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Toxicity of Ultraviolet Radiation Exposure to the Lens Expressed by Maximum Tolerable Dose

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Abstract

The maximum tolerable dose ($MTD_{2.3:16}$) for avoidance of cataract on exposure to ultraviolet radiation (UVR)-300 nm in the rat was here estimated at 3.65 kJ/m^2. Sprague-Dawley rats were unilaterally exposed to UVR in the 300 nm wavelength region. One week after the exposure, the intensity of forward light scattering was measured. Toxicity for continuous response events can be estimated with MTD. Current safety standards for avoidance of cataract after exposure to UVR are based on a binary response event. It has, however, recently been shown that UVR-induced cataract is a continuous dose-dependent event. MTD provides a statistically well-defined criterion of toxicity for continuous response events.

Introduction

In the current paper, a new index for toxicity of ultraviolet radiation (UVR) to the lens will be developed.

There is a substantial body of epidemiological information indicating an association between cataract and exposure to UVR [1–5].

It has been known since the end of the last century that an acute overdose of UVR causes cataract [6]. It has been shown that the acute development of cataract after exposure to UVR [7] is related to a sodium potassium shift that causes swelling [8]. It was shown in 1915/1916 that there is a maximum sensitivity to UVR at around 300 nm [9]. This was later confirmed with a more elaborate methodology [10, 11].

Current safety standards for the avoidance of cataract after exposure of the eye to UVR [12] are based on an experimental qualitative determination of the toxicity of UVR to the lens [10] and a comparison with environmental exposure of the human eye and skin to provide an adequate margin of safety. The toxicity estimation in the latter experiment was based on the assumption that the occurrence of cataract after an exposure to UVR is a binary response event. Classically, the ED_{50} strategy [13] is used for toxicity estimation for binary response events.

It has, however, been shown with quantitative measurement of cataract that the dose-response function for UVR-induced cataract is continuous [14]. Therefore, it was attempted here to develop a strategy for toxicity estimation for continuous dose-response functions.

Materials and Methods

Cataract was induced experimentally in rats with UVR-300 nm. Thereafter, a strategy for the estimation of the maximum tolerable dose (MTD) for the avoidance of cataract was developed.

UVR Exposure

Six-week-old Sprague-Dawley rats were anesthetized with an intraperitoneal injection of xylazine (14 mg/kg) and ketamine (94 mg/kg) 10 min prior to exposure. Both eyes were dilated with tropicamide (5 mg/ml) 5 min prior to exposure. The rats were unilaterally exposed to UVR-300 nm (T-max: 300 nm, half-width: ±5 nm). The UVR-300 nm was generated with a high-pressure mercury arc source filtered with a water filter. The UVR-300 nm was spectrally selected with a double monochromator. The rats were sacrificed after 1 week in order to allow for maximum intensity of light scattering to develop [15, 16]. Both eyes were enucleated. For each eye, the lens was isolated and transferred to a cuvette containing a balanced salt solution (BSS, Alcon, USA). The intensity of forward scattered light was measured [17].

Experimental Design and Statistics

Altogether, 20 rats were divided into five groups of 4 rats each. The rats from the first group were put on the exposure bench, as the rats from all the other groups, but did not receive any UVR. The other groups received 1, 2, 4 or 8 kJ/m^2.

The light scattering data obtained were then analyzed with linear regression.

Ethical Approval

The study had been approved by the local ethical committee for experimental animals.

MTD Strategy

If the intensity of forward light scattering is measured in both eyes in normal non-exposed rats with the method cited above [17], the difference of light scattering will be normal distributed around 0 (fig. 1).

The probability of finding a difference of light scattering between the lenses in a rat in the population of rats of 2σ above 0 is 2.3%.

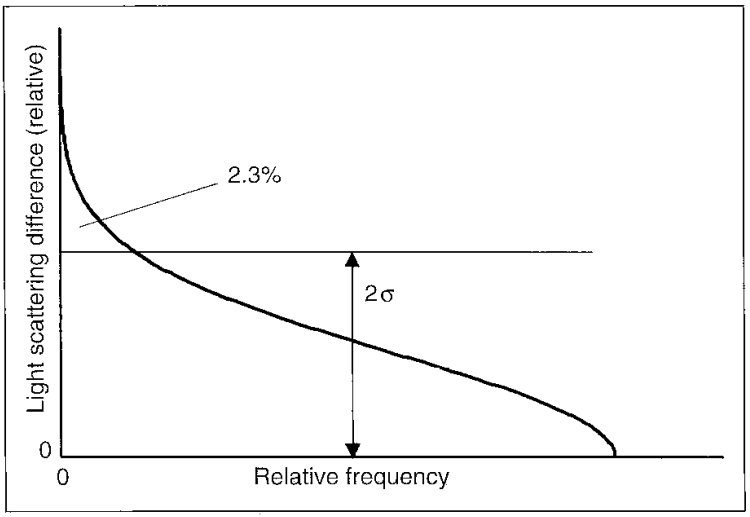

Fig. 1. Frequency distribution of a difference of intensity of light scattering in the normal eyes of a rat. The difference of intensity of forward light scattering is approximately normal distributed with a standard deviation, σ, and the mean 0, $N(0, \sigma)$.

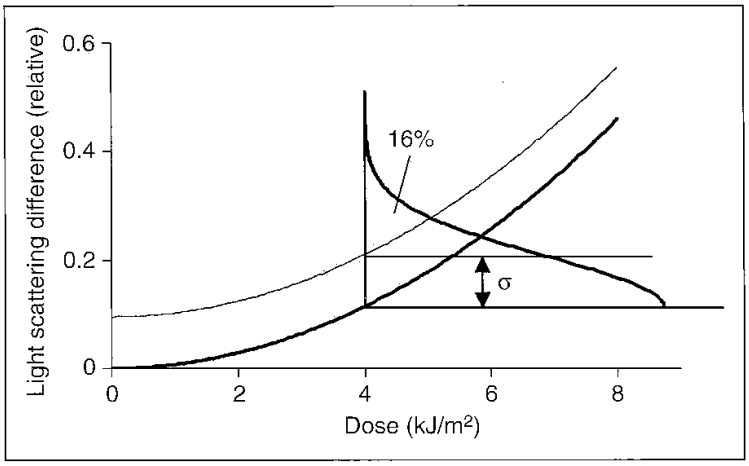

Fig. 2. Dose-response function for UVR-induced cataract close to a dose that induces an insignificant increase of light scattering (—). Juxtaposed the limit describing 1 standard deviation (σ) more intense light scattering has been drawn (—).

It is known from previous work [14] that the dose-response function, expressed as difference of intensity of forward light scattering between the exposed and contralateral non-exposed eye, for UVR-induced cataract at doses close to the level where no cataract is induced can be simplified to a 2nd order polynomial, omitting the first order term (equation 1).

$$Y = kx^2 + \varepsilon \tag{1}$$

where ε belongs to a normal distribution, $N(0, \sigma)$.

This is illustrated in figure 2.

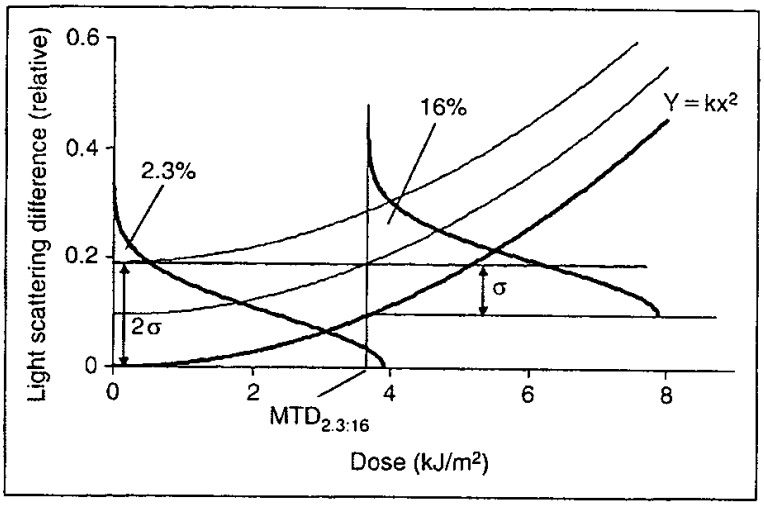

Fig. 3. Definition of $MTD_{2.3:16}$. Dose-response function for UVR-300 nm radiation induced cataract (—) and 1 standard deviation (σ) and 2 standard deviations above (—).

In each individual rat, it is expected that there is a 16% chance to find a difference between the exposed and contralateral side greater than 1 standard deviation above the dose-response function at any dose. If figure 1 and figure 2 are combined, figure 3 is obtained.

The MTD then may be defined as the dose corresponding to the crossover between 2 standard deviations above no difference of light scattering at zero dose, and 1 standard deviation above the dose-response curve for the difference of light scattering between the exposed and contralateral nonexposed side.

From figure 3 it is seen:

$$2\sigma = k(MTD_{2.3:16})^2 + \sigma \tag{2}$$

or

$$MTD_{2.3:16} = \sqrt{\frac{\sigma}{k}} \tag{3}$$

Results

The sensitivity, k (equation 1), was estimated to be 7.17×10^{-3} $_t$EDC/$(kJ/m^2)^2$ and the residual standard deviation was estimated to be 9.56×10^{-2} $_t$EDC. $MTD_{2.3:16}$ was therefore estimated to be 3.65 kJ/m^2.

Discussion

In the present study, we developed a strategy for the estimation of toxicity of UVR to the ocular lens with small sample experiments.

The interpretation of the current finding of $MTD_{2.3:16}$ of 3.65 kJ/m^2 is that there is a 16% probability that an individual exposed to the MTD will have a difference of intensity of forward light scattering between the exposed and the nonexposed contralateral lens exceeding the level found in 97.7% of eyes from individuals that have not been exposed to UVR. The currently found MTD provides a limit for avoidance of cataract that is very close to the threshold limit of 5 kJ/m^2 for permanent lens damage that was previously published by Pitts et al. [10] based on a binary response event model.

The strategy for MTD estimation can be generalized to all continuous response events. However, depending on the specific dose-response curve, the formula for calculation will vary. Further, the probability levels may be chosen differently but that will then also modify the formula for the calculation of the MTD.

In the current strategy, it is assumed that the residual standard deviation is constant regardless of the difference of intensity of light scattering recorded. In some cases, there may be a functional relationship between the residual standard deviation and the difference of intensity of light scattering. If this is the case, it has to be considered.

We are here assuming that the square root of the ratio between the residual standard deviation and sensitivity as estimated from the regression is a correct estimation of the expected value for the square root of the ratio between the residual standard deviation and the real population sensitivity (equation 3). The uncertainty of the estimation of the MTD may be expressed e.g. as a confidence interval. For this, it is necessary to derive the expression that describes the estimation of the standard deviation for MTD. This expression is currently not available.

One of the most significant drawbacks of current safety limits is that these have been derived from acute experiments. Those results are then extrapolated to long-term exposure. The currently derived strategy can also be used for the determination of toxicity in long-term experiments. With such experiments it will be possible to predict safety levels for long-term exposures.

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