Radiation induced cataracts are models for studying mechanisms of lens opacification. WR-77913, S-3-(amino-2-hydroxypropyl) phosphorothioate (NCS-318809), has been identified as a radioprotective agent. Injection of WR-77913 (1160 mg/kg, i.p.) 15 to 30 min before exposure to 15.3 gray appears to be an effective inhibitor of radiation cataractogenesis.


Inhibition of Lens Opacification in X-Irradiated Rats Treated With WR-77913

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Radiation induced cataracts are models for studying mechanisms of lens opacification. WR-77913, S-3-(amino-2-hydroxypropyl) phosphorothioate (NCS-318809), has been identified as a radioprotective agent. Injection of WR-77913 (1160 mg/kg, i.p.) 15 to 30 min before exposure to 15.3 gray of x-irradiation inhibited rat lenses from developing radiation cataracts. Irradiated rats which did not receive the drug developed dense cataracts. Lenses from control rats which received no radiation remained transparent. Individual lenses were weighed, homogenized, and assayed for protein content using the Lowry method. The molecular weight distribution of soluble protein was determined by HPLC. Mean lens weights were: controls 48.2 mg; irradiated, drug-treated 45.9 mg; and irradiated, nontreated 45.5 mg. Protein accounted for over 40% of the lens weight in control and drug-treated rats and less than 20% for the nontreated cataractous lenses. Water was less than 60% of the lens weight in control and drug-treated rats and over 80% in cataractous lenses. Insoluble protein ranged from 12 to 17% of the total lens weight for each group. The ratio of insoluble to soluble lens protein was 0.40 for control, 0.65 for drug-treated, and 11.28 for cataractous rat lenses. HPLC confirmed a dramatic loss of soluble protein and a complete absence of protein below 25K daltons in cataractous lenses. Proteins below 25K daltons accounted for over 25% of the soluble protein in control and drug-treated rat lenses. WR-77913 stabilizes protein composition and appears to be an effective inhibitor of radiation cataractogenesis.


References

irradiation has become a useful model for studies on the mechanisms of lens opacification. Previous studies have indicated that differences between the physical properties of x-irradiated and non-irradiated lenses can be detected in vivo and in vitro as early as two weeks following irradiation, long before opacity can be observed and before significant biochemical changes are measurable. These findings led to the proposal that the chemical treatment of cataracts at the earliest stages of development might protect against progressive opacification and thereby delay or prevent cataract formation. The potential use of the radioprotective agent WR-77913 as an inhibitor of cataract formation was first recognized by Menard. This study reports the effect of WR-77913 on opacification, and the protein and water content of rat lenses following exposure to x-irradiation.

Materials and Methods. Animals: Male Sprague-Dawley rats, 7–9 weeks old, were divided into three experimental groups: irradiated and drug treated, irradiated only, and controls. Irradiated and drug-treated rats (n = 4) received a single intraperitoneal (i.p.) injection of WR-77913, S-3-(amino-2-hydroxypropyl)-phosphorothioate, 15–30 min before receiving a single exposure to 15.3 gray (0.63 gray/min) of γ-irradiation to the head. WR-77913 was dissolved (300 mg/ml) in calcium–magnesium free phosphate buffered saline (PBS, 0.13 M, pH = 7.24) and administered at a dose of 1160 mg/kg. This dose was approximately one half the LD50 and is based on previous studies of biodistribution and toxicity. Rats from the group receiving radiation without drug (n = 5) were given i.p. injections of an equivalent volume of PBS prior to exposure to equal doses of radiation. Irradiation was performed using a 137Cs teletherapy unit equipped with a 18 cm by 18 cm collimator. The unanesthetized rats were confined in a lucite device and positioned to receive whole head irradiation extending to the clavicles. Dosimetry was measured with a 100 R ionization chamber (Victoreen Model 570, Victoreen Instruments, Cleveland, OH). Control rats (n = 4) were given an equivalent volume of PBS by i.p. injection but were not exposed to radiation. The investigations reported in this manuscript conform to the ARVO Resolution on the Use of Animals in Research.

Photographic documentation: The general appearance of lenses from each group of rats was photographically documented with anterior view and slit lamp photographs using a Zeiss slit lamp photo-biomicroscope (Zeiss, Thornwood, NY). Rats were anesthetized with 30 mg/kg i.p. injections of pentobarbital before photographs were taken. The pupils were dilated with an equal mixture of 5% Neo-Synephrine™ (Winthrop Laboratories, Thornwood, NY) and 0.5% Mydriacyl™ (Alcon, Fort Worth, TX). All photographs were taken and the film processed under the same conditions to represent the appearance of the lenses as accurately as possible.

Protein analysis: Rats from each group were sacrificed in ether chambers up to 210 days after receiving treatments, and the eyes enucleated and placed on ice. Lenses were dissected from the eye within 30 min and weighed. To study the content and molecular weight distribution of rat lens proteins, extracts were prepared by homogenizing individual lenses in 1 ml Wheaton homogenizers (Wheaton, Millwood, NJ) with 0.5 ml of 0.10 M sodium sulfate/0.02 M potassium phosphate elution buffer (pH 6.9). The lens homogenate was transferred to microcentrifuge vials and the homogenizers rinsed twice with 0.25 ml of elution buffer. The total volume of 1.0 ml was vortexed for 5 sec and centrifuged for 20 min at 17,000 rpm. The supernatant was decanted and filtered through 0.22 micron Millipore filters (Millipore, Bedford, MA) and saved for protein analysis and size exclusion high performance liquid chromatography (HPLC). The pellet was resuspended in 0.1 N NaOH. The amount of protein in the soluble and insoluble fractions was measured using the Lowry method standardized against bovine serum albumin.

The size-exclusion HPLC system used for these studies consisted of a Perkin-Elmer Series 4 liquid chromatography module (Perkin-Elmer-Norwalk, CT), a Hewlett-Packard HP 1040A photodiode array spectrophotometer (Hewlett, Packard, Palo Alto, CA), and a TSK G3000SW, 30 cm by 7.5 mm column with a 10 cm guard column. The buffer used for homogenization also served as the mobile phase with a flow rate of 0.5 ml/min. The injection volume for each sample was 10 μl and absorbance was monitored at 280 nm. The TSK column was calibrated with known molecular weight standards.

Biodistribution: Biodistribution of the drug was measured in eye tissues using (35S)-WR-77913 which was synthesized as described previously. Rats were sacrificed by cervical dislocation 15, 30, and 60 min after receiving a single i.p. injection of the labeled drug at the dose used in the protection experiments. The eyes were enucleated and dissected into three portions; the lenses were removed, and the vitreous and aqueous were separated from the choroid, retina, and sclera. Each sample was solubilized (1.0 ml Soluene [Packard Instruments, Downs Grove, IL], 0.2 ml 30% H2O2, 1.0 ml 1 N HCl) and the concentration of the labeled drug measured by liquid scintillation counting with appropriate quench corrections. Uptake of radiolabel was calculated as percent injected dose per gram of tissue (percent ID/g).

Results. Figure 1 is a photographic representation of the protective effect that WR-77913 has against cat-
aract formation in x-irradiated rat lenses. Anterior and slit lamp views of rat eyes from control, irradiated, and irradiated and drug-treated animals are shown 154 days after treatment. Control rat lenses remained transparent throughout the study. Irradiated rats which received no drug treatment developed moderate lenticular opacities within 90 days of receiving gamma radiation. Lens opacification progressed to mature cataracts by 120 days post-irradiation in all animals which received no WR-77913. Rats protected by WR-77913 were noted to have very slight lenticular opacities when photographed 154 days after irradiation. The appearance of the opacities in the drug-treated rats remained stable until the animals were sacrificed at 210 days post-irradiation.

Figure 2 summarizes the protein content of rat lenses in relation to the total lens weight for each experimental group, and shows that WR-77913 protected irradiated rat lenses from the loss of soluble protein. The y-axis represents the mean lens content measured in milligrams. The mean weight of control lenses was 48.2 ± 5.7 mg, cataractous lenses averaged 45.5 ± 5.1 mg, and lenses from irradiated, drug-treated rats weighed 45.9 ± 5.9 mg. The combined weight of soluble and insoluble protein was 20.0 ± 3.6 mg in control lenses and 18.5 ± 3.6 mg in drug-treated lenses, which accounted for over 40% of the lens weight in each group. The total protein content in cataractous lenses was 8.6 ± 1.7 mg which accounted for only 19% of the lens weight. Water accounted for less than 60% of the lens weight in control and drug-treated rats and 81% of the lens weight in nonprotected lenses. Since the cataractous lenses of nonprotected rats did not show an appreciable drop in lens weight, hydration of the lens appears to compensate for the loss of protein. The amount of protein measured in the insoluble lens fractions averaged 5.7 mg in control, 7.3 mg in drug-treated, and 7.9 mg in cataractous rats. The amount

Fig. 1. Photographs showing the general appearance of control (a, d), irradiated nonprotected (b, e), and irradiated and drug-treated (c, f) rats’ eyes 154 days post-treatment. Whole eye (top row) and slit lamp photographs (bottom row) were taken of individual rats representative of each experimental group. The rat used for Figures 1c and 1f had the most severe lenticular opacification of all irradiated and drug-treated animals.

Fig. 2. A histogram showing the mean lens protein content of the three experimental groups demonstrates that WR-77913 protects lens proteins from changes associated with radiation cataract formation. Protein is separated into insoluble and soluble fractions. Irradiated rats which received no drug treatment had a dramatic loss of soluble protein. Soluble protein accounted for 68% of the total protein content in controls, 53% of the protein in irradiated and drug-treated rats, and less than 5% of the total protein found in the cataractous lenses of irradiated rats which received no drug treatment.
of protein measured in the soluble lens fractions averaged 14.3 mg in control, 11.2 mg in drug-treated, and 0.7 mg in radiation cataracts. The ratio of insoluble to soluble protein was 0.40 for control, 0.65 for drug-treated, and 11.29 for cataractous rat lenses. The insoluble protein content did not differ significantly between the three groups and ranged from 12-17% of the total lens weight. However, soluble protein represented 30% of the total weight in control lenses, 25% of the weight in drug-treated lenses, and less than 2% of the total weight of cataractous lenses. The hydration and dramatic increase in the ratio of insoluble to soluble protein in the cataract is due primarily to loss of soluble protein.

The effect of WR-77913 on lenticular protein content was also seen in an analysis of soluble lens protein composition. Figure 3 represents the distribution of soluble lens proteins using size-exclusion HPLC. Since injection volumes were equal for each sample, the area under each chromatogram represents the relative concentration of protein from each sample. Thus, drug-treated lenses contained approximately 80% of the soluble protein found in control rat lenses, and cataractous lenses contained less than 10% of the soluble protein found in controls. Both control and drug-treated lenses showed elution peaks at 18, 20.5, 22.5, 25.5, and 27 min, corresponding to proteins with molecular weights of approximately 158K, 43K, 32K, 20K, and 15K daltons. Soluble lens proteins from irradiated rats which received no drug treatment showed peaks at 158K, 43K, and 33K daltons, and contained no protein below 25K daltons. Control and drug-treated lenses had a high content of soluble protein below 25K daltons. The HPLC analysis clearly demonstrates the similarities between the protein composition of control and drug-treated rat lenses, and the marked changes in the protein composition of cataractous lenses in irradiated rats which received no drug treatment.

Based on the effect of WR-77913, it was important to determine the distribution of the drug in the eye. Biodistribution studies using (35S)-WR-77913 showed that maximum concentrations of the drug were found in ocular tissues between 15 and 60 min after i.p. administration of the drug. The greatest concentration of WR-77913 was found in coroid retina and sclera, less in vitreous and aqueous, and least in the lens. The concentration of drug measured by the distribution of the label is 71 ± 10 μg/gm tissue in the whole eye, and approximately one tenth that level in the lens. This measurement assumes that all of the radioactive label remains associated with the drug. The concentration of drug in the lacrimal gland was six times that of the whole eye 30 min after administration of [35S]-WR-77913.

**Discussion.** Intrapitoneal administration of WR-77913 prior to exposure to 15.3 gray of x-irradiation inhibited rat lenses from developing mature radiation cataracts. The protective effect of WR-77913 was apparent in photographs of rat lenses and in analyses of lenticular proteins. WR-77913 protected rat lenses by stabilizing the composition of soluble proteins so that the proportion of insoluble protein did not increase and by preventing lens hydration. This is consistent with recent findings in which the relative loss of low molecular weight crystallins are associated with human opacities. The effect of WR-77913 is to inhibit the formation of scattering centers which are responsible for causing dense opacities in cataractous lenses.

WR-77913 was administered 15 to 30 min before rats were exposed to x-irradiation, which allowed the drug to achieve maximum tissue concentrations at the time the animals were irradiated. The timing of drug administration may be a critical factor to protection against radiation damage. However, if WR-77913 acts on a general mechanism of opacification, then it is conceivable that treatment following irradiation may also protect against insolubilization of proteins, development of scattering elements, and hydration of the lens. It is of interest that the concentration in the lens is well below that in the sclera and the lacrimal gland. We speculate that the concentration of WR-77913 in the lens epithelium was higher than the whole lens and approximated the concentration in the aqueous. Protection of the lens epithelium alone may be necessary for inhibition of opacification.

Additional studies are necessary to define the conditions of maximum effectiveness of WR-77913 and the molecular mechanism of protection that WR-77913 confers to irradiated rat lenses. Our preliminary results demonstrate that WR-77913 can be expected to provide significant protection against the formation of scattering elements in the lens. Our results also emphasize the need to examine the radioprotective mechanism of WR-77913 and related compounds on the early development of lenticular opacification, not only in radiation cataracts but in other cataract models as
well. Further studies with WR-77913 may lead to a better understanding of the chemical and physiological mechanisms of cellular damage resulting from exposure to x-irradiation.

Key words: cataract, x-irradiation, WR-77913, lens proteins, radioprotection

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