Lens Lactate Dehydrogenase Inactivation after UV-B Irradiation: An In Vivo Measure of UVR-B Penetration

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PURPOSE. To elucidate the spatial distribution of inactivation of lactate dehydrogenase (LDH) in ultraviolet-B radiation (UVR-B)–exposed eyes. To determine the in vivo penetration depth of UVR-B in the lens.

METHODS. LDH activity in cornea and lens was investigated with an enzyme histochemical technique. Thirty rats were exposed in vivo to UVR-B of approximately 300 nm, and the eyes were enucleated and frozen at 0, 2, and 6 hours after exposure. LDH activity in frozen sections was determined quantitatively in the corneal epithelium and four different regions in the lens. UVR-B penetration depth was estimated by using a calculated Lambertian absorption coefficient.

RESULTS. The LDH activity was decreased in the cornea and the outer anterior lens cortex at all three time points. The average decrease in enzyme activity in the time range was 35% in the cornea and 20% in the outer anterior lens cortex. UVR-B inhibition of LDH was immediate and not dependent on an inflammatory reaction within the eye. Penetration depth, corresponding to 1/e² (~14%) residual UVR-B intensity, was 0.45 mm.

CONCLUSIONS. UVR-B does not exhibit any significant effect on LDH activity in the major part of the lens, and this is attributed to the shallow penetration (0.45 mm) of UVR-B into the anterior parts of the lens. (Invest Ophthalmol Vis Sci. 2001;42:1833–1836)

The lens transmits and focuses light onto the retina to produce an image. The lens is highly transmissive of light in persons of young age, but with increasing age, both the transmitting and the focusing properties deteriorate. The major cause of blindness in the world is cataract, which is defined as opacity in the lens leading to impaired vision. Since the 19th century, it has been known that experimental exposure to ultraviolet radiation (UVB) damages the lens and induces cataract.1,2 Epidemiologic studies show a correlation between exposure to solar UVR-B and increased incidence of cataract.3,4 Our hypothesis for acute experimental UVB-induced cataractogenesis is that a shortage of energy inhibits the ion pumps. It is known that UVR inhibits Na,K-ATPase triphosphatase (ATPase)5 and induces an accumulation of water and sodium in the lens.6 There are studies supporting the idea of inhibition of Na,K-ATPase through UVR-induced energy depletion: decreased Na,K-ATPase activity after prolonged exposure,7 reduced ATP content in lenses,8 decreased hexokinase activity,9 decreased glycolytic activity in situ10 and decreased lactate dehydrogenase (LDH) activity in vitro11 after exposure to UVR. The lens relies mainly on anaerobic glycolysis for production of ATP with the exception of the lens epithelium and outermost cortex, where the mitochondrial aerobic systems dominate.

The wavelength range shown to be most harmful for the lens is 300 to 305 nm,12,13 in the middle of the UVR-B spectrum (280–320 nm). The current safety limits for lens UVR-B exposure are mainly based on a study by Pitts et al.,12 in which animal cataract was used as the end point. To further understand the wavelength-dependent development of UVR-B cataract, it is essential that the absorption characteristics of UVR-B in the lens be known. Currently, there is only one study on regional absorption characteristics of 300 nm UVR-B within lenses. In 1999, Dillon et al.14 presented photometrically obtained transmission data on sectioned lenses.

Transmittance studies of different species have shown that less than approximately 10% of 300-nm UVR-B falling on the cornea reaches the anterior surface of the lens and of that, practically all radiation is attenuated within the lens.14–17 The human and rabbit lens capsules are poor filters, transmitting more than 50% of 300-nm UVR-B.18,20–22 The purposes of the current study were to further elucidate whether in situ UVR-B–induced inhibition of glycolysis in the lens is caused by LDH inhibition and to find out whether enzyme histochemistry25 could be applied to obtain information on the in vivo penetration of 300-nm UVR-B in the lens. LDH is supposedly suitable for this because of its presence and high activity in all parts of the lens.

METHODS

Animals

All experiments were approved by the Northern Stockholm Animal Experiments Ethics Committee. The experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Thirty 6-week-old female outbred albino Sprague-Dawley rats (Bkl/SD; M&K Universal, Sollentuna, Sweden) of approximately 150 g were used.

UVR-B Exposure

Thirty rats were divided into three groups of 10 rats each. They were anesthetized intraperitoneally with 11 mg/kg xylazine and 80 mg/kg ketamine.24 Before irradiation, tropicamide (0.5%) was instilled in both eyes to dilate the pupils. One eye in each rat was exposed to 90 kJ/m2 300-nm UVR-B (19 minutes’ exposure), measured in the corneal plane. This dose is approximately 90 times the threshold dose for UVR-B cataract in rats.13 The UVR-B source was a 200-W high-pressure mercury arc lamp equipped with water filter, interference filter (λmax 300 nm, full width at half maximum), and collimating optics. The irradiance peaked at 302 nm (Fig. 1) and was quantified with a thermopile calibrated by the Swedish National Bureau of Standards. The contralateral eye served as a nonexposed control. The rats were killed by CO2 asphyxiation at 0, 2, and 6 hours after exposure.
Enzyme Histochemistry

The histochemistry technique has been described in detail previously. The eyes were enucleated, embedded in gel (Cryo-Gel; Instrumentics, Inc., Hackensack, NJ) and frozen in precooled isopentane. Frozen sections were obtained close and parallel to the visual axis. Each eye, exposed or nonexposed, contributed 18 serial sections distributed on three test and three control slides with 3 sections on each slide.

The sections on the test slides were covered with 0.2 ml test medium containing 4 mM nicotinamide dinucleotide (NADH), 2 mM nitroblue tetrazolium (NBT), 0.25 mM phenazine methosulfate (PMS), 5 mM sodium azide, 150 mM lactate, and 10 mM NADPH (NEM). The sections on the control slides were covered with 0.2 ml control medium, which was the same as the test medium but without lactate and with 20 mM pyruvate added. The use of NEM and pyruvate in the medium differs from our earlier description of the histochemical technique. Pyruvate was included in the control medium as a product inhibitor. To minimize the staining reaction in the lens nucleus in the control sections due to endogenous lactate. Without addition of pyruvate the endogenous lactate would cause a false low test-minus-control activity. Sulphhydryl groups are known to reduce NBT into colored formazan, and for that reason NEM was added to both media to block this nonspecific staining reaction. The chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and Kebo Laboratory (now Merck Eurolab AB, Stockholm, Sweden).

Densitometry

Formed NBT-formazan dye in the sections was quantified with a microscope-based densitometer (Ernst Leitz, Wetzlar, Germany) with a measuring spot 9 µm in diameter. Transmittance through a section was calculated as the ratio between intensity transmitted through the section and through a region just outside the section. The transmittance data were then transformed into absorbance. On each slide two sections with acceptable morphology were selected for the absorbance determinations. In each section the basal corneal epithelium (CE), the outer anterior lens cortex (OAC), the inner anterior cortex (IAC), the nucleus (N), and the posterior cortex (PC) were measured on (Fig. 2). The distances from the outer surface of the anterior lens capsule to the different lens regions were 210 µm to the OAC, 630 µm to the IAC, 1460 µm to the N, and 2500 µm to the PC. The regions were situated along the visual axis and three measurements were obtained for each region. The mean absorbance value for each region in the control slide sections was subtracted from the mean value for the corresponding region in the test slide sections. The mean of the three paired test-minus-control differences per lens was considered to be the relative enzyme activity and served as primary data in the statistical analyses.

Lens UVR-B Penetration Model

The inactivation (%) of LDH at 0 hours’ latency (Ω) was used as a measure of the intensity of UVR-B in the tissue during exposure. Inactivation was calculated as 1 minus the ratio of relative activity in the exposed lens (absorbance units, Ae), and the relative activity in the nonexposed contralateral lens (Ac; equation 1).

\[
\Omega = 1 - \frac{A_e}{A_c}
\]  

(1)

According to the second law of photochemistry (only one molecule or atom can be activated by a quantum of light) and the law of reciprocity (same effect with constant dose, regardless of changes in the relative proportions of irradiance and exposure time), the inactivation of LDH (Ω) is, at each position in the lens, directly proportional to the dose of UVR-B (H). The proportionality constant is k (equation 2).

\[
H = k\Omega
\]  

(2)

According to the Lambert-Beer law, the linear absorption coefficient (α) can be estimated if the intensity of the radiation is known at two points along the path of the radiation (equation 3), the distance between the points being \( l_2 - l_1 \).

\[
\alpha = \frac{\ln(H_{l_2}) - \ln(H_{l_1})}{l_2 - l_1}
\]  

(3)

Substituting \( H_{l_2} \) in equation 3 according to equation 2, it is seen that the linear absorption coefficient (α) can be calculated from the inactivation of LDH at the two points along the path of the radiation (equation 4).

\[
\alpha = \frac{\ln(\frac{\Omega_{l_2}}{\Omega_{l_1}})}{l_2 - l_1}
\]  

(4)

Penetration depth is here defined as 2/α, corresponding to a transmittance of 1/e² (−14%) at the penetration depth.

Statistical Analysis

For each region the radiation effect—that is, the paired difference between the rat’s exposed and nonexposed eyes at the three time
points—was analyzed with one-way analysis of variance (ANOVA).

Because of the unequal variances in different regions (Levene’s test),
the nonparametric Friedman’s ANOVA with multiple comparisons
was used to analyze differences in radiation effect between the regions.

The analysis was performed for each time point separately. The signif-
icance level was set to 5%, and mean values are expressed with 95%
confidence intervals.

RESULTS

Six hours after exposure, all exposed eyes showed signs of
inflammation. The relative levels of enzyme activity in the
exposed and the nonexposed eyes are presented in Figure 3.

For each time point (0, 2, and 6 hours), the enzyme activity in
nonexposed eyes was significantly higher in the corneal epi-
thelium and in the lens nucleus than in the remaining lens
regions. The UVR-B–exposed eyes showed the same pattern.

In Figure 4 the radiation effect is shown for each time point
and eye region. There was, except for the lens nucleus, no
time-dependent radiation effect. The immediate radiation ef-
fect in the cornea was significantly different from all other
regions except the nucleus. The radiation effects, seen over
the whole time span, were largest in the cornea and the outer
anterior lens cortex.

In Figure 5 the penetration of 300-nm UVR-B between the
outer and inner lens anterior cortex regions is estimated from
the inactivation of LDH at 0 hours’ latency, according to equa-
tion 4. The penetration depth (2/α) was estimated to 0.45 mm,
corresponding to approximately 14% residual UVR-B intensity.

Also plotted in the figure is the attenuation of 300-nm UVR-B as
expected from in vitro measurements of the linear absorption
coefficient in whole monkey lenses and sectioned 70-year-old
human lens. Maher’s monkey data suggest a considerably
shallower penetration than our rat data, whereas the human
lens data of Dillon et al. in the anterior-most 0.5 mm had a
penetration depth of 0.36 mm, slightly shallower than our
0.45 mm.

DISCUSSION

This study is, to our knowledge, the first to use a biochemical
marker as a measure of in vivo UVR-B transmittance in the lens.

The first law of photochemistry states that only those photons
that are absorbed can give rise to chemical alterations. Conse-
quently, the use of a UVR-B–sensitive marker within the lens
cells provides true information on the absorptive properties
of the tissue, compared with traditional transmittance measure-
ments of tissue in which transmittance is known, but the
relative proportions of absorption and scattering remain more
or less unknown.

The distribution pattern of LDH activity in young rat lens
presented herein is in accordance with a biochemical study by
Schmidt et al.—“... the LDH which in young rats, as is well
known, shows higher activities in the nucleus than in the
cortex”—and our histochemical study method. Two studies
on LDH isoenzymes have shown an increase in LDH-5 activity
in the lens nucleus. Thus, the LDH isoenzyme pattern
may explain the high LDH activity in the lens nucleus.

The LDH activity in the cornea and the outer anterior part
of the lens decreased immediately after UVR-B exposure,
which suggests a photochemical mechanism. There was no
significant difference between the three time points. Therefore
the effect of the radiation-induced ocular inflammation on the
enzyme activity was small.

The fact that lenticular LDH activity decreased only in the
anterior part and not the posterior part is reasonable, consid-
ering the low transmittance of 300-nm UVR-B through the lens.

General knowledge indicates a short penetration depth of 300-nm UVR-B. Biophysical studies on human, monkey, rabbit,
and rat lens have shown that the transmittance of 300-nm
UV-B through the lens is, in principal, 0.14–0.19

The decrease in lens LDH activity in the outer anterior
cortex during the 6 hours of the study was approximately 20%,
which conforms with the small effect on total lens lactate
production seen after UV-B irradiation. If an inhibition of ion
pumps in the lens is caused by decreased ATP production, the
UVR-B effects ought to be localized to an area competent in
ATP production and located close to the anterior surface—
namely, the epithelium and the outer anterior cortex. The
epithelium is also the part of the lens that contains the highest concentration of ion pumps and receives the highest dose of UV-R-B. Evidence has been put forward indicating that the anterior, and not the posterior, parts of the lens are responsible for the development of UV-R-B cataract. Hightower and McCready showed that only anterior and not posterior UV-B irradiation of lenses had an effect on the ion pump activity in the lens epithelium and the development of cataract.

The apparent UV-R-B effect in the lens nucleus at 0 hours (Fig. 3) is most likely an artifact caused by suboptimal morphology in this region and the technical measurement problems associated with it. There is always a statistical 5% risk that the confidence interval excludes the true mean, in this case, hypothetically 0. Nuclear recovery from 0 to 6 hours after exposure is also unlikely. Recovery was not present in any other region and because there are no cell organelles in the nucleus, there can be no de novo production of new enzyme.

More research is needed before our hypothesis of inhibition of ATP production and ion pumps in the lens as a mechanism behind acute UV-R-B cataract can be confirmed. The shallow penetration depth of 300-nm UV-R-B in the lens necessitates a focus on detailed areas within the lens epithelium and anterior cortex and shows whole-lens measurements to be less efficient.

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