The effect of d-isoproterenol on intraocular pressure of the rabbit, monkey, and man


D-isoproterenol d-bitartrate applied topically lowers intraocular pressure (IOP) in normal albino rabbits and rabbits with alpha-chymotrypsin-induced glaucoma. This effect is independent of any effect on systemic blood pressure or pulse rate. A similar response could not be obtained in monkey or human eyes. Subconjunctival injection of d-isoproterenol d-bitartrate to monkey eyes did not alter IOP.

Key words: d-isoproterenol, tachycardia, blood pressure, intraocular pressure, cAMP, facility of outflow, rabbit, monkey, human.

Isoproterenol lowers intraocular pressure (IOP) in rabbits,1-4 primates,5 and man.6-11 Topical application of racemic isoproterenol lowers intraocular pressure in human eyes.6-11 However, a significant percentage of the patients develop tachycardia.6-9 Recently, Seidehamel, Dungan, and Hickey12 reported that d-isoproterenol lowered intraocular pressure in rabbits without producing tachycardia. The present experiments were designed to explore this possibility further.

Methods

Rabbits. Albino rabbits, 2 to 3 kilograms in weight, received topical 2 per cent lidocaine to both eyes. The IOP was measured with a Mackay-Marg tonometer or an Alcon Pneumotonograph every 15 minutes until stable readings were obtained. Two drops of 10 per cent d-isoproterenol d-bitartrate were placed in the superior cul-de-sac of one eye. Saline diluent was applied to the other eye. The lids were held open for 30 seconds forming a trough for the solution. The IOP was measured at 30, 60, 90, 120, 150, 180, 210, 240, and 300 minutes after drug administration.

Albino rabbits had chronic elevations of intraocular pressure in one eye months to years following a posterior chamber injection of alpha-chymotrypsin by a technique previously described.13 In a similar fashion to that described above, topical application of the following drugs was employed to the affected eye: 5 per cent dl-isoproterenol HCl, (Sigma) 5 per cent 1-isoproterenol HCl (Sigma), and d-isoproterenol d-bitartrate in concentrations of 0.1, 1, 5, and 10 per cent.

Six albino rabbits were pretreated with intravenous propranolol, 5 mg. per kilogram, (Sigma) 30 minutes prior to receiving topical d-isoproterenol. Four rabbits were pretreated with intravenous phenoxybenzamine, 25 mg. per kilo-
were anesthetized with intravenous urethane, 2 Gm. per kilogram. Both anterior chambers were cannulated 60 minutes after topical application of d-isoproterenol d-bitartrate to one eye. The aqueous humor samples collected were assayed for cyclic adenosine monophosphate (cAMP) by a technique previously described.14-15

Five albino rabbits underwent paracentesis of both anterior chambers 60 minutes after topical application of d-isoproterenol to one eye. The pupillary diameters were measured with a visual gauge for the next 30 minutes to determine the completeness of the alpha blockade.

Five albino rabbits were anesthetized with intravenous urethane, 2 Gm. per kilogram. Both anterior chambers were cannulated 60 minutes after topical application of d-isoproterenol to one eye. Outflow facility was measured by perfusion at two levels of constant pressure above the prevailing IOP by a technique previously described.14-15

Four albino rabbits, weighing 2 to 3 kilograms, were anesthetized with intravenous urethane, 2 Gm. per kilogram. A 10-gauge polyethylene catheter was placed in the lateral carotid artery and connected to a Sanborn recorder via a blood-pressure transducer. Continuous measurements of blood pressure and pulse rate were recorded. Intravenous injections of various doses of d-isoproterenol d-bitartrate, and d,l-isoproterenol HCl were made through a marginal ear vein.

Monkeys. Rhesus monkeys, weighing 5 to 10 kilograms, were anesthetized with intramuscular phencyclidine, 1 to 2 mg. per kilogram. The IOP was measured with the Mackay-Marg tonometer or the Alcon Pneumotonograph until stable readings were obtained. D-isoproterenol d-bitartrate 10 per cent was applied topically to one eye, saline diluent to the other in a similar fashion to that described above. The IOP was measured at 15, 30, 60, 90, 180, and 240 minutes after drug administration. Another group of rhesus monkeys received 1 mg. of d-isoproterenol d-bitartrate subconjunctivally to one eye and saline diluent to the other eye.

Humans. The IOP of eight normal, human volunteers was measured with a Goldmann tonometer after topical 0.5 per cent proparacaine anesthesia. Two drops of 10 per cent d-isoproterenol d-bitartrate were placed in the superior cul-de-sac of one eye. Saline diluent was given to the other eye. The intraocular pressure was measured 15 and 30 minutes after the medication and then hourly for the next 8 hours. Five normal, human volunteers received 20 per cent d-isoproterenol HCl topically to one eye in a similar fashion.

Analysis of purity. The purity of the d-isoproterenol was checked by chromatography on a Zipax SCX column (1 meter by 2 mm.) using a water-0.5 M sodium perchlorate gradient at a rate of 5 per cent per minute and a flow rate of 60 ml. per hour at 1,400 PSI. A solution of the hydrochloride was prepared from the bitartrate by first dissolving the bitartrate in water containing a small amount of sodium sulfite (for stability purposes) and then adding an equal molar amount of ammonium hydroxide. The free base crystallizes out upon cooling. The free base was then re-crystallized from isopropyl alcohol-water 60/40 mixture. The hydrochloride was prepared by dissolving the free base in water and adding an equimolar amount of hydrochloric acid. The optical rotation of the d-isoproterenol was determined using a Perkin Elmer polarimeter.

Results

Table I. Reduction of IOP 60 minutes after topical d-isoproterenol d-bitartrate in rabbits with alpha-chymotrypsin glaucoma

<table>
<thead>
<tr>
<th>Concentration of d-isoproterenol d-bitartrate (%)</th>
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<td>-0.3 ± 1.6*</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>3.7 ± 2.5*</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4.7 ± 2.8*</td>
</tr>
<tr>
<td>10</td>
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Rabbit. Topical application of 10 per cent d-isoproterenol d-bitartrate lowered IOP in normal albino rabbit eyes (Fig. 1). The effect reached a maximum 60 minutes after topical treatment and was essentially gone by five hours. No significant contralateral effect was noted. Pretreatment with either intravenous propranolol or phenoxybenzamine did not alter the response of IOP to topical d-isoproterenol. No influence of topical d-isoproterenol was noted on pupillary size.

Topical application of 10 per cent d-isoproterenol d-bitartrate lowered intraocular pressure in rabbits with alpha-chymotrypsin glaucoma (Fig. 1). There was a pattern of increasing response of IOP to higher concentrations of d-isoproterenol d-bitartrate (Table I). However, only the 10 per cent concentration gave a statistically significant decrease in IOP when compared
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Fig. 1. IOP after topical 10 per cent d-isoproterenol d-bitartrate in normal albino rabbits and rabbits with alpha-chymotrypsin induced glaucoma. At time zero, 10 per cent d-isoproterenol d-bitartrate is applied topically. ○ Normal albino rabbits (n = 11), ● alpha-chymotrypsin glaucoma rabbits (n = 17), and □ control rabbits (n = 11).

to pretreatment pressures. The pretreatment pressures were quite similar in all four treatment groups.

Four rabbits with alpha-chymotrypsin glaucoma received topical 5 per cent l-isoproterenol HCl. Two of the animals died within five minutes and the other two demonstrated a decrease in IOP similar to that caused by 10 per cent d-isoproterenol d-bitartrate.

Topical application of 5 per cent d,l-isoproterenol HCl produced a similar decrease in IOP in rabbits with alpha-chymotrypsin glaucoma as 10 per cent d-isoproterenol d-bitartrate.

The mean ± S.E.M. aqueous humor concentration of cAMP one hour after topical d-isoproterenol d-bitartrate in six normal albino rabbits was 30.0 ± 4.5 nmoles per liter. The concentration in the control eyes was 27.8 ± 1.6 nmoles per liter.

The mean ± S.E.M. facility of outflow 60 minutes after topical 10 per cent d-isoproterenol d-bitartrate in five normal albino rabbits was 0.52 ± 0.02 μl per minute per millimeter of Hg. The outflow in the control eyes was 0.28 ± 0.04 μl per minute per millimeter of Hg. This difference was statistically significant (p < 0.01).

Intravenous injection of 20 μg of d, l-isoproterenol HCl lowered blood pressure of albino rabbits by 20 to 45 mm. Hg for two to five minutes. Pulse rate increased by 60 to 120 beats per minute. Intravenous injection of 20 μg of d-isoproterenol d-bitartrate did not affect blood pressure or pulse rate. Intravenous doses of 40 to 200 μg of d-isoproterenol d-bitartrate lowered blood pressure by 6 to 10 mm. Hg for 10 to 30 seconds and increased pulse rate by 10 to 30 beats per minute. No change in IOP
was noted. An intravenous injection of 1 mg. of d-isoproterenol d-bitartrate lowered blood pressure 20 mm. Hg for less than five minutes. Intraocular pressure declined 3 to 4 mm. Hg for two to five minutes.

**Monkeys.** Topical 10 per cent d-isoproterenol d-bitartrate did not produce a reduction of IOP in rhesus monkeys. Subconjunctival injection of 1 mg. of d-isoproterenol d-bitartrate did not lower IOP in rhesus monkeys.

**Humans.** Topical 10 per cent d-isoproterenol d-bitartrate did not lower IOP in human volunteers at any time, over an eight-hour time course. Mild conjunctival hyperemia and irritation were briefly noted after administration of the drops. Topical administration of 20 per cent d-isoproterenol HCl produced marked conjunctival hyperemia and mild miosis that persisted for several hours. No reduction in IOP was noted.

**Analysis of purity.** The chromatographic results of d-isoproterenol can be seen in Fig. 2. The isoproterenol peak is removed from the peaks of the other catecholamines. Also, the d-isoproterenol hydrochloride gives identical results with the d,l-isoproterenol hydrochloride standard. Both the d-isoproterenol d-bitartrate and the d,l-isoproterenol hydrochloride plus d-bitartrate, show a slight shoulder as seen in Fig. 2. This may be due to a complex between the isoproterenol and the bitartrate which causes the perturbation of the chromatographic results. Thus, there are no detectable impurities in the d-isoproterenol d-bitartrate.
The optical rotation of the d-isoproterenol was determined to be \([\alpha]_{D}^{20} + 39.2^\circ\) \((c = 5 \text{ in } \text{H}_2\text{O})\). This is in accordance with the expected results.

**Discussion**

Clinicians have long searched for additional adrenergic agents to treat glaucoma. Racemic isoproterenol HC1 has been employed to lower intraocular pressure in humans.\(^6\)\(^-\)\(^11\) However, a high incidence of tachycardia following topical therapy to one eye has been reported.\(^1\)\(^-\)\(^4\) It was presumed that the activity was due to the biologically active 1-form.

Recently, Seidehamel, Dungan, and Hickey\(^12\) reported d-isoproterenol lowered IOP in rabbits without significant effect on the cardiovascular system. We were able to confirm that d-isoproterenol d-bitartrate lowers IOP in the rabbit. This compound appears to have only slight and transient effects on blood pressure and pulse rate, even when injected intravenously in large doses.

Topical d-isoproterenol appears to increase the facility of outflow in the rabbit eye. While the mechanism by which this occurs is obscure at the present time, there is suggestive, although not conclusive evidence, that the compound does not exert its effect by classical alpha or beta mechanisms. The lack of an increase in aqueous humor cAMP concentration after topical application of d-isoproterenol differs from the results reported for l-epinephrine, l-norepinephrine, and l-isoproterenol.\(^3\) Finally, the chromatographic analysis and the optical rotation data rule out the presence of another catecholamine or l-isoproterenol as a contaminant of the agonist.

Monkey and human eyes did not respond to topical administration of d-isoproterenol d-bitartrate nor did subconjunctival injection of the agonist lower IOP in the monkey eye. The reason for this lack of response is unclear at the present time. It is possible the species difference relates to the varying patterns of autonomic innervation of the trabecular meshwork.\(^1\)\(^-\)\(^10\)

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