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Dose-response relationship for α-tocopherol prevention of ultraviolet radiation induced cataract in rat

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ABSTRACT

The purpose of this study is to establish the dose response relationship for α-tocopherol protection of ultraviolet radiation (UVR) induced cataract in the rat. Four groups of 20 six-week-old albino Sprague Dawley rats received 5, 25, 50, and 100 IU/day α-tocopherol, whilst another group of 20 rats without any α-tocopherol feeding was the control group. After 4 weeks of feeding, each rat was unilaterally exposed to 8 kJ/m^2 UVR-300 nm for 15 min. At 1 week after exposure, the rats were sacrificed and lens light scattering was measured quantitatively. Lens total reduced (GSH) and oxidized (GSSG) glutathione; glutathione reductase (GR) and peroxidase (GPx) were determined spectrophotometrically. The UVR-exposed lenses in the α-tocopherol fed groups developed superficial cataract, whereas lenses in the control group developed cortical and equatorial opacities. Light scattering in lenses from the α-tocopherol-supplemented rats was lower than in lenses from the control group. The difference of light scattering between the exposed and contralateral non-exposed lens decreased with increasing doses of α-tocopherol to an asymptote level. UVR-exposure caused a significant depletion of lens GSH in rats without or at low α-tocopherol supplementation. The depletion of GSH became less with higher α-tocopherol supplementation. There was no detectable difference in lens GSSG, GR or GPx at any level of α-tocopherol supplementation. Orally administered α-tocopherol dose dependently protects against UVR-induced cataract. The protection is associated with an α-tocopherol dose-dependent GSH depletion secondary to UVR exposure. UVR-induced light scattering only occurs if the GSH depletion exceeds a threshold.

1. Introduction

In this project, we aimed to determine the dose response relationship for α-tocopherol protection of in vivo ultraviolet radiation (UVR) induced cataract.

Cataract is the major cause of blindness in the world (West, 2000; Thylefors, 2001). The increasing size and age of the world population is predicted to cause an escalating health economic burden of cataract management, particularly in the developing countries where cataract occurs at an earlier age and cataract surgery is often inaccessible (Brian and Taylor, 2001). In a world perspective, it is therefore important to find a method that prevents or delays the onset of cataract.

Cataract is known to be associated with hereditary factors, metabolic disorders and exposure to ultraviolet radiation (UVR) (McCarty et al., 2000). In recent years, the pollution-related depletion of the atmospheric ozone layer has increased the penetration of UVR to the earth (UNEP, 1998). Clinical and epidemiological studies have demonstrated that solar UVR is the most important avoidable risk factor for human cataract development (Zigman et al., 1979; Taylor et al., 1988; Cruickshanks et al., 1992; West et al., 1998).

UVR causes cataract through oxidative damage. UVR-induced reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide lead to DNA destruction and activation of protein kinases (Nishi et al., 1991). Oxidative damage to lens proteins secondary to UVR exposure has been postulated as one of the mechanisms for cataract formation (Spector et al., 1995; Taylor et al., 1995). Cells have developed an antioxidant defense system against ROS and their destruction. Antioxidants act either by preventing ROS production or eliminating them.

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Glutathione in the reduced form (GSH) is the most important non-enzymatic antioxidant in the lens (Clark, 1994). GSH plays a vital role in defending against exogenous and endogenous ROS and keeps lens protein in a reduced state. A dynamic balance is maintained between GSH synthesis, its recycling from the oxidized form (GSSG), and its utilization. The amount of GSH in the lens diminishes with aging and oxidative stress. Several previous studies have demonstrated that UVR exposure results in a depletion of the GSH concentration in lenses of rabbits (Hightower and McCready, 1992) and rats (Risa et al., 2004, 2005; Tessem et al., 2006; Wang et al., 2010). Hightower exposed rabbit lenses in vitro to UVR-315 nm, but the dose of UVR was not provided. The other studies were in vivo exposures of rats to 2–15 kJ/m^2 of UVR-300 nm. Ayala and co-worker did not find any significant depletion of GSH after in vivo UVR exposure of rats (Ayala and Söderberg, 2004), but this may have been due to insufficient resolution in the GSH measurement. Hightower showed a slight increase in lens GSSG, but argued that the increase was too small to account for the loss of GSH observed based on oxidation of GSH (Hightower and McCready, 1992). The in vivo exposures of rats did not demonstrate a detectable change of total lens GSSG concentration (Risa et al., 2004, 2005; Tessem et al., 2006; Wang et al., 2010).

Alpha-tocopherol (type V vitamin E) is an extrinsic antioxidant molecule with reducing action. It is an important dietary constituent. Alpha-tocopherol functions in vivo as a lipid antioxidant and as a free radical scavenger (Bieri et al., 1983; Burton et al., 1983). It exerts its prevention of lipid peroxidation on biological membranes in vivo by functioning as a chain-breaking antioxidant and helping to maintain glutathione level (Shang et al., 2003; Kutlu et al., 2005). Moreover, as an extrinsic antioxidant molecule with reducing power, α-tocopherol may indirectly prevent the consumption of GSH through oxidation of α-tocopherol, preventing the −SH groups on GSH from being oxidized. Additionally, α-tocopherol may directly stimulate GSH synthesis by up-regulating some of GSH-related enzymes in the lens, such as γ-glutamylcysteine synthetase and GSH synthetase (Seth and Kharb, 1999; Masaki et al., 2002).

The recommended daily allowance (RDA) for vitamin E is 12 IU for females and 15 IU for males (Halliwell and Gutteridge, 1985) (1.49 IU of vitamin E are equivalent to 1 mg α-tocopherol). Vitamin E has a low human toxicity and oral administration is not toxic even at high doses. An intake of 1000 mg/day is without risk and 3200 mg/day has been shown to be without any substantial risk (Diplock et al., 1998). The upper tolerance level (UL) for α-tocopherol was set at 1000 mg/day using data from studies in rats (Food and Nutrition Board and Institute of Medicine, 2000). In addition, studies have suggested that supplementation with at least 100 mg/day of vitamin E may decrease the risk of heart disease (Stampfer and Rimm, 1995). This is well above the current RDA and is far greater than what can be received with a well balanced diet. It has been postulated that vitamin E protects against photoperoxidation of lens lipids (Varma et al., 1982; Libondi et al., 1985; Robertson et al., 1989; Ohta et al., 1996; Karslioglu et al., 2004). The pioneering work done by Bhuyan shows that vitamin E is effective in the therapy of certain forms of cataract in the rabbit and the rat (Bhuyan et al., 1981). The outcome of clinical trials aiming for cataract prevention by vitamin E supplementation is inconclusive. Some studies find a beneficial effect (Rouhiainen et al., 1996; Leske et al., 1998; Lyle et al., 1999; Mares-Perlman et al., 2000), whereas others indicate no significant association (Hankinson et al., 1992; Seddon et al., 1994; Chasan-Taber et al., 1999).

Recent studies from our research group have demonstrated that per oral supplementation with α-tocopherol increases lens α-tocopherol and protects against in vivo UVR-induced cataract in rat (Ayala and Söderberg, 2004, 2005).

The aim of the present study was to determine the dose dependence for α-tocopherol prevention of in vivo UVR-induced cataract in rat lens. Further, the impact of α-tocopherol supplementation on the lens concentration of GSH, GSSG and the activities of glutathione reductase (GR) and glutathione peroxidase (GPx) was to be determined.

2. Materials and methods

2.1. Experimental animal

The albino Sprague Dawley rat (female, six-week-old) was the experimental animal. The animals were kept and treated according to the Association for Research in Vision and Ophthalmology Statement for the use of Animals in Ophthalmic and Vision Research. Ethical approval was obtained from the Northern Stockholm Animal Experiments Ethics Committee. Ethical permission: protocol number 227/03.

2.2. Alpha-tocopherol administration

Each rat in the experimental groups was, depending on group belonging, fed with 5, 25, 50 or 100 IU/day α-tocopherol (diluted in corn oil) from vegetable (T3634-25G; Sigma, Stockholm, Sweden), respectively. Each rat in the control group was fed corn oil only without vitamin E activity (Eldorado, Stockholm, Sweden). The treatment was administrated by intragastric intubation with a rubber catheter (Ayala and Söderberg, 2004).

2.3. Ultraviolet radiation

2.3.1. UVR source

UVR in the 300 nm wavelength region (UVR-B) was generated with a high-pressure mercury lamp, collimated, passed through a water filter and a double monochromator. The emerging radiation, centered at 300 nm with a full width half maximum of 10 nm (Wang et al., 2010) was projected to a spot on the cornea of the exposed eye (Michael, 2000). The intensity of UVR was measured with a thermopile (model 7101; Oriel, Stratford CT) that had been calibrated to a NIST (National Institute of Standard and Technology, USA) traceable source.

2.3.2. Exposure to ultraviolet radiation

The rats began to receive α-tocopherol treatment at the age of 2 weeks. After 4 weeks of intragastric feeding, the six-week-old rats were unilaterally exposed to UVR. Ten minutes preceding the exposure, the animal was anesthetized with a mixture of 94 mg/kg ketamine and 14 mg/kg xylazine injected intraperitoneally. Five minutes preceding the exposure, one drop of tropicamide was instilled into both eyes for pupillary dilation. One eye in each rat was exposed to 8 kJ/m^2 UVR-300 nm (delivered during 15 min). The animal was then returned to the cage at the end of each UVR treatment.

2.4. Lens dissection

At 1 week after exposure to UVR, each rat was sacrificed by carbon dioxide asphyxiation, followed by cervical dislocation, and then both eyes were enucleated. In each eye, the lens was extracted and placed in balanced salt solution. Vestiges of the ciliary body were removed from the lens equator under a microscope.

2.5. Macroscopic imaging

The macroscopic appearance of the lens was documented with digital photography in incident illumination against a dark

background. During photography, the anterior surface of the lens faced the camera.

2.6. Measurement of intensity of forward light scattering

The intensity of forward light scattering was measured with a light dissemination meter (Söderberg et al., 1990). This instrument uses the principal 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 of the detector. If the object scatters light in the forward direction, a defined fraction of the scattered light is collected by the objective and projected on a photodiode that produces a current proportional to the illumination. Each current reading was calibrated to a commercially available lipid emulsion of Diazepam (Stesolid Novum, Dumex-Alphapharma, Denmark). Light scattering was therefore expressed in Equivalent Diazepam Concentration (EDC). In order to assure normal distribution, the readings were log transformed (tEDC) (Söderberg et al., 1990).

2.7. Biochemical analysis

Altogether 50 lenses from 25 rats were randomly selected for GSH/GSSG and GR/GPx determination with a spectrophotometer, based on the procedures described recently (Wang et al., 2010). In short, immediately after the forward light scattering measurement, each lens was weighed and frozen at −80 °C. Thereafter, the whole lens was homogenized in 500 μl of ice-cold lysis buffer (Wang et al., 2010) using a glass-to-glass homogenizer (Duall; Kontes Glass Co., Vineland, NJ) and centrifuged at 15000 g at 4 °C for 5 min. The sample supernatant was collected in a vial. Then, 45 μl of the supernatant was transferred to a new vial for later analysis of protein concentration (BCA Protein Assay, Reagent Kit 23225, Pierce Inc., Rockford, IL, USA). The remaining supernatant was used for GSH, GSSG measurement and determination of GR and GPx activity. All measurements were run in triplicates and normalized to water soluble (w.s.) protein concentration.

2.8. Experimental design

Altogether, 100 rats were divided into five groups of 20. Rats in one group were fed without any supplementation as control, while the other four groups of rats were supplemented with increasing doses of α-tocopherol 5, 25, 50 and 100 IU/day (approximately equivalent to vitamin E 20, 100, 200 and 400 mg/kg). After 4 weeks of special feeding, one eye in each rat was exposed to UVR, while the contralateral eye was left non-exposed. At 1 week after exposure to UVR, each lens was photographed, and the intensity of forward light scattering was measured three times for each lens. Further, the concentrations of GSH, GSSG, and the activity of GR, GPx were measured three times for each lens (both exposed and contralateral non-exposed lens) in five animals from each subgroup.

2.9. Statistical parameters

The mean of paired differences between UVR-exposed and contralateral non-exposed lenses were calculated for the statistical analyses. The significance level and the confidence coefficient was set to 0.05 and 0.95, respectively, considering the sample size and the expected contrasts.

3. Results

3.1. Macroscopic appearance

Photographs of in vivo UVR-exposed and contralateral non-exposed lenses are shown in Fig. 1.

The UVR-exposed lenses from groups that received 25 IU/day or higher α-tocopherol supplementation developed superficial and slight equatorial opacities. Cortical and equatorial cataract was found in rats that were fed with 5 IU/day α-tocopherol. The UVR-exposed lenses in the control group without any α-tocopherol supplementation developed dense cortical and equatorial opacities. Vacuoles were seen in all exposed lenses. No group showed nuclear cataract. All lenses from the non-exposed eyes expressed only barely visible light scattering with a smooth surface as seen with the naked eye.

3.2. Intensity of forward light scattering

Intensity of forward light scattering in UVR-exposed lenses was higher than in contralateral non-exposed lenses for all α-tocopherol dose levels (Fig. 2).

The difference of intensity of forward light scattering between the exposed lens and its contralateral decreased with increasing dosage of α-tocopherol supplementation (Fig. 2). The variance for different α-tocopherol levels are different as judged by Bartletts test (test statistic = 19.2, $\chi^2_{4;0.95} = 9.49$). Therefore, orthogonal tests of contrasts were applied. The orthogonal tests for contrasts indicated a quick drop of light scattering difference between exposed lens and its contralateral non-exposed lens with increasing tocopherol dose (Table 1).

The drop of light scattering intensity related to the change of α-tocopherol dose seemed to be approximately directly proportional to the level of light scattering difference (Fig. 2). Therefore, the light scattering difference as a function of α-tocopherol dose was fitted to an exponential model (Appendix. Eq. (1)). The average light scattering difference at zero dose of α-tocopherol was 0.32 tEDC. Oral supplementation with α-tocopherol provided a dose-dependent protection against UVR-induced light scattering with a rate constant (1/k) of 4.8 IU/day and an asymptote light scattering difference of 0.22 tEDC (Fig. 2).

3.3. GSH concentration

The exposure to UVR caused a depletion of total lens GSH at low α-tocopherol supplementation that recovered towards higher α-tocopherol supplementation (Fig. 3).

The variance for different α-tocopherol levels are different as judged by Bartletts test (test statistic = 12.3, $\chi^2_{4;0.95} = 9.49$).

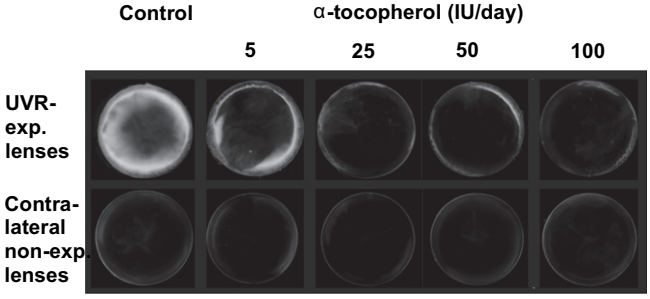

Fig. 1. Photographic images of the anterior surface of the lenses from animals without any a-tocopherol supplementation (control) and lenses from animals with a-tocopherol supplementation at various doses, non-exposed and exposed to UVR in vivo.

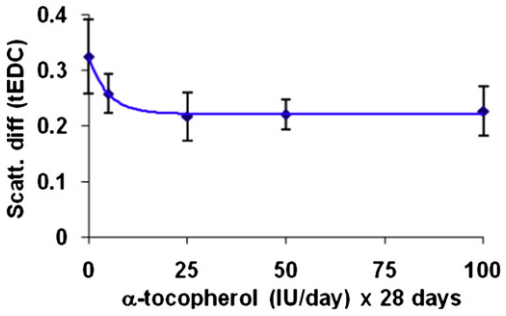

Fig. 2. Difference of light scattering between UVR-exposed and contralateral non-exposed lens as a function of perorally administered a-tocopherol dose. Bars are 95% confidence intervals for the mean ($n = 20$). tEDC refers to Log transformed Equivalent Diazepam Concentration.

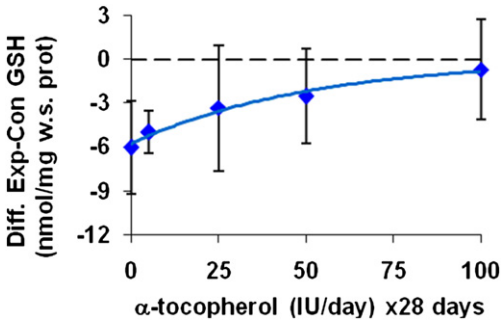

Fig. 3. Difference of GSH concentration (nmol/mg w.s. protein) between UVR-exposed and contralateral non-exposed lens as a function of perorally administered a-tocopherol dose. Bars are 95% confidence intervals for the mean ($n = 5$).

Therefore, orthogonal tests of contrasts were applied. The orthogonal tests for contrasts indicated a quick decrease of the GSH depletion with increasing α-tocopherol dose (Table 2).

The decrease of GSH difference with increasing α-tocopherol dose seemed to be approximately directly proportional to the level of GSH concentration (Fig. 3). The GSH concentration difference as a function of α-tocopherol dose was fitted to an exponential model (Appendix. Eq. (2)). The average GSH difference at zero dose of α-tocopherol was 6.05 nmol/mg w.s. protein. Oral supplementation with α-tocopherol provided a dose-dependent protection against GSH loss with a rate constant (1/k) of 52 IU/day and an asymptote GSH loss of 3.33 nmol/mg w.s. protein (Fig. 3).

The average GSH concentration in the non-exposed lenses from the rats without any α-tocopherol supplementation was 24.31 nmol/mg w.s. protein. This correlates with early findings (Giblin et al., 1976; Devamanoharan et al., 1998; Lou, 2000; Ayala and Söderberg, 2004).

3.4. GSSG concentration, GR and GPx activity

The UVR exposure did not cause any detectable alteration of total lens GSSG, GR or GPx activity at any level of α-tocopherol supplementation.

4. Discussion

In this study, we have demonstrated in the Sprague-Dawley rat that α-tocopherol orally supplemented has a dose dependent preventive effect on in vivo UVR-induced cataract.

The range of doses of α-tocopherol was selected based on a previous experiment demonstrating that oral supplementation of 100 IU/day α-tocopherol for 4 weeks, prior to 8 kJ/m^2 UVR-300 nm exposure, reduced the light scattering in rat lens (Ayala and Söderberg, 2004, 2005). The dose of UVR corresponds approximately to double threshold dose in the Sprague–Dawley rat (Söderberg et al., 2002). The exposure of the human eye to UVR from the sun very strongly depends on the spectral band, the hour of the day, the atmospheric conditions, and the background reflections (Sliney, 1986). The intensity here used, 8.3 W/m^2 is approximately 16 times the intensity of UVR-B reaching the cornea in sunlight, 0.5 W/m^2 (Zigman, 1995). However, since the effect of UVR on the lens is known to be photochemical, it is more elucidating to compare the dose of UVR received. The dose of UVR here used can be estimated to approximately 5 times higher than the dose a human eye would receive for a corresponding spectral band on a sunny day (Sliney, 1986).

The macroscopic appearance of the lenses (Fig. 1) were consistent with previous studies on short delay onset of light scattering in the UVR-exposed lenses from rats with or without vitamin E treatment (Ayala and Söderberg, 2004, 2005). The large variation of light scattering expressed after exposure to UVR as indicated by the uncertainty of the confidence intervals in Fig. 2 is due to a real variation among animals, the measurement error being insignificant in comparison (Söderberg et al., 1990).

In addition to increased light scattering of the rat lens, corneal irregularities were macroscopically observed when the animals were sacrificed at 1 week after the exposure, but not immediately after exposure. However, no quantitative data was collected on corneal light scattering.

It was previously shown that perorally supplemented α-tocopherol at 100 IU/day for 4 weeks reduces the sensitivity to in vivo UVR exposure (Ayala and Söderberg, 2004). In the present study, the data shows that the reduced sensitivity, dose dependently relates to the α-tocopherol orally supplemented (Figs. 1 and 2, Table 1) and the rate constant for α-tocopherol protection (1/k) was estimated to 4.8 IU/day (Fig. 2). This information can be used in future studies for determination of the α-tocopherol dose needed for protection against in vivo UVR-induced cataract, e.g. the minimum dose of α-tocopherol to obtain maximum protection is 25 IU/day (Fig. 2). The currently used dose of 25 IU/day for the 6-week-old rat (150 g) is equivalent to 100 mg/kg α-tocopherol,

Table 1
Orthogonal comparison of difference of light scattering between UVR-exposed and contralateral non-exposed rat lens.

Contrast	I_u(0.95) (tEDC)	D.f
0 versus 5, 25, 50 and 100	0.09 ± 0.07[b]	24[a]
5 versus 25, 50 and 100	0.03 ± 0.04	78
25 versus 50 and 100	0.01 ± 0.05	58
50 versus 100	0.00 ± 0.05	34[a]

[a] Approximate estimation of t-value due to difference of variances.
[b] Confidence interval does not include zero indicating a statistically significant difference.

Table 2
Orthogonal comparison of difference of GSH concentration between UVR-exposed and contralateral non-exposed rat lens.

Contrast	I_u(0.95) (nmol GSH/mg w.s. protein)	D.f
0 versus 5, 25, 50 and 100	−3.16 ± 2.85[b]	23
5 versus 25, 50 and 100	−2.79 ± 1.96[b]	17[a]
25 versus 50 and 100	−1.71 ± 3.50	13
50 versus 100	−1.84 ± 3.92	8

[a] Approximate estimation of t-value due to difference of variances.
[b] Confidence interval does not include zero indicating a statistically significant difference.

which is more than 300 times of RDA's for a human (Food and Nutrition Board and Institute of Medicine, 2000). The dose dependent protective effect of α-tocopherol observed indicates that oxidation of membranes may be one of the key mechanisms of in vivo UVR-induced cataract. Our observation is consistent with earlier studies on oxidation induced cataract showing that vitamin E derivatives are protective (Creighton et al., 1985; Varma et al., 1994; Ohta et al., 1996; Wegener et al., 2002).

In addition to the known antioxidant effects of α-tocopherol, there is a possibility that the protection of α-tocopherol presently observed is due to increased absorption of UVR in the cornea, aqueous humor and the lens, since there is some absorption of UVR in the 300 nm wavelength region in the α-tocopherol molecule (Kagan et al., 1992).

Our current finding that GSH is depleted by in vivo exposure to UVR (Fig. 3, Table 2) is consistent with previous studies (Hightower and McCready, 1992; Risa et al., 2004, 2005; Tessem et al., 2006; Wang et al., 2010). The current study demonstrated an average loss of 25% GSH concentration (Fig. 3) at 7 days after exposure to 8 kJ/m^2 UVR-300 nm. The average loss found by us previously after the same exposure was 14% (Wang et al., 2010). However, in these both studies, there were considerable animal variations and statistical inference which did not reveal any statistically significant difference. Hightower and co-worker found a loss of 40% GSH in the cultured rabbit lenses (Hightower and McCready, 1992), indicating that the dose of UVR was much higher than the presently used just above threshold dose. Hightower's finding provided strong evidence that UVR-induced GSH depletion was a result of GSH leakage out of the lens due to membrane damage which is expected at very high doses (Söderberg, 1988). The fact that the GSH depletion was α-tocopherol dose dependent (Fig. 3, Table 2), less GSH depletion induced with higher α-tocopherol supplementation, is a strong indication that α-tocopherol exerts its effect by preventing oxidation in a dose-dependent manner. The reduction of GSH depletion suggests that α-tocopherol is capable of minimizing lipid damage from peroxidation caused by UVR exposure in lenticular membranes. It is also possible that α-tocopherol decreases GSH leakage out of the lens.

The finding that the asymptote light scattering (Fig. 2) is above zero shows that there is one or several additional factors involved in the UVR-induced light scattering that cannot be altered by α-tocopherol. The difference between the average light scattering difference at zero dose of α-tocopherol (0.32 tEDC) and the asymptote level of light scattering difference (0.22 tEDC) provides information on the fraction of light scattering induced that can be protected by α-tocopherol and was estimated to 30%. Presumably, the protective effect of α-tocopherol is primarily related to prevention of lipid peroxidation (Varma et al., 1982). Additional mechanisms for UVR-induced cataract may be photoxidation of proteins (Spector et al., 1995; Lou, 2003) and DNA-damage (Söderberg et al., 1986).

UVR-induced light scattering approaches an asymptote level at around 25 IU/day supplementation with α-tocopherol (Fig. 2) and simultaneously the loss of GSH concentration decreases asymptotically towards zero at higher supplementation (Fig. 3). This indicates that there is a threshold level of lens GSH for protection of lens clarity after exposure to UVR. If light scattering induced is plotted as function of UVR-induced loss of GSH (Fig. 4) it is seen that losses over a threshold of about 3 nmol GSH/mg w.s. protein is associated with increased light scattering in the lens.

There was no detectable change in total lens GSSG concentration at any level of α-tocopherol supplementation. This finding is consistent with the results of our recent study on evolution of light scattering and redox balance in the rat lens after in vivo exposure to just above threshold dose of UVR-300 nm (Wang et al., 2010). A possible reason for the lack of change of GSSG in the current experiment is that the change of GSSG was too small in relation to the bulk content of GSSG to be detected. It is also possible that some of the reduced GSH may have been protein linked to PSSG. Further, GSH may have leaked out the lens due to UVR-induced membrane damage as previously reported (Hightower and McCready, 1992; Hightower, 1995) although these papers refer to experiments probably exposed to much higher doses of UVR causing extensive membrane damage.

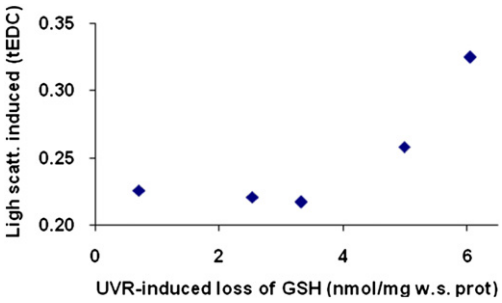

Fig. 4. Light scattering induced as a function of UVR-induced loss of GSH concentration.

No significant change in lens GR or GPx activity was detected at any level of α-tocopherol supplementation. It was previously shown that UVR-300 nm in vivo penetrates only superficially into the anterior surface of the lens (0.45 mm) (Löfgren and Söderberg, 2001). It is therefore possible that a subtle change of GR or GPx activity was masked by non-affected lens bulk biochemistry.

Our experiment relates the protective effect of α-tocopherol on just above threshold dose, short delay onset cataract and provides support for a beneficial effect of α-tocopherol. Further change of light scattering at longer intervals after in vivo exposure to UVR in the current dose domain is not expected (Michael et al., 1996). The potential protective effect of α-tocopherol for long-term, daily, sub-threshold exposure is beyond the scope of the current experiment and the data therefore cannot be directly extrapolated to such exposure in humans.

To the best of our knowledge, this study provides the first experimental evidence that the preventive effect of α-tocopherol on in vivo UVR-induced cataract in the Sprague–Dawley rat is dose dependent. The functional relationship demonstrated strengthens the causal relationship between α-tocopherol and its protection against UVR-induced cataract and the established parameters allows design of future experiments aiming for maximum preventive effect with minimum dose of α-tocopherol. The protection of UVR-induced light scattering is associated with an α-tocopherol dose dependent GSH depletion secondary to UVR exposure without any measurable effects on total lens GSSG concentration, GR or GPx activity. A strategy for estimation of the fraction of α-tocopherol dependent protection was developed. Further, a method to determine the GSH content in the lens associated with protection against UVR cataract was developed.

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Appendix

Model for light scattering difference between exposed and contralateral non-exposed lens after UVR exposure as a function of α-tocopherol dose

The light scattering difference between exposed and contralateral non-exposed lens, I_d, exponentially decreases from 0 dose α-tocopherol towards an asymptote level of light scattering difference, I_1, with increasing α-tocopherol dose, a, the difference of light scattering difference at zero dose and the asymptote being, I_0.

$$I_d = I_1 + I_0 e^{-k_s \cdot a} \quad (1)$$

Here the constant k_s is a measure of the rate of decrease.

Model for GSH concentration difference between exposed and contralateral non-exposed lens after UVR exposure a function of α-tocopherol dose

The GSH concentration difference between exposed and contralateral non-exposed lens, C_d, exponentially increases from 0 dose level, C_0, towards zero difference.

$$C_d = -C_0 e^{-k_g \cdot a} \quad (2)$$

Here the constant k_s is a measure of the rate of increase.

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