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Impact of iris pigment and pupil size in ultraviolet radiation cataract in rat

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ABSTRACT.

Purpose: **To investigate the effect of iris pigment and pupil size in ultraviolet radiation (UVR)-induced cataract.**

Methods: **Brown-Norway rats (pigmented) and Fischer-344 rats (non-pigmented) were unilaterally exposed *in vivo* to 5 kJ/m^2 UVR. Each strain was split into two groups, each receiving either mydriatic (tropicamide) or miotic (pilocarpine) eye-drops. One week after exposure, the degree of ocular inflammation and damage in the anterior segment was determined. The lenses were extracted, photographed and the degree of forward light scattering (cataract) was quantified.**

Results: **The cataract types differed between the two strains. All Fischer rats developed macroscopically identifiable UVR cataract while only 41% of Brown-Norway rats did so. All groups except the miotic Brown-Norway developed significant light scattering. The Fischer rats developed 3–4-fold more lens light scattering than the Brown-Norway rats. The miotic Fischer group exhibited significantly more light scattering than the mydriatic Fischer group. There was no significant difference in light scattering between the two Brown-Norway groups. There was a correlation between ocular inflammation and degree of light scattering, with Brown-Norway rats exhibiting less inflammation and lens light scattering.**

Conclusions: **Pigmented rats develop less UVR cataract and less ocular inflammation than non-pigmented rats. Pupil size plays a smaller role in UVR cataract development in pigmented rats than in non-pigmented. The role of UVR-induced ocular inflammation in cataract development is still ambiguous.**

Keywords: cataract – iris pigmentation – pupil size – rat – ultraviolet radiation

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Introduction

Ultraviolet radiation (UVR) is a major avoidable cause of cataract, indicated by an abundance of experimental studies and several epidemiological studies (Pitts et al. 1977; West et al. 1998; Delcourt et al. 2000; Merriam et al. 2000; Azzam et al. 2004; Meyer et al. 2008; Mody et al. 2008; Simpanya et al. 2008). The attributable risk for cortical cataract because of UV-B radiation ranges from 10% in an Australian population (McCarty et al. 2000) to 13% in a mixed black and white population in Maryland, USA (West et al. 1998) and 20% in a WHO report (Fact sheet 271, 2002). The localization of cataract in the lower nasal quadrant is explained by preferential focusing effects of solar UV radiation into this lens region (Sasaki et al. 2003). In addition, latitude dependence was found, with highest incidence of lower nasal cataract in subjects from Singapore and lower in Melbourne and Reykjavik (Sasaki et al. 2003).

The surgical treatment of cataract where the lens is replaced by an artificial lens is costly and as a consequence not available for the bulk of the world's population. Therefore, much effort is put into finding possible medical treatments or preventive measures for cataract.

The safety levels for UVR exposure are based on animal experiments because human *in vivo* UVR cataract experiments are precluded. Further, the strict regulations for utilization of human eyes and lenses for *in vitro* studies have favoured the use of animal lenses. A diversity of animal species has been used in UVR cataract research, including primates and other mammals, birds and fish. The most commonly used species are rodents and pigs.

With *in vivo* UVR exposure, the individual tissues are allowed to interact with each other. However, sometimes the *in vivo* dynamics may mask the search for specific pathophysiologic

mechanisms. In these cases, the *in vitro* experiment can be a powerful tool, allowing for exclusion of interaction factors.

With *in vivo* UVR exposures, investigators frequently use pupil-dilating eye-drops to reduce the variation in pupil size during exposure. Little is known, however, about the effect of iris pigment and pupil size in the development of experimental UVR cataract. Concern has been raised about the use of sunglasses in humans because the pupil size might increase and – hypothetically but not proven – more sunlight in absolute terms will enter the lens from oblique angles, not passing through the protective sunglass.

A dark brown-pigmented iris absorbs sunlight more than a less pigmented iris (Watts 1971) and should, in theory, better protect the lens and retina. This contrasts to the epidemiological findings of increased incidence of nuclear cataract in individuals with darker irides (Leske et al., 2002). Furthermore, darkly pigmented irides are more common in geographical areas where sunlight is more intense and also in areas where socioeconomic, health and nutritional status are lower, thus complicating cause–effect relationships.

The aim of this study was to investigate the effect of iris pigment and pupil size in experimental UVR cataract development.

Material and Methods

Animals

Eighty-four 6-week-old inbred female rats were used. Non-pigmented Fischer-344 rats [CDF®(F-344)/CrlBR] and pigmented Brown-Norway rats (BN/CrlBR) were purchased from Charles River AB, Uppsala, Sweden. Ethical approval was obtained from the Northern Stockholm Animal Experiments Ethics Committee. The experiments adhered to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Anaesthesia and eye-drops

The animals were anesthetized by an intraperitoneal injection of a mixture of 6 mg/kg xylazine (Rompun Vet.; Bayer AB, Solna, Sweden) and 40 mg/kg ketamine (Ketalar, Parke-Davis, Sweden) 15 min prior to exposure to UVR. Ten minutes prior to exposure, both eyes received either mydriatic eye-drops (tropicamide 0.5%, Mydriacyl; Alcon Sverige AB, Stockholm, Sweden) or miotic eye-drops (pilocarpine 4%, Pilokarpin; Ciba-Vision Nordic AB, Askim, Sweden). The pupil diameter was measured using a ruler.

Irradiation

The UVR source was a 300- W high-pressure mercury lamp (Oriel Instruments, Stratford, CT, USA) equipped with a 10 -cm water filter, double monochromator [set at 300 nm with 9 nm theoretical full width at half maximum (FWHM)] and collimating optics. The spectrum was previously published showing an irradiance peak at 302 nm with 6.2 nm FWHM (Löfgren et al. 2003). The irradiance was measured with a thermopile system (Oriel Instruments) calibrated to an ANSI (American National Standard Institute) traceable source by the Swedish National Bureau of Standards. The 5 kJ/m^2 15 min exposures were unilateral, covering eyelids and eye. Saline solution was instilled in both eyes every 20 seconds until recovery from anaesthesia.

Macroscopic pathologic changes

One week after exposure, the rats were killed by CO_2 asphyxiation and the eyelids and eyes were inspected for pathologic changes and graded from 0 to 3. The corneal changes were epithelial keratopathy, sterile stromal infiltrate or plaque formation, and corneal oedema/haze. The eyes were then enucleated, and the lenses dissected free from remnants of ciliary body, zonular fibres and vitreous. Existence of hyphema was noted during eye dissection. Under the circumstances in this study, hyphema is a sign of intraocular inflammation because mechanical trauma can be outruled. The appearance of cataract was documented by photography with the isolated lens immersed in Ringer's acetate in a Petri dish.

Quantification of cataract

The degree of cataract was quantified with the objective technique developed by Söderberg (Söderberg et al. 1990). A probing white light, distributed from a cold light source via a fibre, was directed from beneath at an angle of 45 degrees through the lens. The forward scattered light from the lens was collected by the optics of a camera equipped with a photo-diode in the film plane. The light-induced current from the photo-diode was measured with an ampere metre. The readings were calibrated against the readings of light scattering from a lipid emulsion of the drug diazepam (Stesolid Novum; Alpharma AB, Stockholm, Sweden). Hence, the unit for light scattering is transformed Equivalent Diazepam Concentration (tEDC) (Söderberg et al. 1990).

Experimental design

Power analysis on paired-sample light scattering data from earlier experiments in our group indicated the need of at least 15 animals in each group to detect a 20% difference between exposed and non-exposed lens, with beta error 0.2 and alpha error 0.05. Calculating for 5% anaesthesia-related mortality and a margin to the minimal sample size, 21 rats from each strain received mydriatic eye-drops in both eyes while the other 21 received miotic eye-drops. The rats were unilaterally exposed *in vivo* to UVR delivered during 15 min. The non-exposed contralateral lens served as internal control. The time interval between UVR exposure and cataract measurement was 1 week. The forward light scattering measurements were made in triplicate.

Statistics

The significance level and confidence interval (CI) coefficient was set to 0.05 and 0.95, respectively. Tukey multiple comparisons were performed when analysis of variance (ANOVA) of group means indicated significant difference in lens light scattering.

Results

Two Brown-Norway rats died during anaesthesia; one lacked lens in one eye; one received too short exposure and one rat's exposed lens was damaged during dissection. All these five Brown-Norway rats were excluded

from data analysis. Two Fischer rats died during anaesthesia.

The average pupil size in the two strains, as measured through the cornea, after instillation of eye-drops was 1.7 mm with pilocarpine and 3.5 mm with tropicamide, with no difference between the two strains.

Non-exposed eyes

Four non-exposed Fischer corneas and five Brown-Norway non-exposed corneas exhibited minor epithelial keratopathy. No correlation to eye-drop medication was seen. There was no difference in light scattering between the four non-exposed lens groups as revealed by ANOVA. All lenses were clear (Fig. 1A). The lens mean light scattering was 0.10 tEDC for both Fischer groups and 0.09 tEDC for both Brown-Norway groups, with confidence interval ±0.01 tEDC for all groups.

UVR-exposed eyes

Cataracts occurred as anterior polar; anterior subcapsular haze; anterior ring; equatorial and posterior cortical cataract (Fig. 1), and the two strains developed almost exclusively different cataract types. All UVR-exposed lenses in the Fischer group developed cataract while only 41% in the Brown-Norway group did so. Fischer cataracts were seen as haze (Fig. 1B) in the anterior subcapsular region and an opaque equatorial region (Fig 1C) with vacuoles and opaque outer cortical spokes reaching posteriorly (Fig. 1D). The predominant Brown-Norway cataract was an anterior polar dot-like opacity (Fig. 1E), and in fewer cases a ring-shaped opacity surrounding the anterior pole (Fig. 1F).

All groups except the miotic-treated Brown-Norway rats developed significantly more light scattering in exposed lenses compared to control lenses (Fig. 2). The Fischer groups differed significantly from each other and also from the Brown-Norway rats (Fig. 2). There was no significant difference in light scattering between the two Brown-Norway groups (Fig. 2).

The UVR-exposed eyes generally exhibited oedematous eyelid margins and conjunctival or mixed injection. The corneas showed epithelial keratopathy, sterile stromal infiltrates and haze. Hyphema was often present.

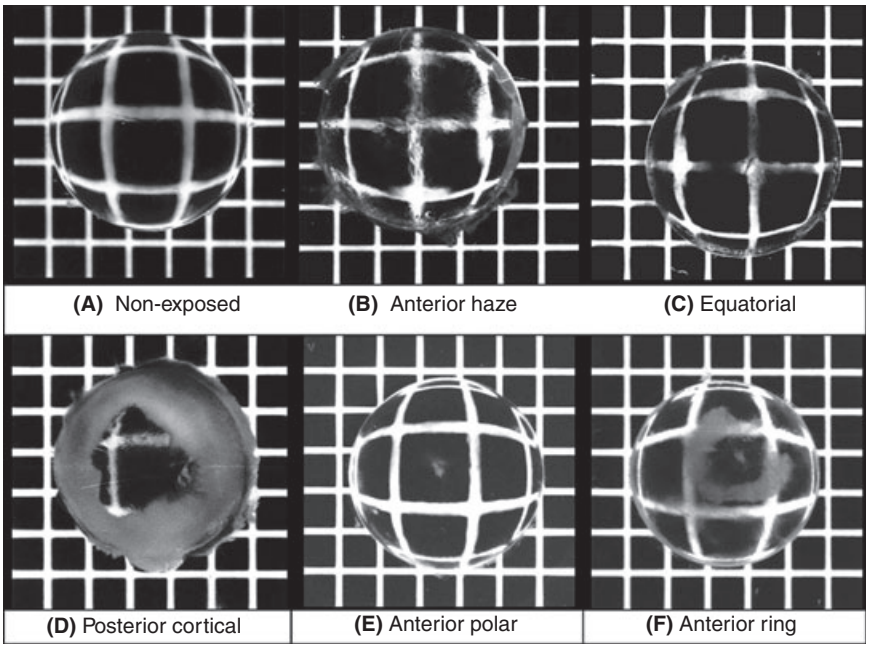

Fig. 1. Ultraviolet radiation-exposed and non-exposed lenses. All lenses except (D) are viewed anterior side up. Besides anterior haze, (B) also has equatorial cataract. (F) has both anterior polar and anterior ring cataract. Grid square diameter is 0.79 mm.

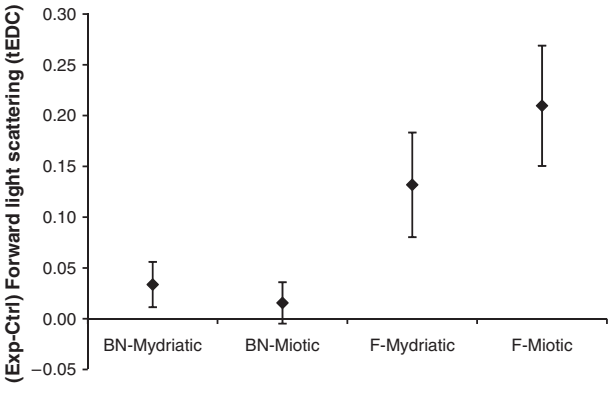

Fig. 2. Forward light scattering in lenses from pigmented and non-pigmented rats after 5 kJ/m^2 ultraviolet radiation-B. Bars are 95% CI for mean paired-sample difference between exposed and non-exposed (ctrl) lenses. The BN-Miotic group did not differ from BN-Mydriatic. The difference between all other groups was statistically significant. BN, Brown-Norway; F, Fischer-344. Sample sizes are 18, 19, 21 and 19, respectively.

There was slightly, but consistently, more external inflammation (corneal damage and eyelid inflammation) in the mydriatic-treated UVR-exposed eyes compared to the miotic-treated (Table 1). The only major species non-lenticular differences were the more prevalent eyelid inflammation and presence of hyphema in the Fischer rats (Table 1).

Discussion

The inbred Brown-Norway rat was chosen as the pigmented strain because it is probably the most widely used pigmented rat strain in UVR eye research, and comparison information is therefore available (Wegener 1994; Wu et al. 1997). The non-pigmented Fischer-344 is also inbred and matches the growth curve of the Brown-Norway.

The finding that pigmented eyes are better protected against UVR than non-pigmented eyes is not surprising because the pigmented iris absorbs more UVR than the non-pigmented iris. Melanins have antioxidant properties (Sarna & Sealy 1984) and also function as UVR heat sink, converting UVR to heat (Forest & Simon 1998).

Table 1. Frequency (%) of ocular inflammation and cataract type in ultraviolet radiation-exposed rats. One individual lens can exhibit more than one cataract type, leading to 100+ sums for the cataracts. All other parameters have a sum of maximum 100%.

	Brown-Norway		Fischer	
	Mydriatic	Miotic	Mydriatic	Miotic
Anterior polar cataract	58	67	0	0
Anterior ring cataract	21	23	0	0
Anterior haze cataract	11	0	90	95
Equatorial cataract	0	0	71	95
Posterior cortical cataract	0	0	33	52
No cataract	58	61	0	0
Eyelid inflammation 3	0	0	19	0
Eyelid inflammation 2	5	6	57	24
Eyelid inflammation 1	58	11	19	62
No eyelid inflammation	37	83	5	14
Corneal haze 3	16	6	0	5
Corneal haze 2	53	33	81	38
Corneal haze 1	32	50	19	57
No corneal haze	0	11	0	0
Epithelial keratopathy 3	11	0	19	5
Epithelial keratopathy 2	21	6	19	5
Epithelial keratopathy 1	26	22	24	29
No epithelial keratopathy	42	72	38	62
Corneal stromal infiltrate	37	28	52	19
Hyphema	16	6	62	67

The co-operation of iris melanin and ascorbate in the aqueous results in UVR-induced production of reactive oxygen species (Rozanowska et al. 1997; Wielgus & Sarna 2008), which in best case are neutralized by the normal antioxidative pathways.

The existence of equatorial cataract in the Fischer rats indicates that the lens equator is UVR-exposed regardless of the pupil size, in *non-pigmented* eyes. It was intriguing to find that the miotic-treated Fischer group exhibited *more* cataract compared to the mydriatic-treated. This is most probably caused by the thinner stretched-out iris transmitting more UVR to the lenticular germinative zone, the cell dividing area in the lens epithelium close to the equator. Contrasting to this is the complete lack of equatorial cataract in the Brown-Norway rats and the localization of the existing anterior opacities within the pupillary border. The non-existing difference in lens light scattering between mydriatic- and miotic-treated Brown-Norway rats is attributed to the efficient protection of the lens equator by the pigmented iris, even with maximally dilated pupil.

The low degree of eyelid inflammation and hyphema in the Brown-Norway rats is explained by the dark melanin in the pigmented rat skin and iris. Even with regular wetting of the corneas, the proptosis and lid retraction caused by the anaesthetics induced a slight and reversible epithelial keratopathy in non-exposed eyes. One week after UVR exposure, there were still nine rats (of 79) that had minor corneal disturbances in the non-exposed eye. The damage occurring in those control eyes was not caused by accidental UVR exposure to the control side because the exposure beam was narrow and projected in such a way that it could not reach the control eye.

The non-existent difference in corneal changes in non-exposed eyes, between the eye-drop medication groups, indicates that there were no drug-related toxic effects on the cornea. The slightly higher degree of external inflammation in the two tropicamide UVR-exposed groups might point towards either a possible photosensitizing effect of tropicamide or a photoprotective effect of pilocarpine in the cornea. On the other hand, the similar light scattering in the two Brown-Norway groups and non-existent difference in hyphema frequency speaks against a photosensitizing effect in the aqueous humour, iris and lens. The high frequency of hyphema in the Fischer rats while low in the Brown-Norway strongly suggests a protection by the iris pigment.

Several studies have identified an association between dark iris colour and development of various cataract types, predominantly nuclear, in African American, Caribbean and Caucasian populations (Leske et al., 2002; The Italian-American Cataract Study Group, 1991; McCarty et al. 1999; Cumming et al. 2000; Delcourt et al. 2000). The reason for this association is not clear, and various theories have been proposed. Heat transfer from iris melanin pigment to the lens might increase ageing processes, as supported by animal experiments with infrared radiation (Langley et al. 1960). Free radicals formed from iris melanin by UV radiation (Mason et al. 1960) and light exposure (Cope et al. 1963) might reach the lens and damage critical molecules. In contrast, melanins can differentially protect or promote against UVR photodamage (Hill et al. 1997). Because of the weak evidence for UVR and melanin cataractogenesis, genetic covariation between cataract development and dark iris colour has received increasing interest as explanatory factor (Hammond et al. 2000). Further, dark iris colour is associated to geographical regions with stronger sunlight. However, we do not believe that solar UV radiation is the culprit in dark iris-associated nuclear cataract, because solar UV-B radiation is predominantly associated with cortical cataract. The low UVR-B penetration into the lens (Löfgren & Söderberg 2001) further argues against UVR-B-induced nuclear cataract. UV-A radiation on the other hand reaches the lens nucleus and is suggested as one cause for human nuclear cataract (Giblin et al. 2002).

Lack of iris or iris pigment in aniridia or ocular albinism is correlated to increased cataractogenesis but it is unlikely that sunlight is involved because these patients are usually photophobic and thus avoids sunlight. Reliable epidemiological data are not available because of the low incidence of these rare diseases.

Even if pigmented rat eyes are more similar to human eyes than non-pigmented rat eyes, the rat is still nocturnal. This holds also for rabbit and mouse. One major argument against nocturnal animals in UVR eye research is that they in general have much less ascorbate in the anterior segment than diurnal animals (Ringvold 1998). The ground squirrel has been proposed as a good model because it is diurnal,

melanin pigmented and has the yellow lenticular pigment that humans have as well (Zigman & Paxhia 1988). However, the difficulties in getting hold of these wild animals, not commercially available, cannot be underestimated. For *in vitro* experiments, the demands can be kept less stringent. There are few known differences in lens biochemistry or structure between non-pigmented and pigmented animals.

The question that must be asked in view of the present results is whether the difference in UVR sensitivity after *in vivo* UVR exposure is still valid after *in vitro* exposure. Potential effects of eye-drops, anaesthetics, ocular inflammation, UVR absorption in cornea and aqueous, iris transmission and iris melanin can then be excluded. There might still be inherent differences in lens UVR sensitivity between the two rat strains.

The small difference in cataract development with small or large pupil in pigmented eyes exposed to axially directed UVR indicates that pupil size might be less important than previously thought. However, the effect of pupil size on oblique rays falling onto the eye and into the lens is unknown and deserves further study.

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