The Effect of Topical Diltiazem on the Intraocular Pressure in Betamethasone-Induced Ocular Hypertensive Rabbits

JOSE´ MELENA, JUAN SANTAFE´ and JOSE´ SEGARRA
Departamento de Farmacologı ´a, Facultad de Farmacia, Universidad del Paı ´s Vasco, Vitoria, Spain
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ABSTRACT
The effect of calcium channel blockers (CCBs) on intraocular pressure (IOP) remains still controversial, although some preliminary reports suggest that these drugs may be effective in the management of ocular hypertension and low-tension glaucoma. The aim of the present work was to assess the effect of topical diltiazem on IOP in an animal model for glaucoma, the betamethasone-induced ocular hypertension in rabbits. IOP was measured with a manometrically calibrated applanation pneumatonograph. Ocular hypertension was produced in 120 rabbits by weekly subconjunctival injection of a betamethasone suspension into the left eye. The experiments examining the ocular actions of diltiazem were carried out in two stages. In the first one, the ability of topical diltiazem to prevent the rise in IOP induced by betamethasone was studied. In a second phase, the effect of topical diltiazem on IOP in betamethasone-induced ocular hypertensive rabbits was assessed. Diltiazem was topically applied once daily for 5 days a week into the left eye. The effect of five different concentrations of diltiazem was evaluated to obtain dose-response curves. Topical diltiazem was found to prevent in a dose-related fashion the betamethasone-induced ocular hypertensive rise as well as to reduce IOP in rabbits made ocular hypertensive by weekly subconjunctival injection of betamethasone. Unilateral topical administration did not produce a clear effect on IOP in the untreated eye. This is the first report describing the ocular hypotensive action CCBs in an animal model for glaucoma. These findings are in agreement with preliminary evidence suggesting that CCBs may have a beneficial effect in human ocular hypertension.

The effect of CCBs on aqueous humor dynamics and IOP remains controversial since a wide range of results have been obtained. After systemic administration, CCBs have generally failed to reduce IOP in both rabbits (Kelly and Walley, 1988; Segarra et al., 1993) and humans (Bose et al., 1995), although several laboratories have reported ocular hypertension (Beatty et al., 1984) and ocular hypotensive (Green and Kim, 1977; Indu et al., 1989; Monica et al., 1983; Payne et al., 1990) responses after oral or intravenous administration of these drugs.

Results of studies on the effect of topically applied CCBs on IOP are also conflicting. Beatty et al. (1984) found that these drugs produced dose-related increases in IOP in albino rabbits and humans, whereas Payne et al. (1990) noted that verapamil, diltiazem and nifedipine had no effect on IOP in rabbits. On the other hand, we have shown (Santafe et al., 1996, 1997; Segarra et al., 1993) that topical application of verapamil, nifedipine and diltiazem effectively lowers IOP in rabbits in a dose-related fashion. In humans, Abelson et al. (1988) and Mooshian et al. (1993) reported a decrease in IOP after a single topical dose of verapamil in ocular hypertensive subjects. Recently, Netland et al. (1995) also found that verapamil significantly lowered IOP in normal human volunteers.

Despite the fact that no consensus has been reached about the effects of these drugs on IOP, evidence suggests that topical application of verapamil and probably other CCBs could be effective in the management of ocular hypertension (Abelson et al., 1988; Goyal et al., 1989; Mooshian et al., 1993) and low-tension glaucoma (Netland et al., 1993, 1995). However, such a potential role in the treatment of glaucoma is largely based on circumstantial evidence and has not undergone an adequate preclinical and clinical evaluation.

The aim of the present work was to study the effect of diltiazem on IOP in an animal model for glaucoma, the betamethasone-induced ocular hypertension in rabbits. The corticosteroid glaucoma is among experimental models more closely resembling human disease since both its clinical features (elevated IOP and gonioscopically open-angle) and underlying mechanism (reduced aqueous outflow) mimic those of human chronic open-angle glaucoma. In contrast to most of the induced experimental models for glaucoma, corticosteroid glaucoma is also observed in ophthalmologic practice after topical, pericocular or systemic administration of corticosteroids, a fact that strengthens the parallel between the animal and human disease. Furthermore, evidence suggesting that

ABBREVIATIONS: CCB, calcium channel blocker; IOP, intraocular pressure.
endogenous glucocorticoids may play a role in the development of ocular hypertension in humans (Sout hern et al., 1985; Weinstein et al., 1985) seems to support the utility of this glaucoma model. Several well known ocular hypotensive drugs, such as pilocarpine (Diepold et al., 1989; Zimmer et al., 1994), alpha and beta adrenoceptor agonists (Bonomi et al., 1978; Lorenzetti, 1970), beta adrenoceptor antagonists (Bonomi et al., 1978) and carbonic anhydrase inhibitors (Lorenzetti, 1970), have been found to effectively lower IOP in this animal model for glaucoma.

Materials and Methods. Experiments were carried out in 120 female New Zealand White rabbits, weighing 3 to 4 kg, which were previously trained to be handled and restrained in boxes in the laboratory environment. IOP was measured with a Mentor model 30 classic pneumatonograph (Norwell, MA) that was calibrated by direct manometry in anesthetized rabbits. To avoid diurnal variations of the IOP, tonometries were always started at the same time of the day (9 a.m.).

To induce ocular hypertension, the animals were treated with a weekly subconjunctival injection into the left eye (Micro-Fine syringes, 29 gauge × ½; Becton Dickinson, Dublin, UK) of 0.7 ml of betamethasone suspension (Celestone Cronodose; Schering-Plough, Madrid, Spain) containing betamethasone sodium phosphate (3 mg/ml) and betamethasone acetate (3 mg/ml). This formulation provides a readily accessible (sodium phosphate) and a sustained release (acetate) fraction of betamethasone. Any bearing of drug vehicle on the IOP was previously ruled out (data not shown).

To avoid corneal epithelium damage through too-frequent tonometry, measures of IOP in both eyes were as a rule repeated twice a week, with the first measure being taken immediately before the weekly betamethasone subconjunctival injection and the second taken after 3 days. Three base-line IOP measures were recorded during the week before betamethasone treatment, with animals exhibiting fluctuations of >2 mm Hg excluded from the experiments. The value observed at zero time (first betamethasone injection) was considered the starting pressure.

Preventive effect. In the first set of experiments, the ability of topical diltiazem to prevent the rise in IOP induced by betamethasone was tested. In the control group, betamethasone subconjunctival injections into the left eye were repeated weekly over a period of 4 weeks in 67 rabbits. In series of 9 to 12 animals, the same schedule of betamethasone administration was performed, and diltiazem was applied topically into the rabbit’s left eye once daily for 5 days a week during the period of betamethasone treatment. Diltiazem administration was started on the same day of the first betamethasone subconjunctival injection. The following concentrations of diltiazem were studied: 4.4 × 10⁻⁴, 1.3 × 10⁻³, 2.8 × 10⁻³, 2.2 × 10⁻² and 8.9 × 10⁻² M.

Hypotensive effect. In the second set of experiments, the effect was studied of topical diltiazem on IOP in betamethasone-induced ocular hypertensive rabbits. All the animals received weekly subconjunctival injections of betamethasone into the left eye over a period of 7 weeks. In the diltiazem-treated groups, the instillation of this drug was started at the 24th day of corticosteroid treatment (3 days after the fourth subconjunctival injection), a time at which the betamethasone-induced ocular hypertension turned out to be stable, and was prolonged up to 25 days. Each experimental series contained 9 to 12 animals. The concentrations of diltiazem tested as well as the time administration schedule were the same as that above.

Diltiazem hydrochloride, which was purchased from Sigma Chemical (St. Louis, MO), was dissolved in distilled water. For each application, one 50-μl drop of the drug solution was instilled in the middle of the inferior cul-de-sac of the left eye (betamethasone-treated eye), followed by lid closure.

The differences between the IOP measurements in the control (betamethasone alone) and diltiazem-treated groups at the corresponding times were considered to be diltiazem effects. For an analysis of the dose-response relationship, the maximum decrease in the IOP was used, independent of the time. Concentration-response curves were fitted with a nonlinear method (GraphPAD Prism 1.0; GraphPAD Software, San Diego, CA) based on the following equation:

$$Y = \frac{B - A}{1 + 10^{[\log EC_{50} - X \times m]}}$$

where Y is the drug effect, X is the logarithm of drug concentration, A is the starting pressure, B is the peak response, log EC₅₀ is the logarithm of drug concentration that produces half the maximum response and m is the slope factor.

Results

Topical diltiazem was found to both prevent the rise in IOP produced by betamethasone and reduce IOP in betamethasone-induced ocular hypertension in New Zealand White rabbits.

In the first set of experiments, the ability of diltiazem to prevent the IOP response to betamethasone was tested. In the 67 rabbits receiving four weekly subconjunctival injections of betamethasone (control group), a gradual increase in IOP throughout the experimental period was observed in the treated eye (fig. 1), which became statistically significant from the third day of treatment (P < .0001) and reached its maximum at the end of the fourth week (4.7 ± 0.1 mm Hg; P < .0001). Statistically significant increases in IOP were also registered in the untreated eye (data not shown), although smaller than that of the treated one, with the maximum response also observed at the end of the fourth week of treatment (1.1 ± 0.1 mm Hg; P < .0001). Once-daily topical application of diltiazem for 5 days a week was shown to attenuate the rise in IOP caused by betamethasone (fig. 1) to such an extent that it was nearly blocked when the highest dose of diltiazem (8.9 × 10⁻² M) was administered. Nevertheless, the lowest dose of diltiazem (4.4 × 10⁻⁴ M) was found to transiently enhance the ocular hypertensive effect of the corticosteroid. If the difference between the maximum increase in IOP measured in the control group (betamethasone-treated) and that registered in the rabbits also receiving diltiazem is considered a CCB effect, a clear concentration-response relationship is obtained (table 1). The log concentration-response curve for diltiazem in the prevention of the IOP rise induced by betamethasone in albino rabbits gave the following parameters (fig. 2: maximum response = 3.25 ± 0.34 mm Hg; \(-\log ED_{50} (pD_{2}) = 2.63 ± 0.17; ED_{50} = 2.32 × 10^{-3} M; \) slope = 1.07 ± 0.44).

In the untreated eye, although statistically significant effects of diltiazem on the betamethasone-induced IOP rise were noted (data not shown), no adequate concentration-response relationship was obtained (table 1).

In the second set of experiments, the effect was studied of diltiazem on IOP in rabbits made ocular hypertensive by the weekly subconjunctival injection of betamethasone. As shown in figure 3, diltiazem significantly lowered IOP in the treated eye of betamethasone ocular hypertensive rabbits. In animals receiving the highest dose of diltiazem (8.9 × 10⁻² M).
Table 1
Effect of topical diltiazem in the prevention and treatment of betamethasone-induced ocular hypertension in albino rabbits

<table>
<thead>
<tr>
<th>Diltiazem concentration (M)</th>
<th>Maximum effect (mm Hg)</th>
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<td></td>
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<tr>
<td></td>
<td>Prevention</td>
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<td></td>
<td>Treated eye</td>
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<td>4.4 × 10^{-4}</td>
<td>-0.2 ± 0.4</td>
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<tr>
<td>1.3 × 10^{-3}</td>
<td>-1.4 ± 0.4</td>
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<tr>
<td>2.8 × 10^{-3}</td>
<td>-1.7 ± 0.4</td>
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<tr>
<td>2.2 × 10^{-2}</td>
<td>-2.8 ± 0.4</td>
</tr>
<tr>
<td>8.9 × 10^{-2}</td>
<td>-3.3 ± 0.4</td>
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<tr>
<td></td>
<td>Treatment</td>
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<tr>
<td></td>
<td>Treated eye</td>
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<tr>
<td>4.4 × 10^{-4}</td>
<td>-0.2 ± 0.4</td>
</tr>
<tr>
<td>1.3 × 10^{-3}</td>
<td>-1.3 ± 0.6</td>
</tr>
<tr>
<td>2.8 × 10^{-3}</td>
<td>-2.5 ± 0.6</td>
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<tr>
<td>2.2 × 10^{-2}</td>
<td>-3.5 ± 0.6</td>
</tr>
<tr>
<td>8.9 × 10^{-2}</td>
<td>-4.5 ± 0.7</td>
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Data represent the mean ± S.E.M. of 9 to 12 individual measurements.

Discussion

In the present study, the IOP-lowering activity is shown of once-daily 5-day-a-week topical application of diltiazem on the betamethasone-induced ocular hypertension in rabbits. Topical diltiazem was found to prevent as well as to lower the rise in IOP after betamethasone administration in a dose-related fashion. This is the first report describing the ocular hypotensive effect of a CCB in an animal model for glaucoma.

The ocular hypotensive effect of topical CCBs had been previously reported in humans and ocular normotensive albino rabbits. In previous reports, we have shown (Sanfà et al., 1996, 1997; Sagarra et al., 1993) that single doses of verapamil, nifedipine and diltiazem produced a dose-dependent decrease in IOP in ocular normotensive rabbits after topical application but not after intravenous administration. Furthermore, the ocular hypotensive effect of diltiazem was remarkable due to its duration (Sanfà et al., 1997), thus permitting the administration frequency used in the present work. In humans, topical verapamil has been found to significantly lower IOP in normal and ocular hypertensive subjects. A single topical application of 0.125% verapamil prompted a 3 to 4 mm Hg IOP decrease in 12 ocular hypertensive patients that lasted up to 10 hr (Abelson et al., 1988), whereas a slight reduction (~1.5 mm Hg) was noted in normal volunteers (Netland et al., 1995). After topical application of 0.125% verapamil for 2 weeks, a 7.0 ± 2.9 mm Hg measured in the rabbits receiving betamethasone alone (control group) and that registered in the diltiazem-treated groups is considered the CCB effect, a dose-response relationship is found (table 1). The log concentration-response curve for diltiazem in the treatment of betamethasone-induced ocular hypertension in albino rabbits showed the following parameters (fig. 2): maximum response = 4.17 ± 0.41 mm Hg; -log ED_{50} (pD_{2}) = 2.64 ± 0.14; ED_{50} = 2.32 × 10^{-5} M; slope = 1.35 ± 0.62.

With regard to the untreated eye, neither statistically significant effects of topically applied diltiazem (data not shown) nor an adequate dose-response relationship (table 1) was found.
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The present study shows that the topical application of diltiazem prevents the betamethasone-induced intraocular pressure rise as well as reduces the IOP in rabbits made ocular hypertensive by the subconjunctival administration of betamethasone. Whether these data can be extrapolated to humans is difficult to say. However, the features of the animal model used, which are very close to those of human chronic open-angle glaucoma, and the fact that corticosteroid glaucoma is not rare in ophthalmological practice appear to support the potential utility of the findings we obtained, especially when endogenous glucocorticoids may play a role in the pathogenesis of human glaucoma (Southren et al., 1985). Our data are in accordance with preliminary reports suggesting that CCBs are effective in the management of ocular hypertension (Abelson et al., 1988; Goyal et al., 1989; Mooshian et al., 1993) and low-tension glaucoma (Netland et al., 1993, 1995), and they might indicate a potentially beneficial effect of these drugs in the prevention of ocular hypertension in subjects undergoing ocular administration of corticosteroids. Nevertheless, further studies are needed to clarify the ocular effects of CCBs and determine their intimate mechanism of action.

References

Fig. 3. Effect of topical diltiazem on IOP in rabbits made ocular hypertensive by weekly subconjunctival injection of betamethasone (arrows). Comparison between the control group (betamethasone alone) and the groups receiving diltiazem. The average IOP level was 26.5 ± 0.2 mm Hg. Each point represents the mean ± S.E.M. of the number of animals shown in parentheses. Values significantly different from the corresponding control time point: *P < .05; **P < .01; ***P < .001.

Diltiazem 4.4 x 10^-4 M (n = 12)
Diltiazem 1.3 x 10^-3 M (n = 10)
Diltiazem 2.8 x 10^-4 M (n = 12)
Diltiazem 2.2 x 10^-4 M (n = 12)
Diltiazem 8.9 x 10^-4 M (n = 11)

decrease in IOP has been measured in ocular hypertensive subjects (Goyal et al., 1989).

These results conflict with those of Beatty et al. (1984), who found an increase in IOP after intravenous and topical application of verapamil, nifedipine and diltiazem in rabbits and after topical verapamil in humans. Because the doses of verapamil used by Beatty et al. (1984) were higher than those applied in most of the aforementioned studies, Abelson et al. (1988) proposed that CCBs may have a biphasic effect on IOP, with an ocular hypotensive action at low and an ocular hypertensive action at high concentrations. Nevertheless, the results reported here do not support this hypothesis because a decrease in IOP was noted even at very high concentrations of diltiazem.

In contrast to previous reports, we have not found a clear bilateral effect of diltiazem when administered to only one eye. Although some statistically significant effects on IOP in the untreated eye were noted when diltiazem was applied to prevent the betamethasone-induced IOP rise, they did not show a dose-response relationship. These findings, as well as the absence of statistically significant IOP changes in the untreated eye of betamethasone ocular hypertensive rabbits receiving unilateral diltiazem, suggest that this CCB lacks a contralateral effect in this animal model for glaucoma. These results conflict with those of Segarra et al. (1993) who found an IOP reduction, although not dose-related, in the contralateral eye after unilateral topical application of verapamil and nifedipine in albino rabbits. Abelson et al. (1988) and Mooshian et al. (1993) also noted a contralateral effect of topically applied verapamil in ocular hypertensive subjects, whereas Netland et al. (1995) reported no effect of verapamil on IOP in the contralateral eye after topical administration in normal subjects.

The mechanism of the ocular hypotensive effect of CCBs remains to be established. Evidence obtained by our group in albino rabbits (Santafe et al., 1997; Segarra et al., 1993) suggests that CCBs decrease aqueous humor secretion, although they also cause a slight, although significant, reduction of tonographic outflow facility. On the other hand, perfusion studies in dissected human eyes showed dose-related increases in outflow facility after verapamil administration (Erickson et al., 1995; Schroeder and Erickson, 1993).

From our results, we must point out the fact that the once-daily 5-day-a-week topical application of diltiazem has been found to be sufficient to prevent and treat the betamethasone-induced ocular hypertension. These findings confirm the persistence of the ocular hypotensive effect of this drug previously described by Santafe et al. (1997) in ocular normotensive rabbits. Such a long-lasting effect may provide a prominent place for diltiazem in antiglaucoma therapy.
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Send reprint requests to: Dr. Juan Santafe, Departamento de Farmacologia, Facultad de Farmacia, Universidad del Pais Vasco, Paseo de la Univer-
sidad No. 7, E-01006 Vitoria, Spain.