Influence of Exposure Time for UV Radiation–Induced Cataract

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Purpose. It is believed that for a certain ultraviolet radiation (UVR) exposure, the biologic effect depends on the product of irradiance and exposure time (the reciprocity Bunsen–Roscoe law). The purpose of this study was to investigate the validity of the reciprocity law for UVR-induced cataract.

Methods. Two experiments were conducted. In the first one, 100 Sprague–Dawley rats were exposed to UVR divided into five groups according to exposure time: 7.5, 15, 30, 60, and 120 minutes. In the second experiment, 80 Sprague–Dawley rats were exposed to UVR divided into four groups according to exposure time: 5, 7.5, 11, and 15 minutes. All the animals were unilaterally exposed to the same dose of UVR (8 kJ/m^2) in the 300-nm wavelength region. One week after exposure both lenses were removed to measure the intensity of forward light scattering and for microphotography. Groups were compared by evaluating the difference between exposed and nonexposed eyes.

Results. The group exposed to UVR for 5 minutes had the lowest intensity of forward light scattering. The highest intensity of forward light scattering was found in the group that was exposed for 15 minutes. With longer exposure intervals, the intensity of forward light scattering decreased as the exposure time increased. No difference in intensity of forward light scattering was found between the groups exposed for 60 and 120 minutes.

Conclusions. Exposure time strongly influenced cataract formation after low-dose UVR. In this model of UVR-induced cataract, the photochemical reciprocity law was modulated by a biologic response. (*Invest Ophthalmol Vis Sci.* 2000;41:3539–3543)

Cataract can be defined as reduced visual performance due to light scattering in the lens. It can occur as a result of a wide variety of factors, including metabolic disorders, exposure to toxic agents, trauma, exposure to radiation, nutritional deficiencies, and hereditary factors.

Global atmospheric changes such as depletion of ozone in the stratosphere are thought to lead to increased levels of ultraviolet radiation (UVR) on the earth. This can have adverse effects on human health. Epidemiologic studies and experimental exposure of animals to UVR show a relationship between UVR exposure and induced lens opacities. Latitude and sunlight hours are also positively associated with cataract incidence.[1–5]

It has been demonstrated that at close to threshold dose, the intensity of forward light scattering reaches maximum 1 week after exposure[6] and then remains essentially constant.[7] It is also known that the dose–response function for UVR-induced cataract is continuous.[8,9]

In 1862 Bunsen and Roscoe formulated the second law of photochemistry, also known as the reciprocity law, which states that the magnitude of time and irradiance are reciprocal for induction of a photochemical effect. If a photobiologic effect depends purely on photochemical events, the biologic effect of a UVR exposure depends on the product of the irradiance and exposure time.[10,11]

Radiologists use the reciprocity law to determine the time and intensity of exposure to ionizing radiation to achieve maximal contrast. Kimme–Smith et al.[12] have shown that the reciprocity law is inaccurate in mammography when large breasts are exposed for more than 1.3 seconds. Large breasts need longer exposure times at equivalent intensity. According to the reciprocity law, for equivalent darkening of the film, the intensity required should decrease if the exposure time is prolonged. However, the contrast found was lower than expected.

In biology, the reciprocity law has been applied to model various photobiologic phenomena. The cultured human skin fibroblast suffers lipid peroxidation after UVA irradiance, and this response obeys the reciprocity law.[13] However, it was shown that the reciprocity law is not valid for in vitro UVA-induced photohemolysis sensitized by psoralens.[14] In the retina, the reciprocity law was used to explain the temporal summation effects of light. To reach a liminal value for retinal stimulation, a light of high intensity requires a shorter time than one of low intensity. In the lens, it is believed that for a certain UVR exposure, the biologic effects depend on the product of irradiance and exposure time. As the product (dose) remains constant, so does the resultant damage. Ocular safety

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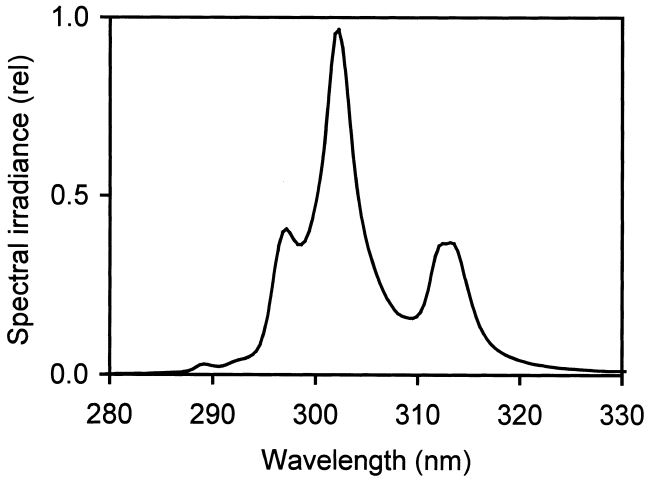

FIGURE 1. Relative spectral irradiance of radiation in the corneal plane.

measures for the industrial use of UVR are based on the postulates of the reciprocity law.

The purpose of this study was to investigate the validity of the reciprocity law in UVR-induced cataract.

METHODS

The Sprague–Dawley rat was the experimental animal. One eye was exposed in vivo to UVR. Intensity of forward light scattering in the lens was measured after in vitro isolation of the lens.

Experimental Devices

The radiation from a high-pressure mercury lamp was collimated, passed through a water filter and an interference filter (λ_{max} = 300 nm, $\lambda_{0.5}$ = 10 nm), and finally projected on the cornea of the exposed eye.[6] The spectral irradiance in the corneal plane was recorded (Fig. 1) with a spectrometer (PC 2000; Ocean Optics, Dunedin, FL), and the total UVR dose at the corneal plane was checked with a thermopile (model 7104; Oriel, Stratford, CT) calibrated to a standard traceable to the United States National Bureau of Standards. The intensity of light was measured before and after each UVR exposure.

The intensity of forward light scattering was measured with a light dissemination meter.[15] This instrument uses the principle of dark-field illumination. The illuminating light transilluminates the measured crystalline lens at 45° against the horizontal plane. At this angle, the light cannot enter the objective aperture. If the object scatters light in the forward direction, a defined fraction of light reaches the objective and is measured by the photodiode.

The opacity standard was a lipid emulsion of diazepam (Diazemuls; Kabi Vitrum, Stockholm, Sweden), and the unit was expressed as transformed equivalent Diazemuls concentration ($_t$EDC).[15] A typical value for a normal rat lens is approximately 0.1 $_t$EDC and for a very opaque lens approximately 1 $_t$EDC. Between 0 and 1 $_t$EDC, the intensity of forward light scattering increases linearly with the concentration of standard in the measurement cuvette.

Experimental Procedure

Female Sprague–Dawley rats were unilaterally exposed at the age of 6 weeks. Ten minutes before the exposure, the animal was anesthetized with 94 mg/kg ketamine plus 14 mg/kg xylazine intraperitoneally.[16] Tropicamide was instilled in both eyes, and after 5 minutes, one eye was exposed to UVR.

Two experiments were conducted with the same experimental procedure, but different exposure times. In the first experiment, 100 Sprague–Dawley rats were unilaterally exposed to 8 kJ/m^2 UVR in the 300-nm wavelength region and divided into five groups according to exposure time: 7.5, 15, 30, 60, and 120 minutes. In the second experiment, eighty Sprague–Dawley rats divided in four groups according to exposure time (5, 7.5, 11, and 15 minutes) were exposed unilaterally to 8 kJ/m^2 UVR in the 300-nm wavelength region. All the animals in both experiments received the same dose. The corneas were moistened every 15 minutes with Ringer solution. The animals were killed 1 week after the exposure with an overdose of carbon dioxide (CO_2) followed by cervical dislocation. Using this system, Michael et al.[17] have shown that forward light scattering peaks 1 week after UVR. The eyes were enucleated, and both lenses were extracted and placed in balanced salt solution (BSS). Vestiges of the ciliary body were removed from the lens equator under a microscope. Photographs were taken of each lens against a dark background with a white grid. During photography the anterior surface of the lens faced the camera. The intensity of forward light scattering of each lens in BSS was measured three times. The animals were kept and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The light-scattering data were first analyzed with the Kolmogorov–Smirnov test for normality. Analysis of variance (ANOVA) was then used to test for significant differences between groups and between the exposed and nonexposed specimens. Thereafter, a multiple comparisons test[18] was performed to reveal differences among the groups. The significance level and confidence coefficients were set to 0.05 and 0.95, respectively. Statistical procedures were the same for both experiments, but each experiment was analyzed separately.

RESULTS

Morphologic changes in the exposed lenses were photographically recorded and evaluated with a stereomicroscope (model MZ6; Leica, Solms, Germany). Photographs of the whole lens were evaluated to identify the different patterns of cataract. A nonexposed lens is shown in Figure 2A. Very mild superficial cataract was found in the groups that were exposed for 5 and 7.5 minutes (Fig. 2B). The group exposed to UVR for 15 minutes showed the densest opacities of any of the groups. Cortical, equatorial opacities (a special geographical cortical cataract) and vacuoles could be seen in lenses exposed for 15 minutes (Fig. 2C). Lenses that were exposed for 11 and 30 minutes showed silky equatorial cataract and vacuoles (Fig. 2D). The groups that were exposed for 60 and 120 minutes showed essentially the same morphologic pattern. Mild superficial cataract was a common finding in both these groups (Fig. 2E). Nuclear cataracts did not develop in any group.

The morphologic findings were classified and graphed (Fig. 3). Vacuoles were seen in all groups. The groups that showed the least number of vacuoles (n = 2) were the 5- and 7.5-minute exposure groups (Fig. 3A). Superficial cataract (epithelial cataract) was a common finding. The incidence of

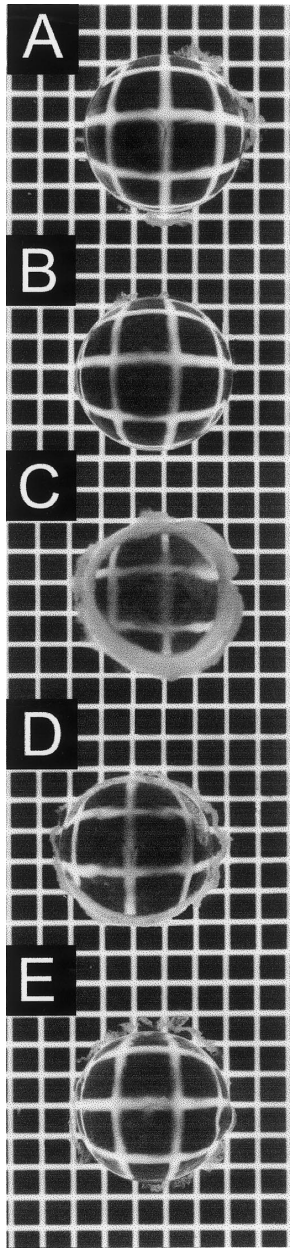

FIGURE 2. Microphotographs of isolated rat lenses corresponding to different exposure time groups. The photographs were taken 1 week after the UVR exposure, against a black background with a white grid. The distance between the white wires is 0.79 mm. (**A**) Nonexposed lens, (**B**) 5 and 7.5 minutes, (**C**) 15 minutes, (**D**) 11 and 30 minutes, and (**E**) 60 and 120 minutes.

superficial cataract was more than 60% in all groups. Lenses exposed for 5 and 7.5 minutes showed the highest number (n = 18) of superficial cataracts (Fig. 3B). Most of the equatorial cataracts were found in the lenses exposed for 15 minutes (n = 14), whereas no equatorial cataract was detected in the lenses exposed for 5 and 7.5 minutes (Fig. 3C). Cortical cataract was found only in lenses exposed for 15 minutes (Fig. 3D).

Groups were compared by calculating the difference in the intensity of forward light scattering between the exposed and nonexposed lenses (Fig. 4). Lenses that were exposed for 5 minutes had the lowest intensity of forward light scattering. Those exposed for 7.5 minutes showed a higher intensity of forward light scattering compared with those exposed for 5 minutes. Lenses exposed for 11 minutes showed a higher intensity of forward light scattering compared with those exposed for 5 and 7.5 minutes. Those exposed for 15 minutes showed the highest intensity of forward light scattering. However, lenses irradiated for 30 minutes showed decreased forward light scattering compared with those exposed for 15 minutes. After exposure times longer than 15 minutes, forward light scattering decreased with increasing time. Lenses exposed for 60 and 120 minutes showed development of the same intensity of forward light scattering.

The Kolmogorov-Smirnov test showed that the data were normally distributed. ANOVA showed a significant difference between exposed and nonexposed lenses and among the var-

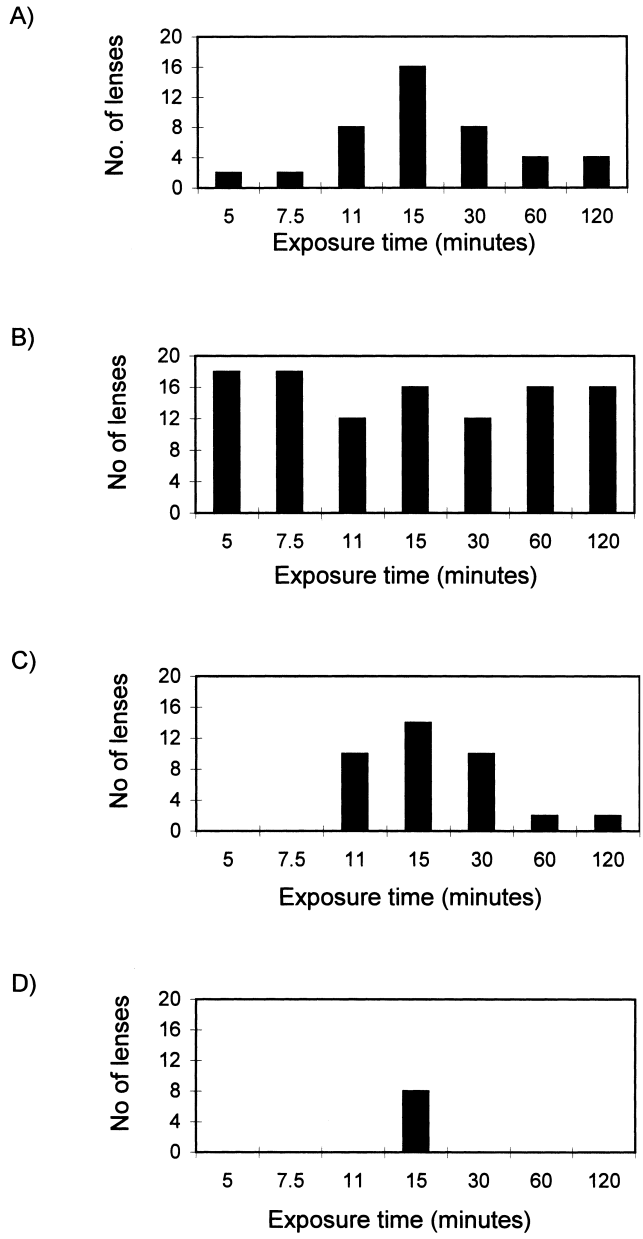

FIGURE 3. Number of lenses and opacity patterns in the different exposure time groups (n = 20). (**A**) Vacuoles, (**B**) superficial cataract, (**C**) equatorial cataract, and (**D**) cortical cataract.

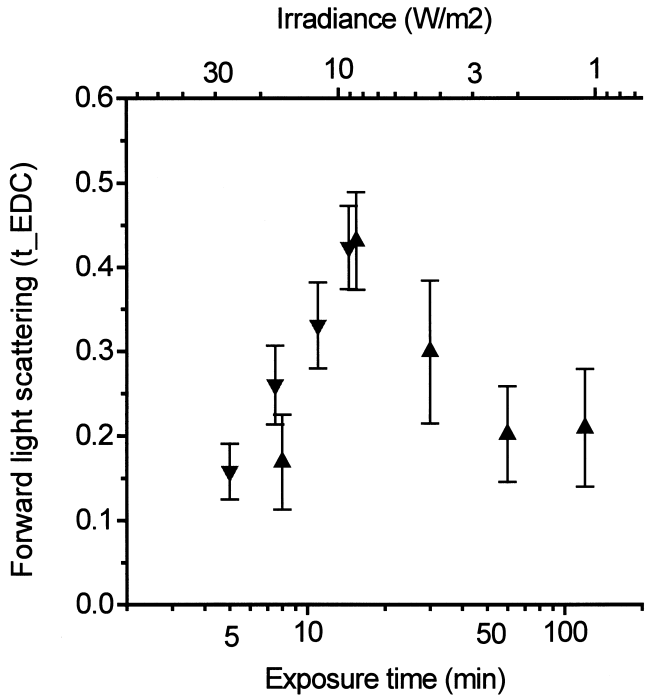

Figure 4. Difference in intensity of forward light scattering between the lenses of exposed and nonexposed eyes after various times of exposure (▲: 7.5, 15, 30, 60, and 120 minutes; ▼: 5, 7.5, 11, and 15 minutes) to UVR. Bars are 95% confidence intervals for the mean difference between the exposed and contralateral nonexposed eyes.

ious exposures groups. Multiple comparisons were used to show the differences between the groups. The results of the both experiment are shown in Table 1. The difference between 15-minute exposure and 7.5-, 30-, 60-, and 120-minute exposures was significant. The difference between the 30-minute exposure and each of the 7.5-, 60- and 120-minute exposures also was significant. No significant difference was detected between the 7.5-minute exposure and each of the 60- and 120-minute exposures. Significant differences were found in all comparisons in the second experiment.

Discussion

The present study shows the results obtained from two experiments. The first experiment was conducted to elucidate the validity of the reciprocity law for UVR-induced cataract. The results of the experiment were unexpected. A second experiment confirmed the results and tested the validity of the reciprocity law for UVR exposure times of less than 30 minutes. The second experiment verified the findings from the first experiment that there is less lens damage when UVR is delivered for 7.5 minutes than when it is delivered for 15 minutes.

UVR at 300 nm is partially transmitted by the cornea. In vitro measurements vary from 2% to 20% for the rat and from 9% for humans to 24% for the rabbit.[19–23] There is a slight attenuation of 300-nm UVR in the aqueous. The radiation that hits the lens is first filtered by the lens capsule. Söderberg et al.[24] have shown that at 300 nm, approximately 60% of the radiation is transmitted by the anterior capsule. The transmitted radiation impacts and damages the lens epithelial cells and thereafter the cortical fibers. Because lens epithelial cells are responsible for maintaining much of the homeostasis of the underlying fibers, damage to lens epithelial cells may also result in abnormalities in lens fibers underlying UVR-altered epithelial cells.[25,26]

If the reciprocity law were directly applicable to UVR-induced cataract, the intensity of forward light scattering would be constant for constant dose, whatever the exposure time, because the eye is exposed to a constant number of photons. However, with exposures of 5 to 120 minutes, maximum damage occurred after exposure of 15 minutes (Fig. 4). This means that the photons have an increasing efficiency in causing biologic damage when exposure times increase from 5 to 15 minutes. However, for exposure times more extended than 15 minutes, efficiency in causing biologic damage decreases toward the same level as that during exposure times of 5 minutes.

Because the primary event, the photochemical effect, is known to obey the reciprocity law (the amount of photochemical effect is directly proportional to the number of photons applied), we hypothesize that the observed increased efficiency in causing a photobiologic effect in the time domain of 5 to 15 minutes is due to increase photosensitization, decreased quenching, or both. Regardless of the process, the kinetics must be biologically driven and independent of the number of photons. It should be possible to improve the understanding of the underlying biologic process by investigating the effect of biologic variables on the relationship between intensity of forward light scattering at 1 week after exposure and the exposure time at constant UVR dose in the time domain of 5 to 15 minutes

The decrease in efficiency in the time domain 15 to 120 minutes may occur because of decreased photosensitization, increased quenching, biologic repair, decreased penetration of the UVR (through the cornea), or a combination of any of these. Again, regardless of process, the kinetics must be biologically driven and independent of the number of photons. Also in this exposure time domain, it should be possible to

Table 1. Multiple Comparisons Measuring Forward Light Scattering for Various Exposure Time Groups in the Two Experiments

Comparisons	Means	Result	LSD
First Experiment (n = 100)			
7.5 vs. 15 minutes	0.169–0.431	−0.262*	0.088
7.5 vs. 30 minutes	0.169–0.299	−0.133*	0.088
7.5 vs. 60 minutes	0.169–0.202	−0.033	0.088
7.5 vs. 120 minutes	0.169–0.209	−0.040	0.088
15 vs. 30 minutes	0.431–0.299	0.132*	0.088
15 vs. 60 minutes	0.431–0.202	0.229*	0.088
15 vs. 120 minutes	0.431–0.209	0.222*	0.088
30 vs. 60 minutes	0.299–0.202	0.097*	0.088
30 vs. 120 minutes	0.299–0.209	0.090*	0.088
60 vs. 120 minutes	0.202–0.209	−0.007	0.088
Second Experiment			
5 vs. 7.5 minutes	0.158–0.260	−0.102*	0.0612
5 vs. 11 minutes	0.158–0.331	−0.173*	0.0612
5 vs. 15 minutes	0.158–0.423	−0.265*	0.0612
7.5 vs. 11 minutes	0.260–0.331	−0.071*	0.0612
7.5 vs. 15 minutes	0.260–0.423	−0.163*	0.0612
11 vs. 15 minutes	0.331–0.423	−0.092*	0.0612

LSD, least-significant difference. Shaded comparisons are nonsignificant.

* P = 0.05.

improve the understanding of the underlying biologic processes by investigating the effect of biologic variables on the relationship between intensity of forward light scattering at 1 week after exposure and exposure time at constant UVR dose.

Little is known about secondary molecular events in the lens after exposure to UVR. Different exposure times of UVR may initiate different gene expression, thus altering protein synthesis in different ways. This could explain differences in damage found in the different groups of exposure time. According to Alberts et al.,[27] a cell can change the expression of its genes in response to external signals. A recent experiment conducted by Iordanov et al.[28] demonstrated a ribotoxic stress response in mammalian cells exposed to UVR. Furthermore, Healy et al.[29] showed increased expression of the *p53* gene in the skin after UVR exposure.

Enzymatic alterations can be produced either directly by UVR exposure or indirectly as a result of changes in gene expression after UVR exposure. Enzymatic changes as well as other biochemical reactions are usually time dependent, possibly explaining the different lens damage when UVR is delivered during different periods. UVR can directly elicit multiple changes in enzymes function. Tung et al.[30] have shown lens hexokinase deactivation by 300 nm UVR. Reddy and Bhat[31] have shown decreased activity of lens phosphofructokinase, isocitrate dehydrogenase, and malate dehydrogenase by irradiating lens homogenate of 3- and 12-month-old rats with 300 nm UVR.

Evaluating the corneal status with slit lamp microscopy and macrophotography, no difference was perceived between exposed and nonexposed eyes in any of the groups. However, the corneas of animals exposed for 60 and 120 minutes had more whitish deposits on the surface of the cornea than the groups irradiated for shorter periods of time, regardless of UVR exposure. Although corneas were moistened every 15 minutes, some desiccation occurred during anesthesia.

In the present study, the first experiment was run to elucidate the validity of the reciprocity law for UVR-induced cataract. Unexpected results were encountered. A second experiment was run to discount any mistake in the first experiment and to test the validity of the reciprocity law for shorter periods of UVR exposure time. The second experiment confirmed that there is less lens damage when UVR is delivered during shorter periods in the time domain of 5 to 15 minutes. The data showed that the photochemical reciprocity law did not apply for UVR-induced cataract when the UVR was delivered during periods shorter than 60 minutes.

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