Iloprost®, A Stable Prostacyclin Analog, Reduces Intraocular Pressure

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Topical application of Iloprost® caused a dose-dependent decrease in intraocular pressure (IOP) in rabbits and ocular hypertensive beagles. In rabbits, the IOP response was biphasic and miosis was observed. In beagles, there was no initial hypertensive phase, and the fall in IOP was more pronounced (up to 37%). In beagles, Iloprost did not influence pupillary diameter. A mild transient hyperemia was noted in both rabbit and beagle eyes. Iloprost led to an increase in the aqueous humor protein concentration in rabbits but not in beagles. The use of artificial tears as vehicle enhanced the effect on intraocular pressure but also aqueous protein in rabbits. The central corneal temperature was increased after application of Iloprost in both rabbits and beagles. In rabbits, tonography revealed an increase in outflow facility during both the hypertensive and the hypotensive phases. Iloprost caused a decrease in mean arterial pressure in beagles; the effect on pulse rate was inconsequential. It is suggested that similar low doses of an analog of Iloprost or carboprostacyclin that does not affect the hemodynamic equilibrium could be of value in the treatment of glaucoma. Invest Ophthalmol Vis Sci 28:470-476, 1987

There is ample evidence that prostaglandins (PGs) can reduce intraocular pressure (IOP). However, a species-dependent initial hypertensive response may occur, and seems to be dose-related. Furthermore, PGs are thought to be mediators of inflammation. Topically administered PGs, particularly when applied in high doses in rabbits, induce conjunctival hyperemia, miosis, dilatation of the iridic vessels, and a breakdown of the blood-aqueous barrier. All of these properties may hinder the development of PGs as an antiglaucoma agent.

Recently it was reported that prostacyclin (PGI₂) can reduce IOP. However, since PGI₂ has a half-life of 3–5 min at a pH of 7.4, a large dose was needed to produce sustained ocular hypotony. It is possible that lower concentrations of a stable PGI₂-analog might produce a similar or even greater reduction of IOP.

Iloprost® is a stable PGI₂-analog with platelet antiaggregation and deaggregation properties. Furthermore, Iloprost might have a vasodepressive effect after intravenous administration.

In the present study, the effect of topically administered Iloprost on IOP was evaluated in rabbits and ocular hypertensive beagles. Special attention was directed toward the ocular and systemic side-effects since they might be sufficient to limit the use of these autacoids for the treatment of glaucoma.

Materials and Methods

Pigmented conscious rabbits (2.5–4 kg, aged 6–12 months) were restrained before the experiments were started. In addition, eight beagles (9.6–15.3 kg, aged 2–5 yr) were selected from a group of 26 beagles because they exhibited ocular hypertension (26–38 mmHg). They were trained for 3 weeks to undergo tonometry while awake. After local anesthesia was induced with 30 μg of 0.2% oxybuprocain (Novesine, Chibret, Riom, France), IOP was measured by means of an Alcon Pneumatonograph. The pneumatonometer was calibrated manometrically for rabbit and beagle eyes according to the closed stopcock method. Three to four IOP readings were recorded one day before the experiments were started.

The horizontal pupillary diameter was measured under normal laboratory lighting conditions, except that no light sources were permitted within the animal’s field of vision, and daylight was excluded. Conjunctival hyperemia was evaluated by gross external examination and with the aid of a Zeiss slit-lamp aqueous flare and cellular response were evaluated. They were graded on a scale of zero to four according to Hogan.

In several experiments the corneal temperature was determined with a Heimann KT 41 infra-red radiation thermometer under controlled environmental condi-
tions. This thermometer gives an almost direct reading (90% of the real value within 1 sec) over the range 22–42°C with a resolution of 0.05°C and accuracy of 0.5°C. The infra-red sensor measures the radiation emitted directly in front of a target area 4 mm in diameter. The censor was held approximately 1 mm from the center of the cornea for 10 sec until a stable reading was obtained. Three readings were taken and averaged.

Iloprost was supplied by Schering AG (Berlin, Federal Republic of Germany) as a 0.5 mg/ml solution in Tris buffer at pH 8.3. Just before application, a known volume was dried under nitrogen; phosphate-buffered saline (PBS) at pH 7.4 or artificial tears (Dura Tears, Alcon-Couvreur, Puurs, Belgium) were added to obtain the desired concentrations.

The following amounts of Iloprost were applied topically: rabbits, 100 ng, 1 μg, 3 μg, and 10 μg in 30 μl PBS, and 200 ng, 400 ng, 600 ng and 1 μg in 30 μl artificial tears (AT); beagles, 3 μg, 6 μg, and 10 μg in 30 μl AT. The drug was applied topically after the first IOP reading taken on the day of the experiment. The contralateral eye received the vehicle alone. IOP measurements were repeated at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hr. There were six groups of eight rabbits, and one group of eight beagles. After an experiment, the animals were not used again for 4 weeks to allow the effect of the drug to wear off.

Tonographic measurement of outflow facility was performed with the aid of the electronic Schiotz tonograph (Berkeley, CA) under general anesthesia induced with thiopental natrium administered via an ear vein. The outflow coefficient was approximated from the 1955 Friedenwald Tables. One day before the experiment, the baseline tonographic value was determined for the right eye of each rabbit. During the experiments the tonographic value was obtained 1.5 or 4 hr after topical delivery to the right eye of 1 μg Iloprost in PBS or AT. There were four separate groups of six rabbits.

One μg of Iloprost in PBS or AT was instilled into one eye of each rabbit; the contralateral eye received the vehicle alone. One or four hr later, a 27-gauge needle was inserted through the cornea (under ketamine anesthesia) and aqueous was aspirated (four groups of six rabbits). Care was taken to avoid touching the iris and lens. In addition, aqueous paracentesis was performed under general anesthesia (IV thiopental sodium) on seven beagles 1.5 hr after 10 μg Iloprost in 30 μl AT was applied topically to one eye (control eye received the vehicle alone). For all aqueous samples the protein concentration was determined in appropriate volumes according to Bradford using human serum albumin as a standard. The ascorbate concentration in 25 μl aqueous was measured immediately after obtaining the sample using a colorimetric test set (Boehringer-Mannheim, Mannheim, Federal Republic of Germany).

In four beagles, pulse rate and mean arterial pressure (MAP) were recorded during anesthesia induced with fluothane, nitrous oxide and oxygen. Intravascular pressure was measured with Elcomatic 751 A transducers and displayed on a Siemens-Elema Mingograf 7 with a seven channel ink-jet recorder along with a Lead I and II electrocardiogram.

After the pulse rate, MAP and anesthesia had remained stable for 30 min, 10 μg Iloprost in 30 μl AT were applied topically to both eyes, and the pulse rate and MAP were recorded over a 90 min period.

Differences between values were tested for significance with the two-tailed paired and unpaired student’s t-test. These experiments adhered to the ARVO Resolution on the Use of Experimental Animals in Research.

Results

Intraocular Pressure

Rabbits: Topical Iloprost produced a biphasic response of IOP. Doses of 2 or more μg Iloprost in 30 μg of PBS induced an initial hypertensive response that was dose-dependent (P < 0.005). All doses used caused a dose-dependent ocular hypotension (P < 0.01) (Fig. 1). When Iloprost was dissolved in AT, similar results were obtained, but at doses that were about five- to tenfold lower (Fig. 2). A consensual effect on IOP was not seen after topical application of Iloprost in PBS or AT.

Beagles: Topical Iloprost induced a dose-related fall in IOP in ocular hypertensive beagles (Fig. 3). No initial hypertensive response was observed, even with the highest dose used (10 μg Iloprost in AT) (Fig. 4). The maximum ocular hypotensive effect was obtained with 10 μg Iloprost in 30 μl AT at 6 and 7 hr. The reduction of IOP was 10.9 mmHg, 37% (P < 0.005). The consensual hypotensive effect, seen at a dose of 10 μg, reached a maximum at 6 hr; the fall in IOP was 4.2 mmHg (P < 0.005) (Fig. 4). The mean fall in IOP between the third and sixth or seventh hr induced by Iloprost was 5.5 mmHg (20%) for 3 μg, 6.9 mmHg (25%) for 6 μg, and 9.8 mmHg (33%) for 10 μg. The reduction of IOP by 10 μg Iloprost lasted more than 24 hr. 3.1 mmHg at 24 hr (P < 0.05) compared to the contralateral control eyes.

Pupil Size

Rabbits: A dose-related miotic response was observed after doses of 1 or more μg in PBS. The maximum response was a change in pupil size of 1.4 ± 0.2
Fig. 1. Effects of topical application of various doses (0.1–10 μg) of Iloprost in PBS at 0 hr on the intraocular pressure in several groups of eight rabbits. Data points represent mean values. The maximum SEM was ± 2.4 mmHg.

Fig. 2. Effects of topical application of various doses (200 ng–1 μg) of Iloprost in artificial tears at 0 hr on the intraocular pressure in several groups of eight rabbits. Data points represent mean values. The maximum SEM was ± 1.6 mmHg.

Hyperemia, Aqueous Flare and Cellular Response

**Rabbits:** A slight significant conjunctival hyperemia was noted 1 and 2 hr after topical application of Iloprost (1 μg in PBS), having its maximum at 1 hr, 1.1 ± 0.2 (P < 0.005). A slight aqueous flare also lasted about 2 hr, maximum at 1 hr, 0.4 ± 0.15 (P < 0.05). The same dose of Iloprost in artificial tears induced a significant hyperemia over 5 hr, maximum 1.3 ± 0.2 (P < 0.005) at 1 hr. Aqueous flare was significant for 2 hr, 0.3 ± 0.1 (P < 0.05) at 1 and 2 hr. No cellular infiltration into the aqueous was observed.

**Beagles:** Iloprost in AT did not affect pupillary diameter. Even 10 μg of Iloprost did not induce a significant miosis.

Hyperemia, Aqueous Flare and Cellular Response

**Rabbits:** A slight significant conjunctival hyperemia was noted 1 and 2 hr after topical application of Iloprost (10 μg in AT). Maximum hyperemia was noted at 1 hr, 1.3 ± 0.2 (P < 0.005). The hyperemia disappeared with time. No significant flare was visible in the aqueous; there was also no cellular infiltration into the aqueous.

In rabbits as well as beagles, no dilatation of the iridial vessels was noted.

**Corneal Temperature**

**Rabbits:** A slight increase in central corneal temperature was noted 1 hr after application of Iloprost (1 μg in PBS). In contrast, 1 μg of Iloprost in AT induced an increase in corneal temperature that lasted 4 hr.
Beagles: The increase in corneal temperature noted after topical administration of 10 μg Iloprost in AT (Table 1) lasted 5 hr.

Rabbits:

- After application of 1 μg Iloprost in PBS, outflow facility was increased at 1.5 hr (48%) and at 4 hr (44%), whereas 1 μg Iloprost in AT caused an increase in outflow facility of 91% at 1.5 hr and 57% at 4 hr. However, the increase in outflow facility after Iloprost in PBS or AT as vehicle was not different (Table 2).

Aqueous Protein and Ascorbate Levels

- Rabbits: Topical 1 μg Iloprost in PBS did not alter the aqueous protein and ascorbate concentrations. However, 1 μg Iloprost in AT caused an increase in the aqueous protein levels at 1 and 4 hr. The difference in aqueous protein levels between Iloprost in PBS and in AT were significant at 1 hr, 1.5 ± 0.5 g/l (P < 0.005), and at 4 hr, 3.1 ± 0.6 g/l (P < 0.005). There was no change in the aqueous ascorbate levels at either point in time.

- Beagles: Topical application of 10 μg Iloprost in AT did not affect the aqueous protein level. The aqueous ascorbate concentration was slightly decreased in the treated eyes (Table 3).

Heart Rate and MAP

- Beagles: In four anesthetized beagles, the pulse rate was not significantly affected by Iloprost (10 μg in AT to both eyes). However, a significant decrease in MAP was noted during the first 15 min after application with a maximum of 23 mmHg at 10 min (Fig. 5).

Discussion

In the present study, the first evidence has been obtained that a stable prostacyclin analog, Iloprost, when topically administered, effectively reduces IOP in both rabbits and ocular hypertensive beagles. In rabbits, the effect on IOP is biphasic at certain doses. An initial dose-dependent hypertensive response is followed by a sustained hypotony. In beagles, no hypertensive phase was seen even at the highest dose of Iloprost used; however, the hypotensive effect, which is also dose-related, is more marked than in rabbits. Beagles also showed a consensual hypotensive effect in the untreated eyes.

It has been reported that PGI2 does not decrease IOP in the rabbit eye. However, recently it was found that in rabbits a low dose of topical PGI2 can reduce IOP, and a high dose causes a biphasic response of IOP comparable to the effects of PGF2α and PGE2. PGI2 is known to have a half-life of 3–5 min at a pH
Table 1. Corneal temperature exp-cont (°C) after topical Iloprost

<table>
<thead>
<tr>
<th>Hours</th>
<th>After 1 μg topical Iloprost in 30 μl PBS (6 rabbits)</th>
<th>After 1 μg topical Iloprost in 30 μl AT (6 rabbits)</th>
<th>After 10 μg topical Iloprost in 30 μl AT (7 beagles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.7 ± 0.2*</td>
<td>1.2 ± 0.2***</td>
<td>1.0 ± 0.2***</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.2</td>
<td>0.8 ± 0.2**</td>
<td>1.1 ± 0.2***</td>
</tr>
<tr>
<td></td>
<td>0.2 ± 0.2</td>
<td>0.6 ± 0.2**</td>
<td>1.2 ± 0.2***</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.1***</td>
<td>0.9 ± 0.2***</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.1***</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
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</tbody>
</table>

All data are mean values ± SEM; significance with respect to contralateral vehicle-treated eye, ie, control eye. *P < 0.05; **P < 0.01; ***P < 0.005.

Table 2. Outflow facility (μl, min⁻¹, mmHg⁻¹) before and after topical application of Iloprost in four groups of six rabbits

<table>
<thead>
<tr>
<th>Hours after Iloprost</th>
<th>Control</th>
<th>1μg</th>
<th>Control</th>
<th>4μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outflow facility after 1 μg Iloprost in 30 μl PBS</td>
<td>0.27 ± 0.03</td>
<td>0.40 ± 0.03***</td>
<td>0.25 ± 0.02</td>
<td>0.35 ± 0.03**</td>
</tr>
<tr>
<td>Outflow facility after 1 μg Iloprost in 30 μl AT</td>
<td>0.23 ± 0.02</td>
<td>0.44 ± 0.04***</td>
<td>0.21 ± 0.03</td>
<td>0.33 ± 0.03*</td>
</tr>
</tbody>
</table>

All data are mean values ± SEM. Significance of difference with respect to control *P < 0.05; **P < 0.01; ***P < 0.005. Control measurements performed the prior day at the same eyes.
Table 3. Aqueous protein and ascorbate concentrations ± SEM after topical application of Iloprost in four groups of six rabbits and one group of seven beagles

<table>
<thead>
<tr>
<th></th>
<th>Protein (gl⁻¹)</th>
<th>Ascorbate (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>1 hr after 1 μg topical Iloprost in 30 μl AT</td>
<td>2.0 ± 0.3**</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td>4 hr after 1 μg topical Iloprost in 30 μl AT</td>
<td>3.5 ± 0.6**</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>1 hr after 1 μg topical Iloprost in 30 μl PBS</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>4 hr after 1 μg topical Iloprost in 30 μl PBS</td>
<td>0.4 ± 0.07</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td>1.5 hr after 10 μg topical Iloprost in 30 μl AT (7 beagles)</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

All data are mean values ± SEM. **Significance: see Table 1.

Dynamics becomes a systematic error, and is therefore of less importance. Determination of outflow facility was not performed in the contralateral untreated eyes since it is known that PGs may have a consensual effect on IOP, and thus on aqueous humor dynamics. For these reasons the tonographic values obtained in this study should only be considered as relative values for changes in outflow facility after application of Iloprost. The baseline values found in the present study are comparable to those previously obtained with the two-level constant pressure method for rabbits. It is shown that topical Iloprost increases outflow facility during both the hypertensive and hypotensive phase.

An increase in corneal temperature was seen after application of Iloprost in rabbits as well as in beagles. No iridial vasodilatation was observed with the slit lamp in either pigmented rabbit or beagle eyes. A 4 mm area in the central part of the cornea was measured; hence the increase in temperature cannot be due to the transient conjunctival hyperemia. Although lid closure may increase corneal temperature, no transient lid closure was seen in beagles after instillation of topical Iloprost. The temperature differences between treated and untreated eyes could be caused by the unequal blood supply to the anterior segments. This has also been observed in conditions affecting the carotid artery in which impaired blood supply to the ophthalmic artery lowers the corneal temperature of the affected eye in humans. Anterior uveitis led to an increased corneal temperature in rabbits and in humans, probably due to the increased blood flow in the iris-ciliary body. A recent report states that in rabbits, ocular blood flow is increased in the iris-ciliary body, but not in the choroid and retina, 30 min after topical application of PGs. Enhanced blood flow through the capillaries of the iris-ciliary body increases the temperature of the circulating aqueous, and consequently the corneal temperature. This may indicate that the prolonged rise in corneal temperature after Iloprost topically was due to an increased flow of blood through the iris-ciliary body. This phenomenon did not seem to affect the simultaneously induced hypotension. It is still questionable if the effects seen after topical instillation of Iloprost are a direct effect of the prostacyclin analog, or are a secondary effect of endogenous induced prostaglandin synthesis. Other studies are needed to investigate this problem. Iloprost has a marked ocular hypotensive effect and increases the outflow facility. The ocular side-effect noted for beagles was a mild hyperemia. However, even in a low dose, topical application of this agent may decrease MAP. It is suggested that similar low doses of an analog of Iloprost or carboprostacyclin that does not affect the hemodynamic equilibrium could be of value in the treatment of glaucoma.

Key words: intraocular pressure, Iloprost, rabbit, beagle, inflammatory response

Fig. 5. Effect of topical application of Iloprost in 30 μl artificial tears to both eyes on mean arterial pressure in 4 anesthetized beagles. The points represent means ± SEM. Asterisk (*) indicates P < 0.05.
Acknowledgments

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References