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The Effect of Exposure Time on Maximum Acceptable Dose for Avoidance of Ultraviolet Radiation-Induced Cataract

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Key Words

Maximum acceptable dose · Ultraviolet radiation B · Exposure time · Cataract · Rat · Light scattering · Dose-response function

Abstract

The effect of exposure time on maximum acceptable dose (MAD) for avoidance of ultraviolet radiation B (UVRB)-induced cataract was investigated. Sprague-Dawley rats were divided into 5 exposure time groups: 7.5, 15, 30, 60, and 120 min. Each exposure time group was divided into 5 dose subgroups: 0, 1, 2, 4, and 8 kJ/m^2. The rats were unilaterally exposed to UVR around 300 nm. One week after the exposure, macroscopic structure was recorded and lens forward light scattering was measured. MAD for avoidance of UVRB-induced cataract was estimated based on the dose-response function. MAD for avoidance of UVRB-induced cataract for 7.5, 15, 30, 60, and 120 min exposures was estimated to be 2.0, 1.4, 1.9, 1.8 and 2.2 kJ/m^2, respectively. In the exposure time domain 7.5–120 min, MAD for avoidance of UVRB-induced cataract depends on exposure time.

Introduction

In the present study, the effect of exposure time on maximum acceptable dose (MAD) for ultraviolet radiation B (UVRB)-induced cataract was evaluated.

The prediction of the impact of environmental UVR exposure on cataract prevalence requires good knowledge of the spectral radiance of the sun since the threshold dose for cataract strongly depends on wavelength [1, 2]. This makes prediction of cataract as a result of chronic exposure even more difficult [3]. Animal models have been used to study UVR-induced cataract [4–8]. Advantages of animal studies are that dosimetry can be controlled and known confounding factors contributing to cataract development can be excluded. Similar experimental studies on humans are ethically impossible. The drawback is the difficulty in translating the results of animal experiments to humans. Therefore, current safety limits for avoidance of UVRB-induced cataract [9] depend on data from animal experiments [1], epidemiological investigations and continuous evaluation of feedback information about the validity of current safety standards in field applications.

For safety purposes, the sensitivity to UVR is expressed as a threshold dose. The threshold dose for UVR-induced cataract is based upon a dichotomous dose-response model, assuming that the outcome of a UVR exposure has a binary response, cataract/no cataract [1]. In that study, cataract was measured qualitatively with a

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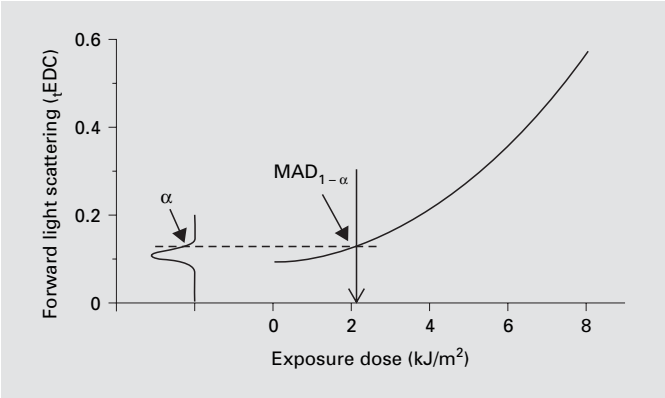

Fig. 1. MAD concept. Left function = Frequency distribution for normal lenses; α = probability for a normal lens to be classified as pathological; dashed line = limit between normal and pathological light scattering; right function = dose-response function for exposed lenses; arrow head = $MAD_{1-\alpha}$ [12].

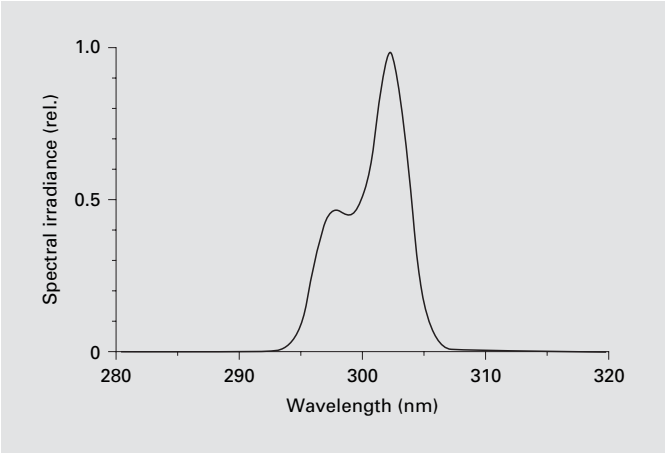

Fig. 2. Spectral distribution of the radiation used.

grading scale by a slit lamp. A method for quantitative measurement of total integrated intensity of light scattering from the lens, irregardless of location, was developed by Söderberg et al. [10]. With this method, it has been shown that UVR-induced cataract follows a continuous dose-response function so that with increasing UVRB dose, there is a continuous increase in forward light scattering in the lens [11].

MAD for avoidance of UVRB-induced cataract (fig. 1) has been developed by Söderberg et al. [10] for estimation of the threshold for UVRB-induced cataract. MAD is based on that UVR-induced cataract follows a continuous dose-response function [12]. An upper limit of normal light scattering is arbitrarily chosen so that it is accepted that a certain percentage of normal lenses, α, are wrongly labelled pathological although they are normal. This limit is projected on the dose-response function and the dose corresponding to the limit on the dose-response function is considered the $MAD_{1-\alpha}$.

The purpose of the current study was to investigate the effect of exposure time on $MAD_{1-\alpha}$ for avoidance of UVR-induced cataract.

Methods

Experimental Animal

The 6-week-old (150 g) Sprague-Dawley rat was the experimental animal. The experiment was approved by the local ethical committee at Karolinska Institutet. The animals were treated according to the ARVO convention for treatment of experimental animals.

UVRB Exposure

Radiation from a high-pressure mercury lamp (350 W, No. 6286, Oriel, USA) was collimated, passed through a water filter and a double monochromator (No. 77 250 × 2, Oriel, USA), and finally projected on the cornea of the exposed eye. The monochromator was set to 300 nm and the entrance and exit slits were adjusted to achieve 9 nm full width half maximum. The actual peak wavelength was 302.6 nm (fig. 2).

Irradiance was measured with a thermopile (No. 7104, Oriel, USA). The system had been calibrated to a standard traceable to the National Institute of Standards, USA.

Light Scattering Measurement

The intensity of forward light scattering was measured with a light dissemination meter. This instrument uses the principle of dark field illumination [10]. A probing white light from a cold light source is directed towards the posterior lens surface at an angle of 45°. The measured light scattered forward from the lens is collected by the optics of a camera equipped with a photodiode in the film plane. The current is voltage converted and read as a voltage. The technique allows detection of less than 1% change in light scattering. The scattering standard was a lipid emulsion of diazepam (Stesolid Novum, Dumex-Alpharma A/S, Denmark) and the unit of reading was expressed as transformed equivalent diazepam concentration ($_t$EDC) [10].

Experimental Procedure

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, the mydriatic tropicamide was instilled in both eyes. After another 5 min, one eye of each animal was exposed to a narrow beam of UVRB covering the cornea and the eyelids. One week after exposure, the animal was sacrificed with CO_2 asphyxiation. The eyes were enucleated. Both lenses were extracted and placed in balanced salt solution (Alcon, USA). Remnants of the ciliary body were removed from the lens equator under a microscope. The intensity of forward light scattering of each lens in balanced salt solution was measured. Then, the macroscopic appearance was photographed.

Experimental Design

Altogether, 100 rats were divided into 5 exposure time groups of 20 rats each: 7.5, 15, 30, 60, and 120 min. Each exposure time group was subdivided into 5 dose subgroups of 4 rats, with UVRB doses designed according to expected $MAD_{0.975}$ [2]. The UVRB doses designed for the current experiment were 0, 1, 2, 4, and 8 kJ/m^2, respectively.

Statistical Parameters

The tolerance limit was set to 97.5% and considering the sample size, the significance limit was set to 0.05.

Results

Macroscopic Appearance

One week after UVRB exposure, a dose-response relationship between UVRB dose and cataract severity was observed macroscopically (fig. 3).

In a nonexposed lens, the grid was clearly seen through the lens and the equator of the lens was clear (fig. 3A, a). After 1 kJ/m^2, the anterior surface of the lens appeared rough and slight equatorial opacities could be detected in all lenses, in all exposure time groups (fig. 3B, b). After exposure to 2 kJ/m^2, equatorial vacuoles could be detected in 1 of 4 lenses in the 7.5-min group, in all lenses in the 15-min group, in 3 of 4 lenses in the 30-min, and in 2 of 4 lenses in the 60- and 120-min groups (fig. 3C, c). After exposure to 4 kJ/m^2, equatorial opacities were found in all five groups (fig. 3D, d). The severest opacities were found in all groups after exposure to 8 kJ/m^2 (fig. 3E, e). There was no nuclear cataract in any of the lenses.

Sensitivity Estimations

The homogeneity of variances of light scattering among the different exposure time groups was tested by Bartlett's test [13]. Using analysis of variance, we found no difference in the level of intensity of light scattering among the different groups. All nonexposed lenses were therefore pooled together to estimate the frequency distribution for light scattering. Thereby, the limit for normal forward light scattering, defined as the limit enclosing 97.5% of normal nonexposed lenses (fig. 1), was found to be 0.190 ($_t$EDC).

The dose-response function was estimated from the light scattering measurements after different doses of UVRB exposure (fig. 4).

The contrast among regression coefficients for different exposure times was analyzed with orthogonal comparison using the t test for independent groups (table 1), according to the strategy: 60 vs. 120 min; 30, 60 and 120 vs. 7.5 min; 7.5, 30, 60 and 120 vs. 15 min. For this, the experimental data for the different subgroups were considered as one group and were pooled together before the regression parameters were estimated with linear regres-

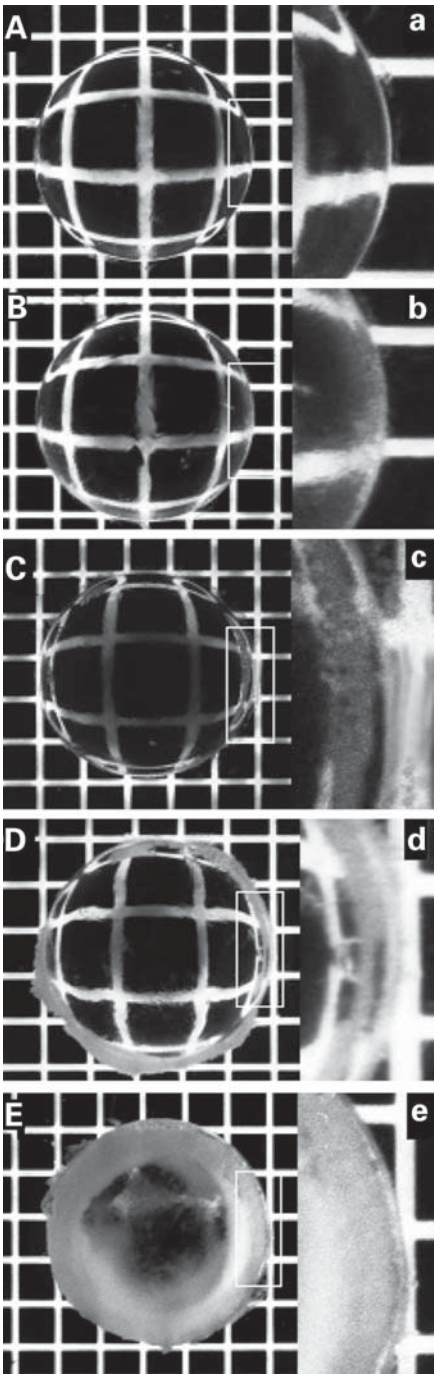

Fig. 3. Macroscopic changes 1 week after unilateral exposure to UVRB. **A**, **a** Nonexposed lens. **B**, **b** 1 kJ/m^2. **C**, **c** 2 kJ/m^2. **D**, **d** 4 kJ/m^2. **E**, **e** 8 kJ/m^2.

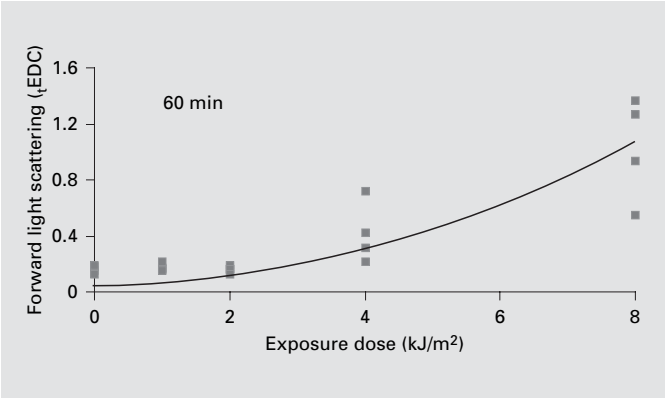

Fig. 4. Dose-response function for UVRB-induced cataract 1 week after 60-min UVRB exposure ($r^2 > 0.8$).

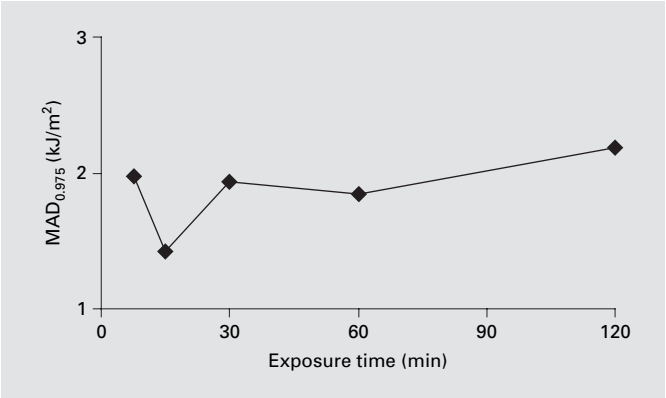

Fig. 5. Exposure time dependence of threshold dose ($MAD_{0.975}$).

Table 1. Orthogonal comparisons of regression coefficients for various exposure times

Comparison	Degrees of freedom	Test statistic[1]	Significance limit ($p < 0.05$)
60 vs. 120 min	38	0.10	2.024
60 and 120 vs. 30 min	56	0.03	2.003
30, 60 and 120 vs. 7.5 min	76	0.00	1.992
7.5, 30, 60 and 120 min vs. 15 min	92	–2.27[2]	1.986

[1] Based on t test for independent groups.
[2] Statistically significant difference.

Table 2. Exposure time dependence of sensitivity to UVRB in the ocular lens in vivo and transformed to in vitro

Exposure time min	In vivo $MAD_{0.975}$ kJ/m^2	Direct $MAD_{0.975}$[a] kJ/m^2
7.5	2.0	0.6
15	1.4	0.4
30	1.9	0.6
60	1.8	0.6
120	2.2	0.7

[a] Transformed from in vivo $MAD_{0.975}$, considering 32% corneal transmittance and 90% aqueous humor transmittance.

sion. There was a significant difference of regression coefficients only when comparing 7.5, 30, 60 and 120 vs. 15 min.

The $MAD_{0.975}$ at exposure times 7.5, 15, 30, 60, and 120 min were 2.0, 1.4, 1.9, 1.8 and 2.2 kJ/m^2, respectively (fig. 5).

There was a minimum $MAD_{0.975}$ at exposure time 15 min as compared to that at exposure times 7.5, 30, 60, and 120 min, respectively.

Discussion

The present experiment was designed to investigate the impact of exposure time on the sensitivity of the ocular lens to UVRB exposure. The threshold dose for avoidance of UVRB-induced cataract, expressed as $MAD_{0.975}$, was estimated as a function of exposure time.

The currently used strategy for threshold estimation, MAD [12], is based on knowledge of the mean and standard deviation for light scattering in normal rat lenses. The mean and the standard deviation in the population of rats currently studied were estimated from a sample of the population of 100 rats. The large sample size ensures that the estimates of the mean and the standard deviation are close to the real parameters.

The finding that the $MAD_{0.975}$ for the 15-min exposure time group was the lowest in all the exposure time groups (fig. 5) agrees with previously published toxicity data [14].

Radiant exposures presently applied refer to exposure in the corneal plane. The corneal transmittance at 300 nm is 32% in the rat [15]. The aqueous transmittance for monkeys has been published to be 90% [16]. Considering an anterior chamber depth of 3 mm in the monkey and 0.5 mm in the rat, and a similar concentration of absorbers

and scatterers as well as a similar specific attenuation coefficient in the rat and the monkey, the estimated in vivo $MAD_{0.975}$ can be transformed to direct in vitro lens exposure according to equation 1, as shown in table 2.

$$MAD_{0.975;\text{in vitro}} = MAD_{0.975;\text{in vivo}} \times 0.32 \times 0.32 \times 0.98 \quad (1)$$

These data could be used as a reference for in vitro studies of UVRB-exposed lenses.

According to the Bunsen-Roscoe law [17], the photochemical effect is determined by the radiant exposure, with a reciprocal relationship between exposure time and irradiance. The present result (fig. 5) demonstrates that in the lens, the photochemical damage induced by UVRB is biologically modified so that for the photobiological expression, the reciprocity law is not applicable in the time domain 7.5 min to 120 min.

The explanation of the finding that 15-min exposure induced the lowest MAD (fig. 5) is not known, but it is the balance between the cellular damage, the biological defense and the biological repair that determines the cataract development. One explanation of the lack of reciprocity for expressed light scattering in the exposure time window 7–15 min is that the exposure of the lens to UVRB triggers endogenous release of photosensitizers. If the delivery of the radiant exposure takes place before any photosensitizers have been released, very limited damage occurs. However, if a lot of photosensitizers have been formed, a lot of damage is expressed. It is further possible that with gradually increasing exposure time, there is a breaking point at which the biological repair rate will exceed the photobiological damage rate. If so, the expressed damage will gradually decrease as was presently found (fig. 5).

Dose-dependent corneal damage also developed in the present study. Most rats developed local corneal opacity and neovascularization after high-dose exposure. Some rats also developed corneal shape change, hyphema and uveitis after a high dose. The corneal structural change and higher intraocular pressure induced by uveitis may be the reason for the corneal shape changes.

In the present study, the dependence of threshold dose on exposure time was estimated. The threshold dose was estimated as $MAD_{0.975}$. The inverse of the threshold dose expresses the sensitivity. In the time domain 7.5–120 min, the sensitivity was found to peak at exposure times close to 15 min. Thus, it is critical to consider exposure time when designing experiments on UVR-induced cataract.

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