Protection by Dimethylthiourea Against Retinal Light Damage in Rats

Daniel T. Organisciak,* Ruth M. Darrow,* Yih-ling Jiang,* George E. Marak,† and Janet C. Blanks‡

The protective effect of dimethylthiourea (DMTU) against retinal light damage was determined in albino rats reared in darkness or in weak cyclic light. Rats maintained under these conditions were treated with DMTU at different concentrations and dosing schedules and then exposed for various times to intense visible light, either intermittently (1 hr light and 2 hr dark) or continuously. The extent of retinal light damage was determined 2 weeks after light exposure by comparing rhodopsin levels in experimental rats with those in unexposed control animals. To determine the effect of DMTU on rod outer segment (ROS) membrane fatty acids, ROS were isolated immediately after intermittent light exposure, and fatty acid compositions were measured. The time course for DMTU uptake and its distribution in serum, retina, and the retinal pigment epithelium (RPE)/choroid complex was determined in other rats not exposed to intense light. After intraperitoneal injection of the drug (500 mg/kg body weight), DMTU appeared rapidly in the serum, retina, and the RPE and choroid. In the ocular tissues, it was distributed 70–80% in the retina and 20–30% in the RPE and choroid. This antioxidant appears to have a long half-life because it was present in these same tissues 72 hr after a second intraperitoneal injection. For rats reared in the weak cyclic light environment, DMTU (two injections) provided complete protection against rhodopsin loss after intense light exposures of up to 16 hr. Only 15% rhodopsin loss was found in cyclic-light DMTU-treated rats after 24 hr of intermittent or continuous light. For rats reared in darkness, DMTU treatment resulted in a rhodopsin loss of less than 20% after 8–16 hr of continuous light and approximately 40% after similar exposure to intermittent light. Irrespective of the type of light exposure, rhodopsin loss in the dark-reared DMTU-treated rats was nearly identical to that found in un.injected cyclic light-reared animals. In rats from both light-rearing environments, DMTU treatment prevented the light-induced loss of docosahexaenoic acid from ROS membranes. As measured by rhodopsin levels 2 weeks later, DMTU was most effective when given as two doses administered 24 hr before and just before intense light exposure. As a single dose given during continuous light exposure, DMTU protected cyclic light-reared rats for at least 4 hr after the start of exposure but was ineffective in dark-reared animals if injected 1 hr after the start of light. It was also ineffective in both types of rats when given after light exposure. The drug DMTU appears to provide complete protection against retinal light damage in cyclic light-reared rats, and to alter the form of retinal damage in dark-reared rats. These results suggest that DMTU is most effective when present during the light-induced initiation of damage in the retina. This pathologic process develops more slowly in cyclic light-reared rats than in dark-reared animals. Invest Ophthalmol Vis Sci 33:1599–1609, 1992

Retinal damage caused by visible light is a complex process, affected by various environmental and genetic factors and influenced greatly by exposure conditions. The intensity of the light and its wavelength, the duration of exposure, and the manner in which light is administered can all affect the extent of retinal damage.1–7 As described earlier,1 multiple short light exposures, followed in each case by a dark period, cause more retinal damage than does a single continuous dose of the same duration. Intermittent light has been used in studies on monkeys8 and rats at both elevated and normal body temperatures.9,10 Environmental or genetic factors that influence light damage

From the *Department of Biochemistry, Wright State University, Dayton, Ohio; †Georgetown University, Washington, DC; and the ‡Doheny Eye Institute, University of Southern California School of Medicine, Los Angeles, California.

Supported by grant EY-01959 (DTO), EY-3042 (JCB), and EY-3040 (core center grant to the Doheny Eye Institute) from the National Institutes of Health (Bethesda, Maryland). JCB is a recipient of the James P. Adams Special Scholars Award given by Research to Prevent Blindness, Inc. (New York, NY).

Submitted for publication: April 2, 1991; accepted November 5, 1991.
Reprint requests: D. T. Organisciak, Department of Biochemistry, Wright State University, Dayton, OH 45435.
in rats include diet, age, genetic makeup, and especially, the prior light-rearing history of the animals.\textsuperscript{11-17} For example, rats reared in darkness have higher levels of rhodopsin and are more susceptible to retinal damage than littersmates maintained in a weak cyclic-light environment before intense light treatment.\textsuperscript{10,12,16} Based on the differing susceptibilities of rats to retinal damage and especially on the degree of involvement of the retinal pigment epithelium (RPE), two types of light damage have been described.\textsuperscript{18} Type I involves damage to the RPE and the photoreceptors and is found typically in dark-reared rats; it is exacerbated by intermittent light exposure.\textsuperscript{10} Type II damage affects primarily the photoreceptors, is seen usually in cyclic light-reared rats exposed to continuous light, and is partially reversible.\textsuperscript{18} Currently, the mechanism of light-induced retinal damage is not well understood for either cyclic light- or dark-reared rats.

Recently, the hypothesis that oxidative reactions are involved in retinal light damage has received considerable attention. During intense light exposure, docosahexaenoic acid (22:6) is lost from rod outer segment (ROS) membranes, and products of lipid peroxidation appear to increase.\textsuperscript{19,20} In addition, rats with lower than normal levels of ROS 22:6\textsuperscript{21-24} are protected to some degree against retinal light damage.\textsuperscript{21,22,24} Furthermore, the retina normally contains high levels of protective antioxidants (such as \alpha-tocopherol, glutathione, and ascorbic acid)\textsuperscript{24-26} and antioxidative enzymes (such as glutathione peroxidase).\textsuperscript{22} However, among the endogenous retinal antioxidants, only ascorbic acid decreases during intense light exposure,\textsuperscript{25-27} and ascorbate supplementation of rats has been shown to protect these animals against retinal light damage.\textsuperscript{21,22,28-30} Recently, the D-stereoisomer of ascorbic acid was shown to have the same protective effect and to prevent or reduce the loss of 22:6 from ROS membranes.\textsuperscript{31}

If oxidation is involved in the process of retinal light damage, then other antioxidants also might reduce or prevent damage. Dimethylthiourea (DMTU) is a water-soluble synthetic antioxidant that reportedly is an effective scavenger of toxic reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, and hypochlorous acid.\textsuperscript{32-34} It can reduce ocular injury associated with uveitis and endotoxin treatment,\textsuperscript{35,36} granulocyte-mediated lung injury,\textsuperscript{32} and reperfusion-induced damage to the brain.\textsuperscript{37} A recent study\textsuperscript{38} showed that DMTU is effective also in reducing retinal light damage in rats. Other evidence suggests that DMTU is more effective than ascorbic acid in preventing light damage.\textsuperscript{31} In our study, we measured the protective effects of DMTU in cyclic light- and dark-reared rats exposed to intense visible light. Our results showed that DMTU is effective in reducing retinal light damage in both types of rats and that, in cyclic light-reared rats, DMTU appears to provide complete protection against retinal damage. A preliminary report of this study has been published.\textsuperscript{39}

**Materials and Methods**

**Animal Maintenance and Light Exposures**

Weanling male albino Sprague-Dawley rats were obtained from Harlan Inc. (Indianapolis, IN) and maintained in a weak cyclic-light environment (20-40 lux, 12 hr/day) or darkness for 40 days. During this time, the animals were fed rat chow (Teklad, Madison, WI) ad libitum and had free access to water. At age 60 days, the rats were dark adapted overnight and then exposed to intense visible light using one of two exposure paradigms: intermittent or continuous light. Intermittent exposure consisted of 1-hr light periods interrupted by 2-hr dark periods; continuous light exposure was uninterrupted.

Light exposures were done in green Plexiglass 2092 cylinders (Dayton Plastics, Dayton, OH) with an effective band pass of 490-580 nm.\textsuperscript{1} During exposures, the light intensity was 1750-2000 lux. The chambers were equipped with automatic timers set for intermittent or continuous light,\textsuperscript{10} and all exposures were started at 9 AM. Continuous light exposures lasted for up to 48 hr; 24 hr of intermittent light required 72 hr of exposure. During light treatment, the rats were unrestrained and had access to food and water. Immediately after exposure, some rats were killed for ROS fatty acid analysis. Others were maintained in a dark environment (along with unexposed control animals) for up to 2 weeks before they were killed. To determine the rate of rhodopsin bleaching, some animals were killed after 5-60 min in the intense light. Others were exposed to light for 1 hr, and then rhodopsin regeneration was measured after various periods in darkness. In these experiments, the rats were killed in carbon dioxide-saturated chambers. The use of animals in this investigation conformed to the ARVO Resolution on the Use of Animals in Research.

**DMTU Treatment and Tissue Analysis**

Twenty-four hours before and again just before light exposure, some rats were injected intraperitoneally (IP) with 1,3-dimethylthiourea (DMTU; Aldrich, Milwaukee, WI), usually at a dose of 500 mg/kg body weight. Saline was used as the vehicle. To determine the dose–response profile for retinal damage, the concentration of DMTU was varied while the dose sched-
uele was maintained. In some experiments, the dose schedule was varied so that the second injection coincided with the end of intermittent light exposure (day 1) or the dark period thereafter (days 2 and 3). In some continuous-light experiments, a single 500 mg/kg dose of DMTU was administered before or during light exposure, after which the light exposures continued for up to 24 hr.

At various times after injection, some rats were killed to determine DMTU levels in their serum, retina, and RPE/choroid tissues. For these measurements, the rats were anesthetized in halothane chambers and maintained under ether during transcardiac perfusion. A sample of cardiac blood was obtained, and then the blood was removed from the ocular tissues by perfusion with saline. The retinas were excised and homogenized in 1 ml of 50 mM phosphate buffer, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid. The eyecup, minus the retina, was everted over a small plastic cone and sonicated (20 sec) in 0.5 ml of buffer to detach the RPE and choroid from the scleral tissue. Whole blood was allowed to clot at room temperature. After centrifugation at 6000 X g for 10 min, tissue supernatants and sera, diluted in buffer, were filtered through a 0.45-μm filter. The DMTU was analyzed by high-performance liquid chromatography using a 4.5 × 250-mm octadecylsilane reverse-phase column (Rainin, Woburn, MA). The antioxidant was eluted with a mobile phase consisting of methanol and water (5:95) at a flow rate of 1 ml/min. The samples were quantified by absorbance at 242 nm, with comparison of samples to DMTU standards of known concentration. Tissue protein levels were determined for selected retinal and RPE and choroid tissues.

**Rhodopsin and Fatty Acid Measurements**

The levels of rhodopsin in the eyes of experimental and control rats were determined with Emulphogene BC-720 detergent 1.5% (GAF, Wayne, NJ). To estimate the extent of photoreceptor cell loss from light exposure, the level of rhodopsin in the eyes of experimental animals was compared with that in unexposed control rats maintained in darkness for the same 2-week period. During the dark recovery period, necrotic photoreceptor cell debris was removed from the light damaged eyes, and rhodopsin in the surviving rod cells was maximized. Techniques for the extraction of rhodopsin and the estimation of retinal light damage have been described.

As determined by rhodopsin measurement, rats reared in a weak cyclic light environment had a lower rhodopsin level than did age-matched rats reared in darkness, and they were less susceptible to retinal damage from continuous or intermittent light. In these experiments, 60-day-old cyclic light-reared rats had 1.8 ± 0.2 nmol rhodopsin per eye before intense light treatment; the dark-reared animals had 2.05 ± 0.2 nmol/eye (n = 8–10). To evaluate the protective effects of DMTU against light damage, the rats were exposed to intense light for a time known to reduce rhodopsin to approximately one half that of unexposed control animals. Both the experimental and control rats then were maintained in total darkness for 2 weeks after intense light exposure. For cyclic light-reared rats, a 40–60% loss of rhodopsin occurred after 8–12 hr of intermittent light exposures or approximately 24 hr of continuous light. For dark-reared rats, a 50–70% loss of rhodopsin occurred after 2–3 hr of intermittent light or 4–6 hr of continuous light. Rhodopsin in the control animals unexposed to light and measured after 2 weeks in darkness was 2.1 ± 0.1 nmol/eye.

**Histologic Findings**

Light microscopic studies were done on an eye from each of two rats under the following experimental conditions: dark-reared animals with or without DMTU after 3 hr of intermittent light followed by a 30-min dark period and cyclic light-reared animals with or without DMTU exposed to 9 hr of intermittent light followed by a 30-min dark period. The eyes were enucleated immediately after the animals were killed, the corneas were excised, and the eyecups were placed in paraformaldehyde 2% and glutaraldehyde 2.5% in 0.1 M cacodylate buffer (pH 7.4). After overnight fixation at 4°C, the eyecup was dissected into 1-mm pieces, osmicated, and dehydrated, followed by embedding in Poly/Bed (Polysciences, Warrington, PA), 1-μm sectioning, and viewing with a Zeiss (Thornwood, NY) photomicroscope.

**Results**

**Retinal Light Damage as a Function of Rearing Environment**

As determined by rhodopsin measurement, rats reared in a weak cyclic light environment had a lower rhodopsin level than did age-matched rats reared in darkness, and they were less susceptible to retinal damage from continuous or intermittent light. In these experiments, 60-day-old cyclic light-reared rats had 1.8 ± 0.2 nmol rhodopsin per eye before intense light treatment; the dark-reared animals had 2.05 ± 0.2 nmol/eye (n = 8–10). To evaluate the protective effects of DMTU against light damage, the rats were exposed to intense light for a time known to reduce rhodopsin to approximately one half that of unexposed control animals. Both the experimental and control rats then were maintained in total darkness for 2 weeks after intense light exposure. For cyclic light-reared rats, a 40–60% loss of rhodopsin occurred after 8–12 hr of intermittent light exposures or approximately 24 hr of continuous light. For dark-reared rats, a 50–70% loss of rhodopsin occurred after 2–3 hr of intermittent light or 4–6 hr of continuous light. Rhodopsin in the control animals unexposed to light and measured after 2 weeks in darkness was 2.1 ± 0.1 nmol/eye.
Dose Response for Protection by DMTU

The optimal concentration (dose) of DMTU for protection against retinal light damage was determined in cyclic light- and dark-reared rats exposed to intermittent light for 8 and 3 hr, respectively. Rhodopsin was measured 2 weeks after the intense light exposure. In unsupplemented or saline-injected cyclic light-reared animals, the rhodopsin loss was more than 40%. It was more than 70% in the unsupplemented dark-reared rats (Fig. 1). Other rats received two IP injections of DMTU at various concentrations and simultaneously were exposed to light for the same 8- or 3-hr periods. In the cyclic light-reared rats, a linear increase in rhodopsin content was found in animals that received DMTU over a dose range of 75-250 mg/kg body weight. At doses greater than 250 mg/kg, rhodopsin levels in the experimental rats were nearly the same as those in the unexposed control animals. A linear spline fit to the data revealed that the point of saturation occurred at 285.7 mg/kg of DMTU. At doses less than this amount, the least-squares regression equation was $y = 1.264 + 0.0025x$, where $y =$ rhodopsin and $x =$ dose. Although retinal damage was greater in dark-reared rats after intermittent exposure for only 3 hr, a similar dose response was found. Nearly complete protection was found for the dark-reared rats when the concentration of DMTU exceeded 375 mg/kg body weight. The actual saturation point was 388.1 mg/kg, with $y = 0.513 + 0.0039x$. For both the cyclic light- and dark-reared rats, a DMTU dose of 500 mg/kg resulted in approximately the same levels of rhodopsin as found in unsupplemented animals.

Effect of DMTU on Retinal Damage as a Function of Light Exposure

Figure 2 shows results for cyclic light- and dark-reared rats that received two IP injections of DMTU (500 mg/kg) and were exposed to intermittent light for up to 24 hr. Rhodopsin was measured 2 weeks after the final exposure. In unsupplemented cyclic light-reared rats, four 1-hr light exposures resulted in 10% less rhodopsin per eye than in the unexposed control rats. As measured by rhodopsin loss, retinal damage was approximately 40% after 8-16 1-hr light exposures. It was 65% after 24 hr of intermittent light treatment. For the cyclic light-reared rats that received DMTU, a protective effect was found. In rats exposed to intermittent light for up to 16 hr (48 hr overall), complete recovery of rhodopsin was measured. After 20 hr of light, only 10% rhodopsin loss occurred. The loss of rhodopsin was only 15% after 24 1-hr light exposures.

In the unsupplemented dark-reared rats, rhodopsin loss was 70% after only three 1-hr light periods. With longer exposure times, 85-90% rhodopsin loss was found in these animals. In addition, DMTU treat-
ment of dark-reared rats provided protection against retinal light damage. Less than 10% rhodopsin loss was found in the DMTU-treated rats exposed to light for three 1-hr periods. The loss of rhodopsin was only 20% after six 1-hr light periods and approximately 40% after intermittent light exposures of up to 24 hr. Overall, for the dark-reared DMTU-treated rats, the loss of rhodopsin was similar to that seen in unsupplemented cyclic light-reared rats. Three-way analysis of variance revealed that the dark-reared DMTU-treated rats and unsupplemented cyclic light-reared rats had rhodopsin values that were not significantly different after 8-, 12-, and 16-hr exposures ($P \geq 0.05$), whereas for the 20- and 24-hr exposures, the dark-reared rats had rhodopsin values that were significantly higher than those of the unsupplemented cyclic light-reared rats.

The protective effect of DMTU was most striking by light microscopic examination of treated compared with untreated animals. Untreated rats exposed to 3 hr of intermittent intense light followed by a 30-min dark period showed obvious photoreceptor cell damage and a large number of phagosomes in the RPE (Fig. 3A). By contrast, DMTU-treated rats under the same experimental conditions appeared to have normal retinal morphology (Fig. 3B). When untreated, cyclic light-reared rats were exposed to 9 hr of intermittent intense light followed by a 30-min dark period, the damage was more pronounced in the RPE and outer nuclear layer (Fig. 3C). In the RPE, numerous phagosomes and vacuoles were present, and the nuclei appeared swollen. Both ROS and rod inner segments contained dark cytoplasm; many photoreceptor nuclei were pyknotic. The retinas of DMTU-treated animals under similar conditions appeared normal (Fig. 3D). Therefore, prior treatment with DMTU appears to protect the retinas of either dark-reared or cyclic light-reared animals exposed to intense light.

For comparisons with light damage from intermittent light and to extend the period of light exposure, other rats were exposed to continuous light for up to 48 hr. The results for unsupplemented and DMTU-treated rats are shown in Figure 4. As expected, less rhodopsin per eye was found in all rats after longer light exposures than after shorter light periods. Damage was also greater in the dark-reared rats than in those reared in the weak cyclic-light environment.

Overall, DMTU treatment was effective in protecting against retinal damage from continuous light. For the cyclic light-reared rats treated with DMTU, approximately 2.0 nmol of rhodopsin per eye was found 2 weeks after 8 or 16 hr of light. After 24 hr of light, a loss of approximately 15% of the rhodopsin occurred. This rhodopsin level was similar to that in rats exposed to intermittent light for 24 hr (Fig. 2). Furthermore, the level of rhodopsin in the DMTU-treated rats was more than twofold higher than that in unsupplemented animals. After 48 hr of continuous light, DMTU-treated rats had almost twice as much rhodopsin per eye as unsupplemented animals.

Similar results were obtained for the dark-reared rats. In these animals, however, DMTU treatment resulted in three to fourfold more rhodopsin per eye than in the unsupplemented rats. Furthermore, the losses of rhodopsin in dark-reared rats treated with DMTU were statistically the same as those of unsupplemented cyclic light-reared rats at all times. Whereas DMTU protection was greater after 8 or 16 hr of continuous light than in comparable intermittent light-treated rats (Fig. 2), 20% less rhodopsin was found after 24 hr of continuous light than in the 24-hr intermittent light-treated animals.

### Tissue Levels of DMTU After IP Administration

The uptake and distribution of DMTU in the serum, retina, and the RPE/choroid complex was determined at various times after the standard injection procedure. Tissue levels of DMTU were measured after a single 500 mg/kg dose or after a second IP injection given 24 hr later. In these experiments, DMTU uptake was the same in both the cyclic light-and dark-reared rats. These rats were not exposed to intense light, and all animals were perfused with saline before analysis. As shown for the 5-min period in Table 1, DMTU appears rapidly in serum, retina, and RPE/choroid after one or two doses. In serum, 7–8 μmol/ml was found; the retinas contained 50–70 nmol/mg protein. In the RPE/choroid tissues, the concentration of DMTU was about eightfold higher than in retina. Generally, tissue levels of DMTU were highest 4 hr after injection and declined thereafter. However, the concentrations of DMTU 4 hr after the second injection were almost double those found in the same tissues 4 hr after a single dose. They were significantly higher than after longer periods. Despite the loss of DMTU over time, 2.7 μmol/ml and 37 nmol/mg protein remained in the serum and retina, respectively, 24 hr after a single injection. The average DMTU concentrations were nearly the same in these tissues 24 hr after the second injection. In the RPE/choroid, however, four to fivefold higher concentrations of DMTU were found than in retina 24 hr after one or two doses. Table 1 also shows that DMTU has a long half-life; it was present in all tissues 3 days after the second injection.

### ROS Fatty Acid Composition After Light Exposure

Table 2 shows that DMTU treatment effectively prevented the loss of ROS 22:6 during intense light
Fig. 3. Representative light micrographs of toluidine blue-stained 1 μm sections from retinas of dark-reared (A and B) or cyclic light-reared (C and D) rats with or without DMTU after exposure to either 3 hr (A and B) or 9 hr (C and D) of intermittent light followed by a 30 min dark period (×864). A section of retina from a dark-reared control rat exposed to 3 1-hr light doses (A) shows darkened, disoriented rod inner (RIS) and outer (ROS) segments. The RIS region has large vacuoles (arrows). Photoreceptor nuclei in the outer nuclear layer (ONL) appear to be degenerating (arrows). Numerous phagosomes are present in the retinal pigment epithelium (RPE). In contrast, the RPE, RIS, ROS, and ONL appear normal in dark-reared rats treated with DMTU and exposed to 3 1-hr light doses (B). Damage is more severe in the RPE and the photoreceptor cell layer in cyclic light-reared rats without DMTU (controls), exposed to 9 hr of light followed by a 30 min dark period (C). Note disorganized RPE (filled with phagosomes; arrows), ROS, and RIS. Many pyknotic nuclei are present in the ONL (arrows). In contrast, cyclic light-reared rats treated with DMTU and exposed to the same lighting regime (9 hr) have normal RPE and retina. OSL, outer synaptic layer; INL, inner nuclear layer.
exposure. In both cyclic light- and dark-reared rats, the major ROS unsaturates were oleic acid (18:1), arachidonic acid (20:4) and 22:6. In the unexposed control rats, 22:6 represented more than 51 mol% of the ROS fatty acids; 18:1 and 20:4 combined were less than 8 mol%. The major saturated fatty acids were palmitic (16:0) and stearic (18:0) acid, which together comprised approximately 36% of the total. When unsupplemented and DMTU-treated rats were exposed to intermittent light, ROS 22:6 levels decreased or were significantly lower in only the unsupplemented animals. In comparison with the unexposed control, unsupplemented cyclic light-reared rats lost 7% and 29% of the 22:6 after 8 and 16 hr of light (P < 0.1 and P < 0.001, respectively). In dark-reared rats, the losses were 16% and 24%, respectively (P < 0.001). In all unsupplemented rats, 16:0 was found to be higher after light treatment. The ROS 18:1 was also higher in the 16-hr light-exposed rats, but it was unchanged after 8 hr of intermittent exposure.

Irrespective of the length of intense light exposure, ROS from the DMTU rats had fatty acid profiles that were essentially the same as those in the unexposed control rats. In each case, more than 50 mol% of 22:6 was present, and the levels of the other fatty acids were similar to those found in control ROS. Three-way analysis of variance revealed that exposure time interacts significantly with both DMTU and rearing environment (P < 0.0391). At both 8 and 16 hr, DMTU-treated rats had significantly more 22:6 than did the unsupplemented cyclic light- or dark-reared animals (P = 0.0001 at 8 hr and P < 0.0001 at 16 hr). In all ROS from light-exposed rats, the average cholesterol values were higher than in control animals.

Protection as a Function of Time of DMTU Administration

Table 3 contains data showing that DMTU is effective in reducing retinal damage when given before the start of intermittent light treatment. In both cyclic light- and dark-reared rats, rhodopsin levels measured 2 weeks after eight 1-hr exposures were highest when DMTU was given the day before (day −1) and just before light treatment (day 0). If the injections were given on days 0 and 1, rhodopsin in the cyclic light-reared rats remained at 100% of control; it was only 50% of control in the dark-reared rats. However, when DMTU was given after the light treatment period (days 1, 2, or later), rhodopsin levels were the same as in the untreated rats.

Because DMTU appeared to be most effective when given before or during the period of light exposure, we further subdivided the exposure period by giving a single 500-mg/kg DMTU injection at various times before or during light treatment. For these experiments, cyclic light-reared rats were exposed to con-
Table 2. Major fatty acids in ROS of light-exposed rats (mol%)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Control</th>
<th>Cyclic light-reared rats (n = 4-8)</th>
<th>Dark-reared rats (n = 4-8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+ or -</td>
<td>8 hr light</td>
<td>16 hr light</td>
</tr>
<tr>
<td>16:0</td>
<td>DMTU</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>12.2 ± 1.3</td>
<td>12.2 ± 1.3</td>
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<tr>
<td>18:0</td>
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<td>3.2 ± 1.4</td>
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</tr>
<tr>
<td>20:4</td>
<td></td>
<td>4.0 ± 0.3</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>22:6</td>
<td></td>
<td>51.4 ± 2.0</td>
<td>53.9 ± 2.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td>15 ± 6</td>
<td>15 ± 11</td>
</tr>
</tbody>
</table>

* Results are the mean ± standard deviation for the number of determinations shown in parentheses. For each experiment, the retinas from three rats were used to prepare ROS.

Table 3. Rhodopsin levels in rats treated with DMTU at various times before or after intense light exposure (nmol/eye)*

<table>
<thead>
<tr>
<th>DMTU injections†</th>
<th>Cyclic light-reared</th>
<th>Dark-reared</th>
</tr>
</thead>
<tbody>
<tr>
<td>uninjected</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Days -1, 0</td>
<td>2.2 ± 0.2</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Days 0, 1</td>
<td>2.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Days 1, 2</td>
<td>1.3 ± 0.4</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Days 3, 4</td>
<td>1.4 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>un.injected</td>
<td>1.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

* Results are expressed as the mean ± standard deviation for n = 4-8 separate determinations.
† DMTU was given 2 times intraperitoneally at 500 mg/kg body weight at 24-h intervals with the first and second injections coinciding with the days shown. Day 0 refers to the start of light exposure. Rhodopsin was measured 2 wk after 8 hr intermittent light exposure.

Fig. 5. Rhodopsin levels in rats given a single 500 mg/kg injection of DMTU at various times before or during continuous light exposure. Cyclic light-reared (C) or dark-reared rats (●) were injected at the times shown. Light exposures were started at time 0 and continued for a total of 24 hr or 8 hr, respectively. Rhodopsin levels were measured 2 wk later and compared to saline-injected or uninjected rats (△, ▲) simultaneously exposed. Results are the averages ± standard deviation for 3–8 pairs of eyes from four separate experiments.
had 0.4–0.5 nmol per eye. Four hours after the 1-hr light exposure, dark-reared rats (treated or untreated) had an average of 2.0 nmol rhodopsin per eye; cyclic light-reared rats had 1.6 nmol per eye.

Discussion

We found a protective effect against retinal light-induced damage in rats by administering a synthetic antioxidant. Irrespective of the light exposure paradigm used, the duration of light treatment, or the prior light-rearing history of the animals, DMTU reduced the loss of rhodopsin. Because the loss of rhodopsin is a measure of photoreceptor cell death and disappearance after damage, we concluded that DMTU effectively protects visual cells from light damage. For rats reared in weak cyclic light, this protection, as measured by rhodopsin, was complete after intense light treatment for up to 16 hr; it was greater than 85% after 24 hr of light (Figs. 2, 4). In the dark-reared rats, DMTU protection was even more remarkable. Despite their high degree of susceptibility to retinal light damage, dark-reared rats given DMTU had rhodopsin losses similar to those of unsupplemented cyclic light-reared rats, and the histologic appearance of the retina was normal (Fig. 3B). Thus, DMTU decreased retinal light damage in dark-reared rats to the levels found in rats previously maintained in a weak cyclic-light environment. In previous studies with ascorbic acid-supplemented rats, a similar change in the form of retinal light-induced damage was reported, but in no study to our knowledge, has the effect of antioxidant treatment been so completely protective as in these DMTU-treated rats. A recent comparative study with DMTU and L- or D-ascorbate treatment in retinal light-induced damage confirmed the greater protective effect of DMTU relative to ascorbic acid. The protective effects of the two antioxidants also suggests that oxidative reactions are involved in the light damage process, but whether oxidation is the primary cause or the result of another earlier event is unknown.

Numerous studies have reported losses of the polyunsaturated 22:6 from ROS during intense light exposure with an apparent increase in organic peroxide levels. Furthermore, rats with low levels of ROS 22:6 are protected against retinal light-induced damage, as shown by dietary depletion of the essential linolenic acid precursor of 22:6 or by rearing the rats under different light intensities. Our study confirms earlier findings by showing that ROS 22:6 is lost in the unsupplemented rats exposed to intermittent light and extends those findings by demonstrating that DMTU prevents that loss (Table 2). These observations allow some general statements to be made about the relationship between 22:6 levels and retinal light-induced damage. For example, in dark-reared rats, the loss, or oxidation, of 22:6 during light exposure cannot be the only factor associated with retinal damage. Whereas 22:6 levels in the ROS of the DMTU-treated rats were unchanged immediately after intense light, approximately 40% rhodopsin loss was measured 2 weeks later (Table 2, Fig. 2). Previous studies have shown that ROS 22:6 loss occurs primarily during light and that, except for overt photoreceptor cell loss, the ROS of rats subsequently maintained in darkness show no additional 22:6 loss. Furthermore, rhodopsin losses in the dark-reared DMTU-treated rats were statistically the same as in the unsupplemented cyclic light-reared rats exposed to continuous light (Fig. 4) and for intermittent exposures of 8, 12, and 16 hr (Fig. 2). For the 20- and 24-hr intermittent light exposures, the dark-reared DMTU-treated rats had significantly higher rhodopsin levels than the unsupplemented cyclic light-reared animals. As reported earlier, adaptive changes in “photon-catch” and light damage susceptibility were attributed, in part, to altered rhodopsin levels in rats reared in different light intensities. Recently, changes in the levels of rhodopsin, α-transducin, and retinal S-antigen have been found in rats changed from one rearing environment to another and after intense light exposure. Cyclic light-reared rats, which have higher S-antigen levels and lower α-transducin levels than dark-reared rats, were more resistant to retinal light damage. Thus, adaptive changes in transduction protein levels may be related to the overall metabolic state of the visual cell and its susceptibility to damage from intense light. By preventing the loss of ROS 22:6 during light exposure, DMTU may allow the photoreceptors of dark-reared rats to accommodate higher light levels and adapt metabolically to some degree during intense light.

In the cyclic light- and dark-reared rats, DMTU completely protected against retinal light damage, as measured by rhodopsin levels 2 weeks later, and totally prevented the loss of ROS 22:6 during light exposure. This suggests that the loss, or oxidation, of 22:6 may be related more directly to light exposure in these animals. Whereas our study does not prove a causative relationship between 22:6 oxidation and retinal light-induced damage in cyclic light-reared rats, it does not exclude this possibility. Additional studies will be required to clarify the role of 22:6 oxidation in retinal light-induced damage.

In both the cyclic light- and dark-reared rats, DMTU was most effective when given before or early during the course of light exposure; there was no pro-
tection when it was administered after light treatment (Table 3). This suggests that pathologic or toxic reaction(s) are associated with retinal damage and that they are light initiated. In studies with ascorbic acid-supplemented rats, we found a similar time dependence and dose response for protection and a rapid ascorbate uptake by ocular tissues. In earlier studies and in the current investigation, the greatest protection occurred when antioxidant was present in the retina and/or RPE (Table 1) at the time light exposure began.

In the cyclic light-reared rats given a single dose of DMTU at various times during light exposure, the time course for rhodopsin loss (protection) was longer than in the dark-reared rats (Fig. 5). Thus, the pathologic process appears to develop more slowly in cyclic light-reared rats. In the dark-reared rats, DMTU was effective only if administered within 60 min after the start of light. The pathologic process in these rats therefore affects the visual cells sooner than in cyclic light-reared rats and, based on the relative levels of protection by DMTU, is more severe. Electroretinographic measurements done on DMTU-treated rats showed that cyclic light-reared rats have a higher degree of functional retinal capacity than do dark-reared animals after intermittent light exposure (Noell WK, personal communication, 1990).

Previous studies using intense light-exposed cyclic light- and dark-reared rats have provided electrophysiologic and histologic evidence for differences in the extent of damage in the RPE. In both studies, the RPE of dark-reared rats showed changes associated with the damaged visual cells that were not seen to the same degree in the cyclic light-reared rats. Our results (Figs. 3B, 3D) suggest that DMTU prevents damage to the RPE in both types of rats exposed to various doses of intermittent light. However, the different rates of rhodopsin loss in cyclic light- and dark-reared rats given DMTU during light exposure (Fig. 5) suggest that other biochemical or electrophysiologic differences may exist between rats reared in weak-light or dark environments. Whether such differences can be related mechanistically to the type I and II forms of retinal light-induced damage or whether retinal light damage in these rats is a matter of degree will require further analysis of the damage incurred by the RPE.

Currently, it is too early to speculate on the mechanism by which DMTU prevents retinal light damage in rats. Although reactive oxygen species are likely to be involved in the overall process, as suggested by studies with DMTU in other systems, understanding the light-initiated process by which massive rhodopsin bleaching triggers retinal damage will require additional experimentation. Future studies with animal models of retinal light damage should help to determine the mechanism of action of DMTU. Whether synthetic antioxidants (such as DMTU) or natural antioxidants (such as ascorbic acid) will be effective in preventing light-induced retinal damage encountered during ocular surgery or that occurring as a result of natural chronic high light exposures is unknown.

Key words: rhodopsin, retinal light damage, antioxidants, rats

Acknowledgments

The authors thank W. K. Noell for doing the electroretinographic measurements, H. Khamis and S. Balkar of the Wright State Statistical Consulting Center for statistical analysis, Michael Pickford and Christine Spee for technical assistance, and Ann Dawson for editorial comments.

References


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