Exp. Eye Res. (1987) **44**, 217–226

The Ocular Pharmacokinetics of Eicosanoids and their Derivatives. 1. Comparison of Ocular Eicosanoid Penetration and Distribution Following the Topical Application of $PGF_{2\alpha}$, $PGF_{2\alpha}$-1-methyl ester, and $PGF_{2\alpha}$-1-isopropyl ester

L. Z. Bito and R. A. Baroody

Department of Ophthalmology, Research Division, College of Physicians and Surgeons, Columbia University, NY 10032, U.S.A.

(*Received 11 July 1986 and in revised form 18 August 1986*)

These experiments were undertaken to determine whether the increased ocular hypotensive potency of topically applied prostaglandin (PG) $PGF_{2\alpha}$ esters, as compared with that of $PGF_{2\alpha}$ free acid, can be accounted for by increased penetration of the eicosanoid moiety of the esterified PG into the eye. One hour after the topical application of [^{3}H]$PGF_{2\alpha}$-1-methyl ester (ME) in peanut oil, the ^{3}H activities in the cornea, aqueous humor, and ciliary body of the rabbit eye were 32-, 22-, and 8-fold higher, respectively, than they were following the topical application of [^{3}H]$PGF_{2\alpha}$ free acid. ^{3}H activity during the first 3 hr declined rapidly in the cornea and more slowly in the aqueous humor, but remained essentially constant in the ciliary body for up to 6 hr, declining rapidly only between 6- and 24 hr. ^{3}H activity in eyes that received [^{3}H]$PGF_{2\alpha}$ ME was also several-fold higher in the anterior sclera and iris than in eyes that were treated with [^{3}H]$PGF_{2\alpha}$ free acid, but this difference was much smaller in the conjunctiva. At 1 hr, most of the ^{3}H activity in the aqueous humor was associated with $PGF_{2\alpha}$, as determined by chromatography, but at 2- and 3 hr other peaks, presumably reflecting metabolites of $PGF_{2\alpha}$, became apparent. The penetration and intraocular distribution of ^{3}H activity was similar when [^{3}H]$PGF_{2\alpha}$ ME was applied to the eye in normal saline rather than in peanut oil or when the isopropyl rather than the methyl ester of $PGF_{2\alpha}$ was used. These studies indicate that esterification of the carboxyl group of $PGF_{2\alpha}$ greatly enhances the penetration of the $PGF_{2\alpha}$ moiety into the eye and suggests that effective de-esterification of the $PGE_{2\alpha}$ ester occurs in the cornea, resulting in the delivery of $PGF_{2\alpha}$ free acid into the aqueous humor. It is concluded that topically applied PG esters act as pro-drugs and that the increased ocular penetration of these esters may account for the previously reported increase in their ocular hypotensive potency as compared to that of PG free acid or salts.

Key words: eye; prostaglandin, pharmacokinetics; $PGF_{2\alpha}$; prostaglandin esters; glaucoma; corneal permeability.

1. Introduction

Based on reports from many laboratories, it has become generally accepted that prostaglandins (PGs) have adverse effects on the eye, such as breakdown of the blood–aqueous barrier and a sharp increase in intraocular pressure (IOP), and therefore must be regarded as mediators of ocular inflammation (see Eakins, 1977). However, in a recent review, it was concluded that early results on the ocular effects of PGs, which were obtained mostly from rabbits, should not be generalized; that these reports tended to overstate the adverse effects of PGs on the eye; and that the primary ocular effect of PGs in most species, especially primates, is a reduction, rather than an increase in IOP (Bito, 1984a).

In recent studies, significant reductions of IOP, produced as a result of daily or twice daily PG application, were maintained in both cats and rhesus monkeys – although not in rabbits – for as long as the treatment was continued (Bito, Draga, Blanco and Camras, 1983). This long-term treatment was not associated with progressively, clinically significant, local or systemic side effects (Bito, Srinivasan, Baroody, and Schubert, 1983). It has been suggested, therefore, that PGs and eicosanoids in general

0014–4835/87/020217+10 $03.00/0

should be considered as a new class of potential anti-glaucoma agents (Bito, 1984a; 1985).

In fact, PGs appear to be ideally suited for the treatment of glaucoma by topical application. Because PGs are natural compounds that are normally present in the eye, they are unlikely to be toxic, and because they are not effectively metabolized by intraocular tissues (Eakins, Atwal and Bhattacherjee, 1974; Bito and Baroody, 1974), topically applied PGs can be expected to reach tissues of the anterior segment in an active form. On the other hand, their accumulation in the extracellular fluids of the retina is minimized by the PG transport function of the ciliary epithelium and the blood–retinal barriers (Bito and Salvador, 1972; Bito and Wallenstein, 1977). Furthermore, topically applied PGs that enter the venous drainage of the eye are unlikely to produce systemic side effects because PGs are actively removed from the circulation and are effectively inactivated by the lungs and kidneys (Bito, 1986).

It has been shown, however, that the corneal – presumably by virtue of the tight-junctional surface layer of the corneal epithelium – is not readily permeable to PGs, which are highly polar compounds that do not readily penetrate the basic cell membrane (Bito and Baroody, 1975, 1982). It has been argued, therefore, that less polar PG derivatives may be more effectively delivered to intraocular tissue compartments from topically applied solutions than naturally occurring free acids can be (Bito, 1984a). Indeed, some esters of $PGF_{2\alpha}$ exhibit a 10- to 30-fold greater ocular hypotensive potency than $PGF_{2\alpha}$ itself (Bito, 1984b). The present investigation was undertaken, therefore, to compare the relative efficacy of eicosanoid delivery into the aqueous humor and other intraocular compartments after the topical application of ^3H-labeled $PGF_{2\alpha}$ and its methyl and isopropyl esters.

2. Materials and Methods

Twenty-five microliters of peanut oil or physiological saline containing 0·2 μCi of [^{3}H]$PGF_{2\alpha}$ (New England Nuclear Co.; specific activity 12·2 Ci mmol^{-1}) and either [^{3}H]$PGF_{2\alpha}$ 1-methyl ester ($PGF_{2\alpha}$-ME) or isopropyl ester ($PGF_{2\alpha}$-IE) (Pharmacia, Uppsala, Sweden; specific activity 1·40- and 0·69 Ci mmol^{-1}, respectively) was applied with a micropipet to the cornea of either eye of conscious female New Zealand White rabbits (2·5- to 3·0 kg). All solutions contained sufficient carrier to bring the total PG concentration to 5 μg $PGF_{2\alpha}$ equivalent per 25 μl of solution. For preparation of the saline solution of the $PGF_{2\alpha}$ free acid or its methyl or isopropyl esters these compounds were first dissolved in a small volume of ethyl or benzyl alcohol; the final solution contained approximately 1 % alcohol. The peanut oil preparations contained no other solvents. Both eyes of each rabbit were given drugs in the same vehicle solution. However, one eye received [^{3}H]$PGF_{2\alpha}$, while the contralateral eye received either [^{3}H]$PGF_{2\alpha}$-ME or [^{3}H]$PGF_{2\alpha}$-IE. The procedure was adopted in order to reduce the number of rabbits that had to be killed, since it had been shown earlier that following the topical application of [^{3}H]$PGF_{2\alpha}$ to one eye, the ^{3}H activity in the contralateral eye is less than 0·1 % of that in the treated eye (Bito and Baroody, 1982).

The rabbits were killed with an overdose of sodium pentobarbital (Diabutol, Butler Co., Columbus OH) at 0·25-, 0·5-, 1-, 2-, 3-, 6-, or 24 hr after the application of these solutions. The aqueous humor was withdrawn and the globe and as much conjunctival tissue as possible were removed. The globes were then dissected. All tissues and fluids were weighed separately and then either oxidized in a Packard Tri-Carb Model 306 sample oxidizer or eluted overnight into 10 ml of Aquasol (New England Nuclear). All samples were counted in a Packard Model 3003 liquid scintillation counter; appropriate corrections were made for quenching.

In experiments involving chromatographic identification of the tracer in the aqueous humor, the total amount of the $PGF_{2\alpha}$-ME was kept constant, but the amount of [^{3}H]$PGF_{2\alpha}$-ME per 25 μl of applied solution was increased to 1·0 μCi. After 1-, 2-, or 3 hr, animals were killed and samples were collected as above, except that only a 25-μl aliquot of the aqueous

humor was used for direct isotope counting; the remainder of each sample (about 200- to 250 μl) from two to four identically treated eyes was pooled and extracted by the procedure of Stamford and Unger (1972). Aliquots of the aqueous phase and the chloroform extracts were counted as above, and showed an extraction efficiency from rabbit aqueous humor of greater than 97% for both $PGF_{2\alpha}$ and its methyl ester. Half of the remaining extracted material was chromatographed according to the A1 system of Green and Samuelsson (1964), using a solvent system of chloroform:benzene:methanol:acetone:acetic acid (100:100:25:25:5). The other half of the material was chromatographed under similar conditions, using acetone:triethylamine (99:1) as the solvent system.

In most of these experiments, the concentration of the ^3H-labeled eicosanoids or eicosanoid derivatives in ocular tissues or fluids after the topical application of 5 μg of [^3H]$PGF_{2\alpha}$ or [^3H]$PGF_{2\alpha}$ esters was calculated on the basis of the ^3H activity in each sample and the specific activity of the $PGF_{2\alpha}$ or its esters in the topically applied solution. In both the $PGF_{2\alpha}$ free acid and its esters, the ^3H label was on C9 in the cyclopentane ring, a position at which the label remains associated with the eicosanoid moiety in all initial metabolites and even in most urinary excretion products of $PGF_{2\alpha}$. Since intraocular tissues exhibit little or no capacity to metabolize PGs, it is reasonable to assume that the ^3H activity in all these tissues represented the concentration of an eicosanoid or its initial metabolite. On the other hand, the $PGF_{2\alpha}$ esters are expected to be de-esterified relatively rapidly and, as will be seen later, most of the ^3H activity in the aqueous humor was shown by paper chromatography to be associated with $PGF_{2\alpha}$ free acid after the topical application of $PGF_{2\alpha}$-ME. For these reasons, '$PGF_{2\alpha}$*' will be used throughout this paper to denote all ^3H-labeled $PGF_{2\alpha}$ and-or $PGF_{2\alpha}$-ME or IE derivatives found in each sample, calculated on the basis of $PGF_{2\alpha}$ equivalents.

3. Results

Fifteen to 60 min after the topical application of 5-μg [^3H]$PGF_{2\alpha}$-ME in 25 μl of peanut oil, 2- to 3% of the total topically applied ^3H activity was found in the cornea as compared with 0·04- to 0·07% of the total topically applied activity in corneas that received $PGF_{2\alpha}$ free acid in the same vehicle (Fig. 1A). At 1 hr after topical application, the concentration of $PGF_{2\alpha}$* was some 30-fold higher in corneas that received $PGF_{2\alpha}$-ME than in those treated with the same dose of $PGF_{2\alpha}$ free acid (Table I). During the first 3 hr, there was a rapid exponential decline in the $PGF_{2\alpha}$* concentration of the cornea; this was followed by a much slower rate of loss between 3- and 24 hr (Fig. 2). At 6 hr after the topical application of [^3H]$PGF_{2\alpha}$-ME, the $PGF_{2\alpha}$* concentration in the cornea was still high (approximately 0·2 ng per mg tissue), some 10-fold higher than the simultaneously measured concentration of $PGF_{2\alpha}$* in the contralateral eyes, which received the same dose of [^3H]$PGF_{2\alpha}$ free acid (Fig. 1A). Up to 6 hr, the $PGF_{2\alpha}$* concentration in the aqueous humor was 10- to 20-fold higher in the $PGF_{2\alpha}$-ME treated eyes than in those treated with $PGF_{2\alpha}$ free acid. In general, the concentration of $PGF_{2\alpha}$* in the conjunctiva of $PGF_{2\alpha}$-treated eyes was only slightly lower than that in the conjunctiva of $PGF_{2\alpha}$-ME-treated eyes (Fig. 1, panels B vs. A).

The $PGF_{2\alpha}$* concentration in the ciliary body was remarkably high at 15 min after the topical application of $PGF_{2\alpha}$-ME. There was a small, statistically insignificant increase at 1- and 2 hr, followed by only a slight decrease at 3- and 6 hr. The amount of $PGF_{2\alpha}$* in the ciliary body at any time during the first 6 hr equalled about 0·2- to 0·3% of the total amount of $PGF_{2\alpha}$-ME contained in the topically applied solution (Fig. 1D). In these eyes, the $PGF_{2\alpha}$* concentration in the ciliary body was 8- to 10-fold higher at all time periods than in those treated with $PGF_{2\alpha}$ free acid (Fig. 1D; Table I). A similar time-course was observed in the concentration of $PGF_{2\alpha}$* in the iridial portion of the anterior uvea (Fig. 1F).

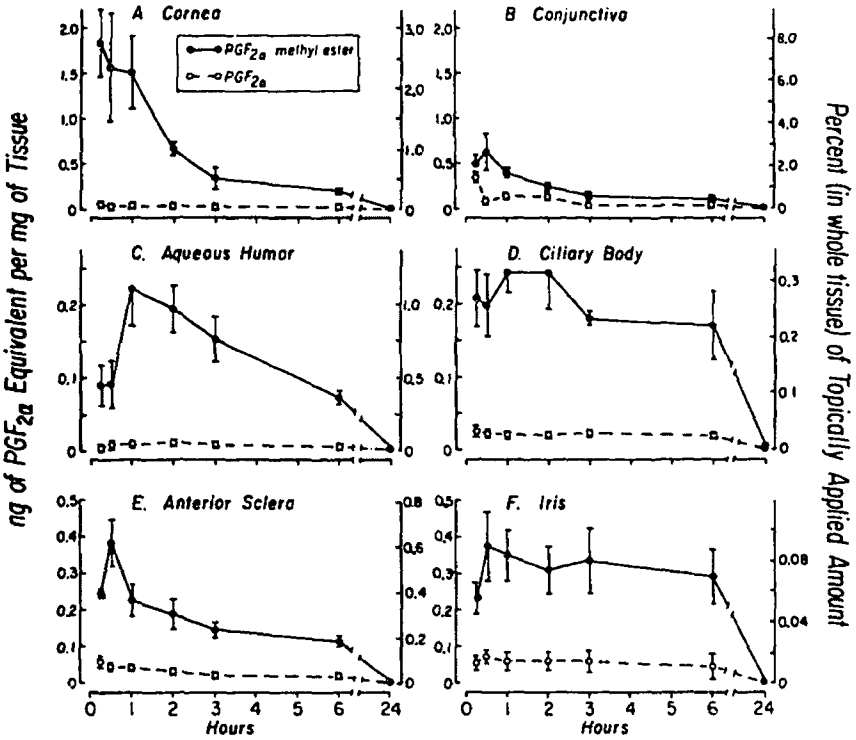

FIG. 1. The distribution of ^{3}H-labeled eicosanoids ($PGF_{2\alpha}$*) in ocular tissues at various intervals after the topical application of [^{3}H]-$PGF_{2\alpha}$ or [^{3}H]$PGF_{2\alpha}$-ME to the eyes of rabbits. The left abscissa shows the ^{3}H activity expressed as ng of $PGF_{2\alpha}$ equivalent per mg of tissue. The right abscissa indicates $PGF_{2\alpha}$ equivalents per total tissue weight expressed as percentage of the total $PGF_{2\alpha}$ equivalent applied to the eye at time zero. Points represent means obtained on 4 to 10 eyes and the limits represent ±1 S.E.(M.).

TABLE 1.

Concentration of $PGF_{2\alpha}$ in all ocular tissues at 1 hr after tropical application of 25 µl of peanut oil or saline containing 5 µg of $PGF_{2\alpha}$ equivalent of either $PGF_{2\alpha}$-Me or [^3H]$PGF_{2\alpha}$ free acid*

	ng of $PGF_{2\alpha}$* per g of tissue					
	$PGF_{2\alpha}$		$PGF_{2\alpha}$		$PGF_{2\alpha}$-ME:$PGF_{2\alpha}$	
	(A) Peanut oil	(B) Saline	(C) Oil	(D) Saline	(A:C) Oil	(B:D) Saline
Aqueous	222±50	223±37	12±6	11±3	19	20
Vitreous	11±4	4±1	3±1	1±1	4	4
Cornea	1470±390	973±71	47±9	35±8	31	28
Anterior sclera	228±41	27[illegible]±37	38±8	39±7	6	7
Posterior sclera	89±14	7[illegible]±9	27±3	25±4	3	3
Iris	349±72	20[illegible]±28	62±23	13±[illegible]	6	16
Ciliary body	242±35	1[illegible]±15	21±3	11±1	12	13
Lens	42±17	[illegible]±1	12±1	1±1	4	1
Retina	69±12	10±2	19±3	6±1	3	2
Choroid	144±27	38±[illegible]	31±7	13±1	5	3
Total globe	128±22	74±6	14±1	6±1	9	12
Conjunctiva	398±49	266±56	138±8	105±16	3	3

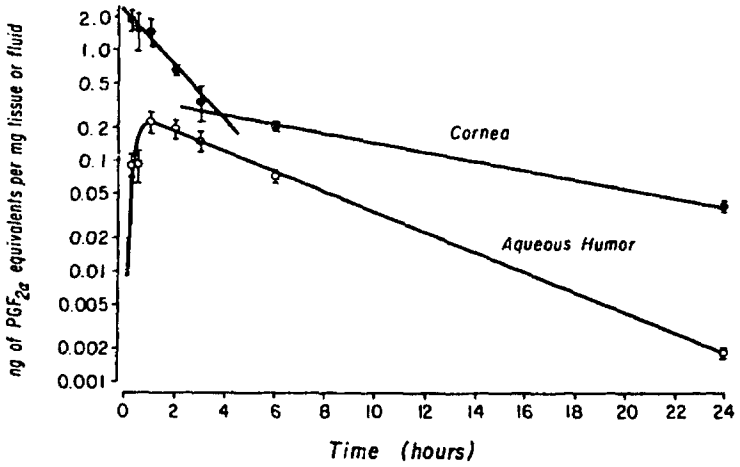

FIG. 2. Semilogarithmic plot of the time course of eicosanoid concentration, given in $PGF_{2\alpha}$ equivalents, in the cornea and aqueous humor after the topical application of 5 μg of [^{3}H]$PGF_{2\alpha}$-ME to rabbit eyes. See also legend to Fig. 1.

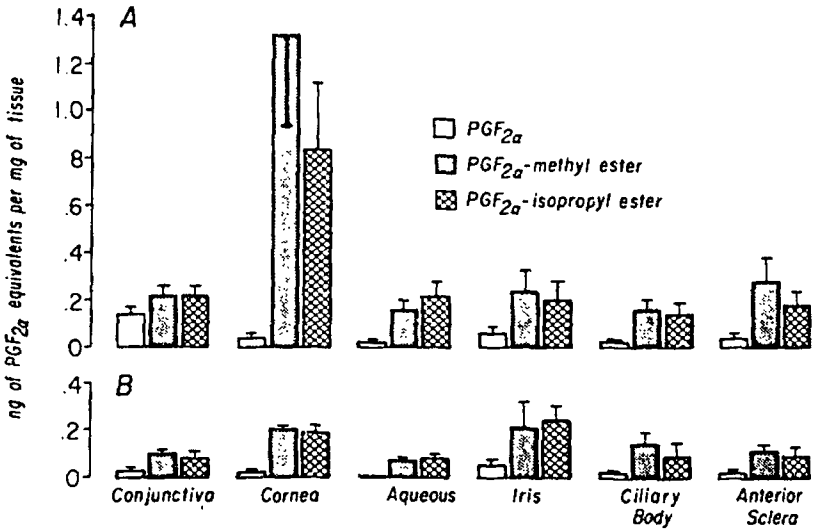

FIG. 3. Comparison of $PGF_{2\alpha}$* concentrations in various tissues 1 hr (Panel A) and 6 hr (Panel B) after the topical application of peanut oil containing [^{3}H]$PGF_{2\alpha}$, [^{3}H]$PGF_{2\alpha}$-ME, or [^{3}H]$PGF_{2\alpha}$-IE. There was no significant difference in the $PGF_{2\alpha}$* concentration of tissues obtained from eyes that were treated with the methyl ester or the isopropyl ester.

After the administration of $PGF_{2\alpha}$-ME, the time course of the $PGF_{2\alpha}$* concentration in the anterior portion of the sclera was similar to that in the conjunctiva, although at the peak concentration (30 min) and at most other time periods, the $PGF_{2\alpha}$* concentration in the anterior sclera was some 30% lower than that in the conjunctiva (Fig. 1E vs. 1B; Table 1). However, in the anterior sclera, unlike the conjunctiva, there was a large difference in the $PGF_{2\alpha}$* concentration between eyes that received $PGF_{2\alpha}$-ME and those that received $PGF_{2\alpha}$-free acid. The penetration of $PGF_{2\alpha}$* into the globe and its distribution within ocular tissues following the topical application of the isopropyl ester of [^{3}H]$PGF_{2\alpha}$ was not significantly different from that observed after the topical application of the methyl ester of [^{3}H]$PGF_{2\alpha}$ (Fig. 3).

In all of the experiments presented above, peanut oil was used as the vehicle solution. However, a similar picture was obtained when $PGF_{2\alpha}$-ME was applied to the

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.