Ultraviolet radiation-B-induced cataract in albino rats: maximum tolerable dose and ascorbate consumption

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ABSTRACT.

Purpose: To investigate the maximum tolerable dose (MTD) for cataract induced by ultraviolet radiation-B (UVB) in 7-week-old albino rats and to study the effect of UVB eye exposure on lens ascorbate content.

Methods: Fifty 7-week-old albino Sprague Dawley rats were unilaterally exposed in vivo to 300-nm UVB under anaesthesia, receiving 0, 0.25, 3.5, 4.3 and 4.9 kJ/m². The MTD was estimated based on lens forward light scattering measurements. Lens ascorbate content was determined in the processed lens using high performance liquid chromatography with UVR detection.

Results: Animals exposed to UVB doses ≥3.5 kJ/m² developed cortical cataracts. The MTD for avoidance of UVB-induced cataract was estimated to 3.01 kJ/m². UVB exposure decreased lens ascorbate concentration in the exposed lens in line with UVB dose, according to the models: $C = C_{NonCo} + C_{Co}e^{-kH}$ for exposed lenses; $C = C_{NonCo} + C_{Co}$ for non-exposed lenses, and $C_{d} = C_{Co}(e^{-kH} - 1)$. Parameters for consumable and non-consumable ascorbate were estimated to $C_{NonCo} = 0.04$ and $C_{Co} = 0.11 \text{ mmol/g wet weight of lens}$. For lens ascorbate difference, $\tau = 1/k = 0.86 \text{ kJ/m}^2$. A total of 63% of UVB consumable ascorbate has been consumed after only $\tau = 0.86 \text{ kJ/m}^2$, indicating that ascorbate decrease is in the order of 3.5 times more sensitive to detecting UVR damage in the lens than forward light scattering.

Conclusions: The MTD for avoidance of UVB-induced cataract in the 7-week-old albino Sprague Dawley rat was estimated to be 3.01 kJ/m². In vivo UVB exposure of the rat eye decreases lens ascorbate content following an exponential decline, and suprathreshold doses cause greater effect than subthreshold doses.

Key words: ultraviolet radiation – maximum tolerable dose (MTD) – ascorbate – rat – cataract

Introduction

The purpose of the present paper was to determine the maximum tolerable dose (MTD) for avoidance of cataract induced by ultraviolet radiation-B (UVB) in 7-week-old albino rats. Further, the dependence of lens ascorbate concentration on UVR dose after in vivo exposure to UVR was investigated.

A large amount of epidemiological data supports an association between exposure to UVB and development of cortical cataract (McCarty & Taylor 2002). Experimental studies on animals, including rats, mice and rabbits, link UVB exposure to development of cortical cataract (Pitts et al. 1977; Jose & Pitts 1985; Söderberg 1988, 1990; Hightower & McCready 1993; Wegener 1994; Michael et al. 1996).

Acute development of cataract after in vivo exposure to UVB (Söderberg 1988) is secondary to a sodium potassium shift resulting in lens swelling (Söderberg 1991). The rat has a maximum sensitivity of the lens to UVR in vivo of around 300 nm (Merriam et al. 2000).

The maximum tolerable dose (Appendix I) for avoidance of UVB-induced cataract is a recently developed threshold dose concept for continuous dose-response relationships that provides a statistically well defined estimate of the threshold dose.
for avoidance of toxicity (Söderberg et al. 2002).

Ascorbate

Ascorbic acid, or vitamin C, has two ionizable – OH groups. At physiological pH, one electron and one hydrogen ion is removed under formation of ascorbate. We therefore use the name ascorbate throughout. Ascorbate is essential for collagen synthesis but is also of particular interest because of its role as an antioxidant in a number of tissues, including the lens (Halliwell & Gutteridge 1999). By functioning as a water-soluble antioxidant, ascorbate is capable of preventing oxidative damage to protein, lipid and DNA in a number of tissues, including the lens (Sies et al. 1992; Reddy et al. 1998). Ascorbate protects against eye damage secondary to a number of oxidative causes, including UVR. In tissue culture experiments, ascorbate was found to thwart photoperoxidation of lens lipids (Varma et al. 1982). Further, in in vitro studies, ascorbate conferred protection against UVB inactivation of rat lens enzymes, including the glycolytic pathway enzyme hexokinase, the pentose phosphate shunt enzyme glucose-6-phosphate dehydrogenase, and the action pump Na/K adenosine triphosphatase (ATPase) (Tung et al. 1988; Reddy & Bhat 1999).

In vivo, ascorbate has been found to protect the lens against oxidatively induced cataract induced by selenite in rats (Devamanoharan et al. 1991). Recently, it has also been shown to protect against cataract in aldose-reductase deficient mice in vitro in the xanthine oxidase model (Hegde & Varma 2004). Further, physiological levels of combined antioxidants including ascorbate have been found to increase the viability of UVR-exposed cultured human lens epithelial cells and maintain transparency of rat lenses in vitro exposed to UVR (Sasaki et al. 2000).

There is evidence that oral intake of ascorbate protects against cataract formation in the human lens (Leske et al. 1991; Robertson et al. 1991; Jacques et al. 1997). Further, lenses with increasing degrees of cataract and browning secondary to protein oxidation have been found to be associated with lower ascorbate content (Tessier et al. 1998).

Materials and Methods

Cataract was induced experimentally in rats, with 300-nm UVR. Thereafter, the MTD for avoidance of cataract (Söderberg et al. 2002) was estimated. Further, the content of lens ascorbate concentration was measured with HPLC.

Animals

Fifty 7-week-old female albino Sprague-Dawley rats (B&K Universal AB, Sollentuna, Sweden) were used in the experiment. Three rats died during the course of the experiment. Ethical approval was obtained from the Northern Stockholm Animal Experiments Ethics Committee. The animals were kept and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

UVR exposure

The 50 rats were divided into five dosage groups of 10 rats each. The rats were anaesthetized with an intraperitoneal injection of xylazine (14 mg/kg) and ketamine (94 mg/kg) 10 mins prior to exposure. Both eyes were dilated with 1% tropicamide 5 mins prior to exposure. The rats were unilaterally exposed to UVR at around 300 nm (maximum at 302.6 nm with 8.0 nm full bandwidth at half maximum). The rats in the first group (zero dose group) were sham irradiated. The other groups received doses of 0.25, 3.5, 4.3 and 4.9 kJ/m². The UVR was generated by a high-pressure mercury arc source equipped with a water filter and a double monochromator. The rats were killed 1 week after exposure in order to allow maximum intensity of light scattering to develop (Söderberg 1990; Michael et al. 1996). Both eyes were enucleated and the isolated lenses were transferred to a cuvette containing a balanced salt solution (BSS; Alcon, Fort Worth, Texas, USA). The degree of cataract was quantified by measurement of the intensity of lens forward light scattering. The lenses were then saved for ascorbate measurement.

UVR with UVR detection

Ascorbate was separated on an ion-exchange, reversed phase column, 220 mm × 4.6 mm, 10 µm (Polypropylene H, Applied Biosystems, Foster City, California, USA) using 2 mM sulphuric acid, pH 2.4, as the mobile phase. A 20 µl ultrafiltrate sample was injected by a refrigerated autoinjector set at 6° (CMA/200 Microsampler; CMA/Microdialysis AB, Stockholm,
Experimental design and statistics

For MTD estimation, the 50 rats were divided into five dosage groups of 10 rats each. Each rat was exposed to UVR on one side while the contralateral side served as a non-exposed control.

Forward light scattering was measured three times in each lens. For each rat, three differences between the exposed and contralateral non-exposed lenses were calculated. The average difference in light scattering was used as the observation. All observations were analysed with linear regression according to the model presented in Appendix 1 (Fig. 6).

Ascorbate concentration was measured once in each lens.

The significance levels and confidence coefficients were set at 0.05 and 0.95, respectively.

Results

MTD estimation

Lenses exposed to 0 kJ/m² or 0.25 kJ/m² UVR developed no cataract (Fig. 1).

Lenses exposed to a UVB dose ≥ 3.5 kJ/m² developed significant anterior subcapsular and equatorial cortical opacities (Fig. 1).

The difference in intensity of light scattering between exposed and contralateral non-exposed lenses increased with increasing doses of UVR (Fig. 2). The data were fitted with linear regression to a second order polynomial omitting the zero order term (Appendix 1). The MTD (Söderberg et al. 2002) was estimated according to Appendix 1.

The sensitivity, , was estimated at 9.20 ± 0.19 × 10⁻² tEDC/(kJ/m²)² and the residual standard deviation was estimated at 9.20 × 10⁻² tEDC. The MTD2.3:16 was therefore estimated at 3.01 kJ/m² (n = 46).

UVR-induced ascorbate oxidation

There was no change of ascorbate in UVR-induced ascorbate oxidation contralateral non-exposed lenses within the interval of UVR doses studied (Fig. 3).

This change of ascorbate concentration in the contralateral lens was fitted with non-linear regression (Appendix 3, equation 6), considering the rate k (m²/kg) (for each animal, were fitted with non-linear regression (Appendix 3, equation 6), considering the rate k (m²/kg)).

Fig. 2. Difference in forward light scattering between exposed and contralateral non-exposed lenses as a function of UVB dose. The solid line represents the best least square fit according to the model indicated. The vertical line illustrates MTD2.3:16.
was estimated to 0.86 kJ/m², indicating that after a dose of 0.86 kJ/m² of 300-nm UVR, 63% of the consumable ascorbate is lost.

UVB exposure decreased lens ascorbate concentration in the exposed lens (Fig. 5).

The data were fitted to the model outlined in Appendix 3, equation 2 with non-linear regression. The consumable concentration of ascorbate, \( C_{Ca} \), was estimated to 0.11 \( \mu \text{mol/g} \) wet weight, the non-consumable concentration of ascorbate, \( C_{NonCa} \), to 0.04 \( \mu \text{mol/g} \) wet weight and the inverse of the rate constant to 0.60 kJ/m².

**Discussion**

In the present study, we used MTD strategy (Söderberg et al. 2002) for *in vivo* estimation of threshold for 300-nm UVR toxicity in the rat lens. We further studied the lenticular ascorbate content as a function of *in vivo* exposure to 300-nm UVR.

The resulting MTD\( _{2.3:16} \) of 3.01 kJ/m² (Fig. 2) agrees with a previously published value (Söderberg et al. 2002) of 3.65 kJ/m² for MTD for a 6-week-old albino rat. The above estimates of MTD are both close to the qualitatively estimated threshold limit for permanent lenticular damage of 5.0 kJ/m² in the pigmented rabbit, previously published by Pitts et al. (1977) and based on a binary dose-response model.

The currently estimated baseline ascorbate content in the exposed lenses of 0.15 \( \mu \text{mol/g} \) wet weight of lens at zero UVB dose (Fig. 5), 0.11 \( \mu \text{mol/g} \) wet weight lens consumable and 0.04 \( \mu \text{mol/g} \) wet weight lens non-consumable, is consistent with the baseline concentration in the rat of 0.16 \( \mu \text{mol/g} \) wet weight of lens obtained in a previous study (Mody et al. 2005b) conducted in our laboratory and the 0.08 \( \mu \text{mol/g} \) wet weight of lens reported by Reddy et al. (1998).

In previous studies, we found that dietary supplementation of ascorbate in the rat increases lens ascorbate concentration following a model of linear increase (Mody et al. 2005b). The finding is in contrast to findings in the guinea pig diurnal animal, in which the baseline lens ascorbate concentration is 0.51 \( \mu \text{mol/g} \) wet weight lens and lens concentration of ascorbate increases to saturation with increased drinking water supplementation (Mody et al. 2005a), as is supported by a previous study (Taylor et al. 1997).

The finding that UVB exposure decreases ascorbate in the lens (Figs 4 and 5) is supported by two reports. Firstly, the antioxidant concentrations of \( \alpha \)-tocopherol and \( \beta \)-carotene in the lens were found to decrease significantly in parallel with the decrease in ascorbate concentration after *in vitro* exposure to UVB (Reddy et al. 2001). Secondly, human lenses with increasing degrees of cataract associated with protein oxidation were found to be associated with low ascorbate content (Tessier et al. 1998).

The finding that in the *in vivo* unilaterally 300-nm UVR-exposed animal, the difference in lens ascorbate concentration between exposed and contralateral non-exposed lenses decreases towards a negative asymptote (Fig. 4) indicates that there is one pool of consumable ascorbate and another pool of non-consumable ascorbate. This is also demonstrated by the fact that the concentration of ascorbate in the exposed lenses decreases towards an asymptote (Fig. 5). There are at least three possible mechanisms for the loss of lens ascorbate secondary to exposure to UVR. Firstly, the consumable ascorbate may be directly oxidized by UVB (Reddy 1996). Alternatively, ascorbate may be consumed while serving its function as an antioxidant in the lens. Finally, consumable ascorbate may be lost by leakage from lens cells damaged by UVR.

The fact that 63% of the UVR-consumable ascorbate has been consumed after exposure of only 0.86 kJ/m² (Fig. 4), while the MTD\( _{2.3:16} \) is 3.01 kJ/m² (Fig. 2), indicates that the ascorbate decrease is in the order of 3.5 times more sensitive for detecting UVR damage in the lens than forward light scattering.
In conclusion, the MTD for avoidance of UVB-induced cataract in the 7-week-old albino Sprague Dawley rat was estimated to be 3.01 kJ/m². In vivo UVB exposure of the rat eye decreases lens ascorbate concentration with an exponentially declining decrease, with a suprathereshold dose having a greater effect than a subthreshold dose.

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References

Appendix 1

Maximum tolerable dose

It is known from previous work (Michael, Söderberg and Chen; 1998) that the dose-response function for in vivo UVR cataract, expressed as the difference of intensity of forward light scattering between exposed and contralateral non-exposed eye, \( I_d \) as a function of UVR-300 nm dose, \( H_e \), can be approximated to a 2nd order polynomial, omitting the first order term (Söderberg, Löfgren, Ayala, Dong, Kakar and Mody; 2002). Therefore, experimental data can be analyzed with linear regression assuming the model

**Appendix 1, equation 1**

\[ I_d = k H_e^2 + \varepsilon \]

Here, \( k \) is a proportionality constant that expresses the sensitivity in the dose-response relationship and \( \varepsilon \) expresses the random measurement error that belongs to a normal distribution, \( N(0, \sigma) \).

The MTD\(_{2.3:16}\) is then defined as the dose corresponding to the cross over between 2 standard deviations above no difference of light scattering at zero dose, and 1 standard deviation above the dose-response curve (Appendix 1, Fig. 6).

In the figure it is seen that

**Appendix 1, equation 2**

\[ 2\sigma = k (MTD_{2.3:16})^2 + \sigma \]

or

**Appendix 1, equation 3**

\[ MTD_{2.3:16} = \sqrt{\frac{\sigma}{k}} \]

The interpretation of MTD\(_{2.3:16}\) is that there is a 16% probability that an individual exposed to UVR at MTD will have a difference of intensity of forward light scattering between the exposed and the non-exposed contralateral lens exceeding the level found in 97.7% of eyes from individuals that have not been exposed to UVR.

Appendix 2

Analysis of variance of contrast of ascorbate concentration in contralateral non-exposed lenses to 300-nm UVR

A measurement of lens ascorbate concentration, \( x_{ij} \), and it is assumed that there is no change in consumable ascorbate in the contralateral non-exposed lens, the ascorbate consumption in the non-exposed lens is given by:

**Appendix 3, equation 4**

\[ C = C_{NamCo} + C_{Co}e^{-4H_L} \]

or:

\[ C_{NamCo} = C - C_{Co}e^{-4H_L} \]

If one lens in an animal is exposed to UVR and the contralateral lens is non-exposed and it is assumed that there is no change in consumable ascorbate in the contralateral non-exposed lens, the ascorbate consumption in the non-exposed lens is given by:

**Appendix 3, equation 5**

\[ C = C_{NamCo} + C_{Co} \]

Then, the difference in ascorbate \( C_d \) (\( \mu \text{mol/g} \) wet weight of lens) as a function of dose \( H_L \) (\( \text{kJ/m}^2 \)) is given by:

**Appendix 3, equation 6**

\[ C_d = C_{Co}(e^{-4H_L} - 1) \]