THE PHARMACOKINETICS OF A NEW ANTIGLAUCOMA DRUG, LATANOPROST, IN THE RABBIT

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ABSTRACT:
Latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-prostaglandin F₂α-1-isopropyl ester) is a unique prostaglandin analogue developed for the treatment of glaucoma. To investigate the pharmacokinetics, tritium-labeled latanoprost was administered topically on the eyes of rabbits and intravenously. About 7.7% of the applied dose was found in the cornea at 15 min after the drug administration. The following Cmax and elimination half-life (interval 1–6 hr) values of the total radioactivity in the eye tissues were found: aqueous humor, 0.09 ng eq/ml and 3.0 hr; anterior sclera, 1.49 ng eq/mg and 1.8 hr; cornea, 1.59 ng eq/mg and 1.8 hr; ciliary body, 0.39 ng eq/mg and 2.8 hr; conjunctiva, 1.41 ng eq/mg and 1.4 hr; and iris, 0.39 ng eq/mg and 2.1 hr. Latanoprost was rapidly hydrolyzed, and most of the radioactivity found in the aqueous humor, anterior eye tissues, and plasma corresponded to the pharmacologically active acid of latanoprost. The initial plasma elimination half-life of the acid of latanoprost was 9.2 ± 3.2 min after iv and 2.3 ± 1.9 min after topical administration on the eyes. The plasma clearance of the acid of latanoprost was 1.8 ± 0.3 liters/hr-kg, and the volume of distribution was 0.4 ± 0.1 liter/kg after iv administration. Based on the retention times on HPLC and GC-MS, the main metabolite in urine and feces was identified as the 1,2,3,4-tetranor metabolite of acid of latanoprost. This acid existed in equilibrium with the corresponding α-lactone. The AUC of radioactivity in the eye tissues was approximately 1000 times higher than in plasma AUC. The recovery of radioactivity was complete.

Glaucoma, a disease characterized by optic nerve damage and visual field defect, is often associated with an increase in the intraocular pressure (IOP). If left untreated, the disease can ultimately lead to blindness. Prostaglandin F₂α and its phenyl-substituted analogues have been shown to effectively reduce the IOP in man and animals (Alm et al., 1993; Bito et al., 1993; Nagasubramanian et al., 1993; Stjernschantz and Alm, 1996; Stjernschantz and Resul, 1992). Latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-prostaglandin F₂α-1-isopropyl ester; PhXA41; fig. 1) is a potent prostaglandin analogue developed for the treatment of glaucoma. It reduces the IOP effect-ively with considerably less side effects in the eye compared with prostaglandin F₂α and its phenyl-substituted analogues (Stjernschantz and Resul, 1992). Latanoprost is a lipophilic prodrug in which the carboxylic acid moiety in the α-chain has been esterified to increase the bioavailability of the active drug into the eye. The permeability coefficient of latanoprost in the porcine cornea has been determined as 6.8 × 10⁻⁶ cm × sec⁻¹ (Basu et al., 1994). Latanoprost is hydrolyzed to the acid 13,14-dihydro-17-phenyl-18,19,20-trinor-PGF₂α (PhXA85; fig. 1) by the esterases in the cornea and in the plasma (Basu et al., 1994, Sjöquist et al., 1994). The acid is pharmacologically active. The prostaglandins of the F₂α type are believed to reduce IOP by increasing the uveoscleral outflow of aqueous humor (Gabelt and Kaufman, 1989; Kaufman and Crawford, 1989; Nilsson et al., 1989).

During the development of latanoprost to a drug for treatment of glaucoma, rabbits among other species have been used to study the safety of the drug. In this study, the ocular and systemic absorption, distribution, metabolism, and elimination of tritium-labeled latanoprost have been investigated in rabbits after topical and iv administration.

Materials and Methods

Chemicals. Latanoprost, 13,14-dihydro-17-phenyl-18,19,20-trinor-PGF₂α-1-isopropyl ester; and 13,14-3H-dihydro-17-phenyl-18,19,20-trinor-PGF₂α-1-isopropyl ester were synthesized at Pharmacia & Upjohn (Uppsala, Sweden) (fig. 1). The specific activity was 1.422 MBq/μg. The purity of the radiolabeled substance was tested by reversed-phase high-pressure liquid chromatography (HPLC) and found to be >97%. HPLC-grade acetonitrile, ethyl acetate, and formic acid were purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were of analytical grade. Reference standards, latanoprost (5.5 mg/ml in ethanol), acid of latanoprost (5.46 mg/ml in ethanol), 3H-acid of latanoprost (778.8 KBq/ml in ethanol), 3H-dinor acid of latanoprost (1600 Bq, 2 ng/μl in ethanol), and 3H-tetranor acid of latanoprost (550 Bq, 0.7 ng/μl in ethanol) were produced at the Departments of Medicinal Chemistry and Pharmacokinetic, Glaucoma Research Laboratories (Pharmacia & Upjohn). The chemical structures of the compounds are shown in fig. 1.

N-Methyl-N[(4-butyldimethylsilyl)trifluoroacetylamide (MTBSTFA) was obtained from Pierce, and tertiary butyldimethylchlororosilane (TBDMSCL) was obtained from Fluka Chemie (AG, Buchs, Switzerland). The liquid scintillation cocktail to a flow-through radioactivity detector, coupled on line with the HPLC column, was Flo-Sci II and Ultima Flo M obtained from Packard Radiomatic through Chemical Instruments (AB Lidingö, Sweden). The liquid scintillation cocktail for the liquid scintillation counter was OptiPhase HiSafe III manufactured by Wallac/LKB (Loughborough, England). Enulsifier-Safe, used for liquid scintillation counting, and Monophase® S and 3H-Spec Chec were obtained from Canberra Packard (Pangbourne, Berks). All solvents used were of analytical grade.
Formulation of Substance. Latanoprost, 50 μg/ml, was formulated in a vehicle for topical application to the eye. One milliliter of the vehicle contained benzalkonium chloride (0.200 mg), sodium chloride (4.1 mg), sodium hydrogengemphosphate-1H2O (4.6 mg), disodium hydrogenphosphate-2H2O (5.94 mg), and water for injection (1 ml). Tritium-labeled latanoprost was added to this formulation for ocular administration to the rabbits. The formulation for iv administration contained 40 μg, 0.30 MBq latanoprost/ml saline, and the eye drop solution for the systemic study contained 350 μg, 98.6 MBq latanoprost in a sodium phosphate buffer, pH 6.7, also containing benzalkonium chloride (200 μg/ml), as preservative.

Animal Experiments for the Ocular Pharmacokinetics. A total of 30 Dutch belted female rabbits (weighing 1.4–2.4 kg) were divided into six groups. The rabbits were placed in metabolism cages and acclimatized to the laboratory conditions for 2 weeks before commencement of the experiment. The room temperature was kept at 19–27°C, and relative humidity was kept at 35–55%. The rabbits were given Diet K1 (lactamin) and water ad libitum. All rabbits received 20 μl (1.51 MBq/0.06 μg) of 3H-latanoprost topically on both eyes by a micropipette. The animals were sacrificed by an overdose of nembutal (pentobarbital, 100 mg/ml) at 0.25, 0.5, 1, 4, 6, and 24 hr after the topical application of 3H-latanoprost. For each time point, four animals were treated as follows. Prior to sacrifice, a blood sample was taken from an ear vein of each animal. Aqueous humor was aspirated into a sterile syringe before dissecting the eye globe. The samples from the left eyes were kept at −20°C until the amount of radioactivity was determined. The aqueous humor samples from the right eyes of the animals (four groups/pool) were pooled for each time point and kept at −70°C for the metabolism study. The eyes were then enucleated and dissected (right eye: eye lids, conjunctiva, cornea, iris, ciliary body, and choroid; left eye: eye lids, conjunctiva cornea, anterior sclera, iris, vitreous, choroid, and lens). The ciliary body was collected from the right eye of all animals except those in the 4-hr group. The corresponding tissues of the right eyes from the animals of the same time group were pooled. These pooled tissues were used to study the metabolic profiles of latanoprost in the respective eye tissues at various times after the drug administration. The different parts of the left eye and plasma were kept separately for the determination of total radioactivity. For each time point, both eyes from one animal were enucleated to determine the total radioactivity of the whole eye globe.

Animal Experiments for the Systemic Pharmacokinetics. Six male and six female Dutch belted rabbits (11–17 weeks old) obtained from Foxfield Farms Ltd. (UK) were housed individually in aluminum cages with steel grid floors at a temperature of 16 to 22°C and with light-dark cycles of 14 and 10 hr, respectively. The animals were allowed free access to commercial pellet diet, SQC Standard Rabbit Diet, and mains water. 3H-Latanoprost was administered with a positive displacement pipette (30 μl) onto the right eye of each animal at a dose of 10 μg per animal corresponding to a nominal radioactive dose of 2.96 MBq (80 μCi). For iv administration, the drug was administered via an ear vein with an infusion pump at a rate of 5 ml/min. This resulted in a dose of 200 μg/kg body weight corresponding to a nominal radioactive dose of 2.96 MBq (80 μCi) per animal.

Urine, feces, and blood were collected. Urine intervals included pre-dose, 0–6, 6–12, 12–18, 18–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hr postdose. Feces intervals included pre-dose, 0–12, 12–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hr postdose. Blood (ca. 1 ml), collected by puncture of the jugular vein, was transferred into lithium heparin tubes. The blood was centrifuged to collect plasma. At the end of the collection period, the animals were sacrificed by an overdose of sodium pentobarbital. All samples were stored at ca. −20°C except for urine and plasma (0 to 24 hr), which were stored at ca. −80°C.

Analytical Techniques. Radioactivity determination. The radioactivity of the ocular samples was determined directly by liquid scintillation counting (Rackbeta 1219, Wallac/LKB, Finland) after total combustion of the tissue samples (<500 mg) by a sample oxidizer (Packard sample oxidizer, Tri-Carb 306, Packard). Control and blank samples were also combusted, and the radioactivity was measured. Background values from the blank samples were subtracted from the sample values. To determine the radioactivity of the whole eye, a sample (<400 mg) was homogenized by a Polytron homogenizer (Kinematica AG, Switzerland), and an aliquot was subjected to liquid scintillation counting after combustion as described above.

Extraction, separation, and identification. The different parts of the eye tissues were mixed with 3 ml of ethanol and homogenized by a Polytron homogenizer. The samples were centrifuged, and the radioactivity of an aliquot of the supernatant was counted by liquid scintillation counting. The supernatant was evaporated under N2 and dissolved in ethanol. The aqueous humor samples were acidified to pH 3–4 with 1 M formic acid and extracted with 3 ml of ethyl acetate. The ethyl acetate phase was separated, and the radioactivity was measured. Background values from the blank samples were subtracted from the sample values. To determine the radioactivity of the whole eye, a sample (<400 mg) was homogenized by a Polytron homogenizer (Kinematica AG, Switzerland), and an aliquot was subjected to liquid scintillation counting after combustion as described above.

Chromatographic analysis. Some ocular samples were co-chromatographed with authentic 1,2,3,4-tetranor acid of latanoprost standards. The radioactivity of the respective eye tissues at various times after the drug administration. The different parts of the left eye and plasma were kept separately for the determination of total radioactivity. For each time point, both eyes from one animal were enucleated to determine the total radioactivity of the whole eye globe. The samples were analyzed by reversed-phase high-pressure liquid chromatography using 5-μm Nucleosil C18 columns (Machery and Nagel, Duren, Germany). Two gradient solvent systems of acetonitrile and water with 0.1% acetic acid were utilized. The first gradient consisted of 35% acetonitrile from 0 to 11 min, 46% acetonitrile from 12 to 18 min, and 35% acetonitrile from 19 to 22 min; the second gradient consisted of 25% acetonitrile from 0 to 15 min, 25 to 40% acetonitrile from 15 to 35 min, 40 to 25% acetonitrile from 35 to 40 min, and 25% acetonitrile from 40 to 45 min. The flow rate was constant at 1 ml/min. The column was always equilibrated with the starting solvent system for at least 20 min prior to use and about 10 min before re-use. The column was connected to an on-line flow through radioactivity detector using a flow cell of 0.5 ml (Packard Radiomatic, Illinois). The ratio of the effluent from the HPLC and scintillation cocktail was 1:5 (v/v). Authentic latanoprost and acid of latanoprost standards were routinely used in the HPLC analysis. Some ocular samples were co-chromatographed with authentic 1,2,3,4-tetranor acid of latanoprost standard to identify the metabolite. Fac-
tions from the major peaks were collected and tert-butyl-dimethylsilyl derivatives were prepared with N-methyl-N-(tertiary-butyldimethylsilyl)-trifluoroacetamide dimethylformamide tert-butyl-dimethylchlorosilane. The samples were subjected to GC-MS for identification (Hewlett-Packard, model 5890 GC with a 10 m x 32-mm i.d. fused silica column coated with 0.12-μm cross-linked 5% phenyl methyl silicone, Finnigan MAT 90 mass spectrometer). The ion source temperature was 250°C. The oven temperature was raised to 290°C at a rate of 35°C/min after 1 min. The electron energy was set to 70 eV, and ionizing current was set to 1 nV.

Calculation of the Concentration of the Acid of Latanoprost. The concentration of the acid of latanoprost in cornea, the aqueous humor, iris, ciliary body, and plasma was calculated from the total radioactivity in the sample multiplied by the percentage of acid of latanoprost obtained from the HPLC run divided by the specific activity of the administered latanoprost, according to the following formula:

\[ C = \frac{P \times R}{V \times S \times 60 \times 100} \]

where \( C \) = concentration of acid of latanoprost \( \mu g \) eq/ml, \( P \) = percentage of acid of latanoprost in the chromatogram, \( R \) = total radioactivity in the plasma sample (dpm), \( V \) = sample volume (ml), and \( S \) = specific activity of latanoprost (Bq/μg).

Pharmacokinetic Calculations. The data obtained were fitted using a commercial pharmacokinetic program PCNONLIN 4.2 (SCI software) installed on an IBM compatible personal computer. The pharmacokinetic parameters of total radioactivity and the acid of latanoprost in the ocular tissues were calculated according to the following: \( T_{\text{max}} \) = time of observed maximum concentration; \( C_{\text{max}} \) = maximum observed concentration; \( \beta \) = first order rate constant based on the terminal (log-linear) phase of the curve, estimated by linear regression of time vs. log concentration (\( \beta = \ln 2/\tau_{\frac{1}{2}} \)).

\[
\text{AUC}_{\text{last}} = \sum_{i=2}^{n} (t_i - t_{i-1})(C_i + C_{i-1})/2
\]

\[
\text{AUC}_{\text{inf}} = \text{AUC}_{\text{last}} + C_i/\beta
\]

where \( C_i \) denotes either the observed or predicted concentration at the last sampling time.

For the systemic pharmacokinetics, the data obtained from the topical administration were fitted to model 200, that means an extravascular input and model 8 that is a two-compartment bolus input and first order output. The data from the iv administration were fitted to a model 201 and model 1 reflecting one compartment with bolus input and first order output.

Results

Plasma Pharmacokinetics. Irrespective of the route of latanoprost administration, no significant sex differences were observed for the plasma concentration curves of radioactivity or the acid of latanoprost. Hence, mean data of all animals within each administration group have been calculated.

Following a single iv administration of \(^{3}H\)-latanoprost, mean plasma radioactivity concentrations at 5 min postdose were 704.0 ± 107.0 ng eq/ml, and the mean acid of latanoprost concentration was 401.8 ± 76.9 ng/ml. Following a single topical administration of \(^{3}H\)-latanoprost at a nominal dose level of 10 μg/animal, mean maximum concentrations of both radioactivity and acid of latanoprost in plasma were reached 5 min postdose, 18.36 ± 4.24 ng eq/ml and 12.6 ± 2.3 ng/ml, respectively. Thereafter, the decline was rapid so that by 2 hr the concentrations were about 3% of \( C_{\text{max}} \). The plasma elimination curves of total radioactivity as well as acid of latanoprost are shown in figs. 2 (iv administration) and 3 (ocular administration). The results of the pharmacokinetic parameters calculated are presented in tables 1 and 2. The bioavailability was calculated using \( \text{AUC}_{\text{last}} \).

Ocular Distribution of Radioactivity. About 7.7% of the total applied dose was found in the cornea at 15 min after topical administration of the drug to the eye. The distribution of the radioactivity in the aqueous humor and ocular tissues is presented in fig. 4. One hour after the application of latanoprost, the following rank order of total radioactivity in the various ocular tissues was obtained: cornea > conjunctiva > anterior sclera > iris > ciliary body > aqueous humor > whole eye > choroid > lens. The AUC, elimination half-life (\( t_{\frac{1}{2}} \)), \( C_{\text{max}} \), and AUC ratio (tissue/plasma and tissue/aqueous humor) of the radioactivity in the aqueous humor and the ocular tissues are presented in table 3. The elimination half-life of the radioactivity in the aqueous humor and the eye tissues was between 1.4 and 3.0 hr. The AUC in the cornea was the highest (5.6 ng eq/hr/mg) of all the tissues examined. The anterior sclera and con-
half-life (t½) min

Area under the curve (AUC) ng eq · hr/mg

Plasma clearance (Clp) ml/min · kg

Volume of distribution (V) liter/kg

Cmax = D/V · EXP(−α · t); Co = Cmax = D/V; t1/2 = K10 half-life; Clp = V · K10

AUClast is calculated with model 201.

TABLE 1
Pharmacokinetic parameters of the acid of latanoprost after intravenous administration of ³H-latanoprost to rabbits (PCnonlin, model 1)*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Males (N = 3)</th>
<th>Females (N = 3)</th>
<th>Mean (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (W) kg</td>
<td>2.47 ± 0.06</td>
<td>2.44 ± 0.20</td>
<td>2.46 ± 0.13</td>
</tr>
<tr>
<td>Dose (D) µg/animal</td>
<td>356 ± 13</td>
<td>555 ± 50</td>
<td>560 ± 33</td>
</tr>
<tr>
<td>Initial concentration (Co) ng eq/ml</td>
<td>676 ± 211</td>
<td>579 ± 167</td>
<td>627 ± 178</td>
</tr>
<tr>
<td>Half-life (t½) min</td>
<td>9.2 ± 3.8</td>
<td>9.3 ± 3.3</td>
<td>9.24 ± 3.21</td>
</tr>
<tr>
<td>Area under the curve (AUC) ng eq · hr/mg</td>
<td>165 ± 23</td>
<td>130 ± 10</td>
<td>147 ± 25</td>
</tr>
<tr>
<td>Plasma clearance (Clp) ml/min · kg</td>
<td>28.3 ± 6.5</td>
<td>31.6 ± 1.7</td>
<td>30.0 ± 4.6</td>
</tr>
<tr>
<td>Volume of distribution (V) liter/kg</td>
<td>0.35 ± 0.09</td>
<td>0.42 ± 0.13</td>
<td>0.39 ± 0.11</td>
</tr>
</tbody>
</table>

* Model 1: C(t) = D/V · EXP(−α · t); Co = Cmax = D/V; t1/2 = K10 half-life; Clp = V · K10

TABLE 2
Pharmacokinetic parameters of the acid of latanoprost derived from rabbit plasma after topical administration on the eyes using PCnonlin model 8*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male (N = 3)</th>
<th>Female (N = 2)</th>
<th>Mean (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (W) kg</td>
<td>2.87 ± 0.21</td>
<td>3.11 ± 0.09</td>
<td>2.96 ± 0.21</td>
</tr>
<tr>
<td>Dose (D) µg/animal</td>
<td>9.3 ± 0.6</td>
<td>9.5 ± 0.5</td>
<td>9.4 ± 0.5</td>
</tr>
<tr>
<td>Maximal concentration (Cmax) ng eq/ml</td>
<td>12 ± 3</td>
<td>13 ± 2</td>
<td>12.6 ± 2.3</td>
</tr>
<tr>
<td>Half-life (t½ alpha) min</td>
<td>2.6 ± 2.5</td>
<td>1.7 ± 0.9</td>
<td>2.3 ± 1.9</td>
</tr>
<tr>
<td>Half-life (t½ beta) hr</td>
<td>1.41 ± 0.34</td>
<td>1.43 ± 0.23</td>
<td>1.42 ± 0.27</td>
</tr>
<tr>
<td>Area under the curve (AUC) ng eq · h/mg</td>
<td>2.84 ± 0.44</td>
<td>3.26 ± 0.49</td>
<td>3.01 ± 0.46</td>
</tr>
<tr>
<td>Plasma clearance (Clp) ml/min · kg</td>
<td>8.8 ± 3.2</td>
<td>6.9 ± 1.9</td>
<td>8.1 ± 2.7</td>
</tr>
<tr>
<td>Volume of distribution (V) liter/kg</td>
<td>0.42 ± 0.38</td>
<td>0.33 ± 0.12</td>
<td>0.39 ± 0.28</td>
</tr>
</tbody>
</table>

* Model 8: C(t) = A · exp(−α · t) + B · exp(−β · t); Cmax = D/V; t1/2 = K10 half-life; Clp = V · K10

AUClast is calculated with model 200.

The concentration of total radioactivity (above) and of the acid of latanoprost (below) in the aqueous humor, cornea, iris, and ciliary body at different intervals after topical application of ³H-labeled latanoprost (1.51 MBq; 1.06 µg) to the rabbit eye.
5–7. No unhydrolyzed latanoprost was found in the aqueous humor and eye tissues examined except in the eye lids (data not shown). Latanoprost was completely hydrolyzed to the acid of latanoprost in the cornea, and it was the predominant peak found in the aqueous humor and all eye tissue examined. The retention time of the acid of latanoprost coincided with the authentic latanoprost acid standard in the HPLC analysis. The identity of this substance was further confirmed by GC-MS analysis. A polar metabolite was also seen in the chromatogram in the aqueous humor and all eye tissues (figs. 5–7). The GC-MS identification of this metabolite was not possible owing to the small amount of the substance in the peak. One hour after administration of the drug, 71% of the radioactivity in the aqueous humor represented the acid of latanoprost and 29% the unknown metabolite (fig. 5). The percentage of this polar metabolite increased with time in aqueous humor and in all eye tissue samples.

Excretion of Radioactivity. No apparent sex differences in the amounts of radioactivity in urine, feces, and cage washings were observed. Thus, intergroup comparisons have been made using com-

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**TABLE 3**
The AUC_{inf}, elimination half-life (t_{1/2}), C_{max}, T_{max}, and AUC ratio of total radioactivity (tissue/plasma and tissue/aqueous humor) after the topical application of \(^{3}H\)-labeled latanoprost (1.51 MBq; 1.06 μg) to the rabbit eye

<table>
<thead>
<tr>
<th>Tissues or Body Fluid</th>
<th>AUC_{inf} (pred.)</th>
<th>t_{1/2}a</th>
<th>C_{max}</th>
<th>T_{max}</th>
<th>AUC Ratio Tissue/Plasma</th>
<th>AUC Ratio Tissue/Aqueous Humor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td>0.51</td>
<td>3.0</td>
<td>0.094</td>
<td>1.00</td>
<td>243</td>
<td>1.00</td>
</tr>
<tr>
<td>Cornea</td>
<td>5.60</td>
<td>1.8</td>
<td>1.590</td>
<td>0.25</td>
<td>2667</td>
<td>10.98</td>
</tr>
<tr>
<td>Iris</td>
<td>1.36</td>
<td>2.1</td>
<td>0.387</td>
<td>0.50</td>
<td>648</td>
<td>2.67</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>1.70</td>
<td>2.8</td>
<td>0.392</td>
<td>0.50</td>
<td>810</td>
<td>3.33</td>
</tr>
<tr>
<td>Anterior sclera</td>
<td>3.39</td>
<td>1.8</td>
<td>1.486</td>
<td>0.50</td>
<td>1614</td>
<td>6.65</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>2.77</td>
<td>1.4</td>
<td>1.412</td>
<td>0.25</td>
<td>1319</td>
<td>5.43</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0021</td>
<td>2.4</td>
<td>0.00102</td>
<td>0.25</td>
<td>1.00</td>
<td>4.12 × 10^{-3}</td>
</tr>
</tbody>
</table>

*Time interval (t_{1/2}) = 1–6 hr; first sampling time = 0.25 hr.*

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**TABLE 4**
The AUC_{inf}, C_{max}, T_{max}, and elimination half-life (t_{1/2}) of the acid of latanoprost in the aqueous humor and ocular tissues after the topical application of \(^{3}H\)-labeled latanoprost (1.51 MBq; 1.06 μg) to the rabbit eye

<table>
<thead>
<tr>
<th>Tissues or Body Fluid</th>
<th>AUC_{inf} (pred.)</th>
<th>t_{1/2}a</th>
<th>C_{max}</th>
<th>T_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td>0.37</td>
<td>2.3</td>
<td>0.067</td>
<td>1.00</td>
</tr>
<tr>
<td>Cornea</td>
<td>4.31</td>
<td>1.6</td>
<td>1.433</td>
<td>0.25</td>
</tr>
<tr>
<td>Iris</td>
<td>0.83</td>
<td>1.8</td>
<td>0.306</td>
<td>0.50</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>1.16</td>
<td>2.1</td>
<td>0.339</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Time interval (t_{1/2}) = 1–6 hr; first sampling time = 0.25 hr.*

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FIG. 5. Chromatographic profiles of the radioactivity in the aqueous humor after topical application of \(^{3}H\)-labeled latanoprost (1.51 MBq; 1.06 μg) to the rabbit eye.

The time after administration of the drug is presented in the chromatograms. The separation of latanoprost and the acid of latanoprost standards is shown in the upper left chromatogram.
bined data from males and females. Following a single iv dose of $^3$H-latanoprost, the overall mean recovery of the radioactivity was 102.5%. Renal excretion accounted for the majority of the radioactivity, 98.85% (urine and cage washings). The remainder of the radioactivity over the 144-hr study period was recovered in feces (3.64%). The excretion of radioactivity after a single iv dose is presented in fig. 8.

Following a single ocular dose of $^3$H-latanoprost to male and female rabbits at a nominal dose of 10 μg/animal, overall mean recovery of radioactivity was 103.7%. Mean urinary elimination was 99.9% (urine and cage washings); fecal excretion accounted for 3.8%. The rate of excretion was similar between the sexes. Most radioactivity was renally eliminated within 24 hr. The results are presented in fig. 8.

**Metabolites in Plasma, Urine, and Feces.** The two or three urine samples from each animal containing most radioactivity were analyzed. Two major metabolites more polar than the acid of latanoprost were present in almost every urine sample. A small peak with the retention time equivalent to that of the acid of latanoprost was also present in many urine samples and the relative intensity of this peak increased in samples collected at the later time periods. There was no obvious difference in the metabolic pattern of latanoprost between iv and topical administration, and no sex differences were observed.

In fig. 9, a standard sample of the 1,2,3,4 tetranor acid of latanoprost has been chromatographed separately and mixed with a urine sample. The 1,2,3,4-tetranor acid of latanoprost has a structure that easily forms a δ-lactone, i.e., an internal ester between the carboxylic group and the hydroxyl group on carbon 5 (carbon 9 in acid of latanoprost).
lantanoprost). There is an equilibrium between the free acid and the lactone. In gradient 3, the free acid has a retention time of around 8 min and the less polar lactone 15–16 min. In fig. 9, it is shown that the unknown metabolites in rabbit urine co-chromatographed with the 1,2,3,4-tetranor acid of lantanoprost and its lactone. Only 3–4% of the total radioactivity administered was found in feces. As in urine, two metabolites appeared. These metabolites were judged to correspond to 1,2,3,4-tetranor acid of latanoprost and its lactone.

From urine samples, the radiolabeled peaks were collected, and the tertiary butyldimethylsilyl derivatives were prepared for GC-MS analysis. The radiolabeled peak with a retention time of around 8 min gave a mass spectrum similar to that of 1,2,3,4-tetranor acid of lantanoprost at the same gas chromatographic retention time as this standard (fig. 10). The base peak was m/z 735, i.e. the molecular ion minus a tertiary butyl group (M+ – 57). M+ – 15 = 777 was also present, and 603 = 735 – 132 (a tertiary butyldimethylsilanol group). The peak collected in the HPLC chromatogram with a retention time around 15 min showed a mass spectrum at a shorter GC retention time than 1,2,3,4-tetranor acid of lantanoprost. This MS is shown in fig. 11 together with the MS of the authentic 1,2,3,4-tetranorlactone of the acid of lantanoprost. Characteristic ions for the lactone are m/z 489 (M+ – 57), 397 (489 – 92), 357 (489 – 132), 339 (397 – 58), and 91 (benzyl). The MS of the biological sample is less intense than the MS of the reference compound, thus column bleeding ions like m/z 207 and 465 show up as intense ions.

The two major metabolites in rabbit urine were thus identified as the 1,2,3,4-tetranor acid of lantanoprost in the form of δ-lactone and acid based on the retention times on HPLC and after derivatization, according to retention times and mass spectra on GC-MS analysis.

Clinical Observations. Immediately after iv dosing, the animals displayed a lack of coordination and an unsteady gait. These effects were rapidly transient. During the remainder of the study, no overt pharmacological or toxicological signs were observed in the test animals, which could have been attributed to the administration of lantanoprost.

Discussion

The bioavailability of drugs into the eye tissues after topical application is limited by the residence time in the tear fluid and the tight junctions of the corneal epithelium. The penetration of a drug through the cornea is favored by high lipophilicity. Thus, esterification of the carboxylic acid moiety of prostaglandins has been shown to enhance the in vitro penetration through the porcine cornea (Camber et al., 1986) and the bioavailability of topically applied prostaglandins 20–30 times in the rabbit eye (Bito and Baroody, 1987). An in vitro study in the porcine cornea (Basu et al., 1994) and in vivo studies in several species including monkeys have shown that after topical application of lantanoprost, hydrolysis of lantanoprost occurs rapidly in the cornea and the pharmacologically active acid of lantanoprost is formed (Sjöquist et al., 1993; Sjöquist et al., 1994). Furthermore, no unhydrolyzed lantanoprost was found in the plasma, aqueous humor, or iris-ciliary body. Thus, lantanoprost acts as a prodrug when topically applied to the eye.

The highest concentrations of radioactivity were, as expected, found in the first measurements in cornea, anterior sclera, and conjunctiva 15 min postdose, whereas the maximum concentrations in the aqueous humor and in the iris-ciliary body were found 1 hr after administration. During the entire 24-hr period, the cornea showed substantially higher levels of radioactivity than the iris and the ciliary body. Thus, the cornea functioned as a slow release depot of the drug into the anterior parts of the eye. Bito and Baroody (1987) also found the maximum concentration of PGF2α in the aqueous humor 1 hr after topical administration, whereas the concentration in the cornea was highest 15 min after the administration of PGF2α to rabbits. A polar metabolite was seen with time in the aqueous humor and the ocular tissues. However, both in the plasma and the eye the acid of lantanoprost was the predominating compound. The major metabolite found in urine was 1,2,3,4-tetranor-lantanoprost acid. Higaki et al. (1995) found the tetranor metabolite of S-1033 (15-deoxy PGF2α) in the eye tissues after topical application of S-1033 to the rabbit eye, whereas the uninstilled eye showed neither S-1033 nor its tetranor metabolite. In vitro incubation of S-1033 with the eye tissues also confirmed the formation of the tetranor metabolite. The authors concluded that the tetranor metabolite was formed by β-oxidation, but whether this had
occurred in the ocular tissues was unclear. In our study, the polar metabolite in the aqueous humor and the ocular tissues did not co-chromatograph with the \( \beta \)-oxidation metabolites 1,2-dinor- or 1,2,3,4-tetranor-latanoprost acid, and it is not possible to conclude where the unidentified polar metabolite was formed. A possible metabolic pathway in the cornea is hydroxylation of the phenyl ring by the cytochrome P-450 system. However, the minor amounts of material available did not allow further elucidation of the identity of the polar metabolite.

\(^3\)H-Latanoprost was rapidly absorbed systemically following ocular administration. The pharmacokinetics after ocular administration is in reality quite complex depending on tear fluid dynamics, absorption into the cornea, conjunctiva, and nasal blood vessels and with a part of the dose running all the way down to the stomach. That means a multiple site contribution to the systemic circulation. This might explain the much lower clearance after topical application compared with after iv administration. Investigations in primates confirm that latanoprost is rapidly absorbed following ocular administration and even more efficient than after oral administration (in preparation). In plasma, the acid of latanoprost disappeared rapidly both after iv and topical administration. The concentration in plasma 2 hr after administration was 1 and 3% of the 5-min values after iv and topical administrations, respectively. The systemic bioavailability after topical administration was approximately 100%. The plasma clearance was high after iv administration, probably mainly owing to liver metabolism, as very small amounts of the acid of latanoprost were recovered in urine; after oral administration, an extensive first pass metabolism occurred (unpublished results). A general metabolic pathway of prostaglandins in the liver is \( \beta \)-oxidation of the \( \alpha \)-chain in common with long chain fatty acids (Hamberg 1968; Johnson et al., 1972). This indicates that accumulation of latanoprost and/or metabolites on a once daily dose regimen is unlikely to occur. The volume of distribution was small, and the plasma elimination half-life was short. A low volume of distribution suggests that extravascular distribution is very limited. The acid of latanoprost predominated the plasma profile, but the 1,2,3,4-tetranor acid of latanoprost and its corresponding lactone were observed in addition.

In the rabbit, almost all radioactivity was recovered in urine (urine + cage wash). Only 3–4% of the dose was recovered in feces. In urine and feces, two peaks identified as the \( \beta \)-lactone and acid form of 1,2,3,4-tetranor acid of latanoprost predominated, and the acid of latanoprost accounted for a minor part. The metabolic pattern of latanoprost in the rabbit was thus very simple. After hydrolysis to the acid, the \( \beta \)-oxidation of the \( \alpha \)-chain was both efficient to rapidly remove the active drug from the circulation and extensive because no 1,2-dinor acid of latanoprost and only trace amounts of the latanoprost acid were excreted. PGF\(_{2\alpha}\) is inactivated within seconds by 15-hydroxyprostaglandin dehydrogenase in the lung (Nakano et al., 1969), but the lack of the double bond between carbon 13 and 14 in

![Figure 10](https://example.com/fig10.png)
the latanoprost acid makes it a very poor substrate for 15-hydroxyprostaglandin dehydrogenase (Basu et al., 1994). The phenyl ring at carbon 17 of the acid of latanoprost could be hydroxylated and conjugated, but it excludes the hydroxylation, oxidation, and β-oxidation that takes place in the ω-chain of PGF_2α. After iv administration of 200 μg/kg body weight, the rabbits displayed a rapidly transient lack of coordination and unsteady gait. The plasma concentration recorded was 400 ng/ml, which is approximately 8000 times higher than the maximal levels observed in man (50 pg/ml) after a clinical dose around 0.04 mg/kg (Sjöquist and Stjernschantz, 1995).

In conclusion, latanoprost acted as a prodrug when administered topically to the rabbit eye and released the pharmacologically active acid efficiently into the anterior parts of the eye. The cornea acted as a slow release depot and supplied the acid of latanoprost to the anterior segment during an extended period of time. The AUC of total radioactivity in the ocular tissues was approximately 1000-fold higher than the AUC of the radioactivity in plasma. The topically applied drug was quantitatively absorbed into the systemic circulation and rapidly cleared metabolically through β-oxidation. The metabolites were almost completely excreted in urine. No sex differences were observed in the fate of latanoprost in the rabbit. The recovery of total radioactivity was complete. Thus, the pharmacokinetics of latanoprost in the rabbit demonstrates its almost ideal properties as an antiglaucoma drug.

References


Nagasubramanian S, Sheth GP, Hichings RA and Stjernschantz J (1993) Intraocular pressure...


