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/m^2. 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/m^2 developed cortical cataracts. The MTD for avoidance of UVB-induced cataract was estimated to 3.01 kJ/m^2. UVB exposure decreased lens ascorbate concentration in the exposed lens in line with UVB dose, H_e, according to the models: $C = C_{NonCo} + C_{Co}e^{-kH_e}$ for exposed lenses; $C = C_{NonCo} + C_{Co}$ for non-exposed lenses, and $C_d = C_{Co}(e^{-kH_e} - 1)$. Parameters for consumable and non-consumable ascorbate were estimated to $C_{NonCo} = 0.04$ and $C_{Co} = 0.11$ μmol/g wet weight of lens. For lens ascorbate difference, $\tau = 1/k = 0.86$ kJ/m^2. A total of 63% of UVB consumable ascorbate has been consumed after only $\tau = 0.86$ kJ/m^2, while $MTD_{2.3:16} = 3.01$ 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/m^2. *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

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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 1) 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).

Besides functioning as an antioxidant in the lens, ascorbate in the aqueous humour attenuates UVR by absorption and conversion of the UVR energy into heat or fluorescence, thereby reducing UVR damage (Ringvold 1995).

Therefore, ascorbate in both the aqueous humour and the lens is important for protection against UVR-induced damage. It has been shown that the concentration of lens ascorbate decreases significantly upon *in vitro* UVB irradiation of the lens (Reddy et al. 2001). In the current study, we set out to determine the effect of *in vivo* UVB exposure on lens ascorbate content. A number of techniques are used for ascorbate measurement, the most recent of which is high-performance liquid chromatography (HPLC), which, with electrochemical detection, has been applied to measure ascorbate in lens tissue from Emory mice (Taylor et al. 1995), guinea pigs and humans (Taylor et al. 1997). We used a method for lens sample processing and ascorbate measurement outlined in previous reports using the technique of HPLC with UVR detection (Mody et al. 2005a, 2005b).

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/m^2. 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.

Lens sample preparation

Each lens was homogenized in 1.0 ml of 0.25% metaphosphoric acid with a tissue grinder (Hallström et al. 1989). Metaphosphoric acid prevents oxidation of ascorbate to dehydroascorbate because of high acidity and metal ion chelation (Washko et al. 1992; Koshiishi et al. 1998). The homogenate was centrifuged at 12 000 ***g*** for 10 mins at 4° . Subsequently, 800 µl of supernatant was ultrafiltered through a 5 kDa molecular weight cut-off membrane (Ultrafree CL; Millipore AB, Sundbyberg, Sweden) using centrifugation at 3830 ***g*** for 1 hour at 4°.

HPLC with UVR detection

Ascorbate was separated on an ion-exchange, reversed phase column, 220 mm × 4.6 mm, 10 µm (Polypore 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,

Sweden) using a 15.4 µl sample loop. The mobile phase was delivered at 0.3 ml/min using an isocratic HPLC pump (CMA 250; CMA/Microdialysis AB, Stockholm, Sweden). The exploring beam of the detection module consisted of radiation from a deuterium lamp that was spectrally limited with a monochromator to 254 nm. The absorbance of the column exit was measured using a UVR detector (L7400; Lachrom Merck Hitachi, Darmstadt, Germany).

The calibration procedure for HPLC measurements consisted of running a 10 equi-distance level calibration curve for the measurement range intended. The absorbance was then obtained for two samples of 10-µM L-ascorbate (Merck, Darmstadt, Germany) diluted in 2.5% metaphosphoric acid and prepared from a stock solution. Finally, the absorbance of a 2.5% metaphosphoric acid-only blank was determined. Before each series, the average absorbance for the two samples of 10-µM L-ascorbate was used to set sensitivity and with this, ascorbate was calculated for each measured unknown sample considering a first order relationship between concentration of ascorbate and absorbance, omitting the zero order term.

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/m^2 or 0.25 kJ/m^2 UVB developed no cataract (Fig. 1).

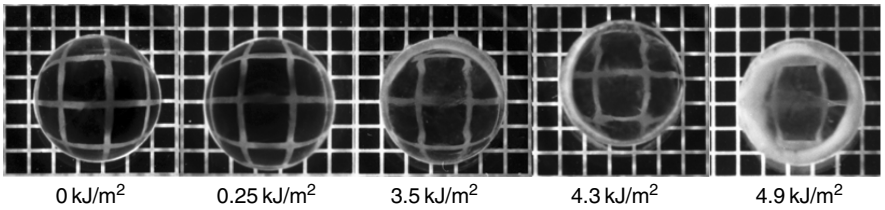

Fig. 1. Rat lenses exposed to UVB at 0, 0.25, 3.5, 4.3 and 4.9 kJ/m^2. The grid square diameter is 0.79.

Lenses exposed to a UVB dose $\geq$ 3.5 kJ/m^2 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, k, was estimated at $1.02 \pm 0.19 \times 10^{-2}$ tEDC/(kJ/m^2)^2 and the residual standard deviation was estimated at 9.20×10^{-2} tEDC. The $MTD_{2.3:16}$ was therefore estimated at 3.01 kJ/m^2 ($n = 46$).

UVR-induced ascorbate oxidation

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

This was confirmed (test statistic = 1.77, $F_{3;19;0.95} = 3.90$, $p = 0.15$) in an analysis of variance using a statistical software program (NCSS Statistical Software, Kaysville, Utah, USA) according to the model in Appendix 2, after excluding variation of variances among the different groups with Bartlett's test (test statistic = 5.23, $\chi^2_{0.05,4} = 9.49$, $p = 0.26$). The mean ascorbate concentration in the contralateral lenses was estimated as a 95% confidence interval to 0.149 ± 0.005 µmol/g wet weight of lens.

UVB exposure increased the difference in lens ascorbate concentration between the exposed lens and the contralateral non-exposed lens (Fig. 4).

As there was no change in ascorbate concentration in the contralateral lens upon unilateral exposure to 300-nm UVR, the model in Appendix 3 was developed. On the assumption that the lens contains one pool of UVR consumable ascorbate, C_{Co}, and another pool of non-consumable ascorbate, C_{NonCo}, the differences in total ascorbate content between exposed and contralateral lenses, C_d (µmol/g wet weight of lens), as a function of exposure to UVR, H_e (kJ/m^2), for each animal, were fitted with non-linear regression (Appendix 3, equation 6), considering the rate k (m^2/kJ). The consumable ascorbate was then estimated to 0.11 µmol/g wet weight of lens and the inverse of the rate constant

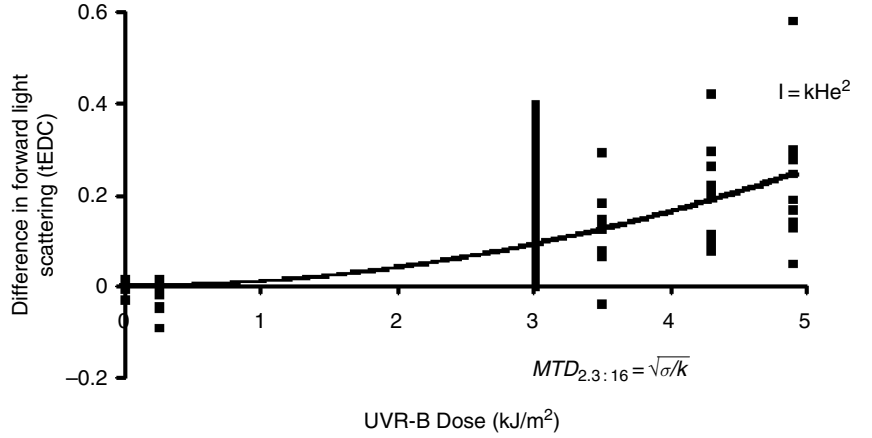

Fig. 2. Difference in forward light scattering between exposed and contralateral non-exposed lenses as a function of UVB dose. The solid line represents the best least square fit according to the model indicated. The vertical line illustrates $MTD_{2.3;\ 16}$.

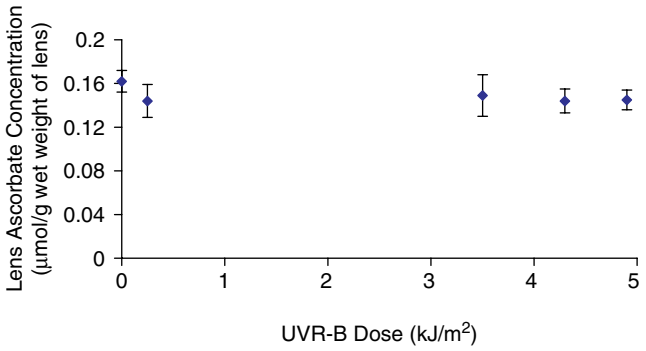

Fig. 3. Lens ascorbate concentration (μmol/g wet weight of lens) in contralateral non-exposed lenses versus UVB dose (kJ/m^2). The bar represents the 95% CI for the mean.

was estimated to 0.86 kJ/m^2, indicating that after a dose of 0.86 kJ/m^2 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_{Co}, was estimated to 0.11 μmol/g wet weight, the non-consumable concentration of ascorbate, C_{NonCo}, to 0.04 μmol/g wet weight and the inverse of the rate constant to 0.60 kJ/m^2.

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/m^2 (Fig. 2) agrees with a previously published value (Söderberg et al. 2002) of 3.65 kJ/m^2 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/m^2 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 μmol/g wet weight of lens at zero UVB dose (Fig. 5), 0.11 μmol/g wet weight lens consumable and 0.04 μmol/g wet weight lens non-consumable, is consistent with the baseline concentration in the rat of 0.16 μmol/g wet weight of lens obtained in a previous study (Mody et al. 2005b) conducted in our laboratory and the 0.08 μmol/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 μmol/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 α-tocopherol and β-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/m^2 (Fig. 4), while the $MTD_{2.3:16}$ is 3.01 kJ/m^2 (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.

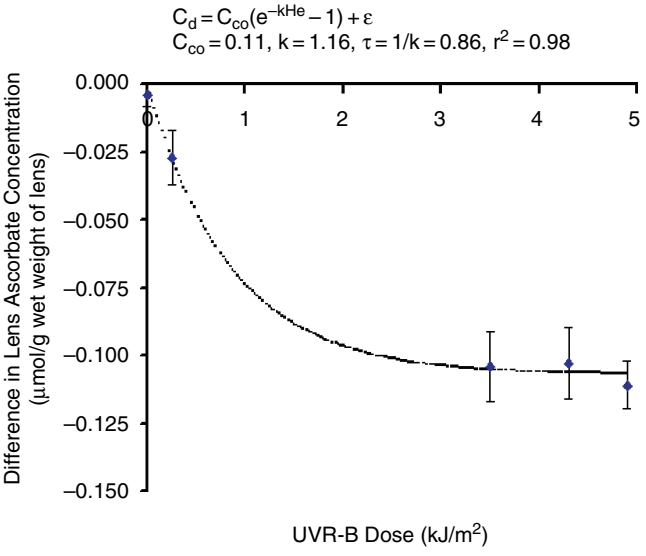

Fig. 4. The difference in lens ascorbate concentration between UVB-exposed and non-exposed lenses. The bar represents the 95% CI for the mean.

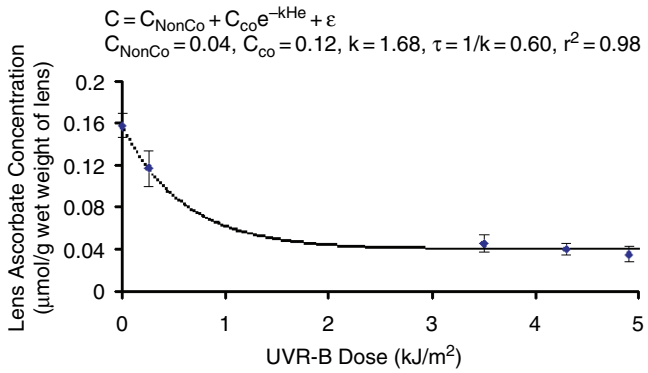

Fig. 5. Lens ascorbate concentration in exposed lenses versus UVB dose. The bar represents the 95% CI for the mean.

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/m^2. *In vivo* UVB exposure of the rat eye decreases lens ascorbate concentration with an exponentially declining decrease, with a suprathreshold dose having a greater effect than a subthreshold dose.

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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

$$I_d = kH_e^2 + \varepsilon$$

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

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},

Appendix 2, equation 1

$$x_{ij} = \mu + \alpha_i + B_{j(i)} + \varepsilon_{j(i)}$$

is the sum of the population mean, μ, a term for the variation of doses, a_i (i = 1..4), a term for the variation of individuals, $B_{j(i)}$ (j = 1..20), and the measurement error, $e_{j(i)}$.

Appendix 3

Kinetics of UVR-induced *in vivo* ascorbate oxidation

On the assumption that the total amount of ascorbate concentration in the lens, C (μmol/g wet weight of lens), can be divided into one pool of UVR non-consumable ascorbate, C_{NonCo} (μmol/g wet weight of lens) and another pool of UVR consumable ascorbate C_{Co} (μmol/g wet weight of lens) and that the consumable ascorbate is consumed with first order kinetics with the rate k (m^2/kJ), the concentration of ascorbate at increasing doses of UVR decreases with an exponential decline towards the concentration of non-consumable ascorbate:

Appendix 3, equation 2

$$C = C_{NonCo} + C_{Co}e^{-kH_e}$$

or:

$$C_{NonCo} = C - C_{Co}e_e^{-kH}$$

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 4

$$C = C_{NonCo} + C_{Co}$$

Then, the difference in ascorbate C_d (μmol/g wet weight of lens) as a function of dose H_e (kJ/m^2) is given by:

Appendix 3, equation 5

$$C_d = (C_{NonCo} + C_{Co}e_e^{-kH}) - (C_{NonCo} + C_{Co})$$

or simplified:

Appendix 3, equation 6

$$C_d = C_{Co}(e^{-kH_e} - 1)$$

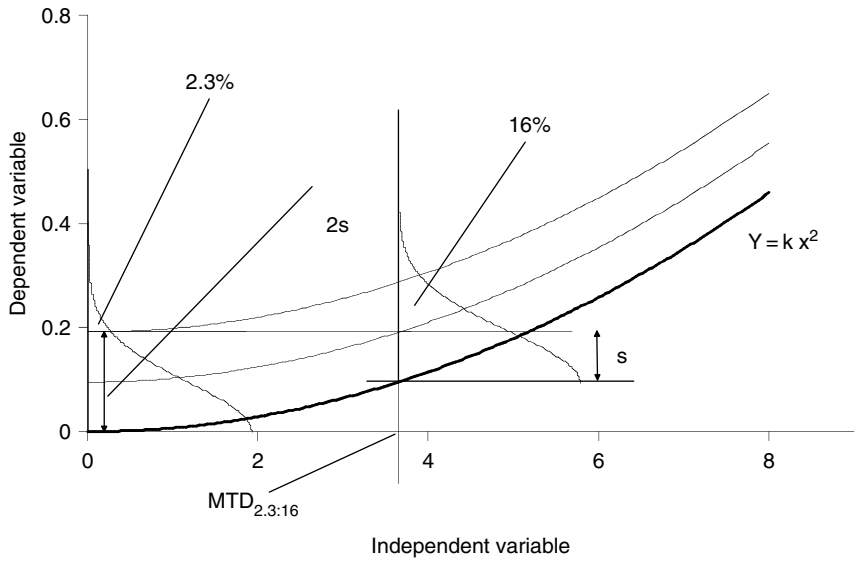

Fig. 6. The MTD concept.