The role of the arachidonic acid cascade in the species-specific X-ray–induced inflammation of the rabbit eye

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To identify the mediator(s) of the apparently species-specific X-ray–induced inflammation of the rabbit eye, inhibitors of the synthesis and/or release of known or putative mediators of ocular inflammation were administered prior to irradiation. The X-ray–induced ocular inflammation, particularly the rise in intraocular pressure, was found to be inhibited by intravenous pretreatment of rabbits with flurbiprofen, indomethacin, or imidazole (1, 10, and 100 mg/kg i.v., respectively), or by combined intravitreal and topical administration of flurbiprofen. Systemic, intravitreal, and/or topical pretreatment with prednisolone or disodium cromoglycate or the retrobulbar injection of ethyl alcohol or capsaicin failed to block the inflammatory response, whereas vitamin E apparently exerted some protective effect. These findings show that the X-ray–induced inflammation of the rabbit eye is mediated, at least in part, by prostaglandins (PGs) and/or related autacoids. In addition, these results suggest that the unique sensitivity of the rabbit eye to X-ray–induced inflammation is due either to the presence in this species of a unique or uniquely effective triggering mechanism for the release of PG precursors or to the greater sensitivity of this species to the ocular inflammatory effects of PGs. Thus the rabbit eye may provide a unique model for studying some aspects of arachidonic acid release or ocular PG effects, but extreme caution must be exercised in generalizing such findings to other species. (INVEST OPHTHALMOL VIS SCI 22:579-587, 1982.)

Key words: rabbit, X-ray, ocular inflammation, intraocular pressure, indomethacin, flurbiprofen, vitamin E, arachidonic acid cascade, phospholipase, prostaglandins
Fig. 1. Effect of systemic pretreatment of rabbits with cyclooxygenase inhibitors and some other agents on the X-ray–induced elevation of IOP. One eye of each rabbit was exposed to X-ray (10 Gy) at 0 time, while the contralateral eye was shielded. The contralateral shielded eyes of these animals showed no measurable X-ray effects and served as controls. The points represent the mean differences ± 1 S.E.M. obtained from four rabbits.

Methods

New Zealand White albino rabbits (2.2 to 3.5 kg) of either sex were placed in rabbit boxes until accustomed to handling and restraint. Their eyes were then examined with a slit lamp, and two or three baseline intraocular pressure (IOP) measurements were taken after the topical application of one drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories, Inc., Fort Worth, Tex.) with a floating-tip pneumatic tonometer (Pneumatonogram; Alcon) calibrated for use on the rabbit eye. Animals that showed any signs of ocular inflammation were rejected. Experimental animals received systemic (intravenous, intraperitoneal, intramuscular, or subcutaneous), intravitreal, retrobulbar, and/or topical pretreatments before their eye(s) were irradiated.

The rabbits were anesthetized with sodium thiopental (25 to 35 mg i.v.; Abbott Laboratories, North Chicago, Ill.), and one eye at a time was irradiated as described previously, with the use of a 3 cm diameter collimator, while the contralateral eye was shielded with a 3 mm lead sheet. The X-rays (180 kVp, 30 mA) were filtered by 0.5 mm Cu and 0.5 mm Al to provide a half value equal to 1 mm Cu and were delivered at a dose rate of 3.7 Gy/min (1 Gy = 100 rads) for 2.7 min to yield a total dose of 10 Gy per eye. IOP and pupillary diameters (using a pupil gauge) were measured and slit-lamp examinations were made, in most cases at 1/4, 1, 1 1/2, 2, 3, and 4 hr after irradiation. The development of flare and iridal hyperemia and the entry of leukocytes into the anterior chamber were assessed by slit-lamp examination and recorded on a scale of 0 to +3 as described previously.

Rabbits that received systemic and, in some cases, topical pretreatment of both eyes were irradiated unilaterally, their shielded contralateral eyes served as controls. Rabbits that received unilateral intravitreal, retrobulbar, and/or topical pretreatments were irradiated bilaterally, one eye at a time, while the fellow eye was shielded so that both eyes received the same known dose.

Most animals were killed 4 hr after irradiation.
with an overdose of sodium pentobarbital. In these cases, the eyes were enucleated and the aqueous humor was removed with a 25-gauge hypodermic needle. The anterior and posterior segments of the vitreous were collected separately by bisection of the globe with a microtome knife over a collecting platform. White cells in the aqueous humor were counted with a hemocytometer, and the concentrations of soluble proteins and ascorbic acid in both the aqueous and vitreous samples were determined. The remaining intraocular fluid (IOF) samples were frozen for future analysis.

**Pretreatments.** Flurbiprofen (Allergan Pharmaceuticals, Irvine, Calif.) and indomethacin (Sigma Chemical Co., St. Louis, Mo.) were converted to their soluble sodium salts by the addition to their suspensions in normal saline of NaOH and Na₂CO₃, respectively (final pH = 8), and were injected (0.1, 1.0, and 10 mg/kg) into the marginal ear vein 1 hr prior to irradiation. Imidazole (Eastman Kodak Co., Rochester, N. Y.) dissolved in normal saline, was administered intraperitoneally (10 or 100 mg/kg), and disodium cromoglycate (Intal; Fisons Corp., Bedford, Mass.) was administered both subcutaneously (100 mg/kg) and topically (0.5 ml) to both eyes (0.05 ml of a 10% solution) 1 hr prior to irradiation. Vitamin E (d-α-tocopherol acetate; Sigma) was administered in repeated combined topical, intraperitoneal, and intramuscular doses for a period of 20 hr prior to irradiation (total of 0.15 ml topically to both eyes, 100 mg/kg i.p. and 100 mg/kg i.m.).

Proparacaine hydrochloride (0.1 ml of a 0.5% solution; Alcon) and prednisolone acetate (0.05 ml of 1%) (Pred Forte, Allergan) were administered topically every 5 min for 30 and 20 min, respectively, so that pretreatment ended ½ to 1 hr prior to irradiation; in most cases the contralateral eye received topical pretreatment with an equal volume of vehicle solution. Prednisolone sodium phosphate (0.4 μg in 20 μl) (Hydeltrasol; Merck, Sharp & Dohme, West Point, Pa.) was injected intravitreally with a clutch-motor pump. Flurbiprofen was administered in a combined intravitreal (10 μl of 5%) and topical (0.2 ml of 0.1%) regimen. Intravitreal injections were performed 2 hr prior to irradiation to ensure adequate distribution of the compounds throughout the intraocular fluids by the time of irradiation, and the contralateral eyes were injected with equal volumes of saline or vehicle solutions. Absolute ethyl alcohol (EtOH) was injected retrobulbarly (0.8 ml/orbit) 12 to 16 days before irradiation as previously described, and the eyes were treated with Neosporin ointment (Burroughs Wellcome Co., Research Triangle Park, N.C.) until 2 to 3 days prior to irradiation. Only animals that showed recovery from the initial EtOH-induced ocular inflammation and that exhibited unilaterally suppressed corneal reflexes were used. A 1% solution of capsaicin (Sigma) was administered retrobulbarly 3 days (0.5 ml) and 4 days (0.8 ml) prior to irradiation; 0.05 ml of this solution was also applied topically 1 hr before irradiation.

**Results**

Within 1.0 hr after exposure to 10 Gy, all irradiated eyes of nonpretreated control rabbits showed a highly significant (p ≤ 0.01) increase in IOP as compared with that of their contralateral shielded eyes (Fig. 1). The irradiated eyes of rabbits systemically pretreated with lower doses of imidazole (10.0 mg/kg), flurbiprofen or indomethacin (0.1 mg/kg), or disodium cromoglycate showed a hypertensive response similar to that of control animals. In contrast, the irradiated eyes of rabbits pretreated with 100 mg/kg imidazole, 10 mg/kg indomethacin, or 1 mg/kg flurbiprofen showed no hypertensive response during the 4 hr observation period. Although the irradiated eyes of the animals pretreated with vitamin E were clearly hypertensive 1 to 2 hr after X-irradiation, the mean peak rise in IOP in these eyes was lower than that of the control animals.

Almost immediately after exposure, irradiated eyes of rabbits that received no pretreatment exhibited a slight iridial hyperemia, which continued to increase in severity, reaching a plateau at 3 hr (Fig. 2). One hour after exposure, these eyes also developed aqueous flare, which continued to increase in severity, reaching a plateau at approximately 3 hr. White cells first appeared in the anterior chambers of the irradiated eyes of control animals 3 hr after exposure and continued to increase in number between 3 to 4 hr. Systemic pretreatment with 10 mg/kg indomethacin or flurbiprofen or 100 mg/kg imidazole greatly reduced the X-ray-induced invasion of cells into the anterior chamber, reduced by half or blocked the development of flare, and either reduced or did not affect development of iridial hypere-
 mia. Systemic pretreatment with lower doses of these compounds was less effective and pretreatment with vitamin E or disodium cromoglycate was entirely ineffective in blocking these biomicroscopic signs of inflammation.

Intraocular fluids taken 4 hr after exposure of the eyes of control rabbits or those pretreated with low doses of flurbiprofen or indomethacin showed as much as a 50% decrease in ascorbic acid concentration of the aqueous humor of irradiated eyes as compared with that of the contralateral shielded eyes (Table I). In addition, the concentration of soluble proteins in the aqueous humor of the irradiated eyes of control rabbits or rabbits pretreated with the lower doses of flurbiprofen or indomethacin was increased to levels 10 to 20 times greater than that of their contralateral eyes. Systemic pretreatment with moderate (1.0 mg/kg) doses of flurbiprofen or indomethacin, or with vitamin E greatly reduced the X-ray-induced changes in both the ascorbic acid and soluble protein concentrations in the aqueous of irradiated eyes. Pretreatment of animals with the higher doses (10.0 mg/kg) of flurbiprofen or indomethacin blocked this decrease in the ascorbic acid concentration and reduced the increase in the protein concentration.

In contrast to the X-ray-induced reduction in the ascorbic acid concentration of the aqueous humor, that of the vitreous was not significantly decreased in any of these unilaterally X-irradiated rabbits. Thus, for example, the ascorbic acid concentration in either the anterior or posterior segments of the vitreous of the irradiated or contralateral eyes of control rabbits was $0.5 \pm 0.1$ mmoles/kg H$_2$O. However, the protein concentration in the anterior but not in the posterior segment of the vitreous was elevated over twofold in the irradiated eyes of rabbits that received no pretreatment ($0.8 \pm 0.1$ vs. $1.8 \pm 0.4$ mg/ml in the control and irradiated eyes, respectively). Pretreatments that reduced the X-ray–induced accumulation of protein in the aqueous humor also reduced or blocked this small but significant accumulation of protein.

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**Fig. 2.** Effect of systemic pretreatment of rabbits with cyclooxygenase inhibitors on the development of iridal hyperemia and on anterior chamber flare and cellular infiltration after exposure to 10 Gy of X-irradiation. See also legend to Fig. 1.
Table I. Concentrations of ascorbic acid and soluble proteins in the aqueous humor of irradiated and contralateral shielded eyes of normal and flurbiprofen-, indomethacin-, or vitamin E-pretreated rabbits 4 hr after exposure of experimental eye to 10 Gy of X-irradiation*

<table>
<thead>
<tr>
<th>Systemic pretreatment</th>
<th>Flurbiprofen (mg/kg)</th>
<th>Indomethacin (mg/kg)</th>
<th>Vitamin E1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>None</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.9</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Experimental</td>
<td>1.4 ± 0.2</td>
<td>1.6 ± 1.2</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

Concentration of ascorbic acid (mmoles/kg H2O) & Concentration of soluble proteins (mg/ml)

- Experimental: 20.7 ± 15.5 mg/ml, 9.7 ± 4.2 mg/ml, 19.2 ± 14.3 mg/ml, 4.7 ± 14.7 mg/ml, 5.3 ± 14.3 mg/ml
- Control: 1.2 ± 1.0 mg/ml, 0.7 ± 0.8 mg/ml, 2.1 ± 0.7 mg/ml, 1.3 ± 0.7 mg/ml, 0.2 ± 0.7 mg/ml

*Values represent means ± 1 S.E.M. obtained on four rabbits.

Miosis was not observed in any X-irradiated rabbit eye at any time during the 4 hr observation period, regardless of the severity of intraocular inflammation or the efficacy of its inhibition.

Discussion

The fact that flurbiprofen and indomethacin, two nonsteroidal anti-inflammatory drugs (NSAIDs) that were shown to be highly effective inhibitors of cyclooxygenase activity in a variety of biological systems, including ocular tissues,12–14 effectively blocked the rise in IOP after exposure of rabbit eyes to 10 Gy of X-rays demonstrates that prostaglandins (PGs) and/or related cyclooxygenase products are important mediators of this X-ray–induced ocular inflammation. The finding that a high dose of imidazole had a similar inhibitory effect supports this conclusion, since at higher concentrations this thromboxane synthesis inhibitor is also known to inhibit cyclooxygenase activity.15 PG concentrations in rabbit IOFs were not measured in these experiments because X-irradiation was previously shown to affect the PG transport capacity of the anterior uvea,1 and therefore a postirradiation increase in PG concentration in the aqueous humor could not be assumed to reflect increased PG synthesis.
The observation that ocular hypertension was more effectively inhibited by these NSAIDs than some other parameters of the inflammatory response does not necessarily imply that mediators other than arachidonic acid derivatives are involved. It has been shown that high doses of topically applied PGF2a, and especially PGE2, cause an initial ocular hypertension in rabbits, whereas lower doses of these PGs, especially PGF2a, actually reduce IOP.16 Thus even partial inhibition of cyclooxygenase activity may be sufficient to reduce the synthesis of PGs to a level below that required to cause a transient rise in IOP but may not be sufficient to prevent other cyclooxygenase-mediated inflammatory responses such as anterior-chamber flare. However, the failure of these NSAIDs to effectively block iridial hyperemia, which develops during the first few minutes after irradiation, suggests that autacoids other than cyclooxygenase products, possibly including other arachidonic acid derivatives such as lipoxygenase products, may play a role in this particular aspect of X-ray-induced intraocular inflammation.

It has been shown that topical application of nitrogen mustard to the rabbit eye causes two distinct phases of ocular hypertension: the second phase is mediated by PGs and/or related cyclooxygenase products, while the first phase is apparently mediated by a neuronal mechanism, possibly by the release of substance P (SP) and/or related neuropeptides.7, 11 There is no indication, however, that any aspect of the ocular inflammatory effects of X-rays is mediated by such peptides or neuronal activity, since none of the parameters observed was affected by prior retrobulbar injection of capsaicin or EtOH or by repeated pretreatment with a topical anesthetic, proparacaine HCl, although similar pretreatments have been shown to block the neurogenic component of the nitrogen mustard–induced7, 11 or laser-irradiation–induced17 ocular irritative responses. Furthermore, X-irradiation produced no miosis, whereas SP and all related polypeptides tested to date were found to have very potent miotic activity both in vivo and in vitro.18–20

The inefficacy of disodium cromoglycate, a putative inhibitor of phospholipase-induced mast-cell degranulation,21 to affect any parameter of X-ray–induced ocular inflammation argues against a primary role for histamine and/or mast-cell products in the mediation of this inflammatory response. Thus we may assume that arachidonic acid derivatives, especially cyclooxygenase products, are the primary mediators of most aspects of this X-ray–induced ocular inflammation, and hence we must consider how X-irradiation of the eye may initiate the local arachidonic acid cascade.

The synthesis of PGs and related autacoids is initiated not by the stimulation of cyclooxygenase activity but rather by activation of specific lipases that cause the liberation of PG precursors, primarily arachidonic acid, from membrane phospholipids.22 The pharmacokinetics of PGs and related compounds is best explained on the basis of a model that regards cyclooxygenase and lipoxygenase products as extracellular autacoids that are released directly into the extracellular environment of cells.23 Thus release of free arachidonic acid from membrane phospholipids is the only control point in the initiation of the arachidonic acid cascade and therefore must be regarded as the primary event in the X-ray–induced ocular inflammation. This release may be a direct effect of X-irradiation on membrane phospholipases or may be mediated by free radicals, which are known to be produced by X-rays and are assumed to mediate many of the detrimental effects of ionizing radiations on biological systems, including the eye.24 Such mediation by free radicals may be supported by our observation that pretreatment with vitamin E, a potent biological antioxidant,25 appeared to reduce the extent of X-ray–induced ocular hypertension. However, antioxidants, including vitamin E, are also known to have direct effects on arachidonic acid metabolism.26 Further studies will therefore be required to assess the role of free radicals in the X-ray–induced initiation of the arachidonic acid cascade.

The present conclusion that the X-ray–induced inflammation of the rabbit eye is
mediated primarily by the arachidonic acid cascade must be reconciled with the fact that all other species studied so far, including chickens,\textsuperscript{2} owl monkeys, cats, ducks, pigeons, guinea pigs,\textsuperscript{3, 5} frogs, and rats,\textsuperscript{4} were found to be refractory to the ocular inflammatory and particularly the hypertensive effects of X-rays. Clearly, we cannot assume that only the rabbit eye is capable of producing PGs or related autacoids, since the capacity to synthesize PGs is a general property of virtually all vertebrate tissues,\textsuperscript{27} including the anterior uvea,\textsuperscript{28} and various forms of ocular inflammation have been shown to be mediated by cyclooxygenase products.\textsuperscript{2, 29}

Although a variety of species show some ocular response to topically applied PGs, considerable species differences exist in the predominant response of the eye to these autacoids and in the doses of PGs required to effect such responses. For example, topical application of as little as 50 \( \mu \)g of PGF\(_2\alpha\) to the rabbit eye causes a brief initial increase in IOP prior to the development of ocular hypotony,\textsuperscript{16} but in owl monkeys,\textsuperscript{30} cats, and rhesus monkeys\textsuperscript{31} the production of ocular hypertony prior to the onset of hypotony requires the topical application of as much as 1 mg of PGs per eye. Thus, depending on the amount of arachidonic acid released from intraocular tissues, some species may be expected to show ocular hypotension rather than hypertension. A small reduction in IOP was, in fact, observed in X-irradiated cat eyes.\textsuperscript{3}

However, guinea pig eyes showed no inflammatory response to a dose of X-ray 24 times that sufficient to produce a marked inflammation of the rabbit eye,\textsuperscript{1, 3} although these two species show comparable, cyclooxygenase-mediated, hypertensive responses to topically applied nitrogen mustard.\textsuperscript{32} Thus known differences in the sensitivity of the eye to the ocular hypertensive effects of arachidonic acid derivatives are unlikely to account for all the observed species differences in the ocular response to X-rays.

We must therefore consider the alternative possibility that although in the rabbit eye an effective dose-dependent link exists between X-irradiation and arachidonic acid release, in other species this link may be ineffective or nonexistent. For example, in species other than rabbits, a more efficient free radical scavenger mechanism may minimize or block the stimulation of X-ray--induced arachidonic acid release. Unfortunately, we do not have sufficient information on the mechanisms of stimulation of arachidonic acid release and on possible species differences in these mechanisms to evaluate this possibility.

The fact that we were unable to block the ocular hypertensive or inflammatory effects of X-rays with topical or intravitreal administration of prednisolone is, however, noteworthy in this respect. It is now generally accepted that corticosteroids such as prednisolone exert their anti-inflammatory effects, at least in part, by blocking arachidonic acid release.\textsuperscript{33} However, it has been shown, for example, that prednisolone pretreatment of rats sufficient to inhibit edema formation produced by subcutaneously implanted carrageenan-soaked sponges did not effectively block the local accumulation of PGs.\textsuperscript{34} These findings suggest that the ability of corticosteroids to effectively block arachidonic acid release may depend on the nature of the stimulation. Thus it is possible that X-irradiation of the rabbit eye stimulates arachidonic acid release by a route that circumvents the steroid-sensitive steps. Clearly, specific experiments will have to be done to identify the mechanism by which X-irradiation stimulates ocular arachidonic acid release in rabbits. This is a particularly pressing problem, since virtually all research on the ocular arachidonic acid cascade and its inhibition has been done on rabbits.

We can, however, conclude at this time that X-ray--induced inflammation of the rabbit eye is mediated largely and possibly entirely by the arachidonic acid cascade, the primary event being lipase activation. The apparent refractoriness of species other than rabbits to the ocular inflammatory effects of X-rays may reflect basic species differences in the mechanism of arachidonic acid release or quantitative differences in the amount of arachidonic acid released or active products
formed. Species differences in the sensitivity of the eye to the inflammatory effect of these autacoids may also contribute to but are unlikely to completely account for the observed species differences in X-ray sensitivity. Thus the rabbit eye may serve as a unique model for elucidating some aspects of arachidonic acid release or for studying the ocular effects of the resultant autacoids. However, results obtained on this atypical animal concerning the arachidonic acid cascade and its therapeutic manipulation should be extrapolated to other species only with great caution.

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