

PRODUCT MONOGRAPH

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BETAGAN[®]

LEVOBUNOLOL HYDROCHLORIDE OPHTHALMIC SOLUTION 0.25% AND 0.5%

GLAUCOMA THERAPY

Noncardioselective beta-adrenoceptor blocking agent

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PRODUCT MONOGRAPH

NAME OF DRUG

BETAGAN^R 0.25% AND 0.5% OPHTHALMIC SOLUTIONS

(LEVOBUNOLOL HYDROCHLORIDE)

THERAPEUTIC CLASSIFICATION

GLAUCOMA THERAPY

NONCARDIOSELECTIVE BETA-ADRENOCEPTOR BLOCKING AGENT

ACTIONS, CLINICAL PHARMACOLOGY

Levobunolol HCl is a noncardioselective beta-adrenoceptor antagonist, equipotent at both beta₁ and beta₂ receptors. Levobunolol HCl is approximately 60 times more potent than the dextro isomer of bunolol in its beta-blocking activity, yet equipotent in its potential for direct myocardial depression. Accordingly, the levo isomer, levobunolol, is used. Levobunolol HCl does not have a significant local anesthetic (membrane stabilizing) effect or intrinsic sympathomimetic activity.

Beta-adrenergic receptor blockade reduces cardiac output in both healthy subjects and patients with heart disease. In patients with severe impairment of myocardial function, beta-adrenergic receptor blockade may inhibit the stimulatory effect of the sympathetic nervous system necessary to maintain adequate cardiac function.

Beta-adrenergic receptor blockade in the bronchi and bronchioles results in increased airway resistance from unopposed parasympathetic activity. Such an effect in patients with asthma or other bronchospastic conditions is potentially dangerous.

Betagan (levobunolol HCl), when instilled into the eye, will lower elevated intraocular pressure (IOP) as well as normal (IOP), whether or not accompanied by glaucoma. Elevated IOP is a major risk factor in the pathogenesis of glaucomatous visual field loss. The higher the level of intraocular pressure, the greater the likelihood of optic nerve damage and visual field loss.

The onset of action with one drop of Betagan can be detected within one hour after treatment, with maximum effect seen between two and six hours. A significant decrease in IOP can be maintained for up to 24 hours with once daily dosing of Betagan 0.5%.

Measurements of aqueous flow and total outflow facility suggest that levobunolol lowers IOP primarily by decreasing aqueous humor production. Betagan (levobunolol HCl) reduces IOP with little or no effect on pupil size or accommodation, in contrast to the miosis which cholinergic agents are known to produce. The blurred vision and night blindness often associated with miotics would not be expected. This is particularly important in patients with central lens opacities who would experience decreased visual acuity with pupillary constriction.

Betagan has been shown to be as effective as timolol in lowering intraocular pressure.

I. controlled clinical studies of up to two years duration, intraocular pressure was well-controlled in approximately 80% of subjects treated with Betagan 0.5% b.i.d. The mean IOP decreases from baseline were between 6.87 mm Hg and 7.81 mm Hg. No significant effects on pupil size, tear production or corneal Sensitivity were observed. Topically applied Betagan at concentrations of 0.5% and 1%, decreased heart rate and blood pressure in some patients. The IOP-lowering effect of Betagan was well-maintained over the course of these studies.

In a three-month controlled clinical study, once-daily application of Betagan 0.5% controlled the IOP of 72% of subjects, producing an overall mean decrease in IOP of 7.0 mm Hg. Once-daily application of timolol 0.5% controlled the IOP of 64% of subjects, producing a mean decrease of IOP of 4.5 mm Hg. The difference in overall mean decreases in IOP was statistically significant.

In two subsequent three-month trials comparing Betagan 0.5% with timolol 0.5% administered once daily, overall differences between the two drugs were not significant. A greater percentage of subjects in both the levobunolol groups and the timolol groups maintained adequately lowered intraocular pressure in the latter two studies, probably because subjects with severe ocular hypertension, unlikely to be controlled by therapy with a beta-blocker alone, were excluded from the study.

In one 3 month study and one 1 year study, Betagan 0.25% twice daily controlled the IOP of approximately 63% and 70% of the subjects, respectively. The overall mean decreases from baseline were 5.4 mm Hg and 5.1 mm Hg respectively.

In another three-month clinical study, the mean decrease in IOP was significantly greater (more than 2 mm Hg) in the 0.25% and 0.5% levobunolol twice daily treatment groups than in the betaxolol 0.5% twice-daily treatment group.

The prophylactic effect of topical 0.5% Betagan (levobunolol HCl) on IOP elevations after neodymium: YAG laser posterior capsulotomies was investigated in a controlled study. One drop was administered 30 to 120 minutes prior to the capsulotomy. Eight subjects (38%) in the vehicle treatment group and none in the levobunolol group experienced increases from baseline in IOP of 10 mm Hg or greater. Mean reductions in IOP from baseline ranged from 2.1-2.9 mm Hg in the levobunolol group, while in the vehicle treatment group, IOP increases (4.4-6.4 mm Hg) were observed at hours 1, 2, and 3 following capsulotomy.

In a controlled study, 0.5% Betagan (levobunolol HCl) or placebo were administered immediately after a unilateral extracapsular cataract extraction and implantation of a posterior chamber intraocular lens. Treatment continued on a once-daily basis for seven days. The incidence of IOP elevations from baseline ≥ 10 mm Hg was eight subjects (40%) in the vehicle group and four subjects (19%) in the levobunolol group. Mean IOP increased from baseline up to 8.6 mm Hg at 24 hours in the vehicle group and up to 2.0 mm Hg at 24 hours in the levobunolol group.

I. another controlled study, levobunolol 0.5% was significantly more effective than betaxolol 0.5% or placebo in preventing increased IOP after cataract extraction and posterior chamber lens placement. Two drops of the assigned medication were administered to the study eye after surgery. A significant mean increase in intraocular pressure from the preoperative to the early postoperative period was noted in the groups treated with betaxolol (6.73 mm Hg), placebo (5.35 mm Hg) and timolol (3.83 mm Hg). Levobunolol-treated eyes showed a mean decrease in pressure of 0.43 mm Hg.

An IOP of 30 mm Hg or greater was found in three placebo-treated eyes (15%), four betaxolol-treated eyes (20%), one timolol-treated eye (5%), and none of the levobunolol-treated eyes. Five placebo-treated eyes (25%), six betaxolol-treated eyes (30%), five timolol-treated eyes (25%), and one levobunolol-treated eye (5%) experienced a pressure rise of 10 mm Hg or greater.

INDICATIONS AND CLINICAL USES

Betagan (levobunolol HCl) 0.25% and 0.5% are indicated for the control of intraocular pressure in patients with chronic open-angle glaucoma or mild to moderate ocular hypertension.

CONTRAINDICATIONS

Betagan (levobunolol HCl) is contraindicated in those individuals with bronchial asthma or with a history of bronchial asthma, or severe chronic obstructive pulmonary disease; sinus bradycardia; second and third degree atrioventricular block; overt cardiac failure; cardiogenic shock; or hypersensitivity to any component of this product.

WARNINGS

As with other topically applied ophthalmic drugs, Betagan (levobunolol HCl) may be absorbed systemically. The same adverse reactions found with systemic administration of beta-adrenergic blocking agents may occur with topical administration.

Contains sodium metabisulfite, a sulfite that may cause allergic-type reactions including anaphylactic symptoms and life-threatening or less severe asthmatic episodes in certain susceptible people. The overall prevalence of sulfite sensitivity in the general population is unknown and probably low. Sulfite sensitivity is seen more frequently in asthmatic than in nonasthmatic people.

Keep out of reach of children. For external use only. Do not touch dropper tip to any surface, since this may contaminate the solution. Protect from light and excessive heat. Discard any unused solution after end of treatment period.

PRECAUTIONS

General: Betagan (levobunolol HCl) should be used with caution in patients with known contraindications to systemic use of beta-adrenoceptor blocking agents. These include abnormally low heart rate and heart block more severe than first degree. Congestive heart failure should be adequately controlled before beginning therapy with Betagan. In patients with a history of cardiac disease, especially arrhythmia and bradycardia, pulse rates should be monitored.

Betagan should be used with caution in patients with known hypersensitivity to other beta-adrenoceptor blocking agents.

Use with caution in patients with known diminished pulmonary function.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Systemic beta-blockers and topical timolol maleate are known to be excreted in human milk. Caution should be exercised when Betagan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

Drug Interactions: Betagan may have additive effects in patients taking systemic anti-hypertensive drugs. These possible additive effects may include hypotension, including orthostatic hypotension, bradycardia, dizziness, and/or syncope. Conversely, systemic beta-adrenoceptor blocking agents may potentiate the ocular hypotensive effect of Betagan.

Close observation of the patient is recommended when a beta-blocker is administered to patients receiving catecholamine-depleting drugs such as reserpine, because of possible additive effects and the production of hypotension and/or marked bradycardia, which may produce vertigo, syncope, or postural hypotension.

ADVERSE REACTIONS

Transient burning, stinging or itching, blepharoconjunctivitis and decreases in heart rate and blood pressure have been reported occasionally with the use of Betagan (levobunolol HCl). Iridocyclitis, headache, transient ataxia, dizziness, lethargy, urticaria and pruritus have been reported rarely with the use of Betagan. Decreased corneal sensitivity has been noted in a small number of patients. The following additional adverse reactions have been reported with ophthalmic use of beta₁ and beta₂ (non-selective) adrenergic receptor blocking agents:

BODY AS A WHOLE: Headache.

CARDIOVASCULAR: Arrhythmia, syncope, heart block, cerebral vascular accident, cerebral ischemia, congestive heart failure, palpitation.

DIGESTIVE: Nausea.

PSYCHIATRIC: Depression.

SKIN: Hypersensitivity, including localized and generalized rash.

RESPIRATORY: Bronchospasm (predominantly in patients with pre-existing bronchospastic disease), respiratory failure.

ENDOCRINE: Masked symptoms of hypoglycaemia in insulin-dependent diabetics.

SPECIAL SENSES: Signs and symptoms of keratitis, blepharoptosis, visual disturbances including refractive changes (due to withdrawal of miotic therapy in some cases), diplopia, ptosis.

Other reactions associated with the oral use of non-selective adrenergic receptor blocking agents should be considered potential effects with ophthalmic use of these agents.

SYMPTOMS AND TREATMENT OF OVERDOSAGE

Overdose has not been reported to date. Should accidental ocular overdosage occur, flush eye(s) with water or normal saline. If accidentally ingested, efforts to decrease further absorption may be appropriate (gastric lavage). The most common signs and symptoms to be expected with overdosage of a systemic beta-adrenergic blocking agent are symptomatic bradycardia, hypotension, bronchospasm, and acute cardiac failure. Should these symptoms occur, discontinue Betagan therapy and initiate appropriate supportive therapy.

DOSAGE AND ADMINISTRATION

The recommended starting dose is one drop of Betagan 0.25% twice a day in the affected eye(s). If the clinical response is not adequate, the dosage may be changed to one drop of Betagan 0.5% twice a day in the affected eye(s). Betagan 0.5% once a day has been found to be effective in controlling IOP in many patients with mild to moderate open-angle glaucoma and ocular hypertension. As with any new medication, careful monitoring of patients is advised.

Dosages above one drop of Betagan 0.5% b.i.d. are not generally more effective. If the patient's IOP is not at a satisfactory level on this regimen, concomitant therapy with dipivefrin and/or epinephrine, and/or pilocarpine and other miotics, and/or systemically administered carbonic anhydrase inhibitors, such as acetazolamide, can be instituted.

PHARMACEUTICAL INFORMATION

DRUG SUBSTANCE

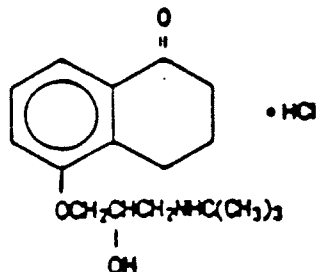
Nonproprietary
Name (USAN):

Levobunolol hydrochloride

Chemical Name:

l-5-(3-(tert-butylamino)-2-hydroxypropoxy)-3,4-dihydro-1(2H)naphthalenone hydrochloride

Structural Formula:



Chemical Formula:

$C_{17}H_{25}NO_3 \cdot HCl$

Molecular Weight:

327.9

Solubility:

(25°C)

Distilled water: > 300 mg/ml

Absolute ethanol: 24 mg/ml

Physical Characteristics:

Fine, white to off-white crystalline powder; odourless; bitter in taste

COMPOSITION

Each millilitre contains levobunolol hydrochloride 2.5 mg or 5 mg, with the following non-medicinal ingredients: benzalkonium chloride 0.0040% as the preservative and edetate disodium; polyvinyl alcohol; potassium phosphate, monobasic; sodium chloride; sodium metabisulfite; sodium phosphate, dibasic, sodium hydroxide or hydrochloric acid to adjust pH.

STABILITY AND STORAGE RECOMMENDATIONS:

Protect from light and excessive heat.

AVAILABILITY OF DOSAGE FORMS

Betagan (levobunolol hydrochloride) ophthalmic solution is supplied on prescription only in a concentration of 0.5% in plastic dropper bottles containing 3 ml (hospital only), 5 ml, 10 ml, and 15 ml, and in a concentration of 0.25% in plastic dropper bottles containing 5 ml, 10 ml, and 15 ml.

PRECLINICAL PHARMACOLOGY

PRIMARY ACTIONS

Ocular Beta-Adrenoceptor Antagonist Activity: The β -adrenergic blocking properties of levobunolol, timolol, and propranolol were assessed in rabbits by determining the topical concentration necessary to block the ocular hypotensive effect of topical 0.5% epinephrine. The IC_{50} (concentration producing 50% inhibition) for topically applied levobunolol was approximately $1 \times 10^{-4}\%$, which was less potent than timolol ($IC_{50} \sim 3 \times 10^{-5}\%$) and approximately 300 times more potent than propranolol ($IC_{50} \sim 3 \times 10^{-2}\%$).

Dihydrolevobunolol, an active ocular metabolite of levobunolol in rabbits, possesses a topical beta-blocker efficacy similar to levobunolol in a rabbit isoproterenol-challenge model.^{1,2}

SECONDARY ACTIONS

Corneal Anesthetic Effects: Topically applied levobunolol (0.03% to 10%) was tested for its acute corneal anesthetic activity in female New Zealand albino rabbits. Peak anesthetic activity occurred 5 to 10 minutes following drug instillation and lasted 15 to 20 minutes. Five minutes after drug administration, the EC_{50} for inducing corneal local anesthesia was 7.8% (95% confidence limits of 3% and 20%). Levobunolol was weakly anesthetic--approximately one-tenth as potent as propranolol and one-hundredth as potent as proparacaine. Timolol (10%) had no effect on corneal sensitivity in this test.

Local anesthetic effects were also assessed in chronically treated rabbits that had received topical levobunolol (0.5%, 1%, or 5%) twice daily for one year. At an examination after one year of treatment, a full blink response was obtained in all rabbits tested, indicating the absence of residual corneal anesthesia. After two additional treatments with topical levobunolol, corneal anesthesia was observed 5 to 10 minutes post-instillation, lasting 15 to 20 minutes. Five minutes after drug instillation, the EC_{50} for local anesthesia was 1.7%. With 95% confidence limits of 0.6% and 4.9% the EC_{50} for chronically treated rabbits was not significantly different from the ED_{50} for acutely treated rabbits.

C Cardiovascular Beta-Adrenoceptor Antagonist Activity

Potency: The ability of intravenously and orally administered levobunolol, racemic bunolol, and propranolol to antagonize isoproterenol-induced tachycardia has been studied in anesthetized, vagotomized dogs.³ Intravenous ED₅₀ values were 2.8 µg/kg for levobunolol, 8 µg/kg for racemic bunolol, and 16 µg/kg for propranolol. Thus, levobunolol was nearly six times more potent than propranolol and three times more potent than racemic bunolol when administered intravenously. Orally, levobunolol was 49 times more potent than propranolol and more than twice as potent as racemic bunolol .

Duration: The duration of beta-blockade following oral administration of equiactive doses of levobunolol and propranolol to conscious dogs were compared in isoproterenol-induced and treadmill exercise-induced tachycardia.⁴ The duration of beta-blockade was greater with levobunolol in both models. The time to 50% recovery of the exercise tachycardia was 24 hours for levobunolol and 6 to 9 hours for propranolol.

Affinity: The affinity of levobunolol for β_1 - and β_2 -adrenoceptors was determined in vitro with a competitive radioligand binding assay.⁵ Like propranolol, levobunolol had nearly equal affinity for β_1 and β_2 receptors.

Intrinsic Beta-Sympathomimetic Activity, Racemic Bunolol: Racemic bunolol, popranolol and pronethalol were evaluated for intrinsic beta-sympathomimetic activity in anesthetized, vagotomized dogs pretreated with reserpine to suppress endogenous sympathetic activity. Racemic bunolol and propranolol caused minor decreases in isoproterenol-induced tachycardia, while pronethalol caused an increase. The investigators concluded that racemic bunolol, like propranolol but unlike pronethalol, is devoid of intrinsic beta-sympathomimetic activity.³

Direct Myocardial Depressant Activity, Various Isomers: In a study of direct myocardial depressant activity, racemic bunolol, levobunolol, and dextrobunolol were administered to vagotomized, reserpinized dogs, in whom an increase in myocardial contractile force had been induced by isoproterenol.⁶ All three compounds were equally effective and potent in reducing contractile force, indicating a lack of stereospecificity in myocardial depressant effect.

Levobunolol was more potent than racemic bunolol as a beta-blocking agent but similar in its myocardial depressant effect. Thus, levobunolol provides a wide separation between the dose that produces beta-blockade and the dose that products direct myocardial depression.

Antiarrhythmic and Antianginal Activity, Racemic Bunolol: In anesthetized dogs, racemic bunolol antagonized isoproterenol-induced arrhythmias. However, it was comparatively ineffective in converting ouabain-induced arrhythmias due to its lack of membrane stabilizing effect.⁷ In the anesthetized dog, racemic bunolol administered intravenously was similar to propranolol and nitroglycerin in increasing endocardial oxygenation.⁸

Pulmonary Resistance Effects, Racemic Bunolol: Racemic bunolol, propranolol, and alprenolol were intravenously administered to anesthetized dogs and were evaluated for their effects on pulmonary resistance.⁹ Propranolol (0.1 mg/kg) increased pulmonary resistance, bunolol (6.4 mg/kg) had no effect, and alprenolol (0.4 mg/kg) decreased resistance. Following histamine- or pilocarpine-induced bronchoconstriction, both bunolol and propranolol potentiated the increase in pulmonary resistance. Alprenolol decreased or had no effect on resistance. All three agents antagonized isoproterenol-induced bronchodilation.

PRECLINICAL METABOLISM AND PHARMACOKINETICS

Ocular Absorption: In a study in which a single 50 µl dose of radiolabeled racemic bunolol formulated as a 0.5% solution was topically administered to female New Zealand albino rabbit eyes, high levels of radioactivity were observed in all the tissues of the anterior segment with highest levels observed in the cornea and iris. Radioactivity was first observed 15 minutes after instillation and was maximal in most tissues at 30 minutes. The half-life of the labelled compound was approximately 60 to 90 minutes in the aqueous humor, cornea, iris, and ciliary body. The higher concentrations observed in the iris and ciliary body suggest that the drug may bind to receptor sites in these tissues to exert its effect on intraocular pressure.¹⁰

Ocular Metabolism: The ocular metabolism of bunolol was investigated in a second study in which a single 50 µl dose of radiolabeled racemic bunolol 0.5% was topically administered to rabbit eyes. Dihydrobunolol was detected in the aqueous humor 15 minutes following dosing and represented 39% of total radioactivity. The concentration of dihydrobunolol increased with time reaching a maximum level of 90% of the total radioactivity in the aqueous humor.¹⁰

Instilled topically into the eyes of rabbits, most of the levobunolol applied is converted to dihydrolevobunolol within the anterior segment within the first four hours after dosing.¹⁰

Ocular Accumulation: A subsequent study investigated the extent of accumulation of radiolabeled racemic bunolol 0.5% in ocular tissue and fluid during four days of twice-daily topical administration to both eyes of rabbits. No significant drug accumulation was observed in any of the tissues or fluid examined.¹⁰

Excretion after Ocular Administration: The excretion of topically instilled levobunolol containing radiolabeled racemic bunolol was investigated over a five day period following a single topical 50 µl dose of the 0.5% solution in six female New Zealand rabbits (2 to 3 kg). Most of the radiolabeled bunolol was excreted in the urine. Peak concentrations of radioactivity were reached at 24 hours in the urine sample and at 48 hours in the fecal sample, with more than 92% of the radioactivity recovered in urine and feces by the fifth day.¹⁰

Systemic Absorption and Excretion: The high percentage of renally excreted products indicates that levobunolol is well-absorbed following oral administration by all species. Following oral administration of ¹⁴C or ³H labelled bunolol, 85% of the administered radioactivity was found in the urine of mice in two days (74% in the first eight hours). In rats, 60% was excreted within four days.

In dogs, 54-75% was found within 72 hours and in humans, 78% was found within four days.^{11,12}

Systemic Metabolism: Studies on bunolol metabolism were performed in mice, rats and humans using levobunolol-³H and also in dogs using bunolol-¹⁴C. Several metabolites were isolated from urine. The elucidation of structure was done using mass spectrometry. Some of the metabolites were identified by means of co-chromatography with authentic substances.

The principal routes of biotransformation were:

1. Reduction of dihydrolevobunol.
2. Oxidation of the side chain.
3. Hydroxylation of the naphthalenone ring system.
4. Conjugation of parent compound and metabolites.

The mechanisms of biotransformation varied among the species tested. Hydroxylation of the ring system with subsequent conjugation with glucuronic acid dominated in mice and rats. Similar oxidative changes in the side chain and in the ring system occurred in dogs. In humans, oxidative processes were less evident. Urinary excretion was largely accounted for by unchanged levobunolol, dihydrolevobunolol and their conjugates.¹²

Active substances in blood and their kinetics: Dihydrolevobunolol is the main metabolite of levobunolol and has similar biological activity. It is produced primarily in the erythrocytes and in the liver.^{13,14} Its concentration in blood is only slightly lower than that of levobunolol. These two substances are eliminated at near equal rates from the blood. In rats and dogs, half-lives of less than 2 hours were determined; while in humans, the drug had an apparent half-life of 5-8 hours.

Elimination: In all animal species examined, levobunolol is eliminated primarily in urine following extensive metabolic transformation. A moderate amount of biliary elimination and evidence of enteroheptic circulation was noted in rats, and was also suggested in dogs and humans.

Unchanged levobunolol and dihydrolevobunolol were found only in small amounts in the urine of mice, rats and dogs. Greater amounts of both substances were excreted by the kidney in humans in comparison to animals.¹⁵

HUMAN PHARMACODYNAMICS

DOSE RESPONSE STUDIES

The onset, magnitude, and duration of ocular hypotensive effect was evaluated in a double-blinded, randomized, parallel study in 49 subjects (22 male and 27 female) with ocular hypertension (intraocular pressure > 23 mm Hg).¹⁶ Mean ages in each dosage group ranged from 49 to 62 years. Prior to study entry, subjects using ocular hypotensive medication were required to undergo a washout period. They each received one of five concentrations of levobunolol (0.03%, 0.3%, 0.6%, 1%, or 2%) or placebo (vehicle). A single 50 µl drop of the test medication was administered to one randomly assigned eye and a drop of placebo to the fellow eye.

In this preliminary evaluation, levobunolol was shown to be an effective ocular hypotensive agent. The ocular hypotensive effect in ocular hypertensive subjects was evident within the first hour after drug instillation and lasted for 24 hours. Maximal hypotensive effect (> 10 mm Hg) occurred between 2 and 12 hours post-instillation and was dose-related, with the 1% dosage producing slightly greater decreases than the 2% dosage, up to 12.0 mm Hg mean decrease at hour 6. The mean decrease in the 1% group at 24 hours was 6.7 mm Hg. Significant reductions from baseline in IOP were seen in the 0.3% and 0.6% groups at one, two, and four hours after administration. The 1% and 2% concentrations of levobunolol produced significant decreases in heart rate during the first two hours following drug administration (up to a mean decrease of 10.8 bpm in the 2% group at two hours, $p = 0.04$).

At hour 2, mean systolic blood pressure was slightly decreased in the group of subjects receiving 2% levobunolol (10.1 mm Hg, $p = 0.02$).

The ocular hypotensive response to 0.5% levobunolol, 1% levobunolol, 0.5% timolol, and placebo was investigated in a double-masked, randomized parallel study of 24 subjects with ocular hypertension or chronic open-angle glaucoma.¹⁷ After a washout of ocular hypotensive medication, the subjects received a single drop of test medication in one eye.

The onset of effect for both medications occurred within the first hour, and intraocular pressure decreases were observed up to 24 hours later. Maximal pressure decreases occurred two to four hours after drug administration and were 8.3 mm Hg for 0.5% levobunolol, 6.8 mm Hg for 1% levobunolol, and 7 mm Hg for 0.5% timolol. Significant decreases in heart rate were observed during the first four hours after treatment in all treatment groups. Significant diastolic blood pressure decreases were observed after treatment with 0.5% levobunolol and placebo, but not after treatment with 1% levobunolol and 0.5% timolol.

MECHANISM OF OCULAR HYPOTENSIVE ACTION

The mechanism of the hypotensive effect of levobunolol in the human eye was evaluated in a double-masked study of 18 ocular hypertensive subjects treated with one 50 μ l drop of 0.5% levobunolol in one randomly selected eye and with vehicle in the other eye.¹⁸ Aqueous flow was measured by fluorophotometry and total outflow facility by tonography. Analysis showed a decrease of up to 35% in aqueous flow (with a mean decrease of 29%), a decrease of 36% in IOP, and no effect on either true outflow facility, episcleral venous pressure, or uveoscleral outflow in the levobunolol-treated eyes. The results of this study suggest that levobunolol lowers IOP by decreasing aqueous humor production.

CONTROL OF CHRONIC ELEVATED IOP

0.5% Betagan (Levobunolol HCl) Administered Twice Daily

Data were analyzed for up to two years of treatment in three long-term, multi-center controlled clinical trials comparing the efficacy and safety of topical levobunolol (0.5% and 1%) and timolol (0.5%).¹⁹ Subjects had chronic open-angle glaucoma or ocular hypertension, with untreated IOP of 23 mm Hg or higher. In all three studies, a single drop of the medications was instilled twice daily in both eyes after a suitable washout of other ocular hypotensive medication.

The ocular hypotensive effects of the three treatments were similar; no significant differences were seen among the groups in the magnitude of IOP reductions or the percentage of subjects with adequately controlled IOP. In all three studies, mean IOP reductions at study visits were in the -6 to -8 mm Hg range for both levobunolol groups, with mean decreases from baseline over 24 months of 27.0% in the 0.5% levobunolol group, 27.3% in the 1% levobunolol group, and 26.3% in the 0.5% timolol group. Approximately 17% (65 of 391) of the subjects were terminated from all treatment groups because of inadequately controlled IOP (22 subjects treated with 0.5% levobunolol, 20 treated with 1% levobunolol, and 23 treated with timolol). A total of 59 subjects with inadequately controlled IOP were continued in the studies following administration of a second antiglaucoma drug (pilocarpine or dipivefrin); of these subjects, 22 had been treated with 0.5% timolol, 19 with 0.5% levobunolol, and 18 with 1.0% levobunolol.

In all three long-term studies, statistically significant decreases in mean heart rate were observed in all treatment groups. Significant among-group differences were noted at four of 13 visits over the two-year study period, with greater decreases observed in one or both levobunolol groups. However, mean heart rate decreases in the levobunolol groups did not become greater, but rather, heart rate decreases in the timolol group were reduced. The effect on blood pressure for all groups was minimal and of limited clinical significance.

0.5 Betagan Administered Once Daily

Once-daily and twice-daily administration of 0.5% Betagan were compared in a controlled clinical trial.²⁰ Subjects had a mild to moderate degree of glaucoma or ocular hypertension (untreated IOP of 22 to 30 mm Hg). Seventy-one subjects were included in the study analysis. Decreases in IOP were slightly greater with twice-daily administration but not statistically significantly different ($p = 0.059$). Overall mean decreases in IOP were 4.5 mm Hg in the once-daily group and 5.6 mm Hg in the twice-daily group over the three month study period. While ocular and systemic safety of the two dosing regimens did not differ substantially, greater decreases in heart rate were observed in the twice-daily group than in the once-daily group. This difference may have been influenced by the difference in time between the last dosage and heart rate measurements in the two treatment groups (12 hours vs. 24 hours).

The efficacy and safety of once-daily administration of levobunolol (0.5% and 1.0%) was compared to timolol (0.5%) in a three-month study.²¹ This study was a multi-center, randomized double-masked parallel trial in subjects with chronic open-angle glaucoma or ocular hypertension characterized by an untreated IOP of 23 mm Hg or higher.

Seventy-two percent (18 of 25) subjects treated with levobunolol 0.5%, 79% (22 of 28) subjects treated with 1% levobunolol and 64% (16 of 25) of the timolol-treated subjects successfully completed the study with adequately controlled IOP. Four subjects in each of the levobunolol groups and seven in the timolol group were dropped from the study because of inadequately controlled IOP. Most of the cases of inadequate control of IOP were evident within the first month. Inadequate control of intraocular pressure with once-daily treatment was more likely to occur in subjects who had previously used two or more medications to reduce IOP.

Three of 30 subjects in the 0.5% timolol group, eight of 30 subjects in the 0.5% levobunolol group, and seven of 32 subjects in the 1% levobunolol group had decreases in heart rate $\geq 15\%$ at some follow-up visit. The effect of either concentration of levobunolol on blood pressure in this study was minimal.

The overall mean decrease in IOP was significantly greater in the levobunolol groups (6.5 mm Hg with 1% levobunolol to 7.0 mm Hg with 0.5% levobunolol) than in the timolol group (4.5 mm Hg).

In a double-masked, randomized, parallel three-month study,²² the efficacy of 0.5% levobunolol administered once-daily was compared with 0.5% timolol once-daily in 69 subjects with ocular hypertension (IOP of 22 mm Hg or greater). Subjects previously treated with multiple glaucoma medications, and those whose IOP was unlikely to be controlled by a single medication were excluded from the study.

Of the total number of subjects included in the statistical analysis (69 patients), eighty-eight percent (30 of 34) of subjects in the levobunolol group and ninety-four percent (33 of 35) of the

Subjects in the timolol group successfully completed the study. Three levobunolol-treated subjects and two timolol-treated subjects were terminated because their IOP was not adequately controlled with the study medication. From mean baseline IOP, the mean overall decreases were 4.8 mm Hg in the timolol group and 5.3 mm Hg in the levobunolol group. The two groups did not differ significantly in either the control rate or in average intraocular pressure reduction except at week 8, when the mean reduction was significantly greater in the levobunolol group.

Mean heart rate at baseline was similar in both treatment groups. There was an overall mean increase in heart rate in the levobunolol treatment group of 0.4 bpm and an overall mean decrease of 0.3 bpm in the timolol treatment group. Five subjects (15%) in the levobunolol treatment group and seven subjects in the timolol treatment group (20%) experienced decreases in heart rate $\geq 15\%$. There were no subjects with heart rates of 55 bpm or less at any follow-up visit.

Both levobunolol 0.5% and timolol 0.5% adequately controlled IOP in this population of subjects with mild to moderate ocular hypertension or open-angle glaucoma. Systemic effects were generally less pronounced than those seen in long-term studies with twice-daily administration of these drugs. In contrast with the previous study, reductions in IOP were not significantly greater with levobunolol. The exclusion of subjects with ocular hypertension unlikely to be controlled by a single medication in this study may have contributed to the lack of significant differences in efficacy between the two drugs.

A study designed identically to the one described above was conducted to compare the ocular hypotensive efficacy and safety of topical levobunolol 0.5% with topical 0.5% administered once-daily.²³ Of the 91 subjects enrolled, 36 of 42 (78%) subjects in the levobunolol treatment group and 40 of the 43 (89%) in the timolol treatment group successfully completed the study. Three subjects in the 0.5% timolol group and six subjects in the 0.5% levobunolol group were terminated from the study due to lack of efficacy. The overall mean decrease in IOP was 5.6 mm Hg (22.8%) in the levobunolol treatment group and 6.7 mm Hg (25.9%) in the timolol treatment group.

There was an overall mean decrease in heart rate of 0.6 bpm in the levobunolol treatment group and 0.7 bpm in the timolol treatment group. Ten subjects (22%) in the levobunolol treatment group and six subjects (13%) in the timolol treatment group experienced decreases in heart rate $\geq 15\%$ from baseline. In this study, there were no significant differences, with respect to safety and efficacy, between levobunolol 0.5% and timolol 0.5% administered once-daily. These results confirm those seen in the study described previously.

Trials with 0.25% Betagan

A three-month double-masked, randomized, parallel titration study²⁴ was designed to determine the effective dose of topically applied levobunolol or timolol necessary to control elevated IOP. Fifty-six subjects with chronic open-angle glaucoma or ocular-hypotension were entered into the study. Subjects had pre-treatment IOP's of 23 mm Hg or greater. Three concentrations each of levobunolol (0.25%, 0.5%, and 1%) and timolol (0.125%, 0.25%, and 0.5%) were evaluated. Following an appropriate washout period without ocular hypotensive medication, one drop was administered twice daily to both eyes starting with the lowest of the three concentrations of either levobunolol or timolol. If IOP was controlled with lowest concentration during the three-month period, the subject was considered to have successfully completed the study. If IOP was inadequately controlled, treatment was increased to the intermediate dose. If still inadequately controlled during the following three-month period, the treatment was increased to the highest dose.

Data from twenty-five subjects in the levobunolol treatment group and 26 subjects in the timolol treatment were analyzed. The mean age of subjects in the levobunolol group, 65.5 years, was greater than that of the timolol group, 58.3 years. This difference approached statistical significance ($p = 0.065$). The incidence of systemic hypertension in the levobunolol group, 32% (8/25), was slightly greater than that in the timolol group, 19% (5/26). No other notable differences were observed among treatment groups in any demographic or medical history variable.

Intraocular pressure was controlled in approximately 63% (15 of 24) of the subjects treated with the lowest dose of levobunolol (0.25%) and 69% (18 of 26) with the lowest dose of timolol (0.125%). Of the remaining subjects whose medications were titrated to higher doses, 33% (3 of 9) had adequately controlled IOP with the intermediate or highest concentrations of levobunolol, and 25% (1 of 4) had adequately controlled IOP with the intermediate or highest concentrations of timolol. The inability to control IOP at any given concentration became apparent during the first month of treatment with that concentration. Mean IOP reductions at follow-up visits ranged from 5.5 to 8.3 mm Hg in the levobunolol group at the lowest concentration tested and from 6.3 to 7.5 mm Hg in the timolol group at the lowest concentration tested. Overall mean decreases (estimated from repeated measures analysis of variance) were 5.4 mm Hg for the levobunolol group and 6.8 mm Hg for the timolol group. Between-group differences were not significant.

Cardiovascular effects were analyzed only for subjects receiving the lowest dosages of levobunolol or timolol because of the small number of subjects treated with the higher concentrations. Both drugs produced reductions in heart rate and blood pressure. The reductions in heart rate with levobunolol 0.25% were significantly greater than the reductions with timolol 0.125% at four of nine return visits. Mean decreased from baseline of up to 11.2 bpm were seen in the levobunolol group. During treatment, two subjects in

t. levobunolol group were diagnosed as having sinus brachycardia during electrocardiography.

In the subjects in which the intermediate and high dosages were used, a relatively small percentage (31%) completed the study period with adequately controlled IOP. Thus, it appears that in a majority of cases, a subject will either adequately respond to a low concentration of a beta-antagonist or will not adequately respond to higher concentrations. In this study, the adequacy of each of the drug concentrations could be ascertained within a month. Therefore, trying the higher concentrations may be of value for some individuals before adding additional drugs to the treatment regimen.

The apparent difference between the effect of levobunolol and timolol heart rate in this study may be related to (a) the greater mean age and incidence of systemic hypertension at baseline in levobunolol-treated subjects, as older and hypotensive subjects may be more susceptible to cardiovascular side effects of topical beta-adrenoceptor antagonists, (b) the study design, in which the concentration of levobunolol used was twice that of timolol during any given period, or (c) a differential effect of topical levobunolol and timolol on heart rate. The effect of levobunolol on blood pressure in all treatment groups in this study was minimal and of limited clinical significance.

The efficacy and safety of 0.25% levobunolol was evaluated in a two-phase titration, double-masked parallel clinical trial with random assignment of subjects to treatment groups.²⁵ To be included in the study, subjects had to exhibit chronic open-angle glaucoma or ocular hypertension with untreated intraocular pressure of 22 mm Hg or greater in at least one eye.

All subjects started the study on the 0.25% concentration of the study medication (either 0.25% levobunolol or 0.25% timolol), instilled as a single drop twice daily. If a subject's IOP was successfully controlled with 0.25% concentration of the assigned study medication, this treatment was continued for twelve months (Phase I). If at any time, a subject's IOP was not successfully controlled on the 0.25% concentration, the subject's medication regimen was titrated up to the 0.5% concentration of the same medication (either levobunolol or timolol). Treatment with the higher concentration was then followed for an additional three months (Phase II).

Phase I Study Results: Data from 78 subjects with open-angle glaucoma or ocular hypertension were analyzed. At all follow-up visits, there was a significant decrease from baseline in mean IOP in the 0.25% levobunolol group. Similar decreases in mean IOP were seen in the 0.25% timolol group. Overall decreases in IOP of 4.6 mm Hg in the 0.25% timolol group and 5.1 mm Hg in the 0.25% levobunolol group were found. No significant difference was observed in the efficacy of the two treatments. Seventy percent (26/37) of the 0.25% levobunolol-treated subjects and 71% (29/41) of the 0.25% timolol-treated subjects successfully completed the one year study period with adequately controlled IOP.

Phase II Study Results: The dosage in 13 of the 78 subjects (9 timolol and 4 levobunolol) was titrated up to the 0.5% concentrations of their assigned study medication. Of these subjects, 8/9 in the timolol group and 3/4 in the levobunolol group successfully completed the Phase II study period with adequately controlled IOP. In both the levobunolol and timolol treatment groups, decreases in heart rate and blood pressure were observed in some subjects. Statistically significant decreases in mean heart rate of 3.6 beats per minute (bpm) at week 1 and of 4.6 bpm at week 24 were observed within the levobunolol group. No significant between-group difference was observed at any visit.

The higher proportion of subjects who maintained adequate control of IOP in this study as compared with the study described previously was probably due to the exclusion of prospective subjects who had previously required two or more drugs to maintain adequately controlled IOP. Of the 13 subjects who required titration of the 0.5% dosages of levobunolol or timolol, 11 successfully completed the study with successfully controlled IOP. This indicates that the 0.5% dosage may be useful in some patients for whom the 0.25% concentration of either drug is not sufficient. However, conclusions about the relative efficacy of the two dosage forms are difficult to make, due to the limited number of subjects treated with the higher dosages.

In a three month controlled study,²⁶ the ocular hypotensive efficacy and safety of twice-daily administration of 0.25% and 0.5% levobunolol compared with 0.5% betaxolol were evaluated in 85 subjects with open-angle glaucoma or ocular hypertension.

Of the 85 subjects analyzed, 72 successfully completed the study with adequately controlled IOP. Six subjects were terminated due to adverse reactions including: two in the betaxolol group (one due to shortness of breath and excessive ocular burning, and the other due to multiple ocular problems); one subject in the levobunolol 0.25% group (due to shortness of breath); and three subjects in the levobunolol 0.5% group (one due to libido problems and headaches, one due to stinging and burning and one due to tachycardia, shortness of breath and heart palpitations). One subject in each group was terminated due to inadequate control of IOP. Four subjects were discontinued due to reasons unrelated to the study medication.

Approximately 89% (24/27) of subjects in the 0.25% levobunolol treatment group, 83% (25/30) of subjects in the 0.5% levobunolol treatment group, and 82% (23/28) of subjects in the 0.5% betaxolol group successfully completed the three-month study period.

The overall mean decreases in IOP was 6.2 mm Hg in the 0.25% levobunolol treatment group, 6.0 mm Hg in the 0.5% levobunolol treatment group, and 3.7 mm Hg in the 0.5% betaxolol treatment group. The overall mean decrease from baseline in both levobunolol groups was significantly greater than in the betaxolol group.

There were no statistically significant differences among the three treatment groups with respect to the evaluations of heart rate or

blood pressure during the study period. For all treatment groups, effects on mean heart rate and mean blood pressure were minimal. Overall mean decreases from baseline in heart rate were 2.67 bpm with 0.5% betaxolol, 1.73 bpm with 0.25% levobunolol and 4.74 bpm with 0.5% levobunolol.

Analysis of overall mean IOP changes from baseline indicated that 0.25% levobunolol and 0.5% levobunolol were more effective in reducing IOP than 0.5% betaxolol at the three-month analysis. A larger percentage of subjects with clinically significant decreases from baseline were seen in the 0.5% levobunolol group than in the other two treatment groups. The effects of betaxolol and levobunolol on mean systemic blood pressure were small and of limited clinical importance. The results of this study indicate that levobunolol is more effective than betaxolol in lowering IOP and equally safe in this patient population with mild to moderate ocular hypertension.

In an open-labelled study of once-daily dosing,²⁷ all subjects started the study using levobunolol 0.25% as a single 50 µl drop in each eye once-daily (Phase I of the study). If a subject's IOP was not successfully controlled on the 0.25% concentration, the concentration of levobunolol increased up to 0.5% (Phase II of the study). The frequency of instillation during Phase II remained once-daily.

Twenty-one (72%) of the 29 subjects were successfully treated with levobunolol 0.25% administered once-daily for the three-month study period. Six of the 29 subjects evaluated (21%) required titration from 0.25% concentration of levobunolol to the 0.5% concentration because of inadequately controlled IOP. These six subjects had varied prior treatment histories: three subjects had previously been treated with two anti-glaucoma medications (timolol and dipivefrin or pilocarpine); two subjects had been treated with timolol 0.5% twice-daily; one subject had not previously been treated with anti-glaucoma medication. The IOP of one subject (previously treated with timolol) was subsequently controlled with 0.5% levobunolol and the subject successfully completed the three-months under Phase II. The IOP of the other five subjects was not controlled on the higher concentration of levobunolol and these subjects were terminated from the study.

At follow-up visits under Phase I, mean decreases in IOP from baseline were 5.3 mm (21.1%) to 7.2 mm Hg (26.8%). The overall mean decrease in IOP was 5.9 mm Hg (23.6%). At follow-up visits under Phase II, mean decreases from baseline of 3.3 to 5.0 mm Hg were seen at follow-up visits. The overall mean decrease in Phase II was 3.4 mm Hg.

Prevention of Post-Surgical IOP Spikes

The prophylactic effect of topical 0.5% Betagan (levobunolol HCl) on intraocular pressure (IOP) elevations after neodymium:YAG (Nd:YAG) laser posterior capsulotomies and extracapsular cataract extractions was investigated in two separate, double-masked,

placebo-controlled studies.²⁸ In the first study, involving 42 patients without evidence of glaucoma, one drop of levobunolol 0.5% or vehicle was instilled into the study eye 30 to 120 minutes prior to the Nd:YAG capsulotomy. The eye of each subject was dilated with 2.5% phenylephrine and 1% tropicamide topically. Late in the evening of the day of surgery, subjects instilled another drop into the study eye. IOP measurements were performed one, two, three, and 24 hours after surgery.

Eight subjects in the vehicle treatment group experienced increases from baseline in IOP of 10 mm Hg or greater. No subject in the levobunolol study group experienced any pressure spikes of 10 mm Hg or greater up to 24 hours following laser capsulotomy. The difference was statistically significant ($p = 0.0034$). Mean reductions in IOP from baseline ranged from 2.1-2.9 mm Hg in the levobunolol group. In the vehicle treatment group, IOP increases (4.4-6.4 mm Hg) were observed at hours 1, 2, and 3 following capsulotomy. Mean IOP at the 24 hours measurement was slightly decreased (0.3 mm Hg) in the vehicle treatment group. The difference between the two treatment groups was statistically significant at each time point measured.

In the second study, 41 subjects without evidence of glaucoma received either 0.5% Betagan (levobunolol HCl) or vehicle immediately after a unilateral extracapsular cataract extraction involving the use of a viscoelastic preparation (sodium hyaluronate) and the implantation of a posterior chamber intraocular lens. Miosis was induced with an intraocular injection of 0.01% carbachol. After surgery, 0.5 ml or 0.1% dexamethasone and 3 mg of gentamicin were injected subconjunctivally. One drop of levobunolol 0.5% or vehicle was instilled in the study eye. IOP was measured 12 hours after surgery and the following morning before subjects again instilled one drop of the study medication. Instillation of the study medication continued on a once-daily basis for seven days.

The overall incidence of IOP elevations from baseline ≥ 10 mm Hg was eight subjects (40%) in the vehicle group and four subjects (19%) in the levobunolol group. IOP spikes ≥ 10 mm Hg were found only at Hour 24 (4/21 subjects) in the levobunolol group. In the vehicle treatment group, IOP spikes ≥ 10 mm Hg were found at Hour 12 (4/20 subjects), Hour 24 (6/20), Hour 36 (4/19) and Day 3 (2/20). The percentage of subjects with IOP spikes ≥ 10 mm Hg was significantly less in the levobunolol group at Hour 12 ($p = 0.048$) and at Hour 36 ($p = 0.047$). The highest IOP values seen in any subject were 32 mm Hg in the levobunolol group and 52 mm Hg in the vehicle group. Mean IOP decreased from baseline up to 8.6 mm Hg at 24 hours in the vehicle group and up to 2.0 mm Hg at 24 hours in the levobunolol group. These studies provided evidence that marked elevations in IOP after posterior capsulotomies or extracapsular cataract extractions may be minimized by prophylactic treatment with levobunolol.

In another study,²⁹ levobunolol 0.5% was more effective than betaxolol 0.5% or placebo in preventing increased IOP after cataract extraction and posterior chamber lens placement with the

u. of sodium hyaluronate and acetylcholine. Two drops of the assigned medication were administered to the study eye immediately before patching after surgery. There were 20 participants in each study group (80 total) in the randomized, double-masked study.

A significant mean increase in intraocular pressure from the preoperative to the early (four to seven hours) postoperative period was noted in the betaxolol-treated group (6.73 mm Hg, $p = .0002$), the placebo-treated group (5.35 mm Hg, $p = .0037$), and the timolol-treated group (3.83 mm Hg, $p = .0039$). However, the levobunolol-treated eyes showed a mean decrease in pressure of 0.43 mm Hg in the early preoperative period.

Analysis of the change in pressure from the preoperative to the early postoperative periods demonstrated a significant increase in betaxolol-treated eyes compared to levobunolol-treated eyes ($p = .0032$). The placebo-treated eyes also had a significant increase compared to levobunolol-treated eyes ($p = .0203$). Although there was a strong tendency for levobunolol to control the early postoperative pressure rise compared to timolol, the difference was not statistically significant ($p = .087$). An intraocular pressure of 30 mm Hg or greater was found in three placebo-treated (15%), four betaxolol-treated (20%), one timolol-treated (5%), and none of the levobunolol-treated eyes. Five placebo-treated eyes (25%), six betaxolol-treated eyes (30%), five timolol-treated eyes (25%), and one levobunolol-treated eye (5%) experienced a pressure rise of 10 mm Hg or greater.

HUMAN PHARMACOKINETICS

Oral Administration in Humans - Metabolism and Excretion

In humans, levobunolol is well absorbed following oral administration, achieving peak plasma levels within one hour of dosing. It is subject to hepatic metabolism and is converted primarily to dihydrolevobunolol, which is an active metabolite, having beta-adrenoceptor antagonist properties similar in potency to levobunolol.^{12,15}

Levobunolol has a plasma half-life of approximately six hours, and dihydrolevobunolol of approximately seven hours. Levobunolol is excreted primarily in the urine, mainly as dihydrobunolol and intact levobunolol.¹² For comparison purposes, the half-life of propranolol and timolol following oral administration is about three hours.³⁰

Plasma Levels of Levobunolol Following Ocular Administration

Plasma levels of levobunolol were determined in a parallel, double-masked study in 12 subjects with normal IOP values. A single 50 µl drop of 0.5% or 1.0% levobunolol was instilled in both eyes twice daily for one week.³¹

Four of six subjects treated with 0.5% levobunolol and two of six subjects treated with 1% levobunolol had plasma levels less than 0.5 ng/ml (the limit of detection). Values for these subjects were recorded as 0.25 ng/ml, the assumed mean. The highest levobunolol plasma level was 1.2 ng/ml in one subject treated with 1% levobunolol. Following one week of twice-daily dosing with either concentration, there was little accumulation of levobunolol in the plasma. When levobunolol plasma levels were 0.3 ng/ml one hour after the last treatment with levobunolol 0.5% and 0.6 ng/ml after treatment with levobunolol 1%. Twenty-four hours after the final dose, levobunolol levels were decreased to 0.1 ng/ml for levobunolol 0.5% and 0.3 ng/ml for levobunolol 1%. Results from this study in normal subjects showed that levobunolol plasma levels following instillation of clinically effective (0.5% and 1.0%) concentrations were less than those reported elsewhere for timolol at similar dosages.³²

PRECLINICAL TOXICOLOGY

ACUTE TOXICITY

Topical Effects--Ocular Administration

The toxic ocular effects of acute administration of topical levobunolol (0.2% and 2%) were investigated in female New Zealand albino rabbits. After one day of multiple topical instillations (one 50 μ l drop every half hour for eight hours), no toxic effects were found with the 0.2% concentration. However, the 2% concentration caused mild corneal damage evidenced by fluorescein staining, involving 25% to 50% of the cornea.

An additional study testing less frequent instillation of 2% levobunolol (one drop every hour for eight hours) demonstrated no fluorescein staining. No signs of ocular discomfort were noted in any of the rabbits in either study.

Acute Systemic Toxicity--Oral and Intravenous Administration

Acute oral (p.o.) and intravenous (i.v.) toxicity studies of levobunolol in mice, rats, hamsters, and dogs have been performed. The following table summarizes the LD₅₀ data obtained for each species tested.

TABLE 1

Acute Oral and Intravenous Toxicity of Levobunolol

Species		LD ₅₀		Asymptomatic	Signs of Toxicity
Strain	Route	Sex	(mg/kg)	Dose (mg/kg)	(oral administration)
Mouse MF ₁	p.o.*	M	344-1,530	200-500	ataxia, loss of righting reflex, seizures; decreased respiration, cyanosis
		F	273-1,220	150-500	
	i.v.	M	78	65	
		F	84	65	
Rat CFN	p.o.	M	700	100	decreased spontaneous motor activity, ataxia; decreased palpebral size; decreased respiration; deaths preceded by seizures
		F	800	100	
	i.v.	M	25	5	
		F	28	5	
Hamster Syrian	p.o.	M	435	100	sedation, ataxia, seizures; deaths attributed to respiratory failure
		F	500	100	
Dog Mongrel	p.o.	M	>100	>10, <100	sedation, ataxia, seizures; decreased respiration, vocalization; autopsies revealed changes related to convulsions (pulmonary congestion and hemorrhage)
		F	>100	>10, <250	

* Three strains of mice were used in oral studies:

MF₁, CD₁ and CF₁

LONG TERM TOXICITY

Subacute Toxicity--Ocular Administration

During the two 28-day studies, one 50 µl drop of 1% or 5% levobunolol topical ophthalmic solution was unilaterally instilled in female New Zealand albino rabbit eyes two, four, or eight times a day (six animals per group). Twice-daily administration of 1% levobunolol produced no apparent ocular discomfort and no significant ocular reactions. Administration four times a day produced ocular discomfort at fewer than 1% of all evaluations and mild hyperemia at fewer than 2%. Administration eight times a day produced ocular discomfort at fewer than 5% of all evaluations, mild hyperemia at fewer than 9%, and tearing at fewer than 8%; ocular discharge and conjunctival congestion were infrequently observed.

Twice-daily administration of 5% levobunolol produced ocular discomfort at 37% of all evaluations and mild hyperemia at 13%; there was one observation of mild corneal damage evidenced by fluorescein staining. Administration four times a day produced ocular discomfort at 75% of all instillations and mild hyperemia at 51%; conjunctival congestion, discharge, and tearing were infrequently observed. Administration eight times a day produced ocular discomfort at 91% of all instillations and mild to moderate hyperemia at 19% of all instillations; mild to moderate conjunctival congestion and ocular discharge were observed at approximately 50% of all slit lamp evaluations.

In both studies, histological evaluation of the eyes receiving treatment eight times a day revealed no structural changes that could be attributed to levobunolol. In these studies, 1% levobunolol was nontoxic and 5% levobunolol was mildly irritating but nontoxic in rabbit eyes.

Chronic Toxicity--Ocular Administration

During a one-year chronic toxicity study, female New Zealand albino rabbits (40 animals per group) received a 50 µl drop of topical levobunolol (0.5%, 1% or 5%) or a vehicle control solution twice daily. No drug related ocular or systemic toxic effects were found.

Variations in blood chemistry and hematology variables occurring during the study were within normal ranges. Histologically, no ocular or systemic drug-related changes were observed in any tissue. The few lesions found were evenly distributed between the placebo-treated and drug-treated groups. Body weight and organ weight did not change significantly during the course of the study.

The incidence of hyperemia, discharge, chemosis, and tearing recorded during daily eye examination was extremely low, occurring at fewer than 1% of all examinations. Lens opacities and corneal damage evidenced by fluorescein staining were observed in some

rabbits during slit lamp examination, but were attributed to extraneous factors because of their occurrences in both treated and untreated eyes or their subsequent disappearance while treatment continued. Ophthalmoscopic examination revealed no drug-related effects on ocular tissue.

Chronic Oral Administration

Levobunolol (0.5, 2, 5, 30, or 180 mg/kg/day) was orally administered to Wistar rats for two years without significant adverse effects. In one study, 80 animals of each sex were included in each dosage group (0.5, 2, or 5 mg/kg/day), with 120 per sex in the control group. In a second study, 70 animals of each sex were included in each dosage group (5, 30, or 180 mg/kg/day), with 120 per sex in the control group. Some animals receiving 180 mg/kg showed suppression of weight gain, and dark-coloured urine was noted in some animals in the 30 and 180 mg/kg groups. Also in those dosage groups, a steel-grey discolouration of exposed skin areas was noted during the last six months of the study. On autopsy, the internal organs of the majority of the animals in the 180 mg/kg group and some of the animals in the 30 mg/kg group showed generalized steel-grey discolouration. Histopathology revealed the presence of small, brownish-yellow granules, which were found to be secondary lysosomes during electron microscopy and histochemistry studies.

During a one-year chronic toxicity study, beagle dogs receiving oral levobunolol (2, 6, or 24 mg/kg/day) showed no significant adverse reactions. Animals receiving 100 mg/kg/day showed significant toxic effects, including decreased food consumption and emesis, which resulted in the deaths of some animals and eventual discontinuation of the high dosage group. No significant behavioral or ophthalmic changes were observed, and no systemic toxicity was noted, except in the high-dose group. Although there were no changes in systolic blood pressure, the mean resting heart rate was lower in the group of treated dogs than in the control group throughout the study.

No adverse ocular effects were observed in rabbits administered Betagan (levobunolol HCl) Liquifilm^R sterile ophthalmic solution topically in studies lasting one year in concentrations up to ten times the human dose concentrations.

CARCINOGENICITY STUDIES

There was no significant difference in the overall tumor rate among groups of Swiss albino CF₁ mice orally treated with levobunolol for 80 weeks. Fifty animals of each sex were included in each dosage group (12, 50, or 200 mg/kg/day), while 100 of each sex were included in the control group.

It was, however, noteworthy that four uterine leiomyomas occurred among the 50 female mice in the high-dose group, while only one such tumor was noted among the 100 females in the control group and none in the mid- and low-dose groups.⁴⁶ The development of leiomyomas as a result of treatment with carcinogenic substances is unknown, and degeneration to a malignant leiomyosarcoma is extremely rare.

During the two-year oral toxicity studies in rats mentioned previously, the carcinogenic potential of levobunolol was also evaluated. With oral administration of levobunolol (0.5, 2, 5, 30, and 180 mg/kg/day), there were few noteworthy differences among the treatment groups in either the frequency or the types of tumors observed. There was a statistically significant ($p \leq 0.05$) increase in the incidence of benign hepatomas in male rats administered 180 mg/kg/day (12,800 times the maximum recommended human dose for glaucoma). Similar differences were not observed in rats administered oral doses equivalent to 350 times to 2,000 times the maximum recommended human dose for glaucoma. There were no significant differences in the overall liver tumor incidence. The results of the two-year studies in rats and the 80-week study in mice give no evidence of carcinogenic risk for levobunolol.

MUTAGENICITY STUDIES

Levobunolol does not appear to have any mutagenic properties as indicated by negative results in several in vitro tests for genetic mutations. Tests include Ames (histidine-dependent) tests on five strains of Salmonella typhimurium; gene mutation tests in Schizosaccharomyces pombe (cell point mutation) and in Saccharomyces cerevisiae (mitotic gene conversion); sister-chromatid exchange tests in Chinese hamster ovary cells; in-vitro point mutation test in Chinese hamster lung cells and chromosome-metaphase analysis in bone marrow cells of Chinese hamsters.

In a lifetime oral study in mice, there were statistically significant ($p \leq 0.05$) increases in the incidence of benign leiomyomas in female mice at 200 mg/kg/day (14,000 times the maximum recommended human dose for glaucoma), but not at 12 or 50 mg/kg/day (850 and 3500 times the maximum recommended human dose). In a two-year oral study of levobunolol HCl in rats, there was a statistically significant ($p \leq 0.05$) increase in the incidence of benign hepatomas in male rats administered 12,800 times the maximum recommended human dose for glaucoma. Similar differences were not observed in rats administered oral doses equivalent to 350 times

of fetal malformations. There were no adverse effects on postnatal development of offspring. It appears when results from studies using rats and studies with other beta-adrenergic blockers are examined, that the rabbit may be a particularly sensitive species. There are no adequate and well-controlled studies in pregnant women. Betagan should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

When results from rat and rabbit studies are compared, it appears that the fetotoxicity noted in rabbits may be a species-specific effect related to beta-blockade.

SUMMARY

In numerous preclinical toxicology studies, levobunolol has been demonstrated to be exceptionally well tolerated in doses well above the therapeutic level.

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