Ovarian Hormone Modulation of Radiation-Induced Cataractogenesis: Dose-Response Studies

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PURPOSE. Epidemiologic data on the effects of female sex hormones in cataract formation are conflicting. With the use of a rat model of radiation-induced cataractogenesis, it was found that estrogen can either enhance or inhibit the progression of radiation cataracts, depending on when the hormone is administered. The present study was performed to further define radiation-hormone interactions during cataractogenesis.

METHODS. In one experiment, rats were left ovary-intact or ovariectomized and were then irradiated with 2.5, 5, 10, or 15 Gy to one eye. In another experiment, ovariectomized rats were treated continuously with three different doses of estradiol through a slow-release capsule implanted subcutaneously, after which one eye was irradiated with 15 Gy. In all animals, cataract formation was followed by slit lamp examination at regular intervals.

RESULTS. Latency to identification of cataracts decreased exponentially with increasing radiation dose. The presence of ovaries enhanced cataractogenesis when the eye was irradiated with 15 Gy, but there was no difference between ovary-intact and ovariectomized rats that were irradiated at lower doses. In ovariectomized rats irradiated with 15 Gy, estradiol increased the rate of progression of cataracts in a dose-dependent manner. The rate of cataract progression increased linearly with increasing estradiol dose; there was no sign of saturation at high estradiol doses, as would be expected from a receptor-mediated effect.

CONCLUSIONS. Ovarian hormones enhance radiation-induced cataract formation; hormone supplementation experiments indicate that estrogen is responsible for this effect. The data suggest that the enhancing effect of estradiol is not mediated by its receptor, but this requires further study. (Invest Ophthalmol Vis Sci. 2009; 50:3304–3310) DOI:10.1167/iovs.08-3262

Ionizing radiation induces the progressive development of cataracts. Cataracts may be a significant adverse effect of radiation therapy if the orbit is included in the treated volume.1–5 Although early studies suggested that a minimum of approximately 2 Gy of x-rays is required for the induction of cataracts,6 recent assessment of atomic bomb survivors argues against the concept that a threshold dose of radiation is required.7 In addition, studies of astronauts suggest that low-dose ionizing radiation encountered in long-term space travel may lead to the premature development of cataracts.8,9 In animal studies, cataractogenesis appears to be a fully stochastic effect of radiation, and the notion that cataracts only form on exposure to a threshold dose was derived from the impact of animal lifespan on the experimental system.10,11

The female sex hormone estrogen may modify rates of cataractogenesis. Epidemiologic data suggest that estrogen may be protective against age-related cataracts.12–15 However, some studies find that estrogen either has no effect16 or actually enhances cataractogenesis.17 With the use of a rat model of radiation-induced cataracts, we found that estrogen both enhances and attenuates the rate of cataractogenesis, depending on the time of hormone administration relative to the cataractogenic insult.18,19

In the present study, we examined the effects of different doses of γ-irradiation on cataractogenesis in female rats that either underwent ovariectomy or had intact ovaries. The effects of different doses of estradiol in ovariectomized rats receiving a set cataractogenic dose of γ-irradiation were also determined. We found that cataracts develop at all doses of radiation tested and that they develop more rapidly in ovari-intact females, but only when the radiation dose is high. In addition, estradiol administered to ovariectomized rats before irradiation enhanced the rate of cataract progression in a dose-responsive fashion.

METHODS

Animals

All animal work was conducted under protocols that adhere to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee. At approximately 56 days of age, female Sprague-Dawley rats (Harlan, Indianapolis, IN) were subjected to various doses of radiation in the control eye or in the animal in general. The animals either were left intact or underwent ovariectomy 1 week before irradiation. In estradiol dose-response studies, a silicone (Silastic; Dow Corning, Midland, TX) capsule was implanted subcutaneously at the time of surgery in each animal that underwent ovariectomy, as described in our earlier studies.18 With this method of irradiation, the left eye received less than 2% of the total dose, and there were no obvious ill effects from the radiation in the control eye or in the animal in general. The animals either were left intact or underwent ovariectomy 1 week before irradiation. In estradiol dose-response studies, the capsule was filled with 20 mg crystalline estradiol or 20 mg of a mixture of estradiol and cholesterol to achieve dilutions of 1:10 (2 mg estradiol) or 1:5 (4 mg estradiol). Each experimental group was initiated with 15 to 16 animals.

Cataractogenesis

Beginning at approximately 7 weeks after irradiation, the irradiated and nonirradiated eyes of each animal were examined every 2–4 weeks for cataract formation. The animal was lightly anesthetized with isoflurane, and its eyes were dilated with 1% tropicamide (Alcon Laboratories, Indianapolis, IN) according to protocol.18

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Inc., Fort Worth, TX) and were examined with a hand-held slit lamp. Cataracts were scored based on estimated percentage surface area of the opacity on a scale of 7.5 (trace) to 90 (complete opacity), as previously described.\textsuperscript{18,20}

Uterine Weight Assay

The rodent uterus is a classical estrogen-responsive organ.\textsuperscript{21–25} Ovariectomy causes the uterus to atrophy; after 2 weeks, uterine weight
reaches its nadir. Estrogen maintains the uterus at its ovary-intact weight by inducing water imbibition, protein synthesis, and cell proliferation. These effects of estrogen are mediated by its receptor, ERα,24,25 and will therefore demonstrate saturation in a dose-response study. It must be noted that it is the rate of growth of the uterus, not its final weight, that necessitates maximum receptor saturation; maximum uterine weight can be achieved at very low doses of estrogen if administered continuously for an extended period (>5 weeks; RMB, unpublished observations, 2008). Therefore, we set up a short-term experiment to determine whether our estrogen dosing method would demonstrate saturability of a receptor-mediated event. Female rats underwent ovarioectomy at 56 days of age and were implanted with a (Silastic; Dow Corning) capsule containing crystalline cholesterol (control), crystalline estradiol (20 mg), or estradiol diluted with cholesterol at 1:10 or 1:5 (2 mg or 4 mg E2, respectively). Two weeks later the animals were killed and weighed, and their uteri were removed and weighed. Uterine weight was calculated as milligram of uterine weight per gram of body weight (gBW), and then the uterine weight increase (mg/gBW) was determined by subtracting the average uterine weight of the control group from each individual uterine weight.

**Statistical Analysis**

Statistical analysis was performed using a desktop computer program (Prism 4.0 for Macintosh; GraphPad Software. San Diego, CA). Cataract incidence was determined when the eye began to consistently display cataract scores of 15 or higher. Incidence was analyzed using the Kaplan-Meier method; incidence curves were compared by the log-rank test. Scores of eyes with cataracts were averaged to assess the rate of progression, and means ± SEM were plotted against time after irradiation. Plotted mean scores were subjected to regression analysis using nonlinear (exponential or sigmoidal) curves according to best-fit determinations; these curves were compared by applying an F-test to the slope or rate constant. In some cases, the slopes of the initial, linear portions of these curves were compared by F-test. Uterine weight increases were averaged for each dose of estradiol and compared against control by ANOVA and Dunnett multiple comparison tests. Rate constants of the curves for cataract scores and uterine weight increases were plotted against the estradiol dose to determine whether the effects were saturable under the conditions of the experiments.

**RESULTS**

Anterior subcapsular cataracts (ASCs) and posterior subcapsular cataracts (PSCs) were induced at all doses of γ-irradiation (Fig. 1). Incidence reached 100% in the 5-, 10-, and 15-Gy groups and was limited to less than 100% in the 2.5-Gy group only by the high attrition rate of animals because of age. The relationship between time to median incidence and irradiation dose is shown in Figure 2. Regression analysis indicated that the data are best described by a single curve ($r^2 = 0.97$), with a plateau at 70 days, indicating the minimum number of days required to detect cataracts induced by γ-irradiation.

Ovarian hormones enhanced cataract formation in lenses irradiated with 15 Gy but not in those irradiated with lower doses. Incidence analyses show that initial cataract formation occurs earlier in ovary-intact rats irradiated with 15 Gy than in their ovarioctomized counterparts (Fig. 1). The median time to form ASC with a score of 15 or greater was 103 and 224 days ($P < 0.05$) in the intact group and the ovarioctomized group, respectively; the hazard ratio (HR) for ASC in ovary-intact animals was 2.3 (95% CI, 1.8–11.8) compared with ovarioctomized animals. Although the median time to form PSC did not differ significantly between the two groups (103 and 117 days in ovary-intact and ovarioctomized animals, respectively), the cataract HR for ovary-intact animals was 2.0 (95% CI, 1.2–9.0). In animals irradiated with <15 Gy, neither the time to cataract formation nor the HR differed significantly between ovary-intact and ovarioctomized groups.

Progression of cataracts was monitored with our standardized scoring system based on the percentage of opacification of anterior or posterior regions.18 Scores shown are the mean (±SEM) of the eyes with cataracts (Fig. 3). All the irradiated eyes of surviving animals had cataracts at the end of the observation period; however, because of the length of the study, animal attrition hampered analysis in the 2.5-Gy groups, in which only 2 of the ovarioctomized animals and 6 of the intact animals were still alive at the end of the experiment. Attrition was less of a problem in the other groups. At the end of the observation periods, there were 8 intact and 11 ovarioctomized animals left in the 5-Gy groups, 13 intact and 15 ovarioctomized animals in the 10-Gy groups, and 16 intact and 15 ovarioctomized animals in the 15-Gy groups. In lenses irradiated with 15 Gy, scores for ASC and PSC progressed more rapidly in the ovary-intact animals than in the ovarioctomized animals. The initial, linear portions of the curves (days 100–350 after irradiation) had slopes of 0.23 ± 0.016 and 0.16 ± 0.022 score units per day ($P = 0.034$) for ASC in ovary-intact and ovarioctomized animals, respectively; similarly, the initial slopes in PSC scores were 0.31 ± 0.018 and 0.23 ± 0.026 ($P = 0.024$) for the ovary-intact and ovarioctomized groups, respectively. There was no effect of ovarian status on the rates of progression of cataracts in lenses irradiated with 5 or 10 Gy. Although analysis was hampered by attrition in the 2.5-Gy groups, it is apparent that cataract formation progressed such that those animals surviving to 1000 days after irradiation had lenses that were nearly completely opaque.

PSC progressed more rapidly than ASC. The initial rate of progression was faster in the PSC than in ASC for ovary-intact animals irradiated with 15 Gy ($P = 0.0039$); the slope for PSC scores in the ovarioctomized group was marginally higher than that of the ASC scores ($P = 0.074$). In the 10- and 5-Gy groups, progression of PSC was also faster than that of ASC ($P < 0.01$), but in the 2.5-Gy groups there was no statistical difference between PSC and ASC, most likely because there were too few surviving animals for this comparison. Thus, it appears that in

**Figure 2.** Relationship between median cataract incidence and radiation dose. The time to 50% incidence determined from each plot in Figure 1 was plotted against radiation dose. Regression analysis indicated that the data were best described by one curve ($r^2 = 0.97$). The curve approximates a plateau (minimum median incidence) of 70 days.
the 15 Gy-irradiated rat eyes, ovarian hormones enhanced the rate of progression of cataracts, particularly for PSC.

Nonirradiated eyes were devoid of radiation-induced cataracts. In the nonirradiated, left eyes of the 2.5-Gy and 5-Gy groups, 90% of the 41 animals remaining in the study at 2 years developed sutural cataracts, which were easily distinguishable from the ASC and PSC that developed in the irradiated eyes. Time of onset and severity of the sutural cataracts were not
affected by ovariectomy. The left eyes of the 10-Gy and 15-Gy groups did not develop any type of cataract throughout the 20- to 22-month observation period.

Given that the effect of ovarian hormones was evident only in eyes irradiated with 15 Gy, we used this dose of irradiation to test the cataractogenic-enhancing effect of different doses of estradiol. In our previous study, we subcutaneously implanted a slow-release (Silastic; Dow Corning) capsule for estradiol treatment; a single capsule contains 20 mg crystalline estradiol. To perform a dose-response study, we implanted capsules of undiluted estradiol (20 mg) or estradiol diluted with crystalline cholesterol at 1:5 (4 mg) and 1:10 (2 mg). The capsules were implanted subcutaneously at the time of ovariectomy, 1 week before irradiation. Estradiol treatment had no discernible effect on the incidence pattern of initial cataract formation (Fig. 4). However, the rate at which cataracts progressed was increased by the addition of estradiol (Fig. 5). Rate constants for the curves describing the cataract scores were progressively higher with increasing estradiol dose, showing no sign of saturation at the highest doses used (Fig. 6A). Estradiol induced increases in uterine weights in a dose-dependent fashion, and this estrogen receptor-mediated effect was saturable over the range of doses used in our experiments (Fig. 6B). Thus, unlike gains in uterine weight, the cataract-enhancing effect of estradiol did not approach saturation, as might have occurred with a receptor-mediated event.

**DISCUSSION**

We had previously shown that estradiol either enhances or attenuates cataractogenesis, depending on when it is administered to ovariectomized rats relative to time of irradiation with 15 Gy to the target eye. Here we show that ovariectomy reduces cataractogenesis when eyes are irradiated with 15 Gy, suggesting that endogenous ovarian hormone is capable of enhancing the progression of cataracts. We expected that the effect of ovarian hormones would be more apparent at the lower doses of radiation because others had shown that biological modifiers, either endogenous or administered, have the potential to increase sensitivity to the cellular killing effects of radiation. However, in the present study, the effect of ovariectomy was apparent only in the group exposed to the highest dose of γ-irradiation. The lack of hormonal effect in animals irradiated with low doses (2.5–10 Gy) may be attributed to the mechanisms involved in the estrogen effect.

Estradiol can act through its receptors or through chemical interactions with cellular proteins or DNA. Receptor-hormone interactions are characterized as high-affinity, saturable events. The effect of estrogen on uterine weight is well established as a receptor-mediated action of the hormone. In addition, as our dose-response study showed, it is a saturable end point. On the other hand, estrogens are metabolized to form catechol estrogens through a reactive quinone intermediate that can form adducts in DNA through a chemical reaction that does not
involve a saturable, high-affinity receptor; this effect provides the basis for the involvement of estrogens in the initiation of cancer and neurodegenerative diseases.\textsuperscript{35,36} DNA adduct formation may play a role in radiation-induced cataractogenesis.\textsuperscript{37,38} Unlike the uterine weight response, estradiol-induced increases in the rate of cataractogenesis did not demonstrate a saturable dose-response effect. Thus, it would appear unlikely that estrogen enhancement of radiation-induced cataracts is a receptor-mediated event. However, the bioavailability of estradiol to the lens is unknown. Certainly, uterine tissues have direct vascular supply though the lens does not. Thus, the distribution of hormone to the lens must be established before these dose-response studies can be fully interpreted.

Although the exact mechanisms of radiation-induced cataractogenesis are not known, it has been argued that cataractogenesis is a stochastic effect of radiation-induced cellular damage.\textsuperscript{11} Furthermore, it has been suggested that the response in animal models only appears to be subject to threshold doses because of the limitation of the lifespan of the animal.\textsuperscript{11} Indeed, in the present study, in the 2.5 Gy-irradiated group, cataracts did not appear until the animals were nearly 2 years old, and our observations were carried out through an additional 10 months. Cataract formation is evident only after a latency period that is dependent on the dose of radiation. This suggests that cataractogenesis is a progressive late effect resulting from the initial damage induced by radiation. It was hypothesized that perturbation of cell proliferation in the equatorial region of the lens and subsequent aberrant migration of the cells to the posterior capsule was responsible for cataract formation.\textsuperscript{39–40} Extrapolation of the radiation dose-response curve for the time to median incidence of cataracts indicates that 70 days is the minimum time required for radiation-induced cataracts to become detectable by slit lamp examination. It would be interesting to track the migration of cells from the equatorial region to the posterior subcapsular region to determine whether that process accounts for the latency in cataract formation. Estrogens have been shown to reduce apoptosis and to increase cell proliferation in other cell types.\textsuperscript{41–44} We speculate that estrogen may enhance radiation-induced cataractogenesis by increasing the survival of damaged cells and promoting their proliferation and migration posteriorly.

In summary, ovarian hormones cause differences in the rate of cataractogenesis induced by γ-irradiation, but these differences occurred only after irradiation with the highest dose (15 Gy). Endocrine ablation and hormone supplementation experiments indicate that estradiol is responsible for the enhanced cataract formation in females. The dose-response studies with estradiol indicate that the hormonal effect on radiation-induced cataracts is not saturable and therefore may be mediated by a nonreceptor mechanism. Additional studies will be required to definitively determine the mechanisms of hormone action in radiation-induced cataractogenesis.

References


