Potent Reduction of Intraocular Pressure by Nipradilol Plus Latanoprost in Ocular Hypertensive Rabbits

Masahiro ORIHASHI,*a,b Yuichiro SHIMA,b Hiroshi TSUNEKI,a and Ikuko KIMURAa

aDepartment of Clinical Pharmacology, Graduate School of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930–0194, Japan; and bTeika Pharmaceutical Co., Ltd.; 1–3–27 Arakawa, Toyama 930–0982, Japan.

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The present study was performed to evaluate the intraocular pressure (IOP)-lowering effect of nipradilol in combination with latanoprost on ocular normotensive and hypertensive rabbits. IOP was measured using an applanation pneumatonograph under topical application of 0.4% oxybuprocaine hydrochloride for corneal anesthesia. Ocular hypertension was induced by injection of 0.1 ml hypertonic saline (5% NaCl) into the vitreous body. Saline, nipradilol, latanoprost, sodium nitroprusside (SNP) or indomethacin was then instilled just after 5% NaCl injection. All drugs were instilled in the inferior conjunctival sac, using 50 μl drops. If more than two drugs were used, they were applied 5 min apart. Nipradilol lowered IOP in both ocular normotensive rabbits and ocular hypertensive rabbits, whereas latanoprost did not lower IOP in either. When nipradilol was applied in combination with latanoprost, the reduction in ocular hypertension was significantly enhanced, compared to the effect of nipradilol alone. A significantly potent reduction in ocular hypertension was also observed by the SNP-latanoprost combination. The IOP-lowering effects of SNP in combination with latanoprost were abolished by treatment with indomethacin. These results indicate that the IOP-lowering effect of latanoprost was enhanced when applied in combination with nipradilol or SNP, both of which have nitric oxide (NO)-donating actions. Since both combined effects were abolished by treatment with indomethacin, the mechanisms by which nipradilol combined with latanoprost lowered ocular hypertension may be related, at least in part, to the production of prostaglandins via the NO-donating action of nipradilol.

Key words intraocular pressure; nipradilol; latanoprost; combination; nitric oxide (NO)

Ocular hypertension is a major risk factor for the progression of glaucoma. Achieving good control of intraocular pressure (IOP) is an important part of the treatment for preventing the progression of optic neuropathy. A number of new topical antiglaucoma drugs have come into clinical use recently, providing ophthalmologists with an increased range of treatment options. It is a common practice to use two or three different topical antiglaucoma drugs in combination, when a favorable response is not obtained with monotherapy. Depending on the mechanisms of pharmacological action of these drugs, there are cases in which even combination therapies are not sufficiently effective. In view of the need to predict the result of multi-drug treatment, it is important to study the effectiveness of the combined use of drugs.

Antiglaucoma drugs are classified into two categories of mechanisms according to their actions on aqueous flow dynamics. One is the group of drugs suppressing aqueous production, and the other is the group promoting aqueous outflow. The former include β-adrenoceptor blockers and carbon dehydratase inhibitors, and the latter are represented by 1° adrenoceptor blockers and prostaglandins (PGs). Drugs in different categories are often used in combination. The combined effects can be achieved by a combination of a drug suppressing aqueous humor production and a drug promoting aqueous humor outflow, e.g. nipradilol and latanoprost.

Nipradilol is a β-adrenoceptor blocker with weak 1° adrenoceptor blocking activity and nitric oxide (NO) releasing activity. We have studied the effects of the combined application of nipradilol and other agents, and our previous data have indicated that combination regimens including nipradilol acted differently from those based on typical β-blockers lacking other activities. For instance, the IOP-lowering effects of nipradilol combined with timolol in rabbits were stronger than timolol applied twice and these were not explained by the additive effect of the β-blocking action. It may be due to the 1°-blocking action or NO. In this study, we evaluated the effects of the combined application of nipradilol and latanoprost, which is a prostaglandin F2,α analogue commonly used in recent clinical practice, by measuring the IOP of rabbits as an index.

MATERIALS AND METHODS

Animals Seventy-six male Japanese white rabbits (2.5—3.5 kg; Aizu Animal Laboratory, Fukushima, Japan) were used in this study after more than 1 week of acclimatization in individual cages at a temperature of 23±1°C and 55±10% humidity under a 12-h light/dark cycle with free access to food and water.

Measurement of IOP Intraocular pressure (IOP) was measured in unrestrained conscious rabbits using an Alcon Applanation Pneumatonograph (Alcon PTG; Alcon Japan, Tokyo, Japan) under topical instillation of 0.4% oxybuprocaine hydrochloride for corneal anesthesia. In the experiments with ocular hypertensive rabbits, ocular hypertension was induced by the same method as reported by Kuribayashi et al. Briefly, while the ocular surface was anesthetized by a topical instillation of 0.4% oxybuprocaine hydrochloride, the basal level of IOP was measured, and hypertonic saline (5% NaCl, 0.1 ml) was injected into the vitreous body with care to avoid lens injury. The test drug, i.e. nipradilol, latanoprost, sodium nitroprusside or indomethacin, was applied immediately after the injection of hypertonic saline. In the experiments with ocular normotensive rabbits, the same procedures were adopted except for the injection of hypertonic saline.
For the application of a single drug, IOP was measured after the drug administration at the time points indicated in the figures. For the combined application of drugs, npradilol or sodium nitroprusside (SNP) was administered 5 min before latanoprost application. Indomethacin was administered 5 min before application of npradilol or SNP, except one case examining the combined effects of indomethacin and latanoprost, in which indomethacin was administered 5 min before latanoprost application. All drugs were instilled in the inferior conjunctival sac, using 50 μl drops. No animals were subsequently reused for the IOP measurement.

**Drugs** Nipradilol (0.25%, Nipronol; Teika Pharmaceutical, Toyama, Japan), latanoprost (0.005%, Xalatan; Pharcia & Upjohn Inc., NJ, U.S.A.), indomethacin (0.1%, Indomelol; Senju Pharmaceutical, Osaka, Japan) and oxybuprocaine hydrochloride (0.4%, Anelocal; Senju Pharmaceutical, Osaka, Japan) were purchased from their respective manufacturer's. Sodium nitroprusside (Sigma Chemical, St. Louis, MO, U.S.A.) was dissolved in phosphate-buffered saline.

**Statistical Analysis** The difference between multiple groups was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple range tests. Values of $p$ less than 0.05 were considered to be significant.

**RESULTS**

The effects of npradilol, latanoprost, and the combination thereof were examined in ocular normotensive rabbits (Fig. 1). The basal IOP levels in the saline, npradilol, latanoprost, and the npradilol+latanoprost combination groups were 20.5±0.2, 19.5±0.8, 21.0±0.7, and 20.5±1.5 mmHg, respectively ($n=6$). No significant differences were detected among the groups. Npradilol significantly decreased IOP during the period from 60 min ($\Delta$IOP $= -4.5±0.6$ mmHg) to 180 min ($\Delta$IOP $= -5.2±0.7$ mmHg) after instillation, while latanoprost caused no decrease in IOP. The combination of the two drugs significantly decreased IOP at 60 min ($\Delta$IOP $= -4.2±0.5$ mmHg) and 180 min ($\Delta$IOP $= -4.5±1.4$ mmHg) after instillation, but no significant difference was noted as compared with npradilol alone.

Next, we examined the effects of the combined use of the two drugs in a rabbit model of ocular hypertension prepared by injecting 5% NaCl into the vitreous body ($n=7$). Without drug administration, the time course of IOP for the period from 15 to 240 min after 5% NaCl injection was as shown in Fig. 2. The IOP was higher than the baseline value by about 33 mmHg during the period from 15 to 60 min. While it started to decrease at 90 min, it remained higher than the baseline IOP until 180 min after injection, and had returned to the baseline level at 240 min.

The effects of the combined application of npradilol and latanoprost were evaluated at 60 and 120 min after 5% NaCl injection. The timing of this evaluation was selected based on the time when the ocular hypotensive effect of npradilol was recognized after instillation and the duration of the ocular hypertension induced by 5% NaCl injection. The basal IOP levels in the saline, npradilol, latanoprost, and the npradilol+latanoprost combination groups were 17.0±1.1, 18.7±0.5, 20.3±1.5, and 20.5±1.2 mmHg, respectively ($n=6$). No significant differences were observed among the groups. At 60 min after instillation, npradilol ($\Delta$IOP $= 14.7±1.7$ mmHg, $n=6$) and npradilol+latanoprost ($\Delta$IOP $= 5.3±2.6$ mmHg, $n=6$) significantly suppressed the increase in IOP as compared with saline ($\Delta$IOP $= 24.8±1.7$ mmHg, $n=6$), while latanoprost ($\Delta$IOP $= 22.7±1.2$ mmHg, $n=6$) showed no suppression of the increase in IOP (Fig. 3A). The suppression achieved by npradilol+latanoprost was also significant in comparison with npradilol or latanoprost alone. Similar results were observed at 120 min after injection (Fig. 3B). Npradilol ($\Delta$IOP $= 7.3±1.6$ mmHg, $n=6$) and npradilol+latanoprost ($\Delta$IOP $= 1.7±2.7$ mmHg, $n=6$) significantly suppressed the increase in IOP as compared with saline ($\Delta$IOP $= 16.2±1.6$ mmHg, $n=6$), while latanoprost ($\Delta$IOP $= 9.8±1.9$ mmHg, $n=6$) showed no suppression of the increase in IOP. The effects of npradilol+latanoprost were slightly less marked at this point in time. The suppression of the IOP increase was not significant in comparison with npradilol alone, although it was significant in comparison with latanoprost.

It is possible that the experimental ocular hypertension in this study may involve a disruption of the blood-aqueous barrier, which is a condition related to acute inflammation. To
We further examined the effect of SNP, which is an NO donor similar to nипрадил и lacks a β- and α1-blocking activity (Fig. 5). The basal IOP levels in the saline, SNP, indomethacin+SNP, SNP+latanoprost and indomethacin+ SNP+latanoprost groups were 17.5 ± 1.0, 18.0 ± 0.7, 18.5 ± 1.0, 20.4 ± 0.9 and 17.0 ± 1.1 mmHg, respectively (n = 5 to 6). There were no significant differences among the groups.

SNP alone tended to suppress the IOP increase (ΔIOP 18.2 ± 2.9 mmHg, n = 6), and the combination with indomethacin enhanced the suppression of the IOP increase (ΔIOP 14.9 ± 1.7 mmHg, n = 6). Moreover, the combination of SNP and latanoprost enhanced the suppression of the IOP increase (ΔIOP 4.8 ± 4.9 mmHg, n = 5). However, the synergistic IOP-lowering effects of SNP and latanoprost were greatly diminished by co-treatment with indomethacin (Indomethacin+SNP+latanoprost, ΔIOP 22.8 ± 2.9 mmHg, n = 5).

DISCUSSION

We examined the effects of the combined application of nипрадил и latanoprost in ocular normotensive and ocular hypertensive rabbits. Although experimental models of ocular hypertension can be achieved by several methods, including water loading, intravenous glucose administration, and chymotrypsin-induced ocular hypertension, these models have drawbacks such as the short duration of ocular hypertension, complicated procedures, and variability in the extent or occurrence of ocular hypertension. In this study, we adopted the model induced by the intravitreal injection of 5% NaCl and observed that long-lasting ocular hypertension was induced by an osmolarity gradient dependent on the salt...
concentration. In ocular normotensive rabbits, nipradilol showed an ocular hypotensive effect, whereas latanoprost had no effect. These results are consistent with previous reports.1,2,7,8

No additive effects on IOP were detected in ocular normotensive rabbits, with the combined application of nipradilol and latanoprost, whereas the synergistic IOP-lowering effect was observed in ocular hypertensive rabbit. In general, IOP could not be lowered below the episcleral venous pressure. Since the IOP seems to be reduced to near the episcleral venous pressure by single application of nipradilol, it might be difficult to detect the combined effects in ocular normotensive rabbits. However, since there is no obvious reason why the IOP-lowering effect was detected exclusively in ocular hypertensive state, further studies are necessary to clarify more precise mechanism behind this effect.

Because the ocular hypertensive rabbit model prepared by intravitreal injection may cause inflammation in the eye, we examined the influence of indomethacin on changes in IOP after the combined drug application. The addition of indomethacin tended to suppress the increase in IOP compared to the saline control, indicating that the IOP increase seems to partially involve inflammatory changes. If the combined effects of nipradilol and latanoprost were mainly due to the suppression of inflammation-induced changes, the combined effects would have been enhanced by the addition of indomethacin. However, the ocular hypotensive activity of the nipradilol+latanoprost combination appears to be abolished by the addition of indomethacin. These results suggest that the synergistic effects of nipradilol and latanoprost may not be due to suppression of the inflammation-induced increase in IOP.

To explore the mechanisms for the combined effects of nipradilol+latanoprost, we performed another experiment using SNP, an NO donor lacking \( \beta \)-blocking or \( \alpha_1 \)-blocking activity, and examined its effect in combination with latanoprost. The combined use of SNP and latanoprost resulted in significant suppression of the IOP increase even in comparison with SNP alone. This result was similar to that for the nipradilol+latanoprost combination. In contrast, the combination effects of SNP+latanoprost were abolished when indomethacin was added (Fig. 5), whereas those of nipradilol+latanoprost were moderately attenuated by addition of indomethacin (Fig. 4). It is anticipated that 5% NaCl-induced ocular hypertension is readily reversed by \( \beta \)-blocking action (i.e., inhibition of aqueous humor production). This may explain the more potent effect of nipradilol than SNP in this ocular hypertensive model.

There have been many reports demonstrating the ocular hypotensive activity of NO donors,9,10 as well as the involvement of NO derived from nipradilol in ocular hypotensive activity.11 NO is known to have various physiological activities, and Salvemini et al.12 have suggested that NO directly activates cyclooxygenase. On the other hand, latanoprost has been reported to activate phospholipase A\(_2\) and promote the generation of endogenous PGs.13,14 Because the combination effect of nipradilol and latanoprost was abolished in the presence of indomethacin, endogenous PG production seems to mediate this combination effect. It has been shown that FP receptors for latanoprost are not contributing to IOP reduction in rabbits, but EP3 receptors for PGE\(_2\) have been reported to play a role in IOP reduction in rabbits. Probably, endogenous generation of PGE\(_2\) may be promoted through synergism between cyclooxygenase activation by NO from nipradilol on one hand and the endogenous induction of arachidonic acid by latanoprost on the other, leading to a greater IOP reduction.

In conclusion, nipradilol combined with latanoprost caused a more potent IOP-lowering effect than nipradilol alone. Thus, this study provides evidence that this combination may be potentially valuable in clinical use for ocular hypertensive diseases such as glaucoma.

REFERENCES