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Comparison of the Ocular Hypotensive Efficacy of Eicosanoids and Related Compounds

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It has recently been shown that prostaglandin (PG)E_2 or $F_{2\alpha}$ reduces the intraocular pressure (IOP) of cats and primates when applied topically to the eye in very small doses, and that reduced IOP can be maintained in these species as long as the topical application of one of these PGs is repeated daily or twice daily. In the present study the ocular hypotensive efficacy and some of the ocular side-effects of 15 eicosanoids and related compounds, especially derivatives of $PGF_{2\alpha}$, were compared and were also compared to some clinically used ocular hypotensive agents. Derivatives of $PGF_{2\alpha}$ were found that had short-term and long-term ocular hypotensive potencies some 10- to 50-fold greater than $PGF_{2\alpha}$ itself.

Key words: prostaglandins; prostaglandin analogues; prostaglandin esters; $PGF_{2\alpha}$; eicosanoids; timolol; carbachol; ocular hypotensives; cat; glaucoma; ocular therapeutics; intraocular pressure; miosis.

1. Introduction

Prostaglandins (PGs) have generally been regarded as ocular hypertensive agents and potent mediators of the ocular irritative and/or inflammatory response (for review, see Eakins, 1973, 1976, 1977; Podos, Becker and Kass, 1973). However, a low dose (5 μg per eye) of topically applied $PGF_{2\alpha}$ has been shown to reduce, rather than increase intraocular pressure (IOP) in rabbits (Camras, Bito and Eakins, 1977), although higher doses (25–200 μg per eye) of $PGF_{2\alpha}$ and, especially, PGE_2 have indeed been found to cause an increase in IOP and breakdown of the blood–aqueous barrier. Thus, at least in rabbits, there is an unacceptably narrow margin between the potentially therapeutic and pathogenic doses of these autacoids. Furthermore, rapid development of subsensitivity or tachyphylaxis to repeated topical applications of a hypotensive dose of $PGF_{2\alpha}$ precluded long-term maintenance of reduced IOP in rabbits (Bito, Draga, Blanco and Camras, 1983a).

In contrast, it has recently been shown that topical application of PGE_2 or $PGF_{2\alpha}$ maintains reduced IOP in cats and Rhesus monkeys as long as a hypotensive dose of one of these PGs is applied at least once a day (Bito et al., 1983a). Although PGs of the E series are reputed to produce more severe adverse effects in the mammalian eye than PGs of the F series (Beitch and Eakins, 1969; Eakins, 1977), topical PGE_2 treatment for up to nine months caused only minimal flare and cellular responses in the anterior chamber of cat eyes and produced no serious local or systemic side effects (Bito, Srinivasan, Baroody and Schubert, 1983b).

In evaluating this potential new class of ocular hypotensive agents, it must be noted that the previously studied naturally occurring PGs may not be the best suited for clinical use. PGE_2, for example, is not sufficiently stable in aqueous solution, while corneal penetration of the much more stable $PGF_{2\alpha}$ is limited by its hydrophilic nature (Bito and Baroody, 1982). The present investigation was undertaken to evaluate the

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ocular hypotensive efficacy of a wide spectrum of eicosanoids representing classes of compounds with different physical, chemical and biological properties.

2. Materials and Methods

Cats of mixed breeds and of either sex (2·5–4·0 kg) were trained daily for four to seven days to accept handling, periodic restraint in animal boxes and the performance of tonometry with only a topical anesthetic (Alcaine; Alcon Laboratories, Inc., Fort Worth, TX). Some cats had been trained and used in a previous investigation (Bito et al., 1983a) that ended more than two months prior to this series of experiments. The eyes of all animals were examined by slit-lamp; only cats that showed no sign of ocular inflammation were used.

Each PG, PG isomer or PG analogue was dissolved in vehicle solution and 50 μl was applied topically to one eye of three to six cats in each experiment. The contralateral eyes which served as controls were untreated or given an equal volume of the vehicle solution alone. The following compounds were dissolved in 0·02 % Na_2CO_3 in water: PGE_2, $PGF_{2\alpha}$ free acid, $PGF_{1\alpha}$, $PGF_{2\beta}$, PGD_2, prostacyclin, 15-keto-$PGF_{2\alpha}$, 16,16-dimethyl $PGF_{2\alpha}$, and the PG analogue U-44069 ([15S]-hydroxy-9α, 11α-[epoxymethano]prosta-5Z, 13E-dienoic acid). The other PG analogue (Merck L-644, 122; 4-{3-[3-[2-(1-hydroxycyclohexyl)ethyl]-4-oxo-2-thiazolidinyl]propyl}benzoic acid) was dissolved in water with a molar excess of $NaHCO_3$. The tromethamine salts of $PGF_{2\alpha}$ and $PGF_{2\beta}$ were dissolved in normal saline, except for the highest concentration of $PGF_{2\alpha}$-tromethamine (1000 μg per eye), which was dissolved in distilled water. $PGF_{2\alpha}$ methyl, ethyl, and isopropyl esters were each dissolved in peanut oil (Sigma Chemical Co., St Louis, MO), as was $PGF_{2\alpha}$-free acid, in some experiments, to allow direct comparison of its effects to those of the PG esters. $PGF_{2\alpha}$ ethyl and ispropyl esters were prepared from $PGF_{2\alpha}$ obtained from The Upjohn Company, Kalamazoo, MI. Carbachol (750 μg per eye carbamylcholine chloride; Sigma) was dissolved in distilled water. An aqueous solution of timolol maleate powder 54·4 mg ml^{-1} (Merck) was prepared to yield a concentration of 40 mg ml^{-1} of timolol equivalent, and was applied in a total 25 μl aliquot. For the lower dose (500 μg), two 50 μl aliquots of a commercially available solution of 5 mg ml^{-1} timolol equivalents (Timoptic; Merck) were applied 1 min apart. The doses of the $PGF_{2\alpha}$- and $PGF_{2\beta}$-tromethamine salt are presented as $PGF_{2\alpha}$ free-acid equivalents (1·34 mg of the salt equals 1 mg of free acid). All other doses are presented as total weight per volume, without correction for differences in molecular weights.

Most compounds were dissolved in vehicle solution immediately before use and discarded thereafter. Exceptions include the $PGF_{2\alpha}$-tromethamine salt and the $PGF_{2\alpha}$ esters, which were stored frozen for several weeks. In one experiment, 200 μg ml^{-1} of $PGF_{2\alpha}$ methyl-ester in peanut oil was allowed to stand in the dark for three months at room temperature before it was retested on a group of cats.

Before the first application of each drug, one drop of 0·5 % alcaine was applied topically to obtain corneal anesthesia and IOP was measured with a floating-tip pneumatic tonometer (Pneumotonograph; Alcon). The shortest median–lateral pupillary diameter of each eye was measured in dim light (< 10 lux), using a mm-scale pupil gauge, and slit-lamp examinations were made. Cellular response and aqueous flare in the anterior chamber were evaluated by the same person throughout the series of experiments.

Aqueous humor flare was rated according to the extent of the Tyndall effect visible with the slit-lamp: none = 0; barely visible using a bright slit = 0·5; easily visible = 1; moderate to dense = 2; and dense with fibrin clots = 3. Cellular response was rated: no cell present = 0; very few cells seen during a through examination of all regions of the anterior chamber = 0·5; some cells easily visible without a thorough examination = 1; many cells in each slit-lamp field = 2; and cells densely dispersed or contiguous (clumps) = 3. In most cases, animals were treated with one dose of a drug and examined periodically for 24 hr thereafter. Pupil diameters were measured under the same conditions as the initial measurement every 30 min for the first 3 hr and at 4, 5, 6 and 24 hr. IOP was measured 1, 2, 3, 6 and 24 hr. Slit-lamp examination of the anterior chamber was repeated, in most cases, at 6 and 24 hr.

In multiple-dose experiments sets of cats received topical application of the same dose of the same drug ($PGF_{2\alpha}$-tromethamine salt, $PGF_{2\alpha}$ methyl, ethyl or isopropyl ester, Merck L-644, 122, or timolol) at 24-hr intervals for four days. In these cases, pupil diameters and IOPs

were measured at 1, 2, 3, 6 and 24 hr after the first and fourth treatments, and at 6 and 24 hr after the second and third treatments; slit-lamp examinations were performed at 6 and 24 hr after each treatment.

3. Results and Discussion

Short-term ocular hypotensive efficacy

Ocular hypotensive potency was found to vary considerably among the eicosanoids, their derivatives, analogues and metabolites. The fact that the initial metabolite of $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, and the isomer of $PGF_{2\alpha}$, $PGF_{2\beta}$ were less potent in reducing IOP than $PGF_{2\alpha}$ itself (Table I) is consistent with the fact that these compounds are less potent than $PGF_{2\alpha}$ in other organ systems (Anggard, 1966). The relative ocular hypotensive potencies of the three compounds suggest that the hypotensive effects of eicosanoids are mediated by stereospecific 'receptors'.

Of the five PGs surveyed (PGE_2, $PGF_{1\alpha}$, PGD_2, PGI_2, and $PGF_{2\alpha}$), PGE_2 showed the greatest ocular hypotensive potency. Although PGI_2 has been reported to be more potent than PGE_2 in many biological systems (Vane, 1978), it was found to be ineffective in reducing IOP at comparable doses. It should be noted, however, that because the half-life of PGI_2 in aqueous solution is only a few minutes (Gryglewski, Bunting, Moncada, Flower and Vane, 1976), only a small fraction of the PGI_2, even when dissolved in its vehicle within 1 min prior to its topical application, can be expected to reach intraocular target sites before inactivation.

The finding that PGE_2 has an apparently greater ocular hypotensive potency than $PGF_{2\alpha}$ or its tromethamine salt should not be taken as an indication that E-type PGs are better suited for clinical use as ocular hypotensive agents than F-type PGs. Indeed, intracameral administration of PGEs has been reported to produce more severe adverse ocular effects than PGFs, including transient ocular hypertension, breakdown of the blood–aqueous barrier, and iridial hyperemia (Beitch and Eakins, 1969; Waitzman and King, 1967). Furthermore, PGEs, but not PGFs have been shown to have adverse effects on retinal electrical activities when administered intravitreally in large doses, particularly in animals pretreated with a PG transport inhibitor (Wallenstein and Bito, 1977; Siminoff and Bito, 1982). Lastly, PGEs are more labile in aqueous solution than PGFs (Roseman, Sims and Stehle, 1972). Thus PGs of the F series must be regarded as potentially more promising therapeutic agents for the long-term treatment of glaucoma. For this reason, most of the remaining compounds selected for this study are related to $PGF_{2\alpha}$.

PG analogues that are protected against enzymatic inactivation as a result of steric hindrance at the site of attack of 15-hydroxy-PG-dehydrogenase, the first step in the enzymatic inactivation of E and F PGs (Anggard and Samuelsson, 1966; Nakano, Anggard and Samuelsson, 1969), have been shown to be more potent than the parent PGs in other biological systems (Wiqvist, Martin, Bygdeman and Green, 1975). In our study, the representative of such sterically protected analogues, 16,16-dimethyl-$PGF_{2\alpha}$ (Hansson and Granstrom, 1976), showed no evidence of enhanced hypotensive efficacy as compared to either $PGF_{2\alpha}$ or its tromethamine salt (Table I). This finding is not surprising in that intraocular tissues cannot effectively metabolize PGs (Eakins, Atwal and Bhattacherjee, 1974; Bito and Baroody, 1974); thus, protection against metabolism is not expected to increase ocular hypotensive potency. Further studies on this sterically hindered derivative were not done at this time because only a limited supply of this analogue was available to us. Furthermore, in the absence of a substantial

TABLE I

Comparison of the ocular hypotensive potency of eicosanoids and other drugs six hours after unilateral top*

	Topically applied dose in μg per eye					
	1000	500	100	50	10	5
PGE_2	−11±2·4 (6)	−8±1·3 (10)	−7±1·1 (9)	−6±0·7 (3)	−1±0·6 (8)	.
$PGF_{1\alpha}$	.	−2±1·1 (11)	−2±1·1 (7)	.	.	.
PGD_2	.	.	−2±0·8 (6)	.	.	.
PGI_2	.	.	0±0·7 (4)	.	.	.
$PGF_{2\alpha}$	.	−4±0·9 (11)	−1±0·3 (6)	.	.	.
$PGF_{2\alpha}$†	.	.	−1±0·4 (4)	.	.	.
$PGF_{2\alpha}$-tromethamine	−9±2·1 (4)	−6±3·8 (2)	−4±0·7 (11)	−3±0·6 (16)	−1±0·9 (14)	0±0·7 (13)
$PGF_{2\beta}$	−3±0·9 (9)	−1±0·5 (7)	−2±2·6 (4)	.	.	.
$PGF_{2\beta}$-tromethamine	.	−1±0·6 (6)	1±0·8 (6)	.	.	.
15-keto-$PGF_{2\alpha}$	.	0±1·6 (4)	−1±0·6 (4)	1±0·9 (4)	.	.
16,16-dimethyl $PGF_{2\alpha}$	.	.	.	0±0·3 (4)	.	0±0·7 (3)
PGF_α methyl ester†	.	.	.	.	−5±0·9 (16)	−3±0 (12)
$PGF_{2\alpha}$ ethyl ester†	.	.	.	.	−5±0·9 (18)	−2±1 (6)
$PGF_{2\alpha}$ isopropyl ester†	.	.	.	.	−4±0·9 (12)	0±0·7 (12)
U-44069‡	.	.	−1±0·9 (5)	.	.	.
Merck L-644, 122§	.	−1±0·8 (6)	−1±2·5 (6)	.	.	.
Timolol	−1±0·8 (6)	−1±0·6 (6)	.	.	.	.
Carbachol (750 μg)	−0·3±0·4 (6)		.	.	.	.

* Mean $(IOP_{exp})-(IOP_{con})$ in mmHg ± S.E.M., (n).
† In peanut oil.
‡ (15S)-hydroxy-9α, 11α-(epoxymethano) prosta-5Z, 13E-dienoic acid.

potentiation of ocular hypotension, protection of PGs against metabolism in the rest of the body, including the lungs, must be regarded as an unnecessary risk since such protection will enhance delivery of active forms of these compounds to other organ systems, and hence would increase the likelihood of adverse systemic side effects.

Studies in our laboratory have shown that the corneal epithelium is an effective permeability barrier to $PGF_{2\alpha}$ (Bito and Baroody, 1982). While penetration of $PGF_{2\alpha}$ through the sclera of the isolated globe was found to be relatively unrestricted, this route of penetration must be hindered in the *in situ* eye by the conjunctiva. These observations suggest that the relative rates of their penetration through the outer coats of the globe is another important criterion in the selection of PG analogues for topical ophthalmic use. It has been well established that the permeability of the cornea to compounds of relatively low molecular weight depends largely on their lipid solubility. In the present study, $PGF_{2\alpha}$ methyl, ethyl and isopropyl esters were used to represent more lipid-soluble PG analogues. Since these compounds are virtually insoluble in water, they were dissolved in peanut oil, a vehicle that has been used clinically for an ophthalmic preparation of diisopropylfluorophosphate (Leopold and Comroe, 1946).

As a result of their lipid solubility, the $PGF_{2\alpha}$ esters are likely to cross the corneal epithelium more readily than the more hydrophilic parent compound. Once such esters have crossed the epithelial barrier, esterases in the cornea (Lee, Morimoto and Stratford, 1982) can be expected to liberate the hydrophilic free acid which, in turn, will diffuse through the corneal stroma with little further hinderance. Because there is no evidence that the three $PGF_{2\alpha}$ esters have greater efficacy *per se*, it is likely that their relatively high ocular hypotensive potencies (Table I) can indeed be attributed to greater penetration through the corneal epithelium, followed by de-esterification. Increased efficacy of delivery to intraocular tissues, combined with effective systemic and pulmonary metabolism, both of which apply to these $PGF_{2\alpha}$ esters, offer the important therapeutic advantage of reducing the possibility of adverse effects on other organ systems.

While all three $PGF_{2\alpha}$ esters were found to reduce IOP at much lower doses than $PGF_{2\alpha}$ or its tromethamine salt, the methyl ester may not be best suited for long-term human use, since its hydrolysis results in the release of methyl alcohol, whose metabolites are known to be toxic to the eye. Although hypotensive doses of $PGF_{2\alpha}$ methyl ester will yield only trace amounts of methyl alcohol per treatment, even such small quantities may be cause for concern when daily treatment must be given for several years. Thus, other lipid-soluble $PGF_{2\alpha}$ esters, such as the ethyl or isopropyl esters, even if they are not more potent ocular hypotensives than the methyl ester, may be more appropriate for long-term ocular use.

Another important factor in the choice of an appropriate PG ester is its relative rate of hydrolysis which, in most biological systems, is determined by the molecular size and steric configuration of the ester, and by the enzymatic profile of the tissues in question. Delayed hydrolysis after penetration into the epithelial surface can be expected to result in a slower release of the free acid from the cornea. Thus the use of higher molecular weight or sterically hindered esters, either alone or in combination with more readily hydrolyzable forms, can be expected to increase the duration of the hypotensive activity of these derivatives.

The PG analogues U-44069 and L-644, 122 were found to be less effective ocular hypotensive agents thatn PGE_2 or $PGF_{2\alpha}$-tromethamine salt, and were much less effective than the $PGF_{2\alpha}$ esters in reducing IOP six hours after their topical

‡ (15S)-hydroxy-9α, 11α-(epoxymethano) prosta-5Z, 13E-dienoic acid.
§ 4-(3-[3-[2-(1-hydroxycyclohexyl)ethyl]-4-oxo-2-thiazolidinyl]propyl] benzoic acid.

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