Prostaglandin synthesis, inhibition, and intraocular pressure

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Arachidonic acid, a precursor of prostaglandin E_2, administered topically to rabbit and monkey eyes in concentrations of 0.2 to 20 per cent, or intravenously to rabbits in doses of 10 to 20 mg., produced a significant elevation of intraocular pressure. A dose-response relationship was demonstrated. Facility of outflow and anterior chamber aqueous protein were significantly higher in eyes treated topically with 2 per cent arachidonic acid than fellow control eyes. These effects of arachidonic acid were similar to those of prostaglandin E applied topically. Pretreatment with indomethacin, given intraperitoneally in doses of 10 mg. per kilogram or greater, or aspirin, as a 600 mg. suppository, prevented completely the elevation of intraocular pressure produced by two drops of 2 per cent arachidonic acid applied to the eyes of rabbits. The elevation of anterior chamber aqueous protein induced by topically applied 2 per cent arachidonic acid also was blocked by prior administration of indomethacin. Indomethacin and aspirin, inhibitors of prostaglandin synthesis from arachidonic acid, had no effect on the rise of intraocular pressure seen after instilling 5 μg of prostaglandins E_1 or E_2.

Key words: prostaglandin synthetase, arachidonic acid, intraocular pressure, indomethacin, aspirin, inflammation, glaucoma.

Prostaglandins, administered topically or systemically, elevated intraocular pressure in the rabbit, cat, and monkey.[1-6] Prostaglandins E_1 (PGE_1) and E_2 (PGE_2) produced the greatest pressure response, while prostaglandins $F_{2\alpha}$, A, and $F_{1\alpha}$ were, respectively, less effective.[2] Extracts of iris have been reported to contain PGE_2 and $PGF_{2\alpha}$.[7] Administration of such extracts elevated intraocular pressure.[8]

The prostaglandins have been characterized as 20 carbon, unsaturated fatty acids. It has been shown that arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid) is the precursor of PGE_2 and dihomo-γ-linolenic acid (8, 11, 14-eicosatrienoic acid) is the precursor of PGE_1.[9, 10] The iris of the pig eye, in vitro, was able to convert fatty acid precursor to prostaglandin.[11]

Arachidonic acid caused slow contractions of isolated guinea pig ileum,[12] similar to the effect of prostaglandin on this preparation. In an initial report arachidonic acid

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applied topically to the eye elevated intraocular pressure in rabbits and monkeys.[13]

It has been suggested that the actions of aspirin-like drugs could be a result of the inhibition of prostaglandin synthesis.[14] Aspirin antagonized the arachidonic acid-induced contractions of isolated guinea pig ileum.[15] Aspirin and indomethacin inhibited the generation of prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 from arachidonic acid added to homogenates of guinea pig lung.[14] The release of PGE from rabbit spleen was abolished by aspirin and indomethacin.[16] Aspirin also inhibited the production of prostaglandin in human platelets.[17] Indomethacin appeared to block the effect of arachidonic acid on the eye in a pilot study.[13]

In order to further explore the modes of action of arachidonic acid and inhibitors of prostaglandin synthesis, further studies were carried out on the effect of indomethacin and aspirin on the arachidonic acid-induced elevation of intraocular pressure.

Materials and methods

1. Dose-response studies. Adult, albino rabbits, 2 to 3 kilograms, were restrained. Proparacaine, 0.5 per cent, was applied topically to both eyes. Intraocular pressure was measured with a Mackay-Marg tonometer. Arachidonic acid (Sigma Chemical Co., St. Louis, Mo.) was prepared freshly each week in concentrations of 0.2 to 20 per cent in peanut oil. Two drops (about 0.05 ml.) of arachidonic acid were administered to one eye, by holding the eyelids open and applying the drops to the cornea, and two drops of peanut oil to the other eye of the rabbits. The active agent was given to equal numbers of right and left eyes for each concentration. Repeat intraocular pressure measurements were made at 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours after instillation of arachidonic acid. Fresh animals were used for each experiment. A similar experiment was carried out applying 2 per cent arachidic acid to one eye and peanut oil to the other eye. (Arachidic acid is a 20 carbon, saturated fatty acid and is not a prostaglandin precursor.) All eyes were examined with slit lamp biomicroscopy before and after using arachidonic acid or other drops.

In other rabbits, 10 or 20 mg. of arachidonic acid in 0.2 ml. of peanut oil were injected intravenously. Control animals received 0.2 ml. of peanut oil. Baseline and repeat intraocular pressure measurements were carried out.

2. Tonographic studies. Tonography was performed with a Crescent tonometer and Leeds-Northrup recorder on rabbit eyes anesthetized topically before and 30 minutes after the administration of two drops of 2 per cent arachidonic acid onto the cornea of one eye and 2 per cent of arachidic acid onto the cornea of the other eye. Equal numbers of right and left eyes were used for each agent. Tonographic values were approximated from the 1955 Friedenwald tables for human eyes.

3. Aqueous humor chemistries. Two drops of 2 per cent arachidonic acid were administered to one eye and two drops of peanut oil to the other eye of awake, restrained rabbits after measuring baseline intraocular pressure. Thirty minutes later, intraocular pressure was remeasured and anterior chamber aqueous humor withdrawn. Determinations of protein[18] and ascorbate[19] in anterior chamber aqueous humor were made. A similar experiment was carried out at two hours after administration of 2 per cent arachidonic acid but measuring aqueous humor ascorbate only.

4. Inhibition studies. Awake, restrained rabbits were given indomethacin in aqueous suspension by intraperitoneal injection in varying doses from 2 mg. per kilogram to 50 mg. per kilogram or aspirin as 600 mg. suppositories.[20] Other uninjected rabbits were used as control animals. Baseline intraocular pressure was measured one hour later. Two drops of 2 or 20 per cent arachidonic acid were administered onto the cornea of one eye and two drops of peanut oil onto the cornea of the other eye of rabbits pretreated with aspirin, indomethacin, or no drug. Equal numbers of right and left eyes were treated with arachidonic acid. Intraocular pressure measurements were repeated at 30 minutes, 1 hour, 2 hours, and 4 hours after topical instillation of arachidonic acid. Similar experiments were carried out applying PGE_1 or PGE_2, 5 μg in 5 μl,[13] to one eye and an equal volume of its diluent to the other eye in rabbits pretreated and not pretreated with aspirin (600 mg.) or indomethacin (50 mg. per kilogram). Some eyes were examined by slit lamp biomicroscopy in each set of experiments.

Similar experiments also were carried out topically pretreating one eye of rabbits with two drops of indomethacin suspension in water in concentrations from 0.0005 to 5 per cent. Thirty minutes later intraocular pressure was determined, both eyes received two drops of 5 per cent arachidonic acid and pressure was followed.

In other awake, restrained rabbits, pretreatment was carried out with intraperitoneal indomethacin (10 mg. per kilogram). Control animals were not injected. One hour later, after measuring baseline intraocular pressures, two drops of 2 per cent arachidonic acid were given to one eye and two drops of peanut oil to the other, alternating right

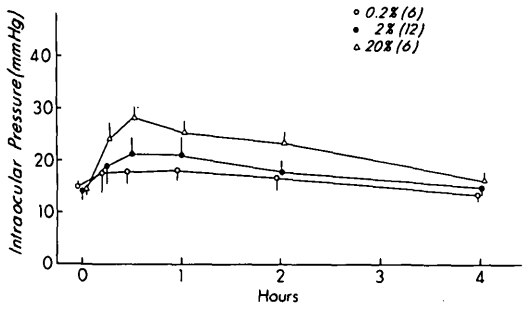

Fig. 1. Mean intraocular pressure (± S.D.) of treated eyes at designated times after topical administration of two drops of various concentrations of arachidonic acid. Numbers in parentheses are numbers of eyes.

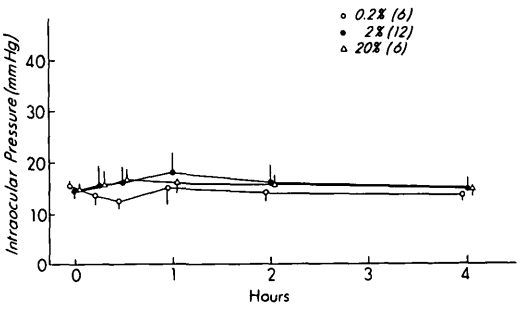

Fig. 2. Mean intraocular pressure (± S.D.) of peanut oil-treated fellow eyes at designated times after topical administration of two drops of various concentrations of arachidonic acid to treated eyes. Numbers in parentheses are numbers of eyes.

and left eyes. Thirty minutes later, intraocular pressure was remeasured and anterior chamber aqueous humor withdrawn for determination of protein.

Results

Arachidonic acid administered topically to rabbit eyes induced an elevation of intraocular pressure of similar magnitude to to prostaglandin. Dose-response relationships could be demonstrated (Fig. 1). The maximum rise of pressure occurred at 30 minutes. Arachidonic acid 20 per cent produced significantly ($p < 0.005$) elevated intraocular pressures at 15 minutes, 30 minutes, 1 hour, and 2 hours after its administration. The elevation of intraocular pressure after arachidonic acid 0.2 per cent was significant ($p < 0.005$) at 15 and 30 minutes. The intraocular pressure approached baseline by 4 hours even after the use of 20 per cent arachidonic acid. The mean intraocular pressure of the fellow eyes that received peanut oil was significantly ($p < 0.005$) elevated at 1 hour only (Fig. 2). On slit lamp examination, eyes that had been treated with 2 per cent arachidonic acid demonstrated external injection, occasional cells in the anterior chamber, and one to two plus aqueous flare.

Arachidonic acid administered intravenously to rabbits in doses of 10 mg. or 20 mg. also produced a significant ($p < 0.001$) elevation of intraocular pressure as compared to intravenous administration of the same volume of diluent (Table I).

The two eyes of 11 rabbits, one treated with 2 per cent arachidonic acid and the other with 2 per cent arachidic acid, were compared tonographically 30 minutes after drug utilization (Table II). The mean intraocular pressure of arachidonic acid-treated eyes was 26.8 ± 4.4 (S.D.) mm. Hg and of arachidic acid-treated eyes was 20.1 ± 3.6 mm. Hg. Outflow facilities were 0.46 ± 0.09 and 0.33 ± 0.07, respectively. Both intraocular pressure and facility of outflow were significantly ($p < 0.001$, $p < 0.01$) higher in the eyes treated with 2 per cent arachidonic acid.

The mean protein level in the aqueous humor of 11 rabbits 30 minutes after two drops of 2 per cent arachidonic acid was 7.5 ± 5.7 mg. per milliliter, significantly ($p < 0.005$) higher than 0.8 ± 0.2 mg. per milliiter in fellow eyes treated with peanut oil (Table III). No differences were noted between the two eyes with respect to anterior chamber aqueous humor ascorbate at 30 minutes or 2 hours after unilateral treatment with 2 per cent arachidonic acid.

Indomethacin, 10 mg. per kilogram to 50 mg. per kilogram intraperitoneally, competely abrogated the rise of intraocular pressure induced by 2 per cent arachidonic acid while a dose of 2 mg. per kilogram did not (Table IV). The latter dose pro-

Table I. Effect of intravenous arachidonic acid on intraocular pressure in rabbits

Treatment (intravenous)	*No. of animals*	*Mean intraocular pressure ± S.D. (mm. Hg)*		
		0 min.	*30 min.*	*60 min.*
Arachidonic acid (10 mg.)	13	13.9 ± 1.5	18.5 ± 2.1*	16.7 ± 2.0*
Peanut oil	5	15.6 ± 2.2	14.1 ± 1.8	13.1 ± 1.4
Arachidonic acid (20 mg.)	9	15.2 ± 1.6	22.3 ± 4.3*	17.6 ± 4.0
Peanut oil	10	15.3 ± 1.4	15.1 ± 1.3	15.5 ± 1.5

*Significant difference between the means of eyes of animals treated with intravenous arachidonic acid and those treated with peanut oil, Student t-test, $p < 0.001$.

Table II. Tonographic results after topical arachidonic acid in 11 rabbits

Treatment	*Intraocular pressure (mm. Hg)*		*Facility of outflow (μl/min./mm. Hg)*	
	0 min.	*30 min.*	*0 min.*	*30 min.*
Arachidonic acid (2%)	20.1 ± 3.0	26.8 ± 4.4*	0.31 ± 0.09	0.46 ± 0.09†
Arachidic acid (2%)	20.6 ± 2.7	20.1 ± 3.6	0.31 ± 0.11	0.33 ± 0.07

*Significant difference between eyes of individual rabbits receiving arachidonic acid to one eye and arachidic acid to the other eye, paired t-test, $p < 0.001$.

†Significant difference between eyes of individual rabbits receiving arachidonic acid to one eye and arachidic acid to the other eye, paired t-test, $p < 0.01$.

Table III. Aqueous humor ascorbate and protein after topical arachidonic acid in rabbits

Treatment	*Time after drug (min.)*	*Aqueous chemistries (mean ± S.D.)*			
		Ascorbate		*Protein*	
		mg. %	*Eyes*	*mg./ml.*	*Eyes*
Arachidonic acid (2%)	30	17.8 ± 3.2	6	7.5 ± 5.7*	11
Peanut oil		17.1 ± 3.1	6	0.8 ± 0.2	11
Arachidonic acid (2%)	120	18.4 ± 4.2	12	—	—
Peanut oil		19.0 ± 5.5	12	—	—

*Significant difference between eyes of individual rabbits receiving arachidonic acid to one eye and peanut oil to the other eye, paired t-test, $p < 0.005$.

duced approximately a 50 per cent reduction in the 2 per cent arachidonic acid effect. The effect of 20 per cent arachidonic acid also was prevented by indomethacin, 50 mg. per kilogram intraperitoneally. The mean intraocular pressures in 8 indomethacin-pretreated rabbits were 14.1 ± 2.6 mm. Hg and 12.1 ± 1.5 mm. Hg 30 minutes after application of 20 per cent arachidonic acid or peanut oil, respectively. The eyes of animals treated with indomethacin in doses that inhibited the arachidonic acid-induced rise of intraocular pressure at 30 minutes exhibited fewer inflammatory signs than those not so pretreated.

Topical administration of indomethacin in concentrations of 0.05 per cent or greater significantly reduced the rise of intraocular pressure produced by 5 per cent arachidonic acid drops (Table V). Complete block was not attained although dose-response relationships were demonstrable.

Thirty minutes after the topical administration of 2 per cent arachidonic acid to one eye of 12 rabbits pretreated with indomethacin, 10 mg. per kilogram intraperitoneally, the mean anterior chamber aqueous humor protein was 1.0 ± 0.6 mg. per milliliter, not significantly different from the peanut oil-treated fellow eyes, 0.8 ± 0.1 mg. per milliliter. A significant ($p < 0.001$) difference existed between the mean protein level of eyes treated with 2 per cent arachidonic acid of animals that did not

Table IV. Dose-response of indomethacin given 1 hour before topical 2 per cent arachidonic acid in rabbits

Indomethacin (intraperitoneal) (mg./Kg.)	*No. of animals*	*Difference of intraocular pressure (mean ± S.D.) arachidonic acid—peanut oil (30 min.)*
50	8	-0.2 ± 3.8
10	8	+0.7 ± 2.8
2	8	+4.4 ± 2.3*
None	8	+9.8 ± 3.3*

*Significant mean difference between eyes of individual rabbits receiving arachidonic acid to one eye and peanut oil to other eye, paired t-test, $p < 0.001$.

Table V. Dose-response of topical indomethacin given 30 minutes before topical 5 per cent arachidonic acid to both eyes of rabbits

Indomethacin (topical) (%)	*No. of animals*	*Difference of change in intraocular pressure (mean ± S.D.) control—indomethacin (30 min.)*
5	8	+5.6 ± 1.5*
0.05	8	+3.0 ± 0.6*
0.0005	8	-0.1 ± 1.5

*Significant mean difference 30 minutes after 5 per cent arachidonic acid between eyes of individual rabbits pretreated with indomethacin to one eye and diluent to the other, paired t-test, $p < 0.01$.

receive indomethacin (7.5 ± 5.7 mg. per milliliter), and those that were pretreated.

Pretreatment with a 600 mg. aspirin suppository had a similar blocking action on intraocular pressure. In 10 pretreated rabbits, the mean intraocular pressure of eyes 30 minutes after topical 2 per cent arachidonic acid was not significantly different from peanut oil-treated fellow eyes, 18.0 ± 5.1 mm. Hg vs. 15.9 ± 2.4 mm. Hg, respectively. Control animals not given aspirin demonstrated a significant ($p < 0.001$) elevation of intraocular pressure after 2 per cent arachidonic acid (Table VI).

Aspirin, 600 mg. suppository, or indomethacin, 50 mg. per kilogram intraperitoneally, failed to prevent the elevation of intraocular pressure caused by 5 μg of topical PGE_1. At 30 minutes after PGE_1 instillation, in the presence of indomethacin or aspirin, the mean intraocular pressure of eight eyes treated with PGE_1 was significantly ($p < 0.001$) higher than that of the diluent-treated fellow eyes. This dose of PGE_1 when given alone or with blocking agent produced a rise of intraocular pressure of similar magnitude to that induced by two drops of 2 per cent arachidonic acid (Table VII). Similarly, indomethacin, 50 mg. per kilogram, did not prevent the elevation of intraocular pressure caused by PGE_2.[13]

Discussion

Arachidonic acid, a precursor of prostaglandin E_2, produces an elevation of intraocular pressure when applied topically or given intravenously to rabbits. Arachidic acid, a 20 carbon, saturated fatty acid that is not a prostaglandin precursor, does not alter intraocular pressure. Thus, only specific fatty acids of this group are capable of elevating intraocular pressure. The time course and duration of this effect of arachidonic acid on intraocular pressure resembles that of prostaglandin E itself.[6]

Prostaglandins mediate certain aspects of the inflammatory response.[21] Perilimbal vasodilatation, miosis, and an increase in aqueous humor proteins accompany the rise of intraocular pressure induced by prostaglandins.[1-6, 22] PGE_1, administered topically, results in an increase in total outflow facility, suggesting that an increase of aqueous production is responsible for the elevation of intraocular pressure. Increased secretion and/or a breakdown of the blood-aqueous barrier may occur. The increase of protein and decrease of ascorbate in anterior chamber aqueous humor that is found after PGE_1[6] points to an enhanced permeability of intraocular vessels.

Topical administration of arachidonic acid also results in significantly increased total outflow facility and aqueous humor protein. Anterior chamber cell and flare are present after topical administration of 2 per cent arachidonic acid, but appear somewhat less than after 5 μg of PGE_1. Reduced ascorbate in the aqueous humor is not seen 2 hours

Table VI. Effect of aspirin on the intraocular pressure response to topical arachidonic acid in rabbits

Treatment	*No. of animals*	*Mean intraocular pressure ± S.D. (mm. Hg)*		
		0 min.	*30 min.*	*60 min.*
Aspirin (600 mg.)	10			
Arachidonic acid (2%)		16.0 ± 2.2	18.0 ± 5.5*	15.2 ± 4.2*
Peanut oil		16.4 ± 2.0	15.9 ± 2.4	16.1 ± 2.1
No aspirin	10			
Arachidonic acid (2%)		15.7 ± 5.8	23.7 ± 3.8*,†	19.7 ± 3.2*,†
Peanut oil		17.1 ± 1.9	17.1 ± 3.4	15.3 ± 2.1

*Significant difference between means of eyes treated with arachidonic acid of animals that did and did not receive aspirin, Student t-test, $p < 0.02$.
†Significant mean difference between eyes of individual rabbits receiving arachidonic acid to one eye and peanut oil to other eye, paired t-test, $p < 0.001$.

Table VII. Effect of indomethacin or aspirin on the intraocular pressure response to topical PGE_1 in rabbits

Treatment	*No. of animals*	*Mean intraocular pressure ± S.D. (mm. Hg)*		
		0 min.	*30 min.*	*60 min.*
Indomethacin (50 mg./Kg.)	8			
PGE_1 (5 μg)		11.5 ± 1.2	28.1 ± 4.6*	23.4 ± 4.2*
Diluent		11.0 ± 1.3	11.6 ± 1.3	12.6 ± 2.7
Aspirin (600 mg.)	8			
PGE_1 (5 μg)		13.6 ± 1.9	30.5 ± 3.8*	27.6 ± 3.9*
Diluent		13.8 ± 2.3	12.6 ± 2.5	11.6 ± 1.8
No drug	8			
PGE_1 (5 μg)		13.8 ± 2.7	27.3 ± 2.7*	24.8 ± 3.0*
Diluent		13.5 ± 2.6	13.6 ± 2.3	12.0 ± 1.6

*Significant mean difference between eyes of individual rabbits receiving PGE_1 to one eye and diluent to other eye, paired t-test, $p < 0.001$.

after treatment with 2 per cent arachidonic acid in contradistinction to the findings with PGE_1.[6] This may relate to peculiarities of exogenously applied as compared to endogenously produced prostaglandin E.

As iris tissue contains the prostaglandin synthetase system,[11] our data on intraocular pressure, outflow facility, and aqueous humor protein suggest that topically applied arachidonic acid may be converted to PGE_2 in vivo and the resultant prostaglandin liberated elevates the intraocular pressure. However, this conversion may take place in other ocular tissues.

Prostaglandin E_1, administered to one eye, is reported to elevate the intraocular pressure of the fellow eye of rabbits.[23] Arachidonic acid appears to have a similar consensual effect. However, the results are less striking with topical application of arachidonic acid. This may be expected as conversion to PGE_2 may have to take place first if the consensual effect is due to systemic absorption of very low levels of precursor.

The results of this study also demonstrate that indomethacin and aspirin block the elevation of intraocular pressure induced by a precursor of PGE_2, arachidonic acid, but not by prostaglandin E itself. The increase of aqueous humor protein produced by the topical application of arachidonic acid to rabbit eyes also is blocked by the prior intraperitoneal administration of indomethacin. It must be noted, however, that the dose of aspirin utilized is much greater than the therapeutic dose in humans. The dose-response data for indomethacin demonstrates an effect in the range used in man.

Inhibition of prostaglandin synthesis from arachidonic acid by aspirin-like drugs is well described and varies in different in vitro systems.[14-17] There may be blockade of

an intermediary substance and/or competition with arachidonic acid for the active site of the transforming enzyme.[14, 24, 25] It is of interest that aspirin-like drugs do not appear to suppress the relaxation of bronchial muscle induced by prostaglandins E_1 and E_2.[26] Thus, our data suggest that the presence and action of ocular prostaglandin synthetase are demonstrable in vivo. The inhibition of the arachidonic acid-induced elevation of intraocular pressure by indomethacin and aspirin represents in vivo evidence that it is the prostaglandin rather than the arachidonic acid that elevates intraocular pressure and that aspirin-like drugs may inhibit the conversion of arachidonic acid to PGE_2 in the eye.

The demonstration of increased levels of aqueous humor prostaglandin after ocular administration of arachidonic acid would be of interest. However, as arachidonic acid provokes an inflammatory response it is possible that the nonspecific release of endogenous prostaglandin is responsible for the elevation of intraocular pressure. The topical application of labeled arachidonic acid and recovery of labeled PGE_2 from aqueous humor would be necessary to prove that conversion takes place.

This inhibition of prostaglandin synthesis may account for many of the therapeutic actions of aspirin-like compounds.[14, 27, 28] Many systemic and ocular inflammatory responses may be mediated by the prostaglandins.[21, 29, 30] The effect of nonsteroidal anti-inflammatory drugs on the carrageenin rat paw edema assay in vivo correlates well with measurement of inhibition of prostaglandin synthetase in in vitro systems.[27, 28] The elevation of intraocular pressure by arachidonic acid applied topically is a simple phenomena that may be applied to the investigation of potential aspirin-like anti-inflammatory agents.

The effect of corticosteroids on prostaglandin synthesis is unclear. In one study, inhibition of PGE_2 and $PGF_{2\alpha}$ synthesis from arachidonic acid in skin homogenates is noted after addition of corticosteroids.[31] However, other investigators find corticosteroid anti-inflammatory agents are relatively poor inhibitors of prostaglandin synthetase, yet very potent against rat paw edema, as compared to indomethacin and aspirin.[28] The effect of corticosteroids on arachidonic acid-induced ocular hypertension is under investigation presently.

Clinical applications are implied with respect to the use of prostaglandin synthesis inhibitors. The disruption of the blood-aqueous barrier that occurs in irritated eyes can be prevented by the previous administration of aspirin to rabbits.[20] Indomethacin and aspirin are relatively ineffective in stemming ocular inflammation as compared to corticosteroids, but no studies indicate whether or not they may reduce the often attendant ocular hypertension. Of note, indomethacin drops do penetrate the eye[32] and have some blocking effect on the arachidonic acid-induced elevation of intraocular pressure. Trials of therapy with aspirin-like drugs in patients with elevation of intraocular pressure that occurs coincident with corneal transplantation in aphakia, glaucomatocyclitic crisis, and uveitis may be indicated.

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