Therapeutic and Systemic Side Effects of Ocular \( \beta \)-Adrenergic Antagonists in Anesthetized Dogs

Alan L. Svec and Arthur M. Strosberg

Beta adrenergic antagonists, while useful in the treatment of glaucoma, can be absorbed transocularly causing a variety of systemic side effects. An adequate animal model which simultaneously indicates the intraocular pressure lowering effects as well as the systemic effects of ocularly applied \( \beta \)-adrenergic antagonists would be highly useful. The pentobarbital anesthetized dog, instrumented to record blood pressure (BP) and heart rate (HR) and in which intraocular pressure (IOP) was measured with a pneumatonometer, was investigated. Isoproterenol iv dose-response curves were run and IOP measured before and hourly for 3 hr following ocular application of 50 \( \mu \)l 0.5\% timolol, 0.5\% RS-52367 (a Syntex \( \beta \)-adrenergic antagonist), or dH\( _2\)O control. Timolol and RS-52367 produced similar decreases in IOP. However, timolol decreased basal BP and HR, markedly inhibited isoproterenol-induced BP and HR responses, and also decreased contralateral IOP. Since both the IOP lowering and systemic \( \beta \)-adrenergic antagonist properties of timolol were elicited, the anesthetized dog appears to be an excellent animal model for examining the effects of \( \beta \)-adrenergic antagonists applied topically to the eye. Invest Ophthalmol Vis Sci 27:401-405, 1986

\( \beta \)-adrenergic antagonists are useful in the treatment of open angle glaucoma by lowering intraocular pressure (IOP)\(^1\) via reduction of the rate of formation of aqueous humor.\(^2\) Clinical studies reveal that timolol maleate, a \( \beta \)-adrenergic antagonist shown to be effective in lowering IOP in glaucoma patients, is absorbed systemically following ocular application and causes a variety of side effects.\(^3\) Of particular significance are the cardiovascular effects, which may intensify failure in congestive heart failure patients, and the pulmonary tract \( \beta \)-adrenergic antagonist effects, which may lead to bronchospasm in asthmatics or patients with pulmonary obstructive disease.\(^4\)

One of the major difficulties in determining the IOP lowering efficacy and potential for systemic side effects of \( \beta \)-adrenergic antagonists is the lack of a suitable experimental animal model. The present study was performed to determine the utility of the pentobarbital anesthetized dog as an experimental animal model for evaluating both the IOP lowering ability and cardiovascular side effects of \( \beta \)-adrenergic antagonists applied topically to the eye. The effects of ocularly administered timolol and RS-52367 (1-t-butylamino-3[S'-5 methyl hexyl aminocarbonyl]-2-thiazoloxylpropan-2-ol HCl, a \( \beta _1 \)-selective Syntex \( \beta \)-adrenergic antagonist\(^5\)) on ipsilateral and contralateral IOP, baseline blood pressure and heart rate, and on isoproterenol-induced blood pressure and heart rate changes (as an indicator of \( \beta \)-antagonism) were determined.

Materials and Methods

Experimental Preparation

Mongrel dogs of either sex (12.6 kg ± 0.8 kg) were anesthetized with pentobarbital Na iv (30 mg/kg) and supplemented by continuous iv infusion (5 mg/kg/hr). A femoral vein was cannulated for injection and a femoral artery for blood pressure measurement. Heart rate was recorded by a cardiograph triggered by the R wave of a limb lead II ECG. Responses were recorded on a Beckman type R polygraph (Sensor Medics Corp., Anaheim, CA). Intraocular pressure measurements were made with a Digilab model 30-D pneumatonometer (Digilab; Cambridge, MA). Respiration was maintained constant with a respirator (Harvard Apparatus; So. Natick, MA).

Experimental Protocol

Isoproterenol was administered iv in half-log doses from 0.0158 \( \mu \)g/kg to 0.5 \( \mu \)g/kg at 10-min intervals (pre-drug control). A 30-min rest period followed. Single 50 \( \mu \)l drops of 0.5\% Timoptic (Merck, Sharp, & Dohme; West Point, PA), 0.5\% RS-52367, or dH\( _2\)O were then instilled into the inferior conjunctival sac of one eye with a micropipette. Isoproterenol dose-response curves were again run hourly for 3 hr. Ipsilateral
IOP was measured immediately before administration of drug, and again hourly, prior to each isoproterenol dose response curve. Blood pressure and heart rate were monitored throughout the experiment.

Studies employing the same protocol were also performed in similarly instrumented dogs to determine the effect of each compound on contralateral IOP following ocular administration.

In additional studies utilizing the same protocol except for the route of administration, the effects of 50 μl intravenous injections of 0.5% timolol and 0.5% RS-52367 were examined in similarly instrumented preparations.

Data were compared for statistical significance using Student’s t-test.6

Drug Preparation

RS-52367 was dissolved in dH2O to a concentration of 0.5% Timoptic solution 0.5% (timolol maleate MSD, Lot. No. 1380J) was obtained commercially. Isoproterenol (Aldrich Chemicals; Milwaukee, WI) was dissolved in saline containing ascorbic acid as a preservative. All solutions were computed as free base concentration.

These studies adhere to the ARVO Resolution on the Use of Animals in Research.

Results

Intraocular Pressure

The effects of ocular 0.5% timolol, 0.5% RS-52367 and dH2O on IOP in treated eyes are shown in Figure 1. Both compounds produced statistically significant (P < 0.005), similar reductions in IOP as compared to dH2O control for 3 hr following a single 50-μl ocular application. At 3 hr, however, RS-52367 affected a slightly greater reduction in IOP than timolol. Mild irritation of the conjunctiva and sclera of treated eyes was noted in some dogs receiving RS-52367.

The effects of 0.5% timolol on IOP in contralateral eyes as compared to treated eyes and dH2O control are shown in Figure 2. Timolol produced statistically significant (P < 0.025) reductions in ipsilateral IOP as compared to dH2O control for 3 hr following a single 50-μl ocular application. Contralateral IOP exhibited a tendency towards reduction (P < 0.10) beginning 1 hr after dosing. The difference between ipsilateral and contralateral IOP was not statistically significant (P < 0.05) at 2 and 3 hr.
Ocular application of 50 μl 0.5% RS-52367 did not produce a reduction in contralateral IOP.

Effects on Blood Pressure and Heart Rate

The effects of ocular 0.5% timolol, 0.5% RS-52367, and dH2O control on baseline blood pressure and heart rate are shown in Figures 3 and 4. Timolol significantly lowered blood pressure and heart rate (P < 0.05) within 30 min after ocular application. Blood pressure and heart rate returned to near pre-drug levels at 3 hr. Intravenous timolol produced similar decreases in blood pressure and heart rate. However, these effects persisted at 3 hr.

Blood pressure and heart rate in dogs treated with ocular RS-52367 were not significantly different from dH2O controls (P > 0.05) over the 3-hr time period (Figs. 3, 4). Intravenous RS-52367 produced no change in blood pressure. A decrease in heart rate was noted 30 min after ocular application. Blood pressure and heart rate responses at 1, 2 and 3 hr after administration (Table 1). The same dose of timolol given intravenously produced a similar inhibition (Table 2). These data indicate that upon ocular application timolol is readily absorbed systemically in the dog and produces significant systemic β-adrenergic antagonism.

In contrast, ocular RS-52367 produced only slight inhibition of isoproterenol challenges (Table 1). Studies with intravenous RS-52367 at 50 μl 0.5% solution (aprox. 20 μg/kg) showed the compound possessed little isoproterenol blocking activity at this dose (Table 2). However, higher doses (>316 μg/kg) produce a complete antagonism of isoproterenol induced cardiac responses with little effect on blood pressure responses.

Discussion

The systemic absorption and resultant side effects of ocularly applied agents has long been a problem in drug therapy. Topically applied β-adrenergic antagonists, specifically timolol, are quite effective in treating open angle glaucoma by lowering IOP. However, timolol is contraindicated for this use in patients with bronchial asthma, obstructive pulmonary disease, second and third degree heart block, sinus bradycardia and cardiac failure. This is because the same cardiac and pulmonary effects seen with systemically admin-

Table 1. Effects of ocular timolol* and RS-52367* on isoproterenol-induced blood pressure and heart rate responses in anesthetized dogs

<table>
<thead>
<tr>
<th>Isoproterenol (μg/kg, iv)</th>
<th>Response</th>
<th>Timolol</th>
<th>RS-52367</th>
<th>dH2O</th>
<th>Timolol</th>
<th>RS-52367</th>
<th>dH2O</th>
<th>Timolol</th>
<th>RS-52367</th>
<th>dH2O</th>
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<tbody>
<tr>
<td>0.0158</td>
<td>mean BP</td>
<td>97.0 ± 1.6ζ</td>
<td>0.0 ± 0.0</td>
<td>1.5 ± 1.5</td>
<td>94.2 ± 1.5ζ</td>
<td>11.5 ± 5.9</td>
<td>0.7 ± 0.7</td>
<td>88.0 ± 4.2ζ</td>
<td>14.7 ± 8.8</td>
<td>3.7 ± 2.9</td>
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<tr>
<td></td>
<td>heart rate</td>
<td>96.7 ± 3.3ζ</td>
<td>15.8 ± 7.8</td>
<td>0.0 ± 0.0</td>
<td>89.7 ± 6.0ζ</td>
<td>13.2 ± 7.4</td>
<td>0.0 ± 0.0</td>
<td>83.6 ± 5.3ζ</td>
<td>28.2 ± 11.8</td>
<td>6.5 ± 5.9</td>
</tr>
<tr>
<td>0.05</td>
<td>mean BP</td>
<td>93.5 ± 2.2ζ</td>
<td>1.1 ± 1.1</td>
<td>3.0 ± 3.0</td>
<td>86.5 ± 2.7ζ</td>
<td>18.3 ± 2.7</td>
<td>3.0 ± 3.0</td>
<td>70.2 ± 5.8ζ</td>
<td>23.8 ± 11.9</td>
<td>5.5 ± 5.5</td>
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<td>heart rate</td>
<td>87.7 ± 4.4ζ</td>
<td>17.0 ± 5.7</td>
<td>3.8 ± 2.1</td>
<td>81.5 ± 6.2ζ</td>
<td>21.7 ± 7.3</td>
<td>4.8 ± 2.8</td>
<td>72.2 ± 6.1ζ</td>
<td>38.3 ± 8.3ζ</td>
<td>8.3 ± 4.7</td>
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<td>0.158</td>
<td>mean BP</td>
<td>76.3 ± 5.8ζ</td>
<td>0.3 ± 0.3</td>
<td>2.2 ± 2.2</td>
<td>66.0 ± 6.5ζ</td>
<td>13.8 ± 9.0</td>
<td>1.8 ± 1.8</td>
<td>55.3 ± 7.0ζ</td>
<td>17.8 ± 10.8</td>
<td>3.2 ± 3.2</td>
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<td></td>
<td>heart rate</td>
<td>75.8 ± 7.0ζ</td>
<td>10.2 ± 3.0</td>
<td>1.8 ± 2.1</td>
<td>64.0 ± 8.3ζ</td>
<td>18.2 ± 8.3</td>
<td>4.7 ± 2.3</td>
<td>57.0 ± 8.4ζ</td>
<td>20.8 ± 7.4</td>
<td>10.3 ± 3.5</td>
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<tr>
<td>0.5</td>
<td>mean BP</td>
<td>60.8 ± 5.7ζ</td>
<td>0.4 ± 0.4</td>
<td>2.0 ± 1.6</td>
<td>50.2 ± 6.5ζ</td>
<td>9.0 ± 7.0</td>
<td>1.0 ± 1.0</td>
<td>39.0 ± 5.3ζ</td>
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<tr>
<td></td>
<td>heart rate</td>
<td>57.6 ± 14.0ζ</td>
<td>10.2 ± 3.3</td>
<td>1.0 ± 1.0</td>
<td>43.8 ± 12.4</td>
<td>11.5 ± 3.6</td>
<td>2.8 ± 1.8</td>
<td>37.0 ± 10.8</td>
<td>13.3 ± 3.4</td>
<td>8.2 ± 3.7</td>
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</table>

* 50 μl, 0.5% solution, ocular administration, as compared to 50 μl dH2O. † mean ± SEM, n = 6
ζ P < 0.001
ξ P < 0.01
istered β-adrenergic antagonists, viz, cardiac depression and antagonism of β₂ receptor mediated bronchodilation, can be produced with ocularly applied timolol, indicating the compound is absorbed transocularly. In rare instances, death by cardiac failure has occurred following ocular timolol administration.

As with many therapeutic agents, a relatively uncommon side effect may not be clearly identified in clinical testing. A statistically significant number of adverse effects show up only when the agent is given to a large enough population of susceptible individuals. With timolol, the cardiovascular and pulmonary side effects were found to be inconspicuous except in patients with pre-existing cardiovascular or pulmonary disease states. As the popularity of using ocularly applied β-adrenergic antagonists to treat open angle glaucoma grows, so does the potential for occurrence of deleterious effects produced by newer agents. These agents will, by default, have had less documentation concerning exposure to a susceptible population. An animal model which simultaneously depicts the IOP lowering effects of β-adrenergic antagonists as well as their potential for cardiovascular side effects would be highly useful in the early identification of compounds which lower IOP but produce minimal systemic side effects.

Several animal models including normotensive and water loaded albino rabbits, cats, and cynomolgus monkeys have been employed to assay the effects of ocularly applied β-antagonists but have met with mixed success. For example, Liu et al, demonstrated a lowering of IOP with timolol applied to the eyes of water-loaded rabbits. However, this did not correlate with decreases in heart rate and blood pressure induced by timolol in these animals. The present study has demonstrated the utility of the pentobarbital anesthetized dog as a model for the study of ocularly administered β-adrenergic antagonists. In this model, ocular timolol produced decreases in IOP similar to those seen in man. The typical cardiovascular side effects seen in man with timolol were also elicited in the dog. The marked antagonism of isoproterenol induced blood pressure and heart rate responses and a tendency toward some lowering of contralateral IOP following ocular timolol administration confirm the systemic absorption of this agent.

The Syntex compound RS–52367 appears useful in lowering IOP. While the compound is less potent than timolol as a β-adrenergic antagonist by intravenous administration, it produces an equivalent lowering of IOP when identical doses are applied ocularly. This may be due to a local high concentration of RS–52367 relative to timolol if RS–52367 is systemically absorbed to a lesser degree than timolol or if RS–52367 is absorbed into the anterior chamber of the eye to a greater degree than timolol. If RS–52367 can be formulated in a nonirritating preparation, the compound may be safe and effective in the treatment of open angle glaucoma.

The pentobarbital anesthetized dog has been used for many years in cardiovascular pharmacology research and remains an accurate model for studying many of the physiological and pharmacological characteristics of man. The adaptation of this model to ocular research will hopefully speed the development of safe and effective new drugs to treat open angle glaucoma.

Key words: β-adrenergic antagonists, timolol, glaucoma, cardiovascular, intraocular pressure

Table 2. Effects of intravenous timolol and RS–52367 on isoproterenol-induced blood pressure and heart rate responses in anesthetized dogs

<table>
<thead>
<tr>
<th>Isoproterenol Response</th>
<th>Hour 1</th>
<th>Hour 2</th>
<th>Hour 3</th>
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<tbody>
<tr>
<td>Mean BP</td>
<td></td>
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<tr>
<td>Heart rate</td>
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<td>Mean BP</td>
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<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
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</tbody>
</table>

* 50 µl, 0.5% solution, intravenous administration; mean ± SEM, n = 3. † P < 0.0025. ‡ P < 0.05.
References