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Estrogen Protects against Radiation-Induced Cataractogenesis

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Abstract

Cataractogenesis is a complication of radiotherapy when the eye is included in the treatment field. Low doses of densely ionizing space radiation may also result in an increased risk of cataracts in astronauts. We previously reported that estrogen (17-β-estradiol), when administered to ovariectomized rats commencing 1 week before γ irradiation of the eye and continuously thereafter, results in a significant increase in the rate and incidence of cataract formation and a decreased latent period compared to an ovariectomized control group. We therefore concluded that estrogen accelerates progression of radiation-induced opacification. We now show that estrogen, if administered continuously, but commencing after irradiation, protects against radiation cataractogenesis. Both the rate of progression and incidence of cataracts were greatly reduced in ovariectomized rats that received estrogen treatment after irradiation compared to ovariectomized rats. As in our previous study, estradiol administered 1 week prior to irradiation at the time of ovariectomy and throughout the period of observation produced an enhanced rate of cataract progression. Estrogen administered for only 1 week prior to irradiation had no effect on the rate of progression but resulted in a slight reduction in the incidence. We conclude that estrogen may enhance or protect against radiation cataractogenesis, depending on when it is administered relative to the time of irradiation, and may differentially modulate the initiation and progression phases of cataractogenesis. These data have important implications for astronauts and radiotherapy patients.

INTRODUCTION

Formation of clinically significant cataracts often occurs if the orbit is included in the treated volume during conventional radiotherapy, brachytherapy or total-body irradiation (TBI) prior to bone marrow transplantation (1–7). Cataractogenic doses of sparsely ionizing radiation (X rays or γ rays) received by radiotherapy patients are usually well in excess of 2 Gy, which until recently was thought to be the threshold for radiation cataractogenesis (8,9). However, astronauts exposed to lower doses of densely ionizing charged-particle radiations during prolonged space missions also represent a population of individuals at an increased risk for cataractogenesis (10,11). Surgery is currently the only cure for cataracts. Surgical procedures do not produce identical outcomes and are associated with some element of risk (12). The development of countermeasures against radiation-induced cataract is therefore of great interest in radiotherapy and for astronauts involved in long-duration missions, notably those involving interplanetary travel. While antioxidants have shown some efficacy in reducing the

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risk of radiation-induced cataracts in animal models, their usefulness has yet to be demonstrated in humans (6).

Unlike spontaneous age-related cataracts, radiation-induced cataracts begin to form in the posterior subcapsular (PSC) region of the lens (13,14) and then gradually progress to the cortex and nucleus until they become indistinguishable from other types of cataracts. The latent period is inversely related to dose and likely corresponds to the time required for damaged lens epithelial cells to migrate from the equatorial region to the posterior pole, where they then accumulate as abnormal nucleated lens fibers. While the exact mechanism remains to be elucidated, it is likely that postirradiation proliferation of surviving cells that have either accumulated or failed to repair DNA damage is a prerequisite for radiation cataractogenesis (14,15). Recent evidence accumulated using mice that were haplodeficient for ATM, a protein kinase involved in the initiation of the radiation-induced DNA damage signal transduction pathway (16), supports the notion that DNA damage in lens epithelial cells, specifically double-strand breaks (DSBs), may lead to the development of radiation-induced opacities (17–20). Mice that are haplodeficient for ATM are hypersensitive to radiation cataractogenesis (20).

Experimental and epidemiological evidence suggests that estrogen can either enhance or retard cataractogenesis, depending on the initiating factor and the type of cataract formed. The prevalence of cataracts increases with age and is slightly higher for women (21). Since there is a higher incidence of cataracts in postmenopausal women compared to age-matched men, it has been hypothesized that the increased risk is due at least in part to the estrogen deficiency that occurs after menopause. Indeed, epidemiological data suggest that estrogens may protect against some forms of cataract. Retrospective studies have found that postmenopausal estrogen replacement therapy reduces the incidence of age-related nuclear, posterior and anterior capsular cataracts in women (22–24). In a small population-based case-control study, women receiving estrogen-based hormone replacement therapy showed a slightly reduced risk of cataract (25). However, previous epidemiological studies of the association between estrogen and cataract have yielded conflicting results. Either no change in or an increased incidence of age-related posterior subcapsular cataracts was observed in two studies involving women receiving estrogen replacement therapy (24,26). Yet another study showed no association between estrogen-based hormone replacement therapy (HRT) and any types of cataract, although oophorectomy resulted in a reduced risk of cataract (27).

Data from animal studies have produced conflicting conclusions, but overall, they suggest that a lack of estrogen is associated with cataractogenesis. Prolonged exposure to tamoxifen, a non-steroidal antiestrogen used in the treatment of breast cancer, increases the incidence of cataracts in rats (28), although there is some question as to whether the drug enhances the development of cataracts in humans (29–31). Interestingly, slightly elevated or physiological levels of 17-β-estradiol (E2), the major secreted estrogen, protected ovariectomized rats from cataractogenesis induced by methylnitrosourea (MNU) (32) and TGF-β2 (33).

Most of the biological effects of sparsely ionizing radiation (X and γrays) are mediated by free radicals. Since estradiol has antioxidant properties (34,35) and also protected rats from cataractogenesis after treatment with potent inducing agents (32,33), some initial experiments were conducted to determine the effects of estrogen modulation on radiation-induced cataractogenesis. Interestingly, we found that if administered prior to, during and after irradiation over the course of study, estradiol reduces the latent period and increases the incidence of radiation-induced cataracts in rats (36).

We now report that estrogen, if administered after irradiation, may decrease the incidence of cataract formation in eyes exposed to ionizing radiation. Thus the modulation of radiation cataractogenesis by estrogen is highly dependent on the time of administration. These data,

which pertain to the timing of the estrogen response, may be relevant to the radiation oncology clinic and the manned program.

MATERIALS AND METHODS

Hormone Treatments

Prior to each experiment, 6-week-old female Sprague-Dawley rats were received from Harlan (Indianapolis, IN). One week after acclimation, all animals except those that were to be left intact and that would not receive an estrogen implant were ovariectomized and assigned randomly to one of four groups described below (15–16 rats per group). Ovariectomies, implants and irradiations were performed while animals were under general anesthesia (12.45 mg ketamine, 0.27 mg acepromazine, 0.06 mg atropine). Three groups received estrogen treatment at various times before or after irradiation. In one group, 1-cm silastic capsules (36) containing 20 mg of crystalline 17-β-estradiol (E2) (Sigma-Aldrich, St. Louis, MO) were implanted subcutaneously on the back of rats at the time of ovariectomy, 1 week prior to irradiation. In this group, the capsule remained in the animal during irradiation and throughout the period of observation (E2-before/after). In the second group, the E2 capsules were implanted in ovariectomized animals immediately after irradiation and remained in the animal throughout the period of observation (E2-after). In the third group, E2 was implanted in animals at the time of ovariectomy, 1 week prior to irradiation, but the capsule was removed immediately after irradiation (E2-before). The fourth group, the control group, consisted of untreated rats that were ovariectomized 1 week prior to irradiation, at the same age as the other animals. An empty capsule was implanted at the time of ovariectomy in this group. Each capsule provided a continuous course of estrogen treatment (~2 μg/day) while implanted (32). Continued estrogen stimulation was verified by measurement of uterine weights at the time animals were killed (not shown). The experimental procedures performed on animals were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine.

Irradiation of Eyes

All animals were irradiated at ~56 days of age. Rats were anesthetized and immobilized ~10 min prior to irradiation with the Leksell Gamma Knife. Each rat received a single fraction of 15 Gy of ^{60}Co γ rays to the right eye as described previously (37). The dose rate for the irradiations was approximately 2 Gy/min. The total exposure time did not exceed 8 min. The unirradiated contralateral eye served as a nonirradiated control; previous dosimetry experiments indicated that the control eye received less than 2% of the total dose to the target eye (37). No rats aged and observed through 550 days showed evidence of opacification in the contralateral eye (all groups).

Measurement of Lens Opacification and Transparency

Animals were observed for cataracts every 2 to 4 weeks postirradiation. In this study, all lenses were examined with a hand-held Kowa SL-15 slit lamp (Tokyo, Japan). ASC and PSC opacities were graded based on the estimated percentage surface area of the opacity as described previously (36). Scores were obtained by calculating the interval midpoint of the following ranges of opacified surface areas: 0, 5–10, 11–20, 21–40, 41–60, 61–80 and 81–100, yielding scores of 0, 7.5, 15, 30, 50, 70 and 90, respectively. Cataract incidence was determined as the time at which an animal exhibited the lowest sustainable score upon examination, i.e., a score of ≥15 over the course of two observations made at least 14 days apart. Observations were carried out through 620 days after irradiation; animals with completely opaque lenses were euthanized. Some animals were removed from the study prematurely due to development of non-ocular pathologies; the scores from these animals were included in the analyses only up to the time at which they were removed.

Statistical Analyses

Cataract scores were analyzed by non-linear regression using a global curve-fitting program (GraphPad Prism 4.0, GraphPad Software, San Diego, CA). Data were fitted to the one-phase exponential equation: $y = \max \times [1 - \exp(-K \times x)]$, where y = cataract score, x = time (days) after irradiation, max is the maximum score possible, 90 (since once cataractogenesis begins, it eventually progresses to full opacification), and K is the rate constant (slope). Estimated values for slope were compared by an F test, and significance was set at $P < 0.01$. Cataract incidence was plotted using the fraction of animals with lens surface area opacification >10% (corresponding to a score of at least 15) and calculated by the Kaplan-Meier method; animals that had been removed from the study prior to developing cataracts or that never developed cataracts were treated as censored data entries. Incidence curves were compared using the logrank test; significance levels were set at $P < 0.05$.

RESULTS

Cataract incidence in irradiated eyes was measured as the time when an animal exhibited any measurable cataract, either anterior subcapsular (ASC) or PSC, with a score of ≥15, as described previously (36). The incidence rate was significantly reduced by estrogen treatment that commenced immediately after irradiation (E2-after) of eyes with 15 Gy (Fig. 1). The median time of cataract appearance was 83 days in the ovariectomized control group and 137 days in the group that received E2 treatment after irradiation; compared to the ovariectomized group, the odds ratio for cataractogenesis in the E2-after group was 0.4 (95% CI: 0.04–0.45). There was also a slight protection afforded by estrogen when the treatment capsule was implanted 1 week before irradiation, and then removed immediately after irradiation (E2-before) (Fig. 1); the median time of cataractogenesis and the odds ratio for this group were 100 days and 0.54 (95% CI: 0.10–0.82), respectively. Compared to the ovariectomized group, there was no change in the rate of incidence when treatment with estradiol commenced at the time of ovariectomy (1 week prior to irradiation) and estradiol was administered continuously through the entire observation period (E2-before/after). The incidence curve for the group of intact animals differed from the ovariectomized control group ($P = 0.011$), suggesting a slight protective effect of endogenous hormones.

Despite the magnitude of the radiation dose, 33% (5 out of 15) of ovariectomized animals that received estrogen only after irradiation (E2-after) with 15 Gy did not develop a significant cataract during the ~1.5-year period of observation; of these, four animals (27%) did not develop any opacification, and one animal had only developed anterior and posterior cataract scores of 7.5 and 15, respectively (data not shown). In contrast, the incidence was greater than 90% at 120 days in the ovariectomized control group and the E2-before/after group and 100% of the E2-before/after group developed significant opacities before 1 year postirradiation.

The rate of progression of cataractogenesis was followed using the scoring system described earlier (36). ASC and PSC cataracts progressed much faster in the E2-before/after group than in any of the other groups (Figs. 2A and B, Table 1). On the other hand, the rate of progression of the E2-after group was considerably slower than ovariectomized controls (Figs. 2A and B, Table 1). The rate of progression in the E2-before group was also slightly reduced compared to the intact group. It should be noted that the incidence curve for the group of intact animals differed from the ovariectomized control group ($P = 0.011$); while a slight protective effect of endogenous hormones is suggested in regard to incidence, there was no significant difference noted in the rate of progression of cataracts when intact animals were compared to the ovariectomized group.

Although treatments affected the rate of cataract progression, the rate of development and progression of PSC cataracts was similar to that of ASC cataracts when the two types of

cataracts were compared within each respective treatment group. That is, ASC and PSC cataracts developed concomitantly during the period of observation.

DISCUSSION

In our previous studies, we found that when lenses of animals were irradiated with 15 Gy of γ rays and evaluated by effective light transmission, the incidence of cataracts was increased when E2 was present, either from an endogenous source in the case of ovary-intact animals or when administered exogenously in the case of ovariectomized animals (36). Radiation induced opacification in the eyes of all ovary-intact and ovariectomized, E2-treated animals during a 25-week period of observation after irradiation, but less than half of the eyes in an ovariectomized placebo group showed any opacification. Light transmission of lenses from irradiated ovariectomized animals that received continuous estradiol was also significantly reduced compared to lenses from irradiated animals that received a placebo. Although the period of observation was much shorter than that in the current study, we concluded that E2, when administered continuously beginning 1 week prior to irradiation, increased the incidence of cataracts. In a subsequent experiment in which a slit lamp was used for analysis of opacities to establish the true time course for cataractogenesis induced after irradiation of rat eyes with 10 Gy, we found that continuous administration of estrogen reduced the latent period for (accelerated the onset of) PSC cataracts and increased the severity and rate of progression of ASC cataracts.

The present study shows that the effect of estrogen is dependent on the time when it is administered relative to the cataractogenic insult. Estrogen, if administered to ovariectomized animals prior to, during and continuously after irradiation (E2-before/after), enhances the rate of progression of cataractogenesis (Fig. 2); this suggests that having estrogen present around the time of irradiation increases the initiating damage that leads to progressive opacification of the lens. On the other hand, if estrogen is absent at the time of irradiation but is present continuously after the initiating insult, the rate and incidence of cataractogenesis are reduced. We conclude that E2 may enhance or protect against radiation-induced cataractogenesis, depending on when it is administered relative to the time of irradiation. Our observations also suggest that cataractogenesis is a two-step process, initiation and progression and that estrogen affects each step differently. A more refined time course study and studies involving the use of estrogens with different oxidant/antioxidant chemical characteristics are warranted to examine this hypothesis.

In the current study, the dose used to induce cataracts (15 Gy) was chosen in part because it induces significant opacification in nearly 100% of ovary-intact rats by 25 weeks after irradiation (36). However, doses of this magnitude also have clinical relevance. The standard treatment for ocular tumors such as uveal melanoma consists of the delivery of five fractions of 10–16 Gy over a period of about 5 days (6); this greatly exceeds the minimum cataractogenic dose. Within 2–6 years after the resulting cataracts are surgically removed from irradiated patients, visual acuity returns to preoperative levels or worsens due to cystoid macular edema, retinal detachment or potential aggravation of radiation retinopathy (7). Thus nonsurgical management of cataracts postirradiation would be preferred.

One-third of the animals that only received estrogen after irradiation with 15 Gy failed to develop significant opacification within the 1.5-year period of observation, while >90% of animals in other treatment groups developed significant opacities within 1 year after irradiation. Further experiments are warranted to determine whether opacification can be inhibited longer, or even completely, after exposure to lower doses, such as in the 2.5–10-Gy range. Such studies would be valuable for determining whether nonfeminizing estrogen derivatives could be applicable to patients receiving treatment for head and neck tumors or ocular melanoma.

A promotive or protective effect of E2 on radiation-induced cataractogenesis could also have important implications for the manned space program, since exposure to even relatively low doses of space radiation may result in a reduced latent period for and an increased incidence of cataractogenesis (10,11). Astronauts aboard the proposed 3-year mission to Mars would be exposed to protons and charged particles of high (H) atomic number (Z) and energy (E) (38). HZE particles are densely ionizing, high-linear energy transfer (LET) particles that are highly penetrating. Over the course of a 3-year mission, it is estimated that the protracted equivalent dose received would be ~1 Sv (39), although solar flare activity and exposure from on-board nuclear power sources could increase the absorbed dose significantly (38). To date, the degenerative changes in the lens induced by radiation have occurred after the active flight careers of astronauts are over (10). However, there is some concern that a reduction in visual acuity could occur during interplanetary missions. At the very least, cataractogenesis represents a health concern after flight.

The relative biological effectiveness (RBE) of a given type of radiation is defined as the ratio of doses of low-LET X rays or γ rays to the test radiation that results in the same biological effect. The estimated RBE for cataractogenesis induced by densely ionizing radiation is high. Therefore, astronauts on long-term missions may be at significant risk for cataract development. Recently, the RBE for cataractogenesis induced by high-energy ^{56}Fe ions was found to range from 5–15 and 5–24 for wild-type mice and mice haplosufficient for ATM, respectively (20). These results are consistent with RBEs obtained from other rodent studies involving low doses of heavy ions (40,41). RBE also increases with decreasing dose up to values in excess of 100 (42). Given the high RBEs obtained for space radiation using animal models, our studies with higher doses of low-LET radiation may be predictive of results obtained after exposure to lower doses of high-LET radiation. Since the RBE for cataractogenesis may be considerably higher in certain subsets of astronauts due to gender-related or age-related differences that predispose them to enhanced radio-sensitivity, our results highlight the need for further ground-based studies.

The dichotomous nature of the E2 effect whereby the hormone enhances or protects against radiation-induced cataractogenesis depending on the time of administration may be related to its physiological effects, the structure of the hormone, or its metabolic breakdown products. Ionizing radiation inhibits cell cycle progression such that DNA damage can be repaired prior to entry of the cell into S or M phase. However, physiological and low pharmacological doses of estrogen may stimulate cell proliferation by reducing cell cycle length through a reduction in the durations of G_1 and S phase (43). In hormone-dependent breast cell lines, E2 also exerts anti-apoptosis effects (44,45). Thus estrogen treatment may both reduce the time available for repair of DNA damage and stimulate proliferation of irradiated lens epithelial cells with damaged DNA; this in turn could lead to retention of damaged cells that would normally be removed through apoptosis, resulting in formation of aberrant differentiated fiber cells. This could explain the potentiating effects of estrogen if it is present systemically prior to, during, and in the hours after irradiation (E2 before/after).

Alternate mechanisms by which estrogen may enhance cataractogenesis if administered before and during irradiation are also worth considering. For example, estrogen metabolism results in the formation of catechol estrogens; continuous redox cycling of catechol estrogens results in the generation of free radicals that can damage DNA by inducing strand breaks and chromosome aberrations (46). Thus, if estrogen is present before *and* after irradiation, the estrogen may add to radiation-induced DNA damage either directly by covalent binding of estrogen metabolites to DNA or indirectly by free radical generation. This additional component of DNA damage resulting from estrogen treatment in irradiated lens cells could interact additively or synergistically with free radicals generated by the indirect action of ionizing radiation and enhance radiation-induced cataractogenesis.

Conversely, estrogens may also act through non-genomic mechanisms to reduce oxidative damage to DNA. The E2 molecule has antioxidant properties and has been shown to decrease DNA damage, reduce mutations and increase cell survival, due at least in part to enhanced DNA repair stimulated by estradiol (34,35,47–50). However, in our case, it is unlikely that the protective effect of estrogen against radiation-induced cataracts, when administered after irradiation, is due to antioxidant effects such as the scavenging of free radicals, since antioxidants are generally effective only if administered during irradiation, when the short-lived free radicals are generated.

We showed previously shown that estrogen is protective against cataracts induced by alkylating agents (32). As in the case of radiation, alkylating agents are effective at inducing DNA damage in lens epithelial cells that could normally lead to the development of lens fiber opacity (51), but the mechanism by which this protection is mediated is not known. Estrogen has also been reported to be protective against some forms of age-related cataracts (22–24,26). It is possible that the mechanism of the protective effect of the hormone on age-related and radiation-induced cataractogenesis may be similar. Significant increases in the level of hydroxyl radicals have been reported in the lenses of cataract patients, indicating a role for oxidative damage in age-related cataract formation (52). An accumulation of or a failure to repair radiation-induced DNA damage from reactive oxygen species in lens epithelial cells may be a precursor to cataractogenesis (14,15). The most protection against cataractogenesis was noted when estrogen was administered after irradiation. However, compared to ovariectomized animals, some protection was afforded by endogenous estrogen or when estrogen was administered after irradiation. It is therefore attractive to speculate that estrogen could play a role in restituting the original local composition of sites within the DNA molecule that may have suffered free radical attack. Further experiments are necessary to determine the mechanism by which estrogen inhibits radiation cataractogenesis and whether the protection by estrogen is mediated via genomic or non-genomic mechanisms.

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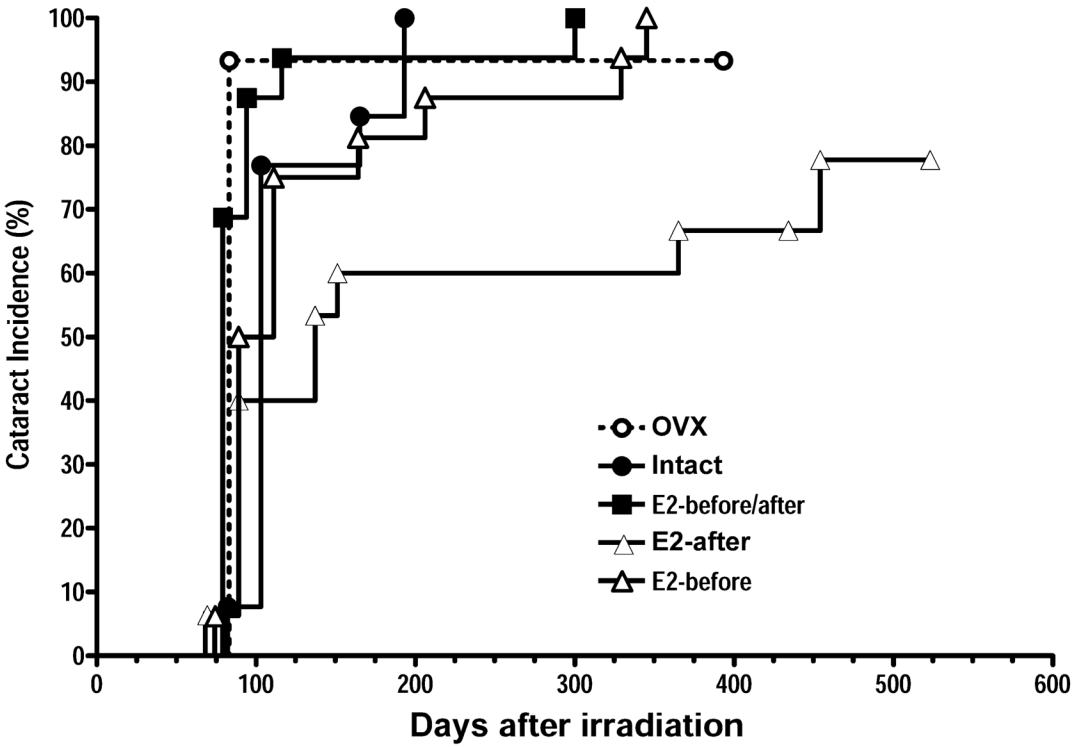

FIG. 1.

Effect of timing of estrogen administration on the incidence of anterior or posterior subcapsular cataracts after 15 Gy irradiation. Ovariectomized rats were treated with continuous estradiol commencing at the time of ovariectomy 1 week prior to irradiation (E2-before/after), received an E2 implant immediately after irradiation (E2-after), received an E2 implant at the time of ovariectomy which was then removed after irradiation (E2-before), or were left untreated prior to and after irradiation (OVX). Data for intact animals are also shown. Animals were scored as positive at the time (day) they exhibited cataracts, either ASC or PSC, with scores of ≥15. Analysis: Incidence curves were generated using the Kaplan-Meier survival curve method and were compared to the curve for the control ovariectomized group by logrank tests. The incidence curves differed from that of the ovariectomized group as follows: E2-before/after, not significantly different; E2-after, $P < 0.001$; E2-before, $P < 0.02$; Intact, $P = 0.011$).

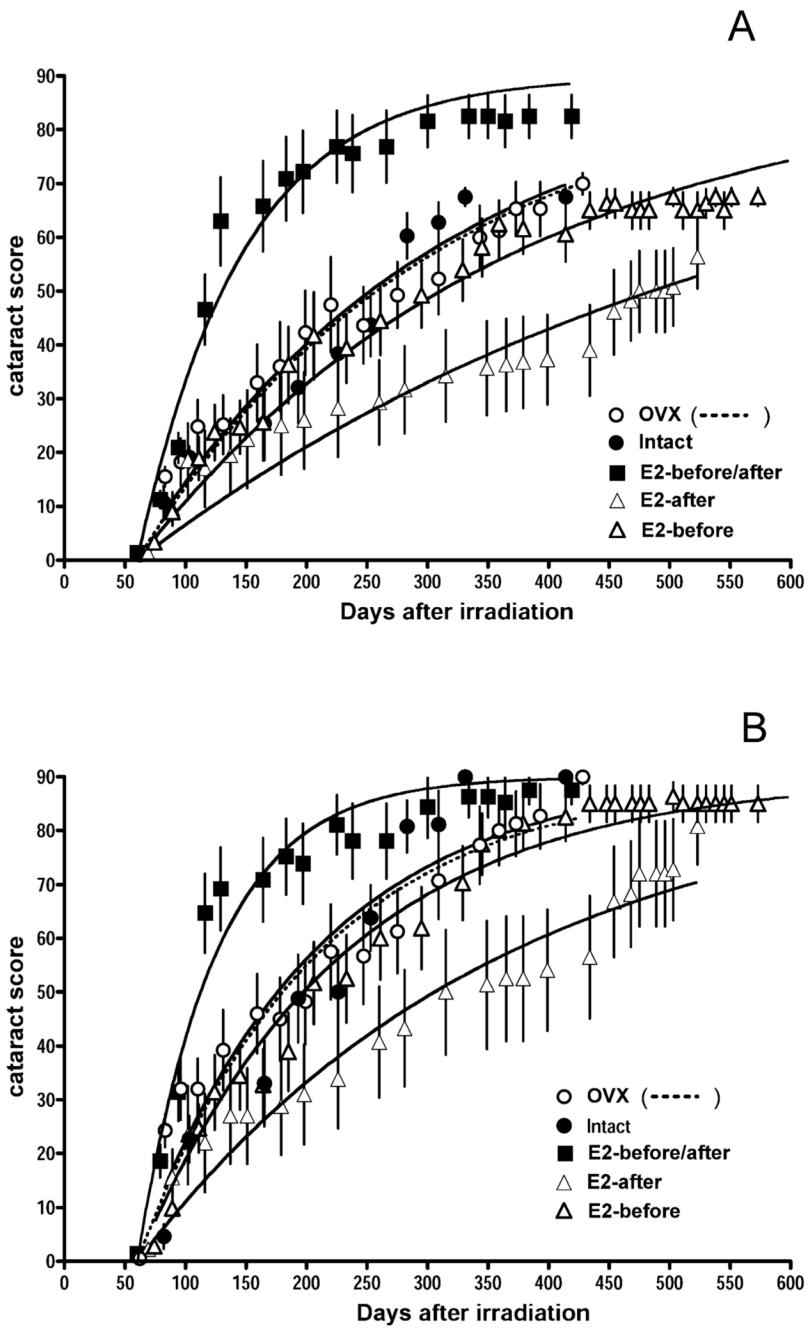

FIG. 2.

Effect of timing of estrogen administration on anterior (panel A) or posterior (panel B) subcapsular cataract progression after irradiation with 15 Gy. Ovariectomized rats either were treated with continuous estradiol commencing at the time of ovariectomy 1 week prior to irradiation (E2-before/after), received an E2 implant immediately after irradiation (E2-after), received an E2 implant at the time of ovariectomy that was then removed after irradiation (E2-before), or were left untreated prior to and after irradiation (OVX). Data for intact animals are also shown. Data represent scores of all eyes ± SEM; groups consisted of 15–16 animals. Analysis: Nonlinear regression with global fitting; *F* test used for comparing individual fitted curves to that of the control, ovariectomized group. The fitted curves differed from that of the

ovariectomized group as follows: E2-before/after, $P < 0.001$; E2-after, $P < 0.001$; E2-before, $P < 0.01$.

TABLE 1

Kinetics of Cataract Incidence and Progression

	Ovariectomized	Intact	E2-before/after irradiation	E2-after irradiation	E2-before irratiation
Incidence (scores ≥ 15)					
Median time to formation (days)	83	103	79	137	100
Odds Ratio (95% CI)	1.0	1.24 (0.77–1.71)	0.66 (0.14–1.14)	0.4 (0.04–0.45)	0.54 (0.10–0.82)
Score Progression (slope)					
ASC	4.0 ± 0.3	4.1 ± 0.3	11.6 ± 0.7**	1.9 ± 0.1**	3.2 ± 0.1**
PSC	6.8 ± 0.5	6.8 ± 0.5	15.5 ± 1.4**	3.3 ± 0.2**	5.9 ± 0.4*

Notes. Values are slopes from curves in Fig. 2 (means ± SEM). Compared to ovariectomized controls:

*, $p < 0.05$;

**, $p < 0.001$.