From PGF$_{2\alpha}$-Isopropyl Ester to Latanoprost: A Review of the Development of Xalatan
The Proctor Lecture

Johan Wilhelm Stjernschantz

The research that led to the concept of using prostaglandins for reduction of intraocular pressure (IOP) has been discussed by Bito and goes back to the early 1980s, when it was shown that PGF$_{2\alpha}$ effectively reduces IOP in monkeys. Because the IOP-reducing effect in primates was found to be profound and of long duration, it was of obvious interest to investigate whether prostaglandins could be developed into drugs for glaucoma treatment. A fruitful collaboration between Columbia University (New York, NY) and Pharmacia (Uppsala, Sweden), a pharmaceutical company, was initiated. As a result of the collaboration, a new glaucoma drug Xalatan was developed. The purpose of this article is to present a review of the research that led to the concept of using prostaglandins and the development of Xalatan. Some relevant recent and previously unpublished data have been included as well. The experimental protocols of all animal studies performed complied with the tenets of the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research, and all protocols were submitted for review and approval to the local Ethics Committee for Animal Experimentation. Protocols for clinical studies were submitted to the appropriate Ethics Committee/Internal Review Board and the Declaration of Helsinki (1964) with subsequent revisions was adopted. Details concerning the experimental procedures of the previously unpublished results are given in the figure and table texts.

PGF$_{2\alpha}$-Isopropyl Ester as a Prototype Prostaglandin Drug for Glaucoma Treatment

The first approach to render prostaglandins suitable for glaucoma treatment was esterification of the carboxylic acid to improve the bioavailability and reduce the side effects. Several esters of PGF$_{2\alpha}$ were synthesized and tested for IOP-reducing effect and side effects. One of the best of these prodrugs of PGF$_{2\alpha}$ was the isopropyl ester (IE), but many other carboxylic acid esters, and di-, tri-, and tetra-esters and lactones of PGF$_{2\alpha}$ were prepared and tested in addition (Bito LZ, Resul B, unpublished results, 1985). Similar experiments were also performed by researchers at Allergan Pharmaceuticals (Irvine, CA). Other companies (e.g., Ueno Fine Chemicals Industry, Osaka, Japan, and Alcon Laboratories, Fort Worth, TX) subsequently adopted the isopropyl ester prodrug concept for their respective prostaglandin analogues (isopropyl unoprostone and travoprost, respectively).

PGF$_{2\alpha}$-IE is a very efficacious and potent IOP-reducing agent in cats, dogs, and monkeys. Comparable reductions of IOP with PGF$_{2\alpha}$ tromethamine salt requires a 10 to 100 times higher dose in monkeys. Thus, esterification of PGF$_{2\alpha}$ with isopropanol increased the bioavailability substantially. Overall, PGF$_{2\alpha}$-IE was found to be a very good IOP-reducing agent in many species except rabbits, in which a pressure increase frequently is induced by a breakdown of the blood-aqueous barrier. It is interesting that whereas cats exhibited distinct signs of ocular pain and discomfort (e.g., closure of the lids and lacrimation) unanesthetized monkeys usually did not (Stjernschantz J, unpublished results, 1988). In addition, in cats and dogs PGF$_{2\alpha}$ and its esters are potent miotics.

In the first clinical trial with PGF$_{2\alpha}$-IE, which had the character of a pilot test, no or a minimal IOP-reducing effect was observed in patients with glaucoma, probably because difficult cases were selected, refractory to all medical treatment (unpublished results, Pharmacia). Despite these discouraging results Villumsen and Alm, in cooperation with Pharmacia, started a systematic investigation to determine the IOP-reducing effect and side effects of PGF$_{2\alpha}$-IE and found that the drug indeed effectively reduced IOP in a dose-dependent manner in healthy volunteers. However, the IOP-reducing effect was accompanied by conjunctival hyperemia and ocular irritation similar to the side effects seen in studies with the tromethamine salt of PGF$_{2\alpha}$. The highest dose (10 μg) of PGF$_{2\alpha}$-IE caused pain and photophobia in all individuals. A dose of 0.5 μg twice daily was chosen for further studies in patients, and this dose, as well as a dose of 1 μg twice daily, was found to reduce IOP significantly, alone and in combination with timolol.

However, many patients reported side effects such as foreign-body sensation and conjunctival hyperemia, and it became questionable whether PGF$_{2\alpha}$-IE would offer any advantage over the already-established glaucoma medications. A particular problem was the irritative response to the drug. In animal experiments, it has been shown that PGF$_{2\alpha}$-IE induces albumin leakage in the iris and the ciliary body of monkeys at a dose of 1 μg and it is possible that the 10 times higher dose previously used in healthy individuals induces edema in the anterior uvea that causes pain and photophobia.

Effect of PGF$_{2\alpha}$-IE on the Uveoscleral Outflow Mode of Action

The first evidence that PGF$_{2\alpha}$-IE and its isopropyl ester reduces IOP through a mechanism based on increased uveoscleral outflow came from the studies by Crawford and Kaufman and Nilsson et al. Both research groups independently of each other found evidence for increased uveoscleral outflow of aqueous humor in monkeys treated with PGF$_{2\alpha}$ tromethamine salt or PGF$_{2\alpha}$-IE and no or minimal effect on the conventional
TABLE 1. Effects of PGF$_{2\alpha}$-IE, PGF$_{2\beta}$-IE, and 11-epi-PGF$_{2\alpha}$-IE on IOP, Pupil Diameter, and Nociception in Cats and on IOP in Monkeys

<table>
<thead>
<tr>
<th>Prostaglandin Analogue</th>
<th>Dose (µg)</th>
<th>Reduction in IOP (mm Hg)</th>
<th>Reduction in Pupil Diameter (mm)</th>
<th>Irritation*</th>
<th>Cat</th>
<th>Dose (µg)</th>
<th>Reduction in IOP (mm Hg)</th>
<th>FP Receptor EC$_{50}$ Value (mole/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF$_{2\alpha}$-IE</td>
<td>1.0</td>
<td>7.5 ± 1.3†</td>
<td>9.3 ± 0.4†</td>
<td>2.7 ± 0.2†</td>
<td>3.0</td>
<td>2.5 ± 0.3†</td>
<td>1.0 × 10$^{-8}$</td>
<td></td>
</tr>
<tr>
<td>PGF$_{2\beta}$-IE</td>
<td>1.0</td>
<td>8.3 ± 1.5‡</td>
<td>0.0 ± 0.0</td>
<td>1.5§</td>
<td>1.5</td>
<td>1.1 ± 0.8</td>
<td>5.6 × 10$^{-6}$</td>
<td></td>
</tr>
<tr>
<td>11-epi-PGF$_{2\alpha}$-IE</td>
<td>1.0</td>
<td>0.2 ± 0.5</td>
<td>7.0 ± 0.7‡</td>
<td>1.0§</td>
<td>2.4</td>
<td>2.0 ± 0.9</td>
<td>3.3 × 10$^{-8}$</td>
<td></td>
</tr>
</tbody>
</table>

The values are based on the maximum difference between the experimental and contralateral control eyes (mean ± SEM; n = 5–6). The FP receptor EC$_{50}$ values are results of in vitro tests performed on cat irides with the corresponding prostaglandin acids. Partly reproduced, with permission, from Resul et al., *Surf Ophthalmol*. 1997;41(suppl):S47–S52.

* Arbitrary scale of 0 to 3 (see Fig. 5).
† P < 0.001.
‡ P < 0.005.
§ Estimated mean value.

Rationale of Receptor Selectivity for Elimination of Side Effects

The preclinical and clinical studies with PGF$_{2\alpha}$-IE demonstrated that prostaglandins of the F type could be useful in the treatment of glaucoma, but it was not realistic to develop PGF$_{2\alpha}$-IE into a glaucoma drug for broader use, because of the side effects. Patients with severe disease could have endured treatment with the drug for some time. The question thus arose of whether it would be possible to separate the IOP-reducing effect from the side effects—primarily the irritative and hyperemic effects—by changing the receptor profile of PGF$_{2\alpha}$ through chemical modification. It should be recalled in this context that the classification of the prostanoid receptors in the mid 1980s was somewhat ambiguous, based on conventional pharmacologic experiments only.25–29 However, early experiments that we had performed with two epimers of PGF$_{2\alpha}$-IE, namely PGF$_{2\beta}$-IE and 11-epi-PGF$_{2\alpha}$-IE indicated that the miotic and IOP responses to PGF$_{2\alpha}$-IE could be distinctly separated by these two epimers in cats (Table 1). PGF$_{2\beta}$-IE reduced IOP with no miotic effect, whereas 11-epi-PGF$_{2\alpha}$-IE was a potent miotic with little effect on IOP. Furthermore, the epimers also differed from PGF$_{2\alpha}$-IE with respect to the nociceptive response (Table 1) and the hyperemic response, PGF$_{2\beta}$-IE being a much stronger vasodilator than both PGF$_{2\alpha}$-IE and 11-epi-PGF$_{2\alpha}$-IE (unpublished results; Pharmacia).

Thus, it appeared possible to separate the different ocular responses from each other, at least partly, and there was also an indication that the FP prostanooid receptor, which mediates miosis in cats, may be involved at least partly in IOP reduction in monkeys.25,26

A critical aspect in the success of the screening work was the selection of appropriate animal models that would allow extrapolation of the results to the human eye. The cat eye seemed very unspecific, in that IOP reduction was brought about by widely different prostaglandin analogues (e.g., PGF$_{2\alpha}$, PGF$_{2\beta}$, PGF$_{2\epsilon}$, PGA$_{2}$, PGB$_{2}$, and PGD$_{2}$). Therefore, we regarded the cat eye as somewhat unpredictable with respect to the IOP effect in humans and used the cat eye primarily for studying the nociceptive and miotic effects, whereas conscious cynomolgus monkeys were used to study the IOP-reducing effect. However, because young healthy monkeys usually have low IOP, often around 11 to 15 mm Hg, the test model was not ideal but was good enough to confirm whether an analogue had an IOP-reducing effect. The hyperemic effect was studied in albino rabbits, but there were no attempts to study anything else in the rabbit, because the rabbit eye is known to react very atypically to prostaglandins.27–29 Thus, the selection of adequate animal models was of paramount importance for the success of the project.

Structure–Activity Approach and Identification of Phenyl-Substituted Prostaglandin Analogues

The first approach to modifying PGF$_{2\alpha}$, included various alterations of the carboxylic acid end of the molecule. The alterations comprised, for example, the alcohol and simple esters but generally did not result in any clear-cut improvement of the pharmacologic profile of PGF$_{2\alpha}$. The second approach was to change the stereochemistry, and the functional groups in the cyclopentane ring. Although this yielded some interesting analogues that offered certain advantages over PGF$_{2\alpha}$, such as 11-epi-PGF$_{2\alpha}$-IE, modifications of the cyclopentane ring resulted in no real breakthrough. The third approach was to alter parts of the ω chain (e.g., the double bond between carbons 13 and 14 and the 15-hydroxyl group) and to substitute part of the chain. However, it was well known that the 15-hydroxyl group is essential for biologic activity of prostaglandins, and dehydration of the hydroxyl group results in marked loss of biologic activity.30–31 Thus, the approach taken by the researchers of Ueno Fine Chemicals Industry (Osaka, Japan) to reduce the side effects of PGF$_{2\alpha}$ by preparing the 13,14-dihydro-15-keto metabolite, or modifications of this metabolite (e.g., isopropyl unoprostone) renders molecules with significantly reduced potency.26

Among a group of different ω-chain-modified prostaglandin analogues, we quite unexpectedly noted that 17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$-IE induced marked miosis in the cat without concomitant irritation, which almost all other prostaglandin analogues had induced. Although there was no significant effect of the analogue on IOP in cats, conceptually, the combination of marked miosis with absence of nociception demonstrated that it was possible to eliminate the nociceptive effect without losing pharmacologic activity. In contrast to cats,32 monkeys responded to the compound with satisfactory IOP reduction.34–35 The compound, which can be regarded as the breakthrough, was assigned the code name PhDH100A and became the lead compound of the group of ω-chain ring-substituted prostaglandan ana-
Influence of \( \omega \)-Chain Length on Pharmacologic Profile

This aspect was studied in detail by attaching a terminal phenyl ring to carbons 15-24 (Fig. 1). The analogues were studied in cats with emphasis on the miotic and nociceptive responses. Of note, attaching the aromatic ring structure to carbon 17 of the prostaglandin skeleton yielded an optimal compound, in that the FP receptor function, as evident from the miotic response, was not compromised, whereas the nociceptive response was completely abolished\(^{34}\) (Table 2). In bizarre contrast, 16-phenyl-17,18,19,20-tetranor-PGF\(_{2\alpha}\)-IE with one additional carbon atom removed from the \( \omega \) chain caused significant irritation, albeit less than PGF\(_{2\alpha}\)-IE. Elongation of the \( \omega \) chain beyond carbon 17 with a terminal phenyl ring attached, reduced the biologic activity\(^{34}\) (Table 2). However, it is noteworthy that most analogues with a terminal ring structure exhibited considerably less nociceptive effect than PGF\(_{2\alpha}\)-IE. Substitution of carbon 17 with oxygen afforded a compound (16-phenoxy-17,18,19,20-tetranor-PGF\(_{2\alpha}\)-IE) with similar pharmacologic profile to that of 17-phenyl-18,19,20-trinor-PGF\(_{2\alpha}\)-IE (unpublished results; Pharmacia).

Influence of Different Ring Structures on the Pharmacologic Profile

A large number of compounds with different ring structures from cyclopropyl to cycloheptyl and aromatic ring structures, such as phenyl, thiophen, and furyl, attached to carbon 17 (Fig. 1) were prepared and tested. Overall, these analogues exhibited an improved side-effect profile compared with PGF\(_{2\alpha}\)-IE, albeit with somewhat different pharmacologic activity. Thus it appears that many different terminal ring structures attached to carbon 17 in the \( \omega \) chain reduce the side effects of PGF\(_{2\alpha}\)-IE.

Influence of Substituents in the Ring Structure on the Pharmacologic Profile

Compounds with various substitutions in the phenyl ring (Fig. 1) were also prepared and tested for pharmacologic activity.\(^{26,34}\) Introduction of a methyl group into the ortho (2) or meta (3) position in the phenyl ring did not appreciably change the miotic activity of 17-phenyl-18,19,20-trinor-PGF\(_{2\alpha}\)-IE, whereas introduction of the methyl group into the para (4) position dramatically reduced the activity.\(^{26,34}\) Obviously, the methyl group in the para position induces a steric hindrance in the receptor ligand interaction. Introduction of an electron-attracting trifluormethyl group into the ortho or para position in the phenyl ring reduced the activity of the compound.

Table 2. Effect of Phenyl-Substituted PGF\(_{2\alpha}\)-IE Analogues with Different \( \omega \)-Chain Lengths on IOP, Pupil Diameter, Nociception, and Conjunctival Hyperemia

<table>
<thead>
<tr>
<th>Prostaglandin Analogue</th>
<th>Monkey Reduction in IOP (mm Hg)</th>
<th>Cat Reduction in Pupil Diameter (mm)</th>
<th>Irritation* (0–3)</th>
<th>Rabbit Hyperemia* (1–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-Phenyl-17,18,19,20-tetranor-PGF(_{2\alpha})-IE</td>
<td>-3.9 ± 0.4‡</td>
<td>-1.0 ± 0.0†</td>
<td>2.2 ± 0.3†</td>
<td>ND</td>
</tr>
<tr>
<td>17-Phenyl-18,19,20-trinor-PGF(_{2\alpha})-IE</td>
<td>-3.9 ± 0.4‡</td>
<td>-0.9 ± 0.3‡</td>
<td>0.0 ± 0.0</td>
<td>1.8 ± 0.3‡</td>
</tr>
<tr>
<td>18-Phenyl-19,20-dinor-PGF(_{2\alpha})-IE</td>
<td>-0.6 ± 0.2‡</td>
<td>-4.3 ± 0.6†</td>
<td>0.7 ± 0.1†</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>19-Phenyl-20-nor-PGF(_{2\alpha})-IE</td>
<td>-0.6 ± 0.2‡</td>
<td>-2.5 ± 0.6‡</td>
<td>1.3 ± 0.2‡</td>
<td>0.6 ± 0.2‡</td>
</tr>
</tbody>
</table>

The values are based on the maximum difference between the experimental and contralateral control eyes. The dose was 3 \( \mu \)g in monkeys, 1 \( \mu \)g in cats, and 0.5 \( \mu \)g in rabbits (mean ± SEM; \( n = 5-6 \); statistical significances determined by paired \( t \)-test). ND, not determined.

* Arbitrary scale.
† \( P < 0.001 \).
‡ \( P < 0.01 \).
§ \( P < 0.05 \).

FIGURE 1. Series of ring-substituted analogues of PGF\(_{2\alpha}\)-isopropyl ester tested for effects in the eye to determine the importance of \( \omega \)-chain length, ring structures, and substituents in the phenyl ring.
whereas introduction of the group into the meta position only slightly reduced the activity. Substituting fluorine for the trifluoromethyl in the ortho, meta, or para position afforded compounds with marked miotic effect and no or very little irritant effect in the cat eye, thus indicating that the trifluoromethyl group may also partly change the pharmacologic activity through a steric effect. Introduction of an electron-donating methoxy group into the ortho position markedly reduced miotic activity, whereas introduction of the group into the meta position only slightly reduced miotic activity. The 16-(4-methoxy)-phenyl-17,18,19,20-tetranor PGF2α-IE analogue had virtually no irritant effect in the cat eye in contrast to 16-phenyl-17,18,19,20-tetranor-PGF2α-IE (unpublished results, Pharmacia). Thus, it appears that the para position, and to some extent the ortho position, in the phenyl ring are sensitive to steric hindrance, whereas the meta position is much less vulnerable. However, in the ortho position electrochemical forces may be important, at least in part, because an electron-attracting trifluoromethyl group reduces the activity in contrast to a neutral methyl group.

Overall, the structure–activity studies indicated that the ring structure on the ω chain is of paramount importance for reducing the side effects of PGF2α-IE, and furthermore that a large number of modifications of the ring structure are possible, still affording useful compounds in the eye.

Saturation of the 13,14-trans double bond of 17-phenyl-18,19,20-trinor-PGF2α-IE was found to further improve the receptor profile somewhat, and 13,14-dihydro prostaglandin analogues in addition exhibited improved chemical stability. The 13,14-dihydro-15R,17-phenyl-18,19,20-trinor-PGF2α-IE analogue was selected as the new candidate drug, and the compound was given the code name PhXA34. Because the 15R epimer is more potent than the 15S epimer, with time the 15R epimer (PhXA41) became the final candidate drug. It was given the generic name latanoprost and is the active principle in Xalatan. The chemical structures of PGF2α-IE, 17-phenyl-18,19,20-trinor-PGF2α-IE, PhXA34, and latanoprost (PhXA41) are presented in Figure 2.

### Latanoprost

As is obvious from the structure–activity studies, the reason for the good therapeutic index of latanoprost in the eye is its pharmacologic receptor profile. It can be seen in Table 3 that latanoprost acid is a much more selective FP prostanoid receptor agonist than PGF2α-IE. In practical terms it is even more selective than 17-phenyl-18,19,20-trinor-PGF2α because it spills less over on the EP1 and TP receptors (Table 3). It is also apparent that latanoprost acid is a full agonist on the FP receptor, and full or near full agonist on the EP1 and EP2 receptors, but has no, or only weak effect on prostanoid receptors EP3, DP, IP, and TP (Table 3). In comparison 17-phenyl-18,19,20-trinor-PGF2α-IE, with the 13,14 double bond intact, is a full agonist on the FP and EP1 receptors, and a partial agonist on the TP receptor, but has no, or only weak effect on the other receptors (Table 3). Thus, increasing the flexibility of the ω chain by saturating the 13,14 double bond has relatively little effect on the interaction with the FP receptor, but reduces the potency on the EP1 and TP receptors, and increases the capacity to stimulate the EP3 receptor, albeit only at very high concentrations.

Latanoprost has virtually no IOP-reducing effect in cats or rabbits, but induces a moderate IOP reduction in conscious normotensive monkeys as measured 3 to 6 hours after topical application. During continuous treatment with 3 μg once daily for 5 days the IOP reduction lasted around the clock (unpublished results; Pharmacia). In ocular hypertensive monkeys a good IOP-reducing effect of latanoprost has also been seen.
matrix components toward catabolism.\textsuperscript{44-46} PGF\textsubscript{2\alpha}\textsubscript{-IE} was found to reduce collagens I, III, and IV in the ciliary muscle and adjacent sclera of the monkey after topical treatment with 2 \textmu g twice daily for 5 days.\textsuperscript{47} Similar results were obtained by Ocklind\textsuperscript{48} who demonstrated a decrease in collagens I, III, and IV; laminin; fibronectin; and hyaluronan in human ciliary muscle cell cultures exposed to latanoprost acid in parallel with an increase in MMP-2 and MMP-3. She also found evidence for reduced collagens IV and VI levels in the ciliary muscle after 10 days of topical treatment with 3 \textmu g latanoprost daily in monkeys.\textsuperscript{49} Furthermore, evidence for a change in the shape of ciliary muscle cells was also found after exposure to latanoprost acid in vitro, with alterations in the actin and vinculin localization in the cells.\textsuperscript{50} Thus, the results indicate that latanoprost may have complex effects on ciliary muscle, the net effect being increased percolation of aqueous humor through the tissue.

### Vascular Effects of Latanoprost

Both the local and systemic vascular effects of latanoprost have been studied in detail. In the rabbit eye latanoprost induced no or minimal change in blood flow,\textsuperscript{19} in sharp contrast to PGF\textsubscript{2\alpha}–IE, which induced marked increase in blood flow to the surface structures and the anterior uvea after topical application.\textsuperscript{51} The hyperemic effect of PGF\textsubscript{2\alpha}–IE seems to be based on a release of nitric oxide (NO), and apparently the mechanism leading to NO release does not involve FP receptors.\textsuperscript{49} Of interest, sensory denervation by electrocoagulation of the ophthalmic nerve, almost completely abolished the hyperemic effect of PGF\textsubscript{2\alpha}–IE in rabbits, implying that the effect is nerve-mediated.\textsuperscript{50} This fits well with the absence of nociceptive effect of FP prostaglandin receptor agonists such as latanoprost. By using selective agonists we studied which of the prostaglandin receptors mediate the nociceptive response to prostaglandins in the cat eye and found that the FP and EP\textsubscript{2} receptors are of little or no importance (Fig. 3). Stimulation of the DP, IP, EP\textsubscript{1}, and EP\textsubscript{3} receptors, on the contrary, induced a nociceptive response in the cat eye (Fig. 3). Whether PGF\textsubscript{2\alpha}–IE-induced conjunctival hyperemia in humans also involves sensory nerves is unknown, but it is quite possible.

Latanoprost and PhXA34 tested at a dose of approximately four times the clinical dose of latanoprost in \textalpha\textlambda\textlambdan had a negligible effect on the regional blood flow in the monkey eye after topical application.\textsuperscript{51} Neither was any effect seen on capillary permeability to albumin in the monkey eye.\textsuperscript{51} Intravenous injection of latanoprost in escalating doses of up to 6 \mu g/kg body weight had no statistically significant effect on the uveal or retinal blood flow in monkeys, although a tendency toward increased blood flow was observed (Table 4).\textsuperscript{49} In aphakic monkey eyes with intact posterior lens capsule, latanoprost induced no capillary leakage in the retina as studied with fluorescein angiography during 6 months of treatment, and similar results were also obtained during shorter treatment periods in pseudophakic patients.\textsuperscript{52} Thus, it appears that the

![Figure 3](https://iovs.arvojournals.org/article-pdfaccess.aspx?url=/data/journals/iovs/933221/ on 11/04/2018)

**Table 3.** Potency Based on EC\textsubscript{50} Values and Estimated Efficacy of PGF\textsubscript{2\alpha}, 17-Phenyl-18,19,20-trinor-PGF\textsubscript{2\alpha} (17-Phenyl-PGF\textsubscript{2\alpha}), and Latanoprost Acid as Measured in Functional Receptor Assays\textsuperscript{24}

<table>
<thead>
<tr>
<th>Prostaglandin Analogue</th>
<th>FP</th>
<th>EP\textsubscript{1}</th>
<th>EP\textsubscript{2}</th>
<th>EP\textsubscript{3}</th>
<th>IP/DP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF\textsubscript{2\alpha}</td>
<td>1.2 \times 10^{-8}</td>
<td>3.2 \times 10^{-7}</td>
<td>6.4 \times 10^{-6}</td>
<td>1.6 \times 10^{-7}</td>
<td>&gt;10^{-4}</td>
<td>0</td>
</tr>
<tr>
<td>17-Phenyl-PGF\textsubscript{2\alpha}</td>
<td>4.5 \times 10^{-9}</td>
<td>6.5 \times 10^{-7}</td>
<td>&gt;10^{-4}</td>
<td>0</td>
<td>&gt;10^{-4}</td>
<td>0</td>
</tr>
<tr>
<td>Latanoprost acid</td>
<td>1.0 \times 10^{-8}</td>
<td>5.0 \times 10^{-6}</td>
<td>&gt;10^{-4}</td>
<td>2.8 \times 10^{-5}</td>
<td>~80</td>
<td>&gt;10^{-4}</td>
</tr>
</tbody>
</table>

The receptor assays were based on the following tissues: FP, cat iris sphincter; EP\textsubscript{1}, bovine iris sphincter in the presence of GR32191B; EP\textsubscript{2}, electrical stimulation of guinea pig ileum; EP\textsubscript{3}, electrical stimulation of guinea pig vas deferens; IP/DP, prevention of adenosine diphosphate–acid as measured in functional receptor assays 24.
FP receptor, if expressed in the vasculature, plays no or only a limited role in the regulation of vessel tone and capillary permeability in the eye.

The effects of latanoprost on the systemic circulation have been studied in monkeys after intravenous administration. With escalating doses of up to 6 μg/kg body weight an increase of the blood flow in parts of the brain as well as the heart was found, whereas very little effect was seen in other vital organs, such as the liver, gastrointestinal tract, and kidneys (Table 4). A tendency towards a slight increase in blood pressure was found. This effect seems to be based on increased cardiac output induced by high doses of the drug in anesthetized animals. It should be emphasized that the highest dose of 6 μg/kg body weight is approximately 100 times the clinical dose of latanoprost in Xalatan (per body weight) applied topically on the eye.

**Effects of Latanoprost on the Respiratory System**

As PGF₂α is a well-known constrictor of human bronchi, the effect of latanoprost on pulmonary function in healthy volunteers and patients who have bronchial asthma was important to investigate. No negative effects on pulmonary function were observed in two specially designed clinical studies. In monkeys, however, intravenous infusion of high doses of latanoprost was found to increase the intrathoracic inspiration–expiration pressure difference consistent with bronchoconstriction (unpublished results; Pharmacia). A study was therefore undertaken to determine the effects of latanoprost on human bronchi in vitro. Both PGF₂α and latanoprost acid contracted the smooth muscle of the bronchi, as measured in small-vessel myographs with an EC50 value of approximately 10⁻⁶ M. Latanoprost acid exerted about half the maximum effect of PGF₂α. Both the contraction to PGF₂α and latanoprost acid was completely abolished by a TP receptor antagonist (GR32191B). Thus, it appears that the effect is mediated primarily by TP receptors and not FP receptors. A concentration of 10⁻⁷ M was necessary to elicit any response at all to latanoprost acid. This concentration exceeds the maximum concentration in plasma of latanoprost acid during long-term treatment with Xalatan by approximately 1000 times. It is therefore unlikely, although not to be excluded completely, that the respiratory function of patients with glaucoma who have severe asthma would be negatively affected by latanoprost.

**Clinical Studies with Latanoprost**

In the phase I clinical trials, four phenyl-substituted PGF₂α analogues were compared: 17-phenyl-18,19,20-trinor-PGF₂α,IE, PhXA34, and latanoprost. Of these analogues PHXA34 and latanoprost appeared to be optimal, when considering the relationship between the IOP-reducing effect and the conjunctival hyperemic effect. None of the compounds had any appreciable irritant effect.

The first phase II clinical trials were performed with PhXA34, and these studies demonstrated a good IOP-reducing effect and advantageous therapeutic index of the drug in the eye. The blood flow in the eye is expressed per whole tissue and represents the right eye. Eye tissue data are in milligrams per minute; other tissue data are in grams per minute per gram tissue weight. (Mean ± SEM; n = 5–7.)

<table>
<thead>
<tr>
<th>Ocular tissues</th>
<th>Baseline</th>
<th>0.6 μg/kg</th>
<th>2 μg/kg</th>
<th>6 μg/kg</th>
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<tbody>
<tr>
<td>Retina</td>
<td>27 ± 3</td>
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<td>30 ± 3</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>Choroid</td>
<td>570 ± 95</td>
<td>574 ± 92</td>
<td>670 ± 119</td>
<td>660 ± 99</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>80 ± 14</td>
<td>83 ± 12</td>
<td>103 ± 19</td>
<td>105 ± 16</td>
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<td>Iris</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
<td>15 ± 2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Other tissues</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal brain</td>
<td>0.66 ± 0.06</td>
<td>0.70 ± 0.07</td>
<td>0.81 ± 0.09</td>
<td>1.09 ± 0.16*</td>
</tr>
<tr>
<td>Occipital brain</td>
<td>1.64 ± 0.34</td>
<td>1.75 ± 0.36</td>
<td>2.00 ± 0.50</td>
<td>1.69 ± 0.29</td>
</tr>
<tr>
<td>Parietal brain</td>
<td>0.56 ± 0.06</td>
<td>0.59 ± 0.07</td>
<td>0.68 ± 0.10</td>
<td>0.88 ± 0.13*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.54 ± 0.07</td>
<td>0.50 ± 0.05</td>
<td>0.55 ± 0.05</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>5.02 ± 0.54</td>
<td>4.94 ± 0.38</td>
<td>5.59 ± 0.60</td>
<td>6.06 ± 0.70</td>
</tr>
<tr>
<td>Myocardium left atrium</td>
<td>0.40 ± 0.09</td>
<td>0.42 ± 0.06</td>
<td>0.73 ± 0.20*</td>
<td>1.29 ± 0.29*</td>
</tr>
<tr>
<td>Myocardium left atrium</td>
<td>0.44 ± 0.11</td>
<td>0.47 ± 0.11</td>
<td>0.60 ± 0.17</td>
<td>0.89 ± 0.22*</td>
</tr>
<tr>
<td>Myocardium right ventricle</td>
<td>1.35 ± 0.18</td>
<td>1.58 ± 0.18</td>
<td>2.28 ± 0.38</td>
<td>4.68 ± 1.10*</td>
</tr>
<tr>
<td>Myocardium left ventricle</td>
<td>1.90 ± 0.21</td>
<td>2.10 ± 0.18</td>
<td>2.79 ± 0.46</td>
<td>4.75 ± 1.01*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.26 ± 0.05</td>
<td>0.26 ± 0.05</td>
<td>0.32 ± 0.12</td>
<td>0.26 ± 0.08</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.24 ± 0.04</td>
<td>0.24 ± 0.04</td>
<td>0.21 ± 0.03</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.82 ± 0.14</td>
<td>0.89 ± 0.14</td>
<td>0.93 ± 0.13</td>
<td>1.36 ± 0.23*</td>
</tr>
<tr>
<td>Colon</td>
<td>0.55 ± 0.10</td>
<td>0.56 ± 0.07</td>
<td>0.55 ± 0.11</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.80 ± 0.58</td>
<td>5.55 ± 0.45</td>
<td>5.42 ± 0.42</td>
<td>5.93 ± 0.42</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with baseline (ANOVA; Tukey’s test).

The first phase II clinical trials were performed with PhXA34, and these studies demonstrated a good IOP-reducing effect and advantageous therapeutic index of the drug in the eye. In the phase I clinical trials, four phenyl-substituted PGF₂α analogues were compared: 17-phenyl-18,19,20-trinor-PGF₂α,IE, 15-keto-17-phenyl-18,19,20-trinor-PGF₂α,IE, PhXA34, and latanoprost. Of these analogues PHXA34 and latanoprost appeared to be optimal, when considering the relationship between the IOP-reducing effect and the conjunctival hyperemic effect. None of the compounds had any appreciable irritant effect.

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The main side effects of latanoprost in the phase III clinical trials comprised slight conjunctival hyperemia and increased pigmentation of the iris, a new side effect. The latter side effect documented by sequential color photographs occurred at different frequencies in the different studies, with highest incidence in the United Kingdom study.\(^77\) The incidence, appearing about 6 months from the start of the treatment, was based on a morning, noon and afternoon measurement. In three of these studies latanoprost reduced IOP significantly better than timolol, whereas the two drugs were about equally effective in the Japan study.\(^76\) The diurnal IOP was found during 12 to 24 months of treatment.\(^79\)–\(^82\)

The main side effects of latanoprost in the phase III clinical trials comprised slight conjunctival hyperemia and increased pigmentation of the iris, a new side effect. The latter side effect documented by sequential color photographs occurred at different frequencies in the different studies, with highest incidence in the United Kingdom study.\(^77\) The incidence, appearance, and dependence on eye color of the side effect have previously been described in detail.\(^83\) Patients with hazel or heterochromic eye color (e.g., blue, grey, or brown) seem to be predisposed to the side effect, whereas the incidence in patients with homogenous eye color (e.g., blue, grey, or brown) was less than 5% during 2 years of treatment.\(^84\) Increased iridial pigmentation has also been reported during isopropyl unoprostone treatment, another prostaglandin analogue used to treat glaucoma.\(^84\) In addition to the iridial pigment side effect, latanoprost has been shown to cause darker and longer eye lashes in many patients.\(^85,86\) The underlying mechanisms of these side effects are discussed in more detail in the following section.

After the introduction of the drug on the market other less frequent side effects have appeared. The most relevant are anterior uveitis\(^87,88\) and cystoid macular edema (CME).\(^89,89\) The exact mechanisms of these side effects remain unknown, but it appears that in the majority of cases the side effects have been confined to predisposed compromised eyes (e.g., aphakic or pseudophakic vitrectomized eyes) that not infrequently previously have exhibited similar symptoms. Even in such eyes, the incidence seems to be relatively low, and the side effects have usually disappeared on termination of the treatment with the drug (unpublished results, Pharmacia). However, CME is a serious side effect, and all ophthalmologists prescribing the drug should be aware of the risk of the side effect, particularly in aphakic and pseudophakic compromised eyes.

Several clinical trials have also demonstrated that latanoprost can successfully be combined with other glaucoma medications, such as timolol,\(^88,89\) acetazolamide,\(^82\) epinephrine,\(^93\) and pilocarpine,\(^94\) the reason for this probably being the unique IOP-reducing mechanism of latanoprost.

**Increased Iridial Pigmentation and Melanogenic Side Effect of Prostaglandins**

The property of latanoprost to induce increased iridial pigmentation was first observed in the chronic toxicity tests. The effect was obvious because cynomolgus monkeys with yellowish irides were used, and one eye only was treated.\(^95,96\) Histologic examination of the iris did not reveal any pathologic changes, and a large well-controlled morphometric study was performed to assess whether the color change was based on a proliferative effect of latanoprost on the iridial melanocytes (unpublished results; Pharmacia). The study showed that a 1-year treatment with doses ranging from 2 to 100 μg of latanoprost per day had no or, at most, a minimal effect on the melanocytes, although the most marked and frequent effects of increased pigmentation were found in this dose group. At the electron microscopic level no or only minute differences between melanocytes in the control iris and the treated iris were seen in another study in rhesus monkeys treated for 2 years with latanoprost (unpublished results; Pharmacia), although at least in some animals there seemed to be a tendency toward larger and more mature melanosomes in the melanocytes of the treated eye (Fig. 5). Many in vitro studies have confirmed that latanoprost (or latanoprost acid) has no significant proliferative effect on human iridial melanocytes or uveal melanoma cell lines.\(^97,99\)

Several studies have also been performed in patients to investigate whether the darkening of the iris color is due to a proliferative or some sinister effect of latanoprost on the iridial melanocytes, but there have been no abnormal findings. For instance, an immunohistochemical study with monoclonal antibodies to proliferating cell nuclear antigen (PCNA) and Ki-67 revealed no signs of cell proliferation in iridectomy specimens of patients treated for 3 months with latanoprost,\(^100,102\) and

### Table 5. IOP-Reducing Effect of 0.005% Latanoprost Once Daily and 0.5% Timolol Twice Daily in Phase III Clinical Trials

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Test Compound</th>
<th>Baseline</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>IOP (mm Hg)</td>
</tr>
<tr>
<td>United States</td>
<td>Latanoprost</td>
<td>128</td>
<td>24.4 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
<td>140</td>
<td>24.1 ± 3.6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Latanoprost</td>
<td>149</td>
<td>25.2 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
<td>145</td>
<td>25.4 ± 3.6</td>
</tr>
<tr>
<td>Scandinavia</td>
<td>Latanoprost</td>
<td>183</td>
<td>25.1 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
<td>84</td>
<td>24.6 ± 3.1</td>
</tr>
<tr>
<td>Japan</td>
<td>Latanoprost</td>
<td>80</td>
<td>23.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
<td>83</td>
<td>23.1 ± 1.7</td>
</tr>
</tbody>
</table>

The values represent mean diurnal IOP except for the study in Japan in which only one IOP measurement was obtained in the morning, representing the trough. The treatment time was 6 months for the trial in Japan in which the treatment time was 3 months (mean ± SD). (Partly reproduced, with permission, from Camras et al.,\(^76\) Watson et al.,\(^77\) Alm et al.,\(^75\) and Mishima et al.\(^78\))

\(* P < 0.001 when comparing latanoprost with timolol (t-test).\)

Four randomized, double-masked phase III clinical trials in which the efficacy and safety of 0.005% latanoprost once daily was compared with 0.5% timolol twice daily were performed: one each in Scandinavia,\(^75\) the United States,\(^76\) the United Kingdom,\(^77\) and Japan.\(^78\) A total of 540 of the 992 patients with primary-open angle glaucoma, ocular hypertension, capsular glaucoma, or pigmentary glaucoma, who enrolled in the studies, were treated with latanoprost, and 496 of the latanoprost-treated patients completed the studies. The treatment time was 6 months, except for the study in Japan in which the treatment time was 3 months. The diurnal IOP was based on a morning, noon and afternoon measurement. In three of these studies latanoprost reduced IOP significantly better than timolol, whereas the two drugs were about equally effective in the United Kingdom study.\(^77\) The average diurnal IOP reduction achieved with latanoprost was 27% to 34% from a baseline diurnal pressure level before treatment of 24 to 25 mm Hg, which can be considered satisfactory and clinically useful. No significant upward drift in IOP was found during 12 to 24 months of treatment.\(^79\)–\(^82\)
neither were any pathologic changes detected in iridectomy specimens from patients treated for 6 months with latanoprost when studied with electron microscopy. Similar to a large histopathologic study in progress no clear-cut pathologic changes in iridectomy specimens from patients treated with latanoprost have been found, except for an apparent increase in pigmentation of the melanocytes in some specimens.

However, the end point of the increase in iridial pigmentation in affected patients is not known, nor is it known whether the newly formed pigment will disperse with time. Evidence for the latter in controlled clinical trials so far has not been found.

The amount of eumelanin in the iridial melanocytes was found to be significantly increased in cynomolgus monkeys that exhibited increased pigmentation after 25 to 38 weeks of latanoprost treatment, whereas no increase of the content of pheomelanin was found. This demonstrates that latanoprost has an eumelanogenic effect on the iridial melanocytes, and the results also argue against a local migration of melanocytes as the main cause of the darkening of the iris, because then the total amount of eumelanin would not have changed significantly. We have also shown an increase of tyrosinase transcription in the iridial melanocytes, both in vivo and in vitro, during latanoprost treatment. In addition, a melanoenic effect of latanoprost in the monkey iris was recently shown by autoradiographic technique with tritiated methimazole which is incorporated into newly formed melanin. No labeling of the iridial pigment epithelium was found, indicating the absence of a melanoenic effect of latanoprost in the pigment epithelium. Thus, there is ample evidence that latanoprost induces melanogenesis in iridial melanocytes of primates including humans.

Because latanoprost is a relatively selective FP receptor agonist, it is reasonable to assume that the melanoenic effect is mediated by FP receptors in the melanocytes. We have found the FP receptor transcript and protein in monkey iridial melanocytes by in situ hybridization and immunohistochemical techniques. At the genomic level, we found no significant variation in the FP receptor protein among 10 randomly selected blood donors and 15 patients who acquired increased iridial pigmentation during latanoprost treatment (Fig. 6).

Only one variation was found at the amino acid level: Isoleucine was exchanged for valine in the carboxyl-terminal part of the receptor. Thus, genetic variation in the FP receptor cannot explain why some individuals display increased pigmentation of the iris, whereas others do not during latanoprost treatment.

Recently, we also found that latanoprost acid consistently induces the production of PGE2 in iridial melanocytes in vitro, an effect possibly mediated by the cyclooxygenase (COX)-2 enzyme. Because EP receptors (e.g., EP1 receptors) have been demonstrated in the nuclear envelope in certain cell systems, it is possible that PGE2 functions as an intracellular signaling substance to promote gene transcription. This hypothetical mechanism could be important for the melanogenic response to exogenous prostaglandins, such as latanoprost and isopropyl unoprostone. In epidermal melanocytes, protein kinase C (PKC)β has been shown to activate the tyrosinase enzyme by phosphorylating two serine residues, and it is possible that latanoprost, which probably releases diacylglycerol in the iridial melanocytes through FP receptor activation, has a similar effect.

**Hypertrichotic Effect of Prostaglandins**

Contradictory reports have previously been published concerning the hypertrichotic effect of prostaglandins. However, as mentioned earlier latanoprost exerts a hypertrichotic effect at least on the eye lashes, and this phenomenon is relatively consistent. We have studied the receptor pharmacology of the hypertrichotic effect of prostaglandins using a...
mouse model in which the fur is shaved and the regrowth of the fur during local treatment with selective prostanoid receptor agonists is studied. The hypertrichotic effect seems to be mediated primarily by the FP receptor (Fig. 7). However, at the cellular level the mechanism in the hair follicle remains unknown, and we have not found, for example, a proliferative effect of latanoprost on fibroblasts or keratinocytes (Stjernschantz J, unpublished results, 2000). A possibility is that latanoprost activates follicular melanocytes, which in turn regulate the proliferation of keratinocytes in the hair follicle.

Prostaglandins as Antiglaucoma Drugs

The concept of using autacoids such as prostaglandins as IOP-reducing agents for glaucoma treatment is very attractive. It seems logical that such local hormones would have a positive synchronized effect in the tissue without untoward effects. However, a disadvantage may be that the receptors to the autacoid are widely distributed in the eye. The FP receptor, for example, has been demonstrated by in situ hybridization and immunohistochemical techniques in the corneal epithelium and endothelium, the lens epithelium, the ciliary muscle and epithelium, and the iridal melanocytes; in different layers of the retina, including the ganglion cell layer; and in the optic nerve and the extraocular muscles. Thus, the tissue selectivity is low. Another problem is the widely different functions of prostaglandins reflected by the many subtypes of receptors. In spite of this, it seems that the local hormone concept can be used successfully with prostaglandins for the treatment of glaucoma, by using selective analogues such as latanoprost, as was predicted by Bito in the early 1980s.

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Figure 6. Genetic variation of the FP prostanoid receptor in 10 healthy individuals and 15 patients in whom increased pigmentation of the iris developed during latanoprost treatment. Genomic DNA was isolated from leukocytes, the gene was amplified by polymerase chain reaction, and the fragments were sequenced using the A. L. F. Express System (Amersham-Pharmacia Biotech, Uppsala, Sweden). The sequences were compared with the cDNA sequence in the EMBL database (European Molecular Biology Laboratory, Heidelberg, Germany; available in the public domain at www.ebi.ac.uk/embl/), and deviations from the latter were defined as genetic variation. Of four variations found in the 25 individuals, only one resulted in a change of amino acid, isoleucine being exchanged for valine in amino acid position 338. Open symbols: absence of genetic variation; shaded symbols: variation at the nucleotide level; filled symbol: variation at the nucleotide and amino acid levels. (Collaboration with Eurona Medical, Uppsala, Sweden.)

Figure 7. Effect of selective prostanoid receptor agonists on hair growth determined by measuring the rate of the regrowth of the fur in adult male CBA-J mice. Approximately 4 cm² of the fur was shaved, and for the following 10 days the animals were treated with selective prostanoid receptor agonists topically on the shaved area once daily. The regrowth of the fur was assessed from photographs taken at regular intervals during 28 days from the beginning of the treatment, and the endpoint at day 28 is depicted. The control animals received vehicle (0.5% Tween-80). The analogues were used as isopropyl or methyl esters except for sulprostone. Mean ± SEM; n = 4 to 8 in each group; *P < 0.01 compared with the control group, t-test.
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