The Effects of *dl*-1-(4-Hydroxyphenyl)-2-isopropylaminoethanol HCl (AL842) on Intraocular Pressure and Pupil Size of Rhesus Monkeys (35460)

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Phenylethylamine derivatives have been used topically for many years in ophthalmology. It is well known that many of these materials will lower intraocular pressure (IOP). Side-effects produced by the drugs used clinically indicate that the ideal agent has not been found. For example, mydriasis with photophobia and blurred vision occurs in most instances with clinical use of topical epinephrine. Consequently, this agent is contraindicated clinically in patients with narrow angle glaucoma due to the mydriasis and secondary blockage of aqueous outflow (1).

A program of pharmacological screening was instituted to detect a potential ocular hypotensive agent which might not demonstrate some of the undesirable side-effects exhibited by drugs of this class in current use clinically. The compound *dl*-1-(4-hydroxyphenyl)-2-isopropylaminoethanol hydrochloride (AL842) is one result of this testing program.

Initial studies undertaken to define the pharmacological properties of AL842 were performed in rabbits. The results of these experiments are reported elsewhere (2). In these studies the test methods utilized to determine ocular hypotensive activity depended upon an IOP which was initially increased artificially. In one system formalin was used to increase IOP secondarily to irritation and/or inflammation (3). The second system was based on a modification of the water-provocative test used clinically in man (4). The nature of these tests, however, precluded the collection of onset–duration data.

A test system in animals was sought in which initial IOP was sufficiently high for potential ocular hypotensive properties to be demonstrated. Rhesus monkeys (*Macaca mulatta*) were chosen for these tests. Preliminary screening of IOP in these animals demonstrated an average IOP of approximately 16 mm Hg while approximately 13% of the animals recorded IOP values of 21 mm Hg or higher in at least one eye.

Twenty-five animals with high IOP were selected for use in obtaining dose–response and duration data for AL842.

Materials and Methods. Three hundred sixteen young adult Rhesus monkeys of either sex, weighing 3.0 to 6.0 kg, under phencylclidine anesthesia, were screened for high intraocular pressure (IOP) by the staff of the Woodard Asiatic Corporation (San Francisco, California) using a Model 6 Mackay Marg Tonometer (Biotronics, Inc., Redding, California). The average IOP was found to be 16.4 mm Hg where pressures in 65% of eyes tested were between 14 and 18 mm Hg. At the time of test, animals were selected in which the IOP of one or both eyes was 21 mm Hg or more by applanation tonometry. Mean control value was 22.47 $\pm$ 0.29 mm Hg for both eyes of 15 test animals in Study I, and 20.1 $\pm$ 0.44 mm Hg for 10 animals in Study II. No attempt was made to calibrate the instruments for monkey eyes. Phencyclidine hydrochloride (Sernylan, Parke Davis) was administered 0.5 to 1.0 mg/kg, intramuscularly, approximately 10 min prior to start of the experiment. In primates, an anesthetic effect is achieved without loss of corneal, palpebral, or pupillary responses (5). Tornquist (6) used the agent in his study of the effect of pilocarpine on the pupil and refraction in monkeys. Animals were placed in the supine position, a single drop of proparacaine (Ophthaine, Squibb) was applied to each eye and pupillary and IOP

TABLE I. Intraocular Pressure and Pupil Size Following Topical Application of Various Concentrations of AL842, Reference Standard, and a Negative Control in Ocular Hypertensive Monkey Eyes.

Treatment	Time (hr):	IOP (mm Hg; applanation) 0	0.5	1	2	4	6	8	Pupil size (mm) 0	0.5	1	2	4	6	8
AL842, 4%		21.7[a] ±0.44 (10)	17.6 ±0.81 (5)	15.4 ±0.67 (5)	17.3 ±0.48 (10)	18.4 ±1.76 (5)	17.0 ±0.45 (5)	18.0 ±0.84 (5)	3.8 ±0.11 (10)	3.3 ±0.20 (5)	3.6 ±0.37 (5)	4.5 ±0.30 (10)	3.8 ±0.20 (5)	4.0 ±0.15 (5)	3.8 ±0.34 (5)
2%		22.6 ±0.71 (5)	17.0 ±1.14 (5)	16.4 ±1.05 (5)	17.6 ±0.92 (5)	— — —	— — —	— — —	3.9 ±0.11 (5)	3.4 ±0.20 (5)	3.8 ±0.12 (5)	4.5 ±0.21 (5)	— — —	— — —	— — —
1%		21.0 ±0.32 (5)	18.4 ±1.21 (5)	17.9 ±1.25 (5)	18.0 ±1.30 (5)	— — —	— — —	— — —	3.7 ±0.12 (5)	3.6 ±0.19 (5)	3.6 ±0.19 (5)	4.9 ±0.49 (5)	— — —	— — —	— — —
Epinephrine bitartrate (1% as base)		21.8 ±0.56 (10)	16.4 ±1.11 (5)	17.0 ±0.84 (5)	18.0 ±0.85 (10)	16.0 ±0.55 (5)	16.2 ±0.67 (5)	15.6 ±0.39 (5)	3.9 ±0.17 (10)	7.5 ±0.51 (5)	7.3 ±0.74 (5)	7.3 ±0.36 (10)	6.5 ±0.28 (5)	5.3 ±0.49 (5)	5.2 ±0.56 (5)
Vehicle control		20.1 ±0.62 (15)	18.8 ±0.79 (15)	18.5 ±0.61 (15)	19.3 ±0.81 (15)	17.0 ±1.14 (5)	18.8 ±0.87 (5)	20.0 ±1.14 (5)	3.8 ±0.17 (15)	4.0 ±0.15 (15)	3.9 ±0.23 (15)	4.0 ±0.26 (15)	3.8 ±0.20 (5)	4.1 ±0.20 (5)	4.1 ±0.20 (5)

[a] $\bar{X} \pm$ SE (n).

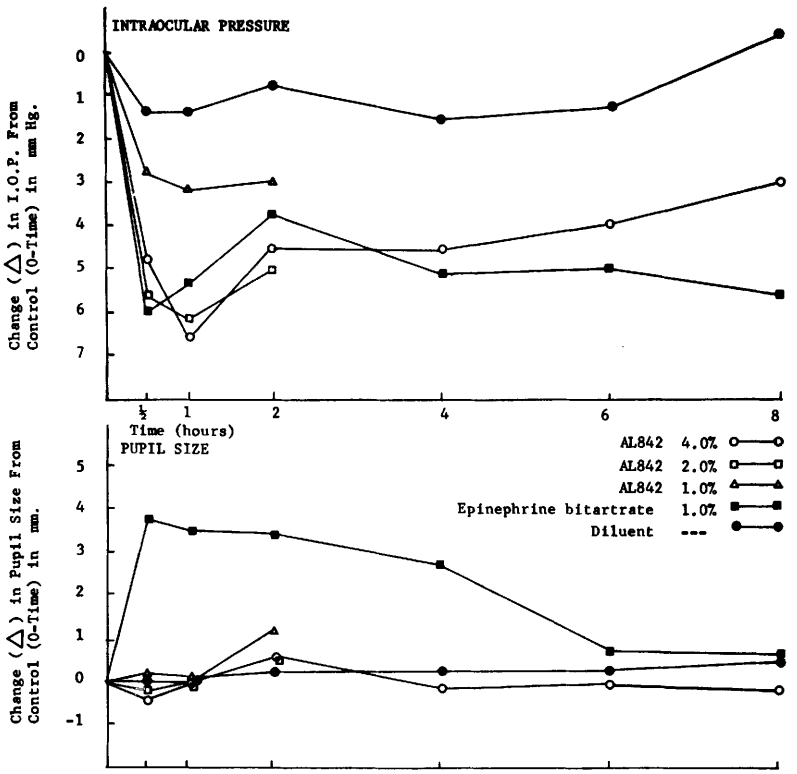

FIG. 1. Intraocular pressure and pupil size responses following topical administration of AL842, epinephrine, or vehicle alone to eyes of rhesus monkeys selected for high initial intraocular pressure.

measurements were made. Both eyes were used and treatment was randomized. Drugs were administered as 1 drop onto the cornea, and eyes were carefully "blinked." Pupillary and IOP measurements were made at 0.5, 1, and 2 hr for concentration response (Study I), and at 2, 4, 6, and 8 hr for duration (Study II).

Intraocular pressure measurements. A Posner-Inglima applanometer (Institute for Glaucoma Research, Inc.) equipped with a 5-g weight was used. Imprint diameter was converted to mm Hg by use of the Posner calibration scale (7–9).

Pupil size measurements. Prior to IOP measurements, a Castroviejo caliper (Storz Instruments) was placed directly over the pupil and diameter was measured to the nearest 0.5 mm.

Analysis of data. Data used in calculation of onset and duration of action of AL842, compared with a marketed antiglaucoma agent, Lyophrin, were the combined data collected during the concentration response study (Study I) and the duration study (Study II) in which changes in IOP were measured at 2, 4, 6, and 8 hr. Statistical significance was calculated by Student's t test (10) for the combined data from both studies ($p = 0.05$).

The following test formulations were employed in this study:

Reference standard—epinephrine bitartrate, Lyophrin 2% (approx 1% as base).

Negative control—Lyophrin diluent.

AL842 hydrochloride (4, 2, 1% base), in isotonic sodium chloride solution, with 0.01% benzalkonium chloride.

Results. Drug effect on intraocular pressure. Mean IOP responses (±SE) to log interval doses of AL842, to the reference standard, and to the vehicle control are shown in Table I. Mean IOP changes (Δ) from control ("0" time) are represented graphically in

Fig. 1. IOP was significantly decreased by epinephrine and by AL842 4% and 2% at 0.5, 1, and 2 hr when compared with vehicle. AL842 1% also decreased IOP at 0.5, 1, and 2 hr but the change was not significant. Equivalent activity was demonstrated by AL842 4%, 2%, and epinephrine relative to onset and intensity of the response through 2 hr. Duration of the IOP response to AL842 was studied through 8 hr. An equivalence in action of AL842 4% and the reference standard was noted through six hours. At eight hours, the reference standard was significantly lower than AL842 4%, although both differ statistically from the negative control. This observation apparently indicates a somewhat shorter duration of action for AL842 than that for epinephrine bitartrate.

Drug effect on pupil size. Mean pupillary responses to log interval doses of AL842, to epinephrine bitartrate, and to a negative control, epinephrine vehicle are tabulated in Table I. Changes (Δ) (mm) are depicted graphically in Fig. 1. Epinephrine bitartrate 2.0% (approx 1.0% as the base) produced a mydriatic effect lasting through 4 hr. AL842 produced no significant increase in pupil size at any of the concentrations tested ($p > 0.05$). These data indicate that concentrations of AL842 which will lower intraocular pressure produce no effect on the pupil size.

Comment. Use of the rhesus monkey as a test animal in which to study aqueous dynamics has been reported previously (11). Preliminary screening of 316 young adult rhesus monkeys (632 eyes) for IOP provided values approximating the classical "bell shaped" normal distribution curve. The mean IOP was 16.4 mm Hg and pressures in 65% of the eyes tested fell between 14 and 18 mm Hg. Of the animals screened, those found to have an IOP in one or both eyes of 21 mm Hg or more comprised 13.0% of the population. These were the animals chosen for testing. This information is provided to explain why the test eyes used in these studies do not necessarily demonstrate a pathologic ocular hypertension. Instead, they comprise that 13.0% of the normal population fitting the upper regions of normal distribution.

The compound AL842 was tested to determine dose–response and onset–duration relationships in the same test model. This was necessary because the test systems initially used to demonstrate potential ocular hypotensive activity of AL842 depended upon artificially-induced increases in IOP and could not be adapted to measure onset and duration of activity (2–4).

The first portion of the present study was designed to determine whether AL842 and/or an agent marketed for its clinical ocular hypotensive effects, epinephrine bitartrate, would be effective in lowering IOP in these animals. If an effect were noted, an estimation of onset of action could also be obtained. IOP readings were taken at "0" time and at 0.5, 1, and 2 hr following drug administration. AL842, (4%, 2% as the base) and epinephrine bitartrate (1% as the base) all reduced IOP by approximately 6 mm Hg at the time of peak effect resulting in pressures near the mean value previously obtained for 632 untreated eyes. Peak pressure effects were recorded at 1 hr following AL842 and at 0.5 hr following epinephrine bitartrate.

Pupil size was also measured at these times. No significant alteration of pupil size was observed following AL842 in any concentration or vehicle control while significant mydriasis occurred following treatment with epinephrine.

Pupil size and IOP data were combined with the responses recorded in the second portion of the study to provide estimate of onset and duration.

The second portion of the study was performed to compare duration of action of AL842 with epinephrine in this test model. AL842 4%, epinephrine 1%, and a negative control (vehicle) were evaluated in this portion of the study through 8 hr. AL842 and epinephrine were equivalent in ocular hypotensive activity through 6 hr. IOP values, in animals receiving epinephrine, however, were significantly lower at 8 hr than those in animals treated with AL842. This indicates a somewhat shorter duration of action for AL842 than that observed for epinephrine bitartrate following topical application of a single dose in this test system.

These studies have demonstrated that rhesus monkeys selected for high IOP may be used as test animals to determine ocular hy-

potensive effect and pupil size changes of potential therapeutic agents. Further, these data indicate that AL842 is an effective, nonmydriatic and nonmiotic hypotensive agent with IOP effects similar to epinephrine bitartrate in this test system when administered at approximately twice the concentration of the epinephrine used clinically. Results of this study are in agreement with the IOP and pupillary effects of AL842 and epinephrine HCl in rabbits, which have been reported (2).

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