

RHMI

Review and Evaluation of Pharmacology and Toxicology Data

Key Words: Levobetaxolol, Isomers, Glaucoma and B-adrenergic antagonist

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Division Name: HFD-550

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Information to Sponsor: (X) No ( )

Sponsor or Agent: Alcon Laboratories

Manufacturer:

Drug: Levobetaxolol

Code Name: AL-1577A (levobetaxolol or S (-) betaxolol hydrochloride); AL-2509B (R + betaxolol) and AL 1401A (RS-betaxolol)

Generic Name: Levobetaxolol Hydrochloride 0.5% ophthalmic suspensions

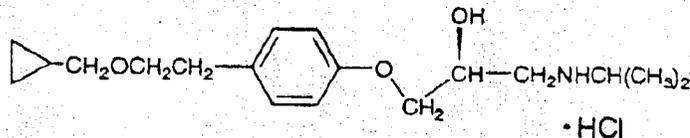
Trade Name: Betaxon™

Chemical Name: (S)-1-[2-(9-cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropylamino)-2-propanol hydrochloride

CAS Registry number: 116209-55-3

Molecular Formula, molecular weight: C<sub>18</sub>H<sub>29</sub>NO<sub>3</sub>.HCl, 343.89

Structure:



Relevant IND: IND [redacted]

Drug Class: β<sub>1</sub>-adrenergic blocker

Indication: Treatment of chronic open angle glaucoma or ocular hypertension

Clinical Formulation:

Levobetaxolol Hydrochloride [redacted]

Benzalkonium chloride [redacted]

Poly (styrene-divinylbenzene) sulfonic acid (PSA) [redacted]

Carbomer 974P [redacted]

Mannitol [redacted]

Boric acid [redacted]

Edetate disodium [redacted]

N-lauroylsarcosine [redacted]

Tromethamine and Hydrochloric acid [redacted]

Purified water [redacted]

Route of administration: Ophthalmic drops

Proposed clinical use: Betaxolol ophthalmic suspensions 0.5% will be used for the treatment of increased intraocular pressure at one drop twice a day in the affected eye.

Previous clinical experience:

Page 3-00011, vol 1:

Betaxolol is a cardioselective beta-adrenergic ( $\beta_1$ ) blocking agent. 0.25% ophthalmic suspension is already approved for the treatment of increased intraocular pressure of the eye. Betaxolol is also approved as 0.5% ophthalmic solution for the reduction of intraocular pressure in glaucoma patients. In the present NDA, the efficacy of the levo isomer (S form of the stereoisomer) is evaluated for the treatment of increased intraocular pressure in glaucoma and ocular hypertension at 0.5% concentration. The levo-isomer of betaxolol is more active than the racemic betaxolol for blocking adrenergic beta-receptors. The sponsor stated that racemic betaxolol preserved visual function and improve ocular blood flow in the previous clinical studies. However, indications and usage section of the label for Betoptic-S indicate that racemic betaxolol is effective in lowering intraocular pressure in-patients with glaucoma and ocular hypertension.

Disclaimer: Pages 5-02669 and 5-02670 vol 8 are attached in the appendix.

Letter of Authorization: To cross-refer to NDA 19-845 on Betoptic S, 0.25% ophthalmic suspension.

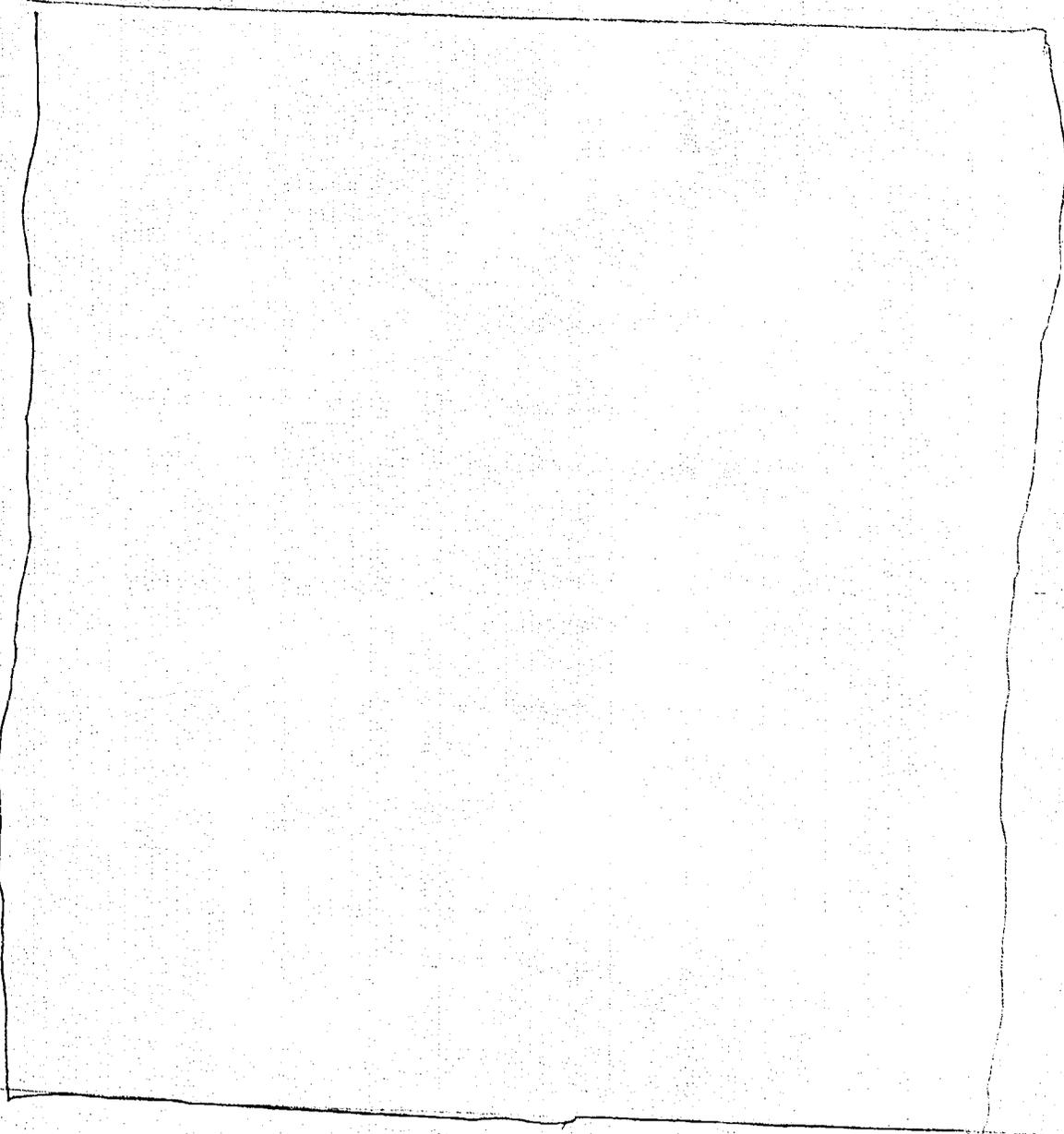
Introduction and Drug History:

Betaxolol ophthalmic solution 0.5% and betaxolol ophthalmic suspensions 0.25% are already approved for the treatment of increased intraocular pressure in glaucoma and ocular hypertension. The present NDA is submitted on the active isomer of betaxolol for blocking adrenergic  $\beta_1$  receptor that may be responsible for the pharmacodynamic effect. It is conceived that the effect of  $\beta$ -adrenergic blocking agents exerts its pharmacodynamic effect by blocking  $\beta$ -adrenergic receptors. On the basis of the preclinical in vitro and in vivo data, the sponsor stated that betaxolol also contributes its efficacy in glaucoma due to its effect on the calcium channels. However, the clinical implication of preclinical data needs to be established in the clinical trials for such claim.

Studies reviewed within the submission:

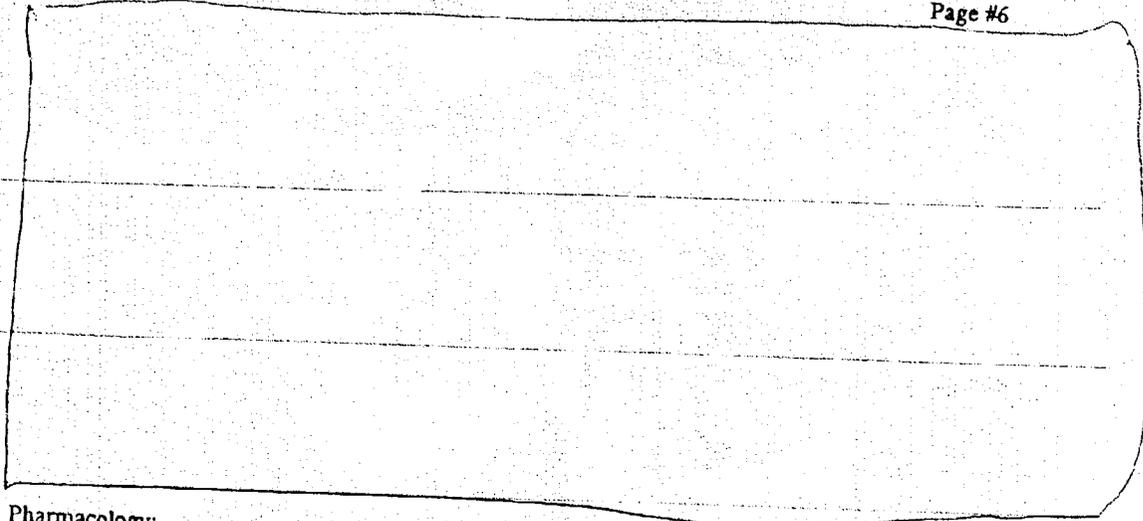
1. Perspective in adrenergic  $\beta$ -receptor blockade. Page 5-00040, vol 2.
2. In vitro and in vivo pharmacological evaluation of betaxolol, a new potent and selective  $\beta_1$  adrenoceptor antagonist. Page 5-00055, vol 2.
3. Determination of  $\beta_1$  and  $\beta_2$  adrenoceptor antagonist potencies of R and S-betaxolol in isolated guinea pig atria and tracheal chain preparations. Page 5-00067, vol 2.
4. Inhibitory action of betaxolol, a  $\beta_1$  selective adrenergic receptor antagonist, on voltage dependent calcium channels in guinea pig artery and vein. Page 5-00078, vol 2.
5. Effect of levobetaxolol and or racemic betaxolol on sodium and calcium conductance and elevations of intracellular calcium of retinal ganglion cells. Page 5-00083, vol 2.
6. In vitro pharmacological studies on racemic betaxolol, levobetaxolol and dextrobetaxolol. Page 5-00096, vol 2.
7. Betaxolol, a  $\beta_1$  adrenoceptor antagonist, has an affinity for L-type calcium channels. Page 5-00115, vol 2.
8. Effect of 150 $\mu$ l S-Betoptic formulation on intraocular pressure in ocular hypertensive monkey following a single topical ocular instillation. Page 5-00138, vol 2.

9. Effect of topical instillation of RS-betaxolol and its R and S-enantiomers on intraocular pressure in laser-induced ocular hypertensive monkeys. Page 5-00148, vol 2.
10. The relaxant action of betaxolol on isolated bovine retinal microarteries. Page 5-00165, vol 2.
11. Calcium channel blocking activity of propranolol and betaxolol in isolated bovine retinal microartery. Page 5-00170, vol 2.
12. The relaxant action of the betaxolol isomers in isolated bovine retinal microartery. Page 5-00177, vol 2.
13. Effect of  $\beta$ -blockers and calcium entry blockers on the ocular vessels. Page 5-00185, vol 2.
14. Effect of betaxolol, timolol and nimodipine on human and pig retinal arterioles. Page 5-00197, vol 2.
15. Assessment of the vasoactive properties of the L- and D-isomers of betaxolol in isolated perfused retinal arteries. Page 5-00206, vol 2.
16. Vasodilating effects of racemic betaxolol and its R and S-isomers in bovine retinal vessels. Page 5-00232, vol 2.
17. The effect of intracarotid administration of betaxolol on optic nerve head blood flow. Systemic blood pressure, heart rate and acid-base status in the anesthetized New Zealand albino rabbits. Page 5-00266, vol 2.
18. Neuropharmacological profiles in mice administered AL-1577A, AL-1401A or AL2509B-01. Page 5-00524, vol 3
19. GI propulsion assay in mice administered AL-1577A, AL-1401A or AL-2509B-01. Page 5-00563, vol 3.
20. Barbiturate sleep time potentiation in mice administered AL-1577A, AL-1401A or AL2509B-01. Page 5-00602, Vol 3.
21. Spontaneous motor activity in mice administered AL-1577A, AL-1401A or AL-2509B-01. Page 5-00642, vol 3.
22. Submaximal electroshock seizure in mice administered AL-1577A, AL-1401A or AL-2509B-01. Page 5-00683, vol 3.
23. Phenylquinone writhing assay in mice administered AL-1577A, AL-1401A, AL-2509B. Page 5-00722, vol 3.
24. Antipyretic evaluation of AL-1577A, AL-1401A or AL-2509B-01 in the rat. Page 5-00759, vol 3. Test of antagonism of acetylcholine, histamine and barium chloride in the isolated guinea pig ileum exposed to AL-1577A, AL-1401A or AL-2509B. Page 5-00835, vol 4.
25. Airway resistance and dynamic lung compliance determination in guinea pig administered AL-1577A, AL-1401A, AL-2509B-01 or AL-1239. Page 5-00886, vol 4.
26. Pharmacodynamic assay of AL-1577A, AL-1401, AL-2509B or AL 1239 in dogs. Page 5-0039, vol 4.
27. Cardiovascular evaluation of AL-1577A, AL-1401A, AL-2509B or AL-1239 in dogs. Page 5-01023, vol 4.
28. One-year chronic topical ocular toxicity evaluation of S (-) betaxolol ophthalmic suspension in rabbits. Page 5-01695, vol 6.
29. A rising dose tolerance toxicity study of S (-) betaxolol (levobetaxolol) and RS betaxolol administered orally by gavage to rats. Page 5-02420, vol 7.
30. A three-month toxicity study of S (-) betaxolol (levobetaxolol) administered orally by gavage to rats. Page 5-02464, vol 8.
31. Developmental toxicity study of S (-) betaxolol administered by gavage to rabbits. Page 5-02932, vol 9.
32. Mutagenicity test with levobetaxolol in the Salmonella, E.coli, mammalian microsome-reverse mutations assay. Page 5-03125 and page 5-03179, vol 9.
33. *In vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese Hamster Ovary (CHO) cells using AL01577A. Page 5-03213, vol 9.
34. Mouse lymphoma forward mutation assay with AL-01577A S (-) betaxolol. Page 5-03290, vol 9.
35. Evaluation of AL-01577A (S - betaxolol) in the *in vitro* transformation assay of BALB/C 3T3 cell assay. Page 5-03326, vol 9.
36. The toxicology of sodium N-Lauroyl sarcosinate. Page 5-03360, vol 9.
37. Ocular tissue distribution of levobetaxolol following one week BID topical ocular administration of 0.5% levobetaxolol ophthalmic suspension to male Dutch belted rabbits. Page 5-03913, vol 11.

38. Ocular tissue distribution of radioactivity following a single topical ocular dose of 0.5% <sup>3</sup>H-levobetaxolol to male Dutch belted rabbits. Page 5-04042, vol 11.
  39. Metabolic inversion study of enantiomers (R and S) of betaxolol in the rabbit eye. Page 5-04084, vol 11.
  40. In vitro protein binding of <sup>3</sup>H-levobetaxolol and <sup>3</sup>H-betaxolol to rat, rabbit, monkey and human plasma proteins. Page 5-04316, vol 12.
  41. Levobetaxolol and racemic betaxolol plasma concentrations from toxicology study N-98-139: A three-month toxicity study of S-betaxolol (levobetaxolol) administered orally by gavage to rats. Page 5-04349, vol 12.
  42. Validation of an [redacted] method for the determination of levobetaxolol or racemic betaxolol in the rabbit plasma. Page 5-04578, vol 13.
  43. Validation of an [redacted] method for the determination of levobetaxolol or racemic betaxolol in rat plasma. Page 5-04646, vol 13.
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Pharmacology:

The sponsor submitted several study reports and publications on the pharmacodynamic activities of levobetaxolol and betaxolol in volumes 2-3 of the NDA. Some of the findings are summarized as follows:

In vitro studies:

Betaxolol is a potent  $\beta_1$  selective antagonist with low membrane stabilizing property and without intrinsic activity. Betaxolol competitively blocks  $\beta$ -adrenergic receptor.  $pA_2$  for antagonism is 8.57 in right atria and 6.18 in tracheal preparations in guinea pigs. The isomeric activity for antagonism of  $\beta_1$  receptor and  $\beta_2$  receptor in heart and trachea of guinea pigs are shown below.

$pK_B$ heart	$pK_B$ heart	$pK_B$ trachea	$pK_B$ trachea
S (-) betaxolol	R(+) -betaxolol	S (-) betaxolol	R (+) betaxolol
$\beta_1$	$\beta_1$	$\beta_2$	$\beta_2$
8.58	7.08	6.79	5.53

Affinity ( $k_i$ , nM) of betaxolol isomers for binding to human recombinant  $\beta_1$  and  $\beta_2$  adrenergic receptors expressed in Chinese hamster ovary cells were investigated using radioligand binding studies *in vitro*. The affinity to  $\beta_3$  adrenergic receptor was examined using membranes of human neuroblastoma cells *in vitro*. Data represented in the following table (page 5-00100, vol 2).

Compound	Cloned human $\beta_1$	Cloned human $\beta_2$	Constitutive human $\beta_3$
RS Betaxolol	29.0	141	60,200
Levobetaxolol	15.7	98.2	95000
Dextrobetaxolol	5720	14400	ND
L-timolol	3.6	1.5	ND

ND not determined.

Above data suggest that levobetaxolol is selective for the human  $\beta_1$  adrenoceptor site with high affinity compared to human  $\beta_2$  and  $\beta_3$  adrenoceptor sites.

In addition to  $\beta$ -adrenoceptor activity, betaxolol inhibited calcium channel currents in the mesenteric artery and portal vein in guinea pigs *in vitro*. However, both S (-) and R (+) betaxolol were equally potent for blocking calcium channels. Therefore, the effect of the drug on the calcium channel is independent of  $\beta$ -adrenergic activity.

The sponsor presented data in page 5-00079, vol 2 for the inhibition of calcium channel current in arterial and portal venous cells in guinea pigs *in vitro*. The  $IC_{50}$  for inhibition for betaxolol is 45-46  $\mu M$ . Propranolol did not inhibit the calcium current in the similar study. However, betaxolol is about 4 log units or 10,000 times more potent for its  $\beta$ -adrenergic blocking activity in the guinea pig heart than calcium channel blockade in vascular tissues. Based on the data the reviewer suggests that the calcium channel blocking activity of betaxolol is weak and its predominant pharmacodynamic effect is mediated by the  $\beta$ -adrenergic receptor system.

The sponsor reported that levobetaxolol and betaxolol inhibited calcium currents in the ganglion cells of salamander retina *in vitro* at 50-100  $\mu M$  (page 5-00084, vol 2). These concentrations are similar to that required for blocking the calcium current in vascular tissues.

Betaxolol also inhibited the binding of diltiazem and nitrendipine in rat cortical membranes with little stereospecificity (page 5-00115, vol 2). The  $IC_{50}$  of betaxolol for inhibition of specific binding of  $^3H$ -diltiazem and  $^3H$ -nitrendipine is 19.7 and 46.3  $\mu M$ , respectively. Levobetaxolol showed similar potency in the system. Timolol and propranolol also possess similar activity in the experimental system but with a lesser potency. Data suggest that calcium channel blocking activity of betaxolol and levobetaxolol in vascular, neuronal and ocular tissues is similar. However, betaxolol and levobetaxolol are more potent for blocking  $\beta$ -adrenergic receptor than blocking calcium channels.

Effect of 30  $\mu M$  concentration of levobetaxolol on acetylcholine, histamine, barium chloride and serotonin-induced contractions of guinea pig ileum was investigated. Levobetaxolol showed 21, 22, 28% and 53% inhibition of contractions induced by acetylcholine, histamine, barium chloride and serotonin, respectively. The effect of levobetaxolol on guinea pig ileum against all agonists except serotonin is non-specific (response was similar irrespective of agonists). Data are provided in page 5-00860, vol 4. Barium chloride induced contractions in guinea pig ileum are mediated by the influx of calcium. The inability of betaxolol to inhibit barium-induced contractions of guinea pig ileum suggests that the effect of betaxolol on the voltage dependent calcium channel is minimal in this preparation.

The sponsor provided several published papers (page 5-00170, vol 2) that indicate betaxolol inhibits contractility of bovine retinal microartery *in vitro*. The tissue lacks adrenergic nerve and functional  $\beta$ -adrenergic receptors. Therefore, the inhibitory effect of betaxolol and levobetaxolol on potassium induced contractions may possibly be due to blockade of the calcium channel in this system. Both betaxolol and propranolol were effective in inhibiting the contractility of the tissue due to potassium in bovine retinal microarterial preparations. Levobetaxolol and dextrobetaxolol did not show any difference in the pharmacological effect in bovine retinal microartery *in vitro*. The  $pD_2$  values were 4.91 and 4.92 for levobetaxolol and dextrobetaxolol, respectively.

Sponsor's comment:

On the basis of the data, the sponsor indicated that levobetaxolol also shares calcium channel blocking activity that can contribute to the vasodilatory activity in the bovine retinal microvasculatures.

However, the reviewer suggests that the calcium channel blocking property of betaxolol and levobetaxolol in the system is much weaker (almost 1000-10,000 times or 3-4 log units) than its  $\beta$ -adrenergic activity in the heart and lung tissues. Therefore, the effect of betaxolol and levobetaxolol on the calcium channel is of little significance.

Page 5-00197 vol 2 provided data on the effect of betaxolol, timolol and nimodipine on perfused human and pig retinal arterioles *in vitro*. ET-1 contracted human retinal arterioles showed dilatation at  $10^{-12}$  to  $10^{-4}$  M concentrations of betaxolol and at  $10^{-12}$  to  $10^{-4}$  M concentrations of nimodipine. A similar data also presented for pig retinal arterioles.

Timolol did not show significant vasodilatation in human retinal arterioles in these experiments.

The sponsor commented that the vasodilatation induced by betaxolol is due to calcium influx because these tissues are unlikely to be responsive to  $\beta$ -adrenoceptors.

However, page 5-00202 vol 2 showed data that norepinephrine induced constriction of pig retinal arterioles. Therefore, the data are not conclusive that the vasodilatory effect of betaxolol in retinal arterioles is due to inhibition of calcium channel rather than its effect on  $\beta$ -adrenergic receptors. The authors also did not show epinephrine and betaxolol interactions in these preparations. Data presented for the effect of betaxolol on human and pig retinal arterioles did not demonstrate that the effect is due to the inhibition of calcium influx.

Page 5-00489, vol 3 provides in vitro data for the effect of levobetaxolol on the viability of cultured transformed photoreceptor cells from rats. There was no effect of levobetaxolol on the viability of cells up to 1mM concentrations.

In vivo studies:

The sponsor conducted several experiments on the effect of levobetaxolol, betaxolol and R (+) betaxolol ophthalmic drops on the intraocular pressure in experimental model of ocular hypertension in the monkey.

Page 5-00138 vol 2 of the submission provides a report that single dose of 150  $\mu$ g (30  $\mu$ l 0.5%) levobetaxolol reduced laser induced intraocular pressure (IOP) in the monkey by about 46% within 3 hours. The vehicle reduced the IOP by about 18%.

A second study is reported on page 5-00148, vol 2. The effect of betaxolol, S (-) betaxolol and R (+) betaxolol on intraocular pressure in the laser-induced ocular hypertensive monkeys was investigated. S (-) betaxolol at 10  $\mu$ l of 0.5% ophthalmic preparation showed 16.3% inhibition of IOP at 3 hours. The control animals showed 2.8% inhibition of IOP.

The R (+) betaxolol up to 50  $\mu$ l of 0.5% showed only 6.8% inhibition of IOP in the eye that undergone laser surgery. Corresponding control animals showed only 2% inhibition.

Betaxolol at 50  $\mu$ l of 0.5% ophthalmic preparation showed 34.5% inhibition of IOP at 3 hours. The control animals showed 5.4% inhibition.

The data suggest that levobetaxolol is effective stereoselectively in inhibiting the IOP in the monkey model.

Several in vivo studies in New Zealand rabbits showed that betaxolol increased the blood flow in the optic nerve head at 1-10  $\mu$ g/kg.

Page 5-00489, vol 3 showed in vivo data on the effect of levobetaxolol and R (+) betaxolol on blue light (435-475 nm) induced retinopathy in Sprague Dawley rats. 20-40 mg/kg oral doses of levobetaxolol or R (+) betaxolol given at 48, 24 and 6 hours before blue light exposure protected rats from retinal damage. The extent of retinal damage was determined by electroretinogram. Levobetaxolol is more potent than R (+) betaxolol at 40 mg/kg. The sponsor indicated that the neuroprotective effect might be due to the upregulation of nerve growth factor expression based on the preliminary experiments. The dose mentioned in the report is about 2,857 times recommended human dose on mg/kg basis considering 7  $\mu$ g/kg as the maximum human dose (2 mg/kg + 7  $\mu$ g/kg). In in vivo system 1 mg/kg dose showed pharmacodynamic effect as an  $\beta$ -adrenergic blocker in the cardiovascular system in dogs. Therefore, it is unlikely that levobetaxolol exerts neuroprotective effect at doses that are effective for reducing intraocular pressure.

In vivo analgesic activity of levobetaxolol in phenylquinone induced writhing test in CD-1 mice was investigated. At 10 mg/kg dose of levobetaxolol did not show significant change in the number of writhes compared to the saline control. It is concluded that levobetaxolol did not show analgesic effect in the mouse model. (Page 5-00723, vol 3). Levobetaxolol also did not show antipyretic effect in rats at 10-mg/kg dose given subcutaneously in the yeast induced fever model. (Page 5-00759, vol 3).

Sponsor's summary and conclusions of pharmacology:

The sponsor presented the summary and conclusion of pharmacology data in page 5-00005, vol 2 of the submission. Levobetaxolol possesses higher affinity and greater potency than R (+) betaxolol in binding studies and functional studies in vitro and in vivo. Betaxolol, levobetaxolol and R (+) betaxolol also showed cardio-selectivity ( $\beta_1$  adrenergic selectivity). It possesses some affinity for L type of calcium channels (7-13  $\mu$ M). No significant interactions were observed at other binding sites in Nova screen at 91 receptors and transporter binding sites except minor affinity at sigma (receptor not specified) and  $H_3$  receptor site (for R isomer only).

Levobetaxolol or betaxolol induced vasorelaxation in ocular and non-ocular blood vessels in several species. Mechanism studies suggest that betaxolol and levobetaxolol exert its vasorelaxing effect probably due to calcium channel blocking action and reduction of entry of excessive calcium into arteries. Based on the relaxation of arterioles of retina and increased blood flow in optic nerve head in experimental studies, it is anticipated that therapeutic effect of levobetaxolol is due to increase retinal and optic nerve blood flow.

The sponsor suggested that the effect of levobetaxolol or betaxolol on the calcium channel would provide neuroprotective action in the retina by blocking the effect of excitatory amino acid receptor.

The sponsor stated that the neuroprotective effect of levobetaxolol in blue light induced retinopathy in rats in vivo was observed at substantially higher doses. However, the sponsor considered that levobetaxolol will be effective against chronic glaucoma degenerative processes. The sponsor also indicated that the experimental glaucoma model used in the submission is not suitable for demonstrating neuroprotective effect of the drug because the concomitant reduction of intraocular pressure. Levobetaxolol did not show analgesic, antipyretic and diuretic effects.

Reviewer's Summary and conclusions of pharmacology:

Levobetaxolol is a stereoselective  $\beta_1$  adrenergic antagonist on the basis of its adrenoceptor blocking activity in the heart and lung (trachea) preparations of guinea pig in vitro. Levobetaxolol also showed lowering of IOP stereoselectively in the experimental model of ocular hypertension in vivo in monkeys. Levobetaxolol showed inhibition of calcium mediated contractions in some smooth muscle and vascular muscle preparations. The effect on the calcium channel is independent of  $\beta$ -adrenergic effect. Several other  $\beta$ -adrenergic blockers also showed inhibitory effect for the calcium channels.

The effect of the  $\beta$ -blockers and also that of levobetaxolol on the calcium channel is not consistent in all tissues examined. The potency for blocking calcium influx through calcium channels was 3- 4 log units lower than that of its  $\beta$ -adrenoceptor blocking effect. Therefore, it is not clear whether the pharmacodynamic effect of levobetaxolol in lowering IOP in glaucoma and ocular hypertension is due to the effect on the calcium channel in addition to blockade of  $\beta$ -adrenergic receptor. The sponsor showed data that S (-) betaxolol and R (+) betaxolol were equally effective in blocking calcium entry to the tissues. If calcium channel blockade would be contributing factor for the efficacy of the drug as suggested in several publications, both the isomers should have been equally potent for lowering IOP. In the absence of such data, the reviewer concludes that  $\beta$ -adrenoceptor blockade contributes most for the reduction of intraocular pressure.

The reviewer also concludes that the neuroprotective effect of levobetaxolol in rats *in vivo* is not the major pharmacodynamic effect of levobetaxolol due to the weak responsiveness of the drug in the system. The sponsor has not provided any data to justify that levobetaxolol will be effective against chronic glaucoma degenerative processes.

The proposed clinical dose is about 25  $\mu$ l (125  $\mu$ g) two times a day for 0.5% ophthalmic suspensions. The total daily dose per eye will be about 250  $\mu$ g. A decrease in the IOP was observed at 150  $\mu$ g single dose in the monkey model. Levobetaxolol did not show analgesic and antipyretic effects in rodents.

Several safety pharmacology studies were reported in the submission. These reports are summarized below.

1. Neuropharmacological study: Single doses of levobetaxolol were administered at 0.1, 1.0 and 10 mg/kg subcutaneously to CD-1 mice. Effects on mean body temperature and neuropharmacological signs were observed. Levobetaxolol did not produce any neuropharmacological changes and did not affect the body temperature. (Page 5-00524, vol 3).
2. GI motility study: Single subcutaneous doses of levobetaxolol were administered in CD-1 mice at 0.1, 1.0 and 10 mg/kg. Thirty minutes after dosing, animals were given 10% activated charcoal by oral route at 10 ml/kg. Animals were sacrificed after 30 min and the intestine was examined for the presence of charcoal. The distance traveled by the charcoal as a fraction of the length of the intestine was determined and compared with the saline treated animals. Levobetaxolol at 10-mg/kg S.C. dose showed only 4% decrease in the motility of the intestine. The effect is statistically-insignificant (page 5-00563).
3. Barbiturate sleeping time: Levobetaxolol pretreatment at 0.1, 1.0 and 10 mg/kg S.C. single doses did not show significant change in the duration of the sleeping time induced by sodium pentobarbital. (Page 5-00615, vol 3).
4. Spontaneous motor activity: CD-1 mice were treated with levobetaxolol at 10-mg/kg S.C. doses. Thirty minutes after the dose, animals were observed for spontaneous motor activity at 0-5, 5-10, 10-15 and 15-20 minute intervals. The motor activity was recorded using photocells. Levobetaxolol did not show changes in the spontaneous motor activity when compared to the saline control. (Page 5-00642, vol 3).
5. Electroshock seizures: CD-1 mice were injected subcutaneously with levobetaxolol at 10-mg/kg dose. Seizures were induced by electroshock techniques at 5-10 mA. Percent changes in the intensity of tonic seizures within 60 seconds were compared to the saline control animals. Mice pretreated with levobetaxolol did not show any change in the tonic seizures. (Page 5-00683, vol 3).
6. Airway resistance and dynamic lung compliance: The study was conducted in anesthetized guinea pigs to determine bronchial-reactivity to IV injections of betaxolol to the lung function. Resistance and compliance to the airways in lungs were measured at 0.3, 1.0, 3.0 and 10.0 mg/kg doses of levobetaxolol. Levobetaxolol did not change the lung function up to 3 mg/kg doses. However, 2 out of 4 guinea pigs died at 10-mg/kg dose. The sponsor did not provide the cause of the death. Page 5-00387, vol 4.
7. Effect of levobetaxolol on the cardiovascular effects of autonomic agents in anesthetized dogs: A dose of 0.02 mg/kg had no effect on the cardiovascular responses of epinephrine, norepinephrine, acetylcholine, histamine and isoproterenol. However, at 0.1 mg/kg IV dose of levobetaxolol, the cardiovascular response to epinephrine was increased without changing the hemodynamic effects of acetylcholine and histamine. It is possible that the effect of alpha-adrenergic receptors were enhanced in the presence of the beta-adrenergic receptor blockade induced by levobetaxolol. Page 5-00939, vol 4. Another experiment is reported in page 5-00986, vol 4. Effect of levobetaxolol at 0.1, 0.3 and 1.0 mg/kg IV doses were examined on the cardiovascular responses of epinephrine, norepinephrine, acetylcholine, histamine and isoproterenol in anesthetized dogs. Levobetaxolol up to 0.3 mg/kg did not show any effect on epinephrine, acetylcholine, histamine and isoproterenol on mean arterial pressure. Significant inhibition of mean arterial pressure induced by norepinephrine was observed at

0.1 and 0.3 mg/kg doses of levobetaxolol. Levobetaxolol inhibited mean arterial pressure to acetylcholine, histamine and isoproterenol at 1 mg/kg dose.

8. Hemodynamic effects of levobetaxolol: Effect of levobetaxolol at 0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg I.V. doses were recorded in anesthetized male and female beagle dogs. Systolic, diastolic and mean blood pressure, heart rate, left ventricular pressure, dp/dt, cardiac output and lead II ECG were recorded in the open chest and artificially ventilated dogs. Results of the experiment suggest that levobetaxolol had no effect on the blood pressure, heart rate and ECG up to 1 mg/kg I.V. doses. However, left ventricular dp/dt was reduced and left ventricular end diastolic pressure was increased at 0.3 and 1.0 mg/kg doses. Page 5-01023, vol 4.

9. Heart rate and mean arterial blood pressure were monitored in conscious New Zealand rabbits after ganglionic blockade. Both levobetaxolol and S (-) timolol showed similar shift of the isoproterenol dose response curves for heart rate to the right. Levobetaxolol and S (-) timolol were equally effective at 0.1 and 0.01 mg/kg IV doses for blocking the heart rate. Therefore, levobetaxolol is about 10 times less potent than S (-) timolol for  $\beta_1$ -adrenergic blockade. Only S (-) timolol shifted the effect on the mean arterial pressure induced by isoproterenol. Data suggest that levobetaxolol is more effective in blocking heart rate than mean arterial pressure in the presence of isoproterenol. Page 5-00510, vol 3.

Above experiment was repeated at 40 and 160  $\mu$ g/kg doses of S (-) betaxolol and S (-) timolol, respectively, administered intraocularly. Within 10 minutes of dosing, S (-) betaxolol reduced the isoproterenol induced changes in the heart rate at 40 and 160  $\mu$ g/kg doses. Timolol showed greater inhibition of heart rate than S (-) betaxolol. S (-) timolol also showed greater effect on the mean arterial pressure than S (-) betaxolol. Data suggest that intraocular S (-) betaxolol showed systemic pharmacodynamic response in blocking cardiovascular responses of isoproterenol. However, compared to S (-) timolol, the effect of S (-) betaxolol is weaker. Page 5-00515, vol 3.

Reviewer's conclusions: It is concluded from the data that levobetaxolol is bioavailable in the systemic circulation at 40  $\mu$ g/kg in rabbits. Considering 500  $\mu$ g per day dose in both eyes for a 70-kg individual, 7  $\mu$ g/kg is the clinical dose. Therefore, levobetaxolol showed blockade of isoproterenol response in the heart and blood pressure at about 7 fold higher doses than the maximum clinical dose. The cardioselectivity of the  $\beta$ -adrenergic blockade is also observed *in vivo*. Levobetaxolol is tolerated up to 0.1-mg/kg IV doses in dogs without any effect on cardiovascular parameters. Levobetaxolol did not show CNS effects and any effect on GI propulsion in the models examined.

Sponsor's summary and conclusions:

Page 5-00033, vol 2:

Levobetaxolol did not show statistically significant or biologically relevant effects on neuropharmacological profiles, Gastrointestinal propulsion, barbiturate sleeping time, spontaneous motor activity and electroshock-induced seizures. No bronchoconstriction was reported up to 3 mg/kg doses (300-fold clinical exposure). Two animals died at 10 mg/kg (1000 fold human exposure) without sign of pulmonary effect. Levobetaxolol up to 0.3 mg/kg (30 fold higher than maximum clinical dose) did not show an effect in any experimental model. At 1 mg/kg (100 fold higher than clinical dose) produced minimal transient decrease in blood pressure and cardiac contractility. The effect is consistent with weak calcium channel blocking activity. Levobetaxolol at 1 mg/kg dose modified cardiovascular responses of isoproterenol, histamine and acetylcholine. In contrast, the cardiovascular responses to epinephrine and isoproterenol were significantly modified by timolol at 0.003 mg/kg.

In conscious rabbits, ocular instillation of 40  $\mu$ g/kg and 160  $\mu$ g/kg doses of levobetaxolol and S (-) timolol showed effect of the drug in systemic circulation after ocular administration. S (-) timolol blocks both the tachycardia and the vasopressor responses to isoproterenol whereas levobetaxolol blocks only the former.



collected from both eyes and radioactivity was counted in liquid scintillation counter. Tissues from untreated animals were collected for background radioactivity. Concentration in  $\mu\text{g}$  equivalents of levobetaxolol per GM of tissue at several hours in the treated eye is shown in the following table.

Tissue	.3 hr	.6 hr	1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr	72 hr	96 hr
Aq. Humor	3.93	1.16	1.01	0.28	0.06	0.03	0.02	0.01	.0064	.0055	.0035	.0043
Choroid	1.56	1.44	2.20	1.99	2.05	1.95	2.03	1.86	0.95	.88	.62	.244
Conjunctiva	15.0	4.18	4.05	1.07	1.74	2.22	2.32	.47	.86	1.0	.94	.22
Cornea	33.7	11.7	9.82	3.88	1.58	1.01	.96	.34	.31	.23	.12	.08
Iris-ciliary body	29.4	20.0	21.1	17.1	7.95	10.0	7.72	5.27	4.55	3.37	1.96	.96
Lens	.19	.15	.23	.11	.07	.06	.05	.03	.02	.01	.006	.003
Optic nerve	0.04	0.03	0.04	0.02	0.02	blq						
Retina	0.27	0.2	0.2	0.13	0.06	0.03	0.04	0.02	0.01	Blq	blq	blq
Whole blood	0.018	0.03	0.03	0.03	0.02	0.01	0.008	0.004	Blq	Blq	blq	Blq
Plasma	0.02	0.03	0.03	0.03	0.02	0.008	0.007	0.004	0.002	0.002	0.002	0.001
Vitreous humor, dosed eye	0.007	0.003	0.004	0.005	0.005	0.005	0.004	0.003	0.003	0.003	0.002	0.002

Blq = below the limit of detection.

The concentrations of radioactivity as  $\mu\text{g}$  equivalent of levobetaxolol/g of tissue in the undosed eye are shown in the following table.

Tissue	.3 hr	.6 hr	1 hr	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr	72 hr	96 hr
Aq. Humor	0.001	0.002	0.003	0.006	0.006	0.004	0.003	0.003	0.002	0.002	0.002	0.002
Choroid	0.637	0.762	0.958	0.918	0.854	0.603	0.623	0.511	0.414	0.206	0.217	0.090
Conjunctiva	0.0274	0.0309	0.0364	0.0288	0.0190	0.0116	0.0086	0.0086	0.0044	0.0026	0.0018	Blq
Cornea	0.0071	0.0111	0.0121	0.0104	0.0126	0.0051	0.0050	blq	blq	blq	blq	Blq
Iris-ciliary body	0.611	0.777	1.01	1.00	1.23	.978	1.07	0.897	0.711	0.329	0.249	0.148
Lens	0.0001	0.0004	0.0007	0.0015	0.0018	0.0022	0.0024	0.0020	0.0022	0.0016	0.0014	0.0010
Optic nerve	0.041	0.0375	0.0400	0.0284	blq							
Retina	0.29	0.204	0.199	0.142	0.0751	0.0456	0.0370	0.0175	0.0174	0.0062	blq	blq
Vitreous humor, undosed eye	0.001	0.002	0.003	0.004	0.003	0.003	0.003	0.002	0.002	0.002	0.002	0.001

The pharmacokinetic parameters calculated from above data are shown in the following table.

Tissue	Dosed eye			Undosed eye		
	$C_{max}$ ( $\mu\text{g}/\text{eq}/\text{g}$ )	$T_{max}$ (hr)	$T_{1/2}$ (hr)	$C_{max}$	$T_{max}$	$T_{1/2}$
Conjunctiva	15.0	0.33	51.5	0.0364	1	37.2
Cornea	33.7	0.33	39.5	0.0126	4	5.51
Aq. Humor	3.93	0.33	101	0.0056	2	148
Iris-ciliary body	29.4	0.33	32.1	1.23	4	33.4
Lens	0.226	1.00	27.4	0.0024	8	73.6
Vitreous Humor	0.0072	0.33	77.9	0.0040	2	103
Retina	0.269	0.33	36.4	0.29	0.33	33.7
Choroid	2.20	1.00	33.8	0.958	1	36.5
Optic Nerve	0.0447	0.33	2.80	0.0411	0.33	3.18
	Body fluids					
Plasma	0.0346	1.00	128			
Whole blood	0.0283	1.00	3.97			

<sup>3</sup>H-Levobetaxolol is formulated similar to Betoptic S. About 150 µg was instilled into the right eye. Tissue distribution following single dose showed that the radioactivity was absorbed in the ocular tissues and distributed mostly in the anterior chamber of the eye. Lower level of radioactivity was detected in the blood and undosed eye. However, due to high potency of the antagonist, the systemic organs and undosed eye may show blockade of beta-receptor as a consequence to the ophthalmic delivery of the drug.

The levels of the drug in the retina and optic nerve were similar for dosed and undosed eyes. Data suggest that levobetaxolol was distributed to the retina and optic nerve through the systemic circulation. Higher concentration of the drug was observed in the ciliary body and aqueous humor compared to posterior chamber of the eye. The drug is available quickly at the site within about 30-min.

Distribution of the drug after multiple doses in the eye:

Ocular tissue distribution of levobetaxolol following one-week BID topical ocular distribution of 0.5% levobetaxolol ophthalmic suspension to male Dutch belted rabbits.

Page 5-03913, vol 11, Protocol: N-98-251.

Levobetaxolol ophthalmic suspensions at 0.5% was formulated with [redacted] Other inactive ingredients were carbopol 0.35%, mannitol 4.0%, boric acid 0.3%, disodium EDTA 0.01%, benzalkonium chloride 0.01%, N-lauroylsarcosine 0.03% and, hydrochloric acid, tromethamine to adjust pH. Purified water was added to adjust the volume.

Male Dutch belted rabbits were used in the study. The body weight of the animal was about 1.67 kg at the time of the dosing. Rabbits were divided into two groups each comprised of 28 animals. Animals in-group one received Betoptic S at 0.25%. Animals for group 2 were given 30 µl of 0.5% levobetaxolol in the right eye. Animals were dosed twice daily for 7 days. Each drop delivered 150µg of levobetaxolol. Four animals per time point were sacrificed at 0.5, 1, 2, 4, 8, 12 and 24 hours. About 6 ml of blood samples were collected prior to sacrifice. Eye tissues and fluids were collected from the right (dosed) and the left (-undosed) animals.

Tissue levobetaxolol levels were determined by [redacted]

Tissue kinetics in the dosed right eye for 0.5% levobetaxolol is shown in the following table.

Tissue	C <sub>max</sub> (ng/g)	T <sub>max</sub> (hr)	T <sub>1/2</sub> (4-12 hr)	T <sub>1/2</sub> (8-24 hr)	AUC 0-12 (ng.hr/g)
Aqueous humor	1420	0.5	16.7	7.2	2060
Iris ciliary body	46800	0.5	10.1	31.3	355000
Anterior sclera	4610	1	ND	59.6	27300
Vitreous humor	4.22	0.5	13	25.7	29.4
Retina	305	1	5.3	20.6	1296
Choroid	4320	12	ND	62.2	47400
Optic Nerve	60.3	0.5	7.1	21.7	242
Optic nerve head	274	2	5.1	ND	1820

Ocular tissue kinetics of the undosed eye and plasma kinetics are shown in the following

Tissue	C <sub>max</sub> (ng/g)	T <sub>max</sub> (hr)	T <sub>1/2</sub> (4-12 hr)	T <sub>1/2</sub> (8-24 hr)	AUC 0-12 (ng.hr/g)
Anterior sclera	31.0	1	13	ND	245
Vitreous Humor	2.53	0.5	3.5	14.4	8.84
Retina	336	1	5.1	12.2	1080
Choroid	1980	0.5	11.5	71.1	16900
Optic nerve	68.0	0.5	9.8	19.6	162
Optic nerve head	212	1	5.8	15.5	1740
Plasma	4.7	0.5	1.6	ND	10.4

Tissue kinetics in the dosed eye for Betoptic-S is shown in the following table.

Tissue	C <sub>max</sub> (ng/g)	T <sub>max</sub> (hr)	T <sub>1/2</sub> (4-12 hr)	T <sub>1/2</sub> (8-24 hr)	AUC 0-12 (ng.hr/g)
Aqueous humor	766	0.5	4.4	18.4	1160
Iris-ciliary body	32500	0.5	14.5	185	250100
Anterior Sclera	4340	0.5	22.2	14.7	28260
Vitreous Humor	2.59	0.5	18.3	nd	18.4
Retina	180	1	9.5	nd	637
Choroid	3880	24	22.4	nd	31742
Optic Nerve	35.7	0.5	11.8	38.1	134
Optic nerve head	119	24	13.9	nd	662

The tissue kinetics in the undosed eye and plasma kinetics for Betoptic S is shown in the following table.

Tissue	C <sub>max</sub> (ng/g)	T <sub>max</sub> (hr)	T <sub>1/2</sub> (4-12 hr)	T <sub>1/2</sub> (8-24 hr)	AUC 0-12 (ng.hr/g)
Anterior sclera	18.1	24	ND	ND	133
Vitreous humor	1.91	0.5	5.2	17.4	4.63
Retina	134	0.5	3.2	39.3	464
Choroid	900	2	13.3	ND	7490
Optic nerve	28.6	0.5	6.9	66.8	91.9
Optic nerve head	99.0	24	ND	ND	509
Plasma	3.05	0.5	1.8	ND	5.22

The ratio of levobetaxolol and racemic betaxolol in the treated and untreated eyes are shown in the following table.

Tissue	C <sub>max</sub> , Treated eye	AUC <sub>0-12</sub> , Treated eye
Aqueous humor	1.8	1.8
Iris-ciliary body	1.4	1.4
Anterior sclera	1.1	1.0
Vitreous humor	1.6	1.6
Retina	1.7	2.0
Choroid	NA	NA
Optic nerve	1.7	1.8
Optic nerve head	2.3	2.8
	C <sub>Max</sub> , Untreated eye	AUC <sub>0-12</sub> , Untreated eye
Anterior sclera	1.7	1.8
Vitreous humor	1.3	1.9
Retina	2.5	2.3
Choroid	2.2	2.3
Optic nerve	2.4	1.8
Optic nerve head	2.1	3.4

ND = Not determined, NA = Comparative values are not appropriate due to analytical error.

Following conclusions can be made from the data presented above.

1. Levobetaxolol and betaxolol were distributed to anterior and posterior chambers of the eye. However, levels in the anterior chamber were higher than the posterior chamber.
2. The drugs were distributed to the untreated eyes via systemic circulation.
3. Both levobetaxolol and betaxolol were bioavailable into the systemic circulation.
4. Similarity in the levels of levobetaxolol in the retina, optic nerve and optic nerve heads between treated and untreated eyes suggest that the systemic bioavailability of the drug contribute to the bioavailability in these tissues.

Metabolic inversion study:

Metabolic inversion study of enantiomers (R and S) of Betaxolol in the rabbit eye:

Page 5-04084, vol 11, Protocol PKDM 56:

Betoptic (Lot # 2K1AA 056:30:0987) and levobetaxolol (lot AHI 099-056:29:0987) were formulated as 0.5% ophthalmic solutions. Doses were given to NZ albino rabbits according to the following schedule.

	Group 1	Group 2	Group 3
Number of animals	3	3	3
Dose volume	30µl x5 times within 100 minutes	30µl x 5 times within 100 minutes	30µl x 5 times within 100 minutes
Concentration	0.5%	0.5%	0.5%
Drug	RS- betaxolol	S (-) betaxolol	S (-) betaxolol
Treatment	Both eyes	Both eyes	Right eye

Aqueous humor, cornea and iris-ciliary body samples were collected twenty minutes after the last dose. Tissue levels of R and S- isomers of betaxolol were determined by

Levels of R and S betaxolol following 5 topical doses of either 0.5% RS or S-betaxolol in rabbit eyes. Results represent application of the drug in both eyes (groups 1 and 2).

Tissues	R-betaxolol levels, given as		S-betaxolol levels, given as	
	RS-form	S-form	RS-form	S-form
Aqueous humor ( $\mu\text{g/ml}$ )	2.33	ND	2.41	4.62
Iris-ciliary body ( $\mu\text{g/g}$ )	2.56	ND	2.31	5.96
Cornea ( $\mu\text{g/ml}$ )	11.08	ND	13.38	23.75

ND = not detected

Above data suggest that there was no inversion of isomers in the ocular tissues during the treatment period.

Data on the concentrations ( $\mu\text{g/g}$  of tissue) of R and S betaxolol given as 0.5% S-betaxolol in the right eye are shown in the following table. Samples were collected 20 minutes after the last dose.

Tissue	Right eye		Left eye	
	R betaxolol	S betaxolol	R betaxolol	S betaxolol
Aqueous humor	ND	5.70	ND	ND
	ND	5.10	ND	ND
Iris-ciliary body	ND	5.98	ND	ND
	ND	7.56	ND	ND
Cornea	ND	48.37	ND	ND
	ND	26.16	ND	ND
	ND	23.53	ND	ND

ND not detected.

Data suggest that there was no inversion from S enantiomer to R enantiomer of betaxolol. Also the untreated eye did not show presence of betaxolol by systemic exposure. The sponsor stated that the level in the untreated eye was lower than the level of detection at  $0.2\mu\text{g/ml}$ .

Other studies:

In vitro protein binding of  $^3\text{H}$ -levobetaxolol and  $^3\text{H}$ -betaxolol to rat, rabbit, monkey and human plasma proteins.

Page 5-04316, vol 12, Protocol N-99-158.

$^3\text{H}$ -levobetaxolol used in the study had specific activity of  $135\mu\text{Ci}/\mu\text{g}$   
The specific activity of  $^3\text{H}$ -betaxolol used in the study was  $147\mu\text{Ci}/\mu\text{g}$

Percent protein binding of  $^3\text{H}$ -levobetaxolol: Rat, rabbit, monkey and human plasma or 1500xg-plasma and 1000 ng/ml final concentrations. Samples were spiked with  $^3\text{H}$ -levobetaxolol to obtain 10, 100 and 1000 ng/ml final concentrations. Samples were incubated for 3 hours at  $37\text{C}$ . One-ml aliquots of plasma sample-levobetaxolol incubation mixtures and 0.5-ml aliquots of levobetaxolol incubation mixtures were taken for Radioactivity of the 100 $\mu\text{l}$  aliquot of incubation mixtures and 100 $\mu\text{l}$  aliquot of were determined by the

Percent protein binding of  $^3\text{H}$ -betaxolol: The procedures used for the plasma protein binding of  $^3\text{H}$ -betaxolol were similar to that for  $^3\text{H}$ -levobetaxolol.  $^3\text{H}$ -betaxolol mixed with the plasma protein or plasma protein to 10-ng/ml final concentration and incubated at 37C for 3 hours. One ml of plasma sample or 0.5 ml of plasma sample was centrifuged for [redacted]. The radioactivity of the 100 $\mu\text{l}$  aliquots of the incubation mixture and 100 $\mu\text{l}$  aliquot of the [redacted] were determined by the [redacted].

Percent protein binding of a 1:1 mixture of  $^3\text{H}$ -levobetaxolol and R-betaxolol:

Rat, rabbit, monkey and human plasma or 1500xg-plasma [redacted] (contains protein less than 30kDaltons) were spiked with plasma or 1500x g-plasma [redacted] (contains protein less than 30kDaltons) to final concentrations of 10, 100 and 1000 ng/ml. The drug protein mixtures were incubated for 3 hours at 37C and centrifuged for [redacted]. Aliquots of 1 ml of plasma and 0.5 ml of [redacted] from the incubation mixtures were taken for determination of radioactivity. The residual filtrates were also taken for the determination of radioactivity.

The optimal equilibration time for the incubation mixtures between the plasma proteins and drugs was determined in a preliminary experiment.

Average protein binding (%) in rat, rabbit, monkey and human plasma is shown in the following table.

Drug	Rat	Rabbit	Monkey	Human
$^3\text{H}$ -levobetaxolol	42.1	68.6	53.6	49.8
$^3\text{H}$ -levobetaxolol/ R-betaxolol (1:1)	44.0	70.5	54.4	51.6
$^3\text{H}$ -betaxolol	39.4	58.0	52.7	46.7

Above data suggest that levobetaxolol is not highly bound to plasma proteins in rats, rabbits, monkey and human plasma. Protein binding for racemic and levobetaxolol was similar.

Reviewer's comments and conclusions of pharmacokinetic studies:

S (-) betaxolol is bioavailable to the systemic circulation, and distributed to anterior and posterior chambers of the eye following ophthalmic delivery as eye drops. S (-) betaxolol is not metabolically inverted to the R-enantiomer. Following ophthalmic delivery of the drug to one eye, the drug is distributed to the undosed eye through the systemic circulation. In vitro plasma protein binding of the drug in rat, rabbit, monkey and human plasma [redacted].

Sponsor's Summary and conclusions of pharmacokinetic studies:

Page 5-03844, vol 11:

Following a single dose of  $^3\text{H}$ -levobetaxolol (0.5%) in Dutch belted rabbits, radioactivity was absorbed rapidly into the eye. Maximum concentrations were measured at the first sampling time of 20 min. Half life of levobetaxolol was relatively long. Radioactivity was detected in the plasma. Local absorption of the drug directly from the topical dose and redistribution of radioactivity in the systemic circulation were observed. Among the tissues of posterior chambers, only choroid showed higher levels in the dosed eye compared to the undosed eye. Retina and optic nerve head did not show higher levels of levobetaxolol in the dosed eye compared to the undosed eye. From the study it was concluded that after a single dose drug reached the retina and optic nerve head via systemic redistribution.

Unlabeled 0.5% levobetaxolol was given twice a day unilaterally for 7 days. In contrast to the single dose study, repeated administration of levobetaxolol in the eye increased bioavailability of the drug in the retina and optic nerve head. From 4 hours through 24 hours post dose, concentrations in the retina of the dosed eye were significantly higher than the undosed eye. These results indicate that drug in the rabbit retina comes from both systemic circulation and local distribution from the topical dose. Differences between dosed and undosed eye retina and vitreous humor were not evident from the single dose study. In the multiple dose study, high concentrations of levobetaxolol were present in the sclera and choroid. It has been proposed that these tissues may be accumulating drug and acting as a depot. Drugs which exhibit significant absorption to the posterior tissues in animals are likely to be bioavailable to the posterior tissues in humans. Ocular pharmacokinetics of the racemate and levobetaxolol are similar after topical dosing.

R-enantiomer and levobetaxolol did not undergo interconversion *in vivo* in the rabbit-ocular tissues. Interconversions were not observed following IV dosing to rats also. Moderate degree of binding to protein over a 1000-fold concentration range indicates that drug interactions due to plasma protein binding are unlikely for levobetaxolol.

Toxicology study #1:

Sponsor's ID: Protocol # N-89-14

Sponsor's Original Title: One-year chronic topical toxicity evaluation of S (-) Betaxolol Ophthalmic suspension in rabbits.

Page 5-01695, vol. 6

Conducting Laboratory: Alcon Laboratories Inc., Fort Worth, Texas

Date of study initiation: March 1, 1989 to Mar 8, 1990

GLP compliance: Yes

QA Report: Yes (X) no ( )

Methods:

Dosing information:

Species: New Zealand albino rabbits

Age: Not mentioned

Body weight: 2.2 to 2.9 kg

Satellite groups: Five male and five female rabbits in each group were designated for three-month interim study. See the study design below.

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Dosage groups in administered unit: Male and female rabbits were randomized into following groups:

Group	Test article	Sub Gr	S(-) Betaxolol %	Animals/Gr		Drops/ Treatment	Treatment /Day	Treated Days	
				M	F			M	F
1	Untreated control	A	-	5	5	-*	2	94	94
		B		7	7			366	367
2	S(-) Betaxolol Vehicle	A	0	5	5	2	2	94	94
		B		7	7			366	367
3	S(-) Betaxolol Suspension	A	0.5	5	5	2	2	94	94
		B		7	7			366	367
4	S(-) Betaxolol Suspension	A	1.0	5	5	2	2	94	94
		B		7	7			366	367
5	S(-) Betaxolol Suspension	A	2.5	5	5	2	2	94	94
		B		7	7			366	367

\*Eyes were physically manipulated to simulate dosing procedure, sham treated animals.

Group A: Animals in the group were designated as the three-month treatment group.  
Group B: Animals in the group were designated as the one-year treatment group.

Although the table on page 5-01720 vol-6 referred that animals were treated for 94 days (subgroup A) or 366-367 days (subgroup B), the body weight data suggest that the last record was made immediately before sacrifice on days 93 and 366 for subgroups A and B, respectively.

Route, form, and volume: Animals were treated topically by ophthalmic drops. Each animal received two drops of the vehicle or respective test article twice a day in the right eye. The left eye of each animal served as the untreated control. Animals were treated at 8:30 AM and 3:00 PM daily.

Drug lot #:

Following table provides the lot number of the drug:

Control or test article	S(-) Betaxolol base (%)	Lot Number
S(-) Betaxolol vehicle	0	ALL-184
S(-) Betaxolol Ophthalmic Suspensions	0.5	ALL-181
S(-) Betaxolol Ophthalmic Suspensions	1.0	ALL-139
S(-) Betaxolol Ophthalmic Suspensions	2.5	ALL-240

Formulation/vehicle: All concentrations are expressed as % w/w.

Ingredient	Lot ALL-184	Lot ALL-181	Lot ALL-139	Lot ALL-240
S(-) Betaxolol HCl	-	0.56 + 5% excess	1.12 + 5% excess	2.80 + 5% excess
EDTA disodium	0.01	0.01	0.01	0.01
Mannitol	4.25	4.25	4.00	1.50
Benzalkonium Chloride	0.01+5% excess	0.01+5% excess	0.01+ 10% excess	0.01+ 25% excess
Sodium hydroxide	To pH=7.6	pH 7.6	pH 7.6	pH 7.6
Purified water	QS 100	QS 100	QS 100	QS 100

Prior to the initiation of the treatment, and after about 1, 3, 6, 9 and 12 months of treatment, S (-) betaxolol and benzalkonium chloride concentrations from betaxolol 0.5%, 1.0% and 2.5% formulations were determined. Benzalkonium chloride concentrations were also determined from the vehicle.

Times at which observations were made:

Clinical signs: Mortality and clinical signs were monitored daily. General health check was also performed on pretreatment days before day 0.

Body weight: All rabbits were weighed on day -5, day 0 prior to the treatment. Body weights were also recorded on days that were scheduled for biomicroscopic examinations. Body weights were also recorded immediately before the necropsy. Data in the appendix J, addendum I showed that the final body weight was taken on day 93 for the subgroups 1A, 2A, 3A, 4A and 5A. Final body weights were recorded on day 366 for groups 1B, 2B, 3B, 4B and 5B, respectively.

Food consumption: Not recorded

Eye examinations:

A biomicroscope and indirect ophthalmoscope examined both eyes on day -5 before dosing. Animals were again assessed on day 0 for enrollment in the study. Animals with 0 score with all parameters except conjunctival congestion were enrolled. Animals with conjunctival congestion score of 0 or 1 was accepted. During the treatment period biomicroscopic evaluations were performed on weekly basis on first month, semi-monthly during 2nd and 3rd months of the study. Biomicroscopic examinations were performed monthly thereafter. Conjunctiva, cornea, anterior chamber, light reflex, lens and iris were examined. Both eyes were examined by biomicroscope. The result showed that the biomicroscopic examinations were conducted on following days:

Days -5, 0, 4, 9, 16, 23, 32, 44, 58, 74, 93, 128, 163, 184, 212, 254, 275, 317, 345 and 366.

Fundus of eyes for each animal was examined for changes in optic nerve head, retinal, choroidal vascular pattern and pigmentation. 1% Mydriacyl was used for the dilatation of pupils prior to indirect ophthalmoscopic examinations. Results of the indirect ophthalmoscopic examinations were indicated as within normal limits (WNL) or abnormal (AB).

**Hematology:**

Blood samples were collected from rabbits in the group A after approximately three months of the treatment. Blood samples were collected from rabbits from group B at approximately twelve months of the treatment. The sponsor has not indicated how they collected the blood samples. Following parameters were analyzed.

Hematocrit, hemoglobin, RBC counts, WBC counts, MCHC, MCV, MCH, platelet counts, leukocyte differential counts.

**Serum chemistry:**

After the collection of blood samples for hematological examinations, serum samples were collected from the serum chemistry analyses. Following parameters were determined.

Albumin, albumin: globulin, alkaline phosphatase, amylase (for gr B), bilirubin indirect for gr B, BUN/creatinine ratio, calcium, cholesterol, CPK, creatinine, GGT (gr B), globulin, glucose, LDH, phosphorus, potassium, SGOT, SGPT, sodium, total protein, triglycerides, BUN, uric acid.

**Handling of moribund and dead animals:**

Animals in the moribund condition was sacrificed and necropsied. The sponsor stated that animals died during the study were necropsied immediately or within 16 hours if refrigerated.

**Gross pathology:**

Animals were sacrificed by I.V injections of commercial euthanasia solution T-61 at the end of three months of treatment (for subgroup A) or twelve months of the treatment (for subgroup B). Following tissues were examined for gross changes and preserved in 10% neutral, buffered formalin. Eyes and adnexa were fixed in Zenker's fixative and preserved in 10% neutral, buffered formalin.

Eyes and adnexa, brain, pituitary gland, thyroid gland, larynx, thymus, heart, aorta, trachea, esophagus, lungs, peribronchial lymph nodes, skeletal muscle, liver, gall bladder, spleen, pancreas, adrenals, kidneys, gonads, prostate gland, uterus, mesenteric lymph nodes, urinary bladder, sciatic nerve, skin, mammary gland, long bone, bone marrow, small intestine, large intestine, colon, caecum, stomach, nasal-lacrimal tissue, spinal cord and salivary glands.

**Organ weights:**

Following organs were weighed at necropsy:

Liver, kidneys, adrenals, gonads, brain and heart.

**Histopathology:** Slides were stained by hematoxylin and eosin.

**Subgroup A (Treated for 3-months):** Histological examinations were conducted on following tissues from all rabbits:

Naso-lacrimal tissue, any gross lesions, eyes, eyelids, nictitating membranes, Harder's gland and lacrimal tissue. Following non-ocular tissues were prepared from each rabbit in the untreated group (gr 1), vehicle control (gr 2) and 2.5% betaxolol (gr 5).

Adrenals, aorta, femur bone, sternum bone marrow, cerebrum, cerebellum, pons, medulla, cecum, esophagus, gallbladder, heart, kidneys, large intestine, larynx, liver, lung, lymph nodes, mammary gland,

skeletal muscle, sciatic nerve, ovary, pancreas, pituitary, prostate, salivary glands, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, and uterus.

Subgroup B (Treated for 12 months): Following tissues were examined for all animals at the end of one year of the treatment.

Eyes, eyelids, nictitating membranes, Harder's gland and lacrimal tissues. All other tissues and organs were fixed and retained for the future examination if needed.

#### Toxicokinetics:

##### S (-) Betaxolol plasma levels:

Blood samples were collected from the ear vein after 1, 3, 6 and 12 months of the treatment. Plasma samples were separated for the determination of S (-) betaxolol levels. Samples were collected approximately half-hour after the last daily dose.

Samples were collected from five rabbits from subgroups A and B for group 1, untreated control. Random samples were collected from four males and one female rabbit from group 1.

Random blood samples were collected from three female and one male rabbits that belonged to group 2 (both subgroups A and B) that received betaxolol vehicle.

##### For 0.5% S (-) betaxolol treated animals:

Blood samples were taken from 5 male and 5 female animals from subgroups A and B at the end of one and three months. Blood samples at the end of 6 months were collected from 6 male and 5 female animals from subgroup B. Blood samples were collected from 4 male and 4 female rabbits from the subgroup B at the end of 12 months.

##### For 1.0% S (-) betaxolol treated animals:

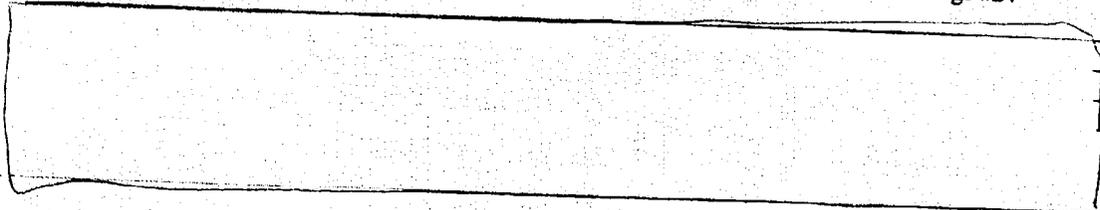
Blood samples were taken from 5 male and 5 female rabbits of subgroups A and B at the end of month one. Blood samples were collected from 4 male and 6 female rabbits from subgroups A and B at the end of three months. Blood samples were taken from 5 male and 5 female rabbits from subgroup B at the end of 6 months. Blood samples were taken from 4 male and 4 female rabbits at the end of 12 months from the subgroup B.

##### For 2.5% S (-) betaxolol treated animals:

Blood samples were taken from 5 male and 5 female rabbits from subgroups A and B at the end of month one. Blood samples were taken from 6 male and 4 female rabbits that belong to subgroups A and B at the end of month 3. Blood samples were taken from 6 male and 6 female rabbits from subgroup B at the end of 6 months of the treatment. The sponsor stated that samples from 4 male and 5 female rabbits were lost during analysis. Blood samples were taken from 2 male and 5 female rabbits from subgroup B at the end of 12 months.

#### Results:

Analytical test of the formulation confirmed the stability of the active ingredient and the preservative. The level of S (-) betaxolol and benzalkonium chloride are shown in the following table.



**Mortality:**

Animal #L6378 (F) from group 4, subgroup B was found dead on day 109. Loss of hair under the chin and ocular discharge (OD) was observed before death. The cause of death was not determined.

**Moribund animals:**

Moribund animals were sacrificed as shown in the following table.

Animal	Group	Subgroup	Remarks
L6357(F)	1	B	Apparently not eating between days 343-354, no stool on days 344 and 351, hair ball observed in stomach at necropsy, sacrificed on day 357
L 6286 (M)	1	B	Not eating on day 273, no stool day 268, ocular discharge OD on day 154, hair ball-observed in stomach at necropsy, sacrificed between day 254-275
L 6271 (M)	2	B	No stool between 304-317, hair ball observed in stomach at necropsy, sacrificed before day 345
L 6312 (M)	2	B	Head tilted on day 112, torticollis, sacrificed on day 115
L 6335 (F)	3	B	Ocular discharge, OD between days 87-206 and nasal discharge between days 204-206, not eating between 203-206, emaciated, hairball observed in stomach at necropsy, sacrificed on day 206
L 6317 (M)	5	B	Not eating, feels cool to the touch, lethargic, sacrificed on day 276
L 6307 (M)	5	B	Ocular discharge, OD, on day 92, apparently not eating between days 283-289, no stool, sacrificed on day 289
L 6325 (M)	5	B	Ocular discharge, OD between days 86-302, mucus in stool, sacrificed on day 317

Above incidences occurred in the treated and untreated animals for the animals allotted to the one year treatment groups.

**Clinical signs:**

Following clinical signs were observed in the untreated control, vehicle control and S (-) betaxolol treated animals.

Group	Subgroup	Ocular discharge	Nasal discharge	Constipation/ no stool	Loss of hair
1	A		1/10		1/10
1	B	3/14 (OD=3)		1/14	2/14
2	A	3/10 (OD=3)			
2	B	7/14 (6=OD, 1=Both eyes)	1/14	3/14	4/14
3	A	4/10, OD=4			1/10
3	B	10/14, OD=6, OS= 1, Both eyes = 3	2/14	1/14	3/14
4	A	6/10 ( OD=5, OS=1)			1/10
4	B	5/14 (OD=5)	1/14	3/14	2/14
5	A	3/10 (OD=2, OS=1)		1/10	1/10
5	B	11/14 (OD=11)		5/14	1/14

Number of animals that showed that an increase in the ocular discharge from the right eye at 2.5% dose (group B) that was higher compared to untreated and the vehicle treated animals.

Body weight:

The body weight (kg) of rabbits on day 0 and day 93 for animals that were treated for 3 months are shown below:

Group	Day 0		Day 93	
	M	F	M	F
1A	2.58	2.64	4.08	4.06
2A	2.84	2.62	4.66	4.64
3A	2.62	2.72	4.24	4.60
4A	2.70	2.54	4.40	4.36
5A	2.68	2.70	4.20	4.56

The weight gain for male rabbits was 1.42, 1.82, 1.62, 1.70 and 1.52 kg for groups 1A, 2A, 3A, 4A and 5A, respectively. The percent increase in the weight gain compared to the untreated control was 28%, 14%, 19% and 7% for groups 2A, 3A, 4A and 5A, respectively.

The weight gain for female rabbits was 1.42, 2.02, 1.88, 1.82 and 1.86 kg for groups 1A, 2A, 3A, 4A and 5A, respectively. The percent increase in the weight gain compared to the untreated control was 42%, 32%, 28% and 30% for groups 2A, 3A, 4A and 5A, respectively.

Male and female rabbits showed weight gain in the vehicle and S (-) betaxolol treated animals compared to the untreated animals. There were no statistically significant time-group interactions. Data suggest that S (-) betaxolol did not have significant effect in the body weight gain in rabbits.

The body weight (kg) of rabbits treated for one year is shown in the following table.

Group	Day 0		Day 366	
	Male	Female	Male	Female
1B	2.68	2.60	5.20	4.75
2B	2.64	2.70	4.78	5.11
3B	2.75	2.66	5.01	5.25
4B	2.73	2.66	4.66	5.15
5B	2.64	2.59	4.65	4.98

The weight gain for male rabbits was 2.52, 2.14, 2.26, 1.93, and 2.01 kg for groups 1B, 2B, 3B, 4B and 5B, respectively. The percent of weight gain compared to the untreated control was 85%, 90%, 76% and 80% for groups 2B, 3B, 4B and 5B, respectively.

The weight gain for female rabbits was 2.15, 2.41, 2.62, 2.59 and 2.39 kg for groups 1B, 2B, 3B, 4B and 5B, respectively. The percent of weight gain compared to the untreated control was 112%, 121.8%, 120.5%, 111.16% for groups 2B, 3B, 4B and 5B, respectively. There was no statistically significant change in the body weight gain in male and female rabbits between untreated and vehicle or S (-) betaxolol treatment.

These data suggest that treatment with 0.5, 1.0 and 2.5% S (-) betaxolol for one year did not affect statistically significant change in the body weight gain compared to the vehicle control in male and female rabbits.

Food consumption: not recorded

Eye examinations:

Slit lamp examination score are reported on the basis of the average of both male and female rabbits. Data were combined for both three and twelve months of observations.

Conjunctival congestion:

The average data for each group are shown in the following table.

Group/ Eye	Day -5	Day 0	Day 4	Day 32	Day 93	Day 184	Day 273- 275	Day 366
1, Right	0.83	1.83	2.25	2.25	1.75	1.57	2.00	1.83
1, Left	0.91	1.66	1.83	1.91	1.33	1.57	2.00	2
2, Right	1.16	1.83	2.08	2.75	1.5	1.53	1.63	1.66
2, Left	1.16	1.75	2	2.25	1.33	1.84	1.69	1.66
3, Right	1.16	1.5	2.5	2.33	1.33	1.29	1.38	1.84
3, Left	0.75	1.41	2.33	2.00	1.5	1.42	0.75	1.84
4, Right	0.83	1.33	2.5	2.33	1.5	1.53	1.38	1.84
4, Left	0.75	1.41	2.25	2.08	1.33	1.53	3.38	1.84
5, Right	0.91	1.75	2.33	2.91	1.66	2	1.71	2
5, Left	0.91	1.83	2.25	2.08	1.58	1.71	1.71	2

Slit lamp examinations showed conjunctival congestion (hyperemia) in the S (-) betaxolol treated, vehicle and untreated control eyes. The effect was considered to be unrelated to any treatment.

Conjunctival swelling:

The average score for conjunctival swelling for all groups was 0 except following differences listed in the table.

Group	Eye	Average Score	Day
1	Right	0.08	93
2	Right	0.15	275
2	Left	0.08	MLC Day 16
4	Left	0.15	254
5	Right	0.08	16
5	Right	0.08	93
5	Right	0.14	254

The data suggest that there was no trend for the development of conjunctival swelling in the S (-) betaxolol or vehicle treated animals.

Conjunctival discharge:

Conjunctival discharge scores are shown in the following table.

Day	Gr 1, OD	Gr 1, OS	Gr 2, OD	Gr 2, OS	Gr 3, OD	Gr 3, OS	Gr 4, OD	Gr 4, OS	Gr 5, OD	Gr 5, OS
-5	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
4	0	0.08	0	0	0.08	0	0.15	0	0.15	0
9	0	0	0	0	0.08	0	0.15	0	0.75	0
16	0	0	0.08	0	0	0	0.08	0	0.50	0.08
23	0	0	0	0	0	0	0.08	0	0.41	0
32	0	0	0.08	0	0.08	0.08	0	0	0.41	0
44	0	0	0	0	0	0	0.15	0	0.15	0
58	0	0	0	0	0.25	0	0.15	0	0.5	0.08
74	0	0	0.16	0	0.08	0	0.25	0	0.33	0
93	0	0	0	0	0.08	0	0.25	0	0.41	0
128	0	0	0	0	0	0	0	0	0.14	0
163	0	0	0	0	0	0	0	0	0	0
184	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	0	0	0	0	0.28	0
254	0	0.14	0	0	0.3	0.30	0	0	0.28	0.28
275	0	0	0.15	0	0	0	0	0	0.42	0.28
317	0	0	0	0	0.30	0.30	0	0	0	0.28
345	0	0	0	0	0.15	0.45	0	0	0.16	0
366	0	0	0	0	0	0.30	0	0.15	0.36	0

Above data suggest that conjunctival discharge was observed at most of the observation points in the treated eye at 2.5% dose. The sponsor stated that the severity was minimal. A low incidences of minimal conjunctival discharge was also observed in 1% S (-) betaxolol treated eyes during days 4 to 93. Rabbit eyes treated at 0.5% S (-) betaxolol also showed minimal conjunctival discharge intermittently. Untreated animals did not show conjunctival discharge. The vehicle treated animals showed conjunctival discharge only four times during the observation period.

Vehicle and S (-) betaxolol treated animals showed occasional changes in the light reflex in treated eyes during the observation period. However, the incidences were minimal.

Treatment related flare, iritis and corneal cloudiness were not observed in any animals. Similarly fluorescein dye staining technique did not reveal treatment-related damage to the cornea in rabbit eyes.

No lenticular changes were observed except following isolated incidences. It is concluded that S (-) betaxolol treatment did not affect the lens.

Group	# Animal	Observation
1	L6283	Bilateral anterior and posterior cataract, OD, days 93-366
2	L6271	Lenticular opacity, OD, days 44-74
3	L 6359	Fibrin strand adhered to anterior lens capsule, OD, days 9-93

No neovascularization was observed in any animals in the control, vehicle and S (-) betaxolol treated groups.

Indirect ophthalmoscopic examinations:

No changes in the untreated control, vehicle control and S (-) betaxolol treated animals were reported. Observations on the optic nerve head, retinal and choroidal vessels did not show any abnormality.

Hematology:

There were no treatment-related changes observed in the animals during the study.

Three-month sacrifice:

Data for some of the parameters are shown below.

Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Basophil (%)	0.2	0	0.2	0.2	0.2	0.8	1.0*	0.8	0.2	0.6
Eosinophil (%)	2.8	2.2	2.2	1.0	2.4	1.6	.8	2.6	2.8	2.4
Lymphocyte (%)	55.0	57.2	53.6	53.4	52.4	57.2	56.6	54.2	66.4	52.8
Neutrophil (%)	38.8	35.6	41.2	41.4	41.4	35.8	39.4	38.8	27.6	41.6
Monocyte (%)	2.8	4.0	2.4	3.8	2.8	4.0	2.2	2.4	3.0	2.2
WBC $10^3/\mu\text{L}$	8.9	8.7	6.9	8.8	7.9	7.6	7.1	6.3	7.5	7.6
RBC $10^6/\mu\text{L}$	6.5	6.2	6.4	5.9	6.4	5.8	6.0	5.6	6.6	5.8
Platelet $10^3/\mu\text{L}$	3.24	3.7	3.8	3.6	4.1	2.9	3.1	2.9	3.2	3.3
Hematocrit (%)	45.6	42.6	44.3	39.9	43.6	39.5	40.2	37.7	46.4	40.3
Hemoglobin (g/L)	14.2	13.3	13.9	13.2	14.0	12.8	12.9	12.5	14.6	13.1

\* Statistically significant

There were no statistically significant changes except isolated changes in the basophil (%) in-group 4 male rabbits. The change in the basophil differential count in group 4 male rabbits was considered to be incidental.

Twelve month sacrifice:

Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Basophil (%)	0	0	0	0	0.14	0	0	0.33	0.25	0
Eosinophil (%)	0.83	1.5	1.0	2.0	0.71	1.0	2.0	0.66	1.5	3.42
Lymphocyte (%)	62.5	63.6	76.0	67.1	65.4	64.6	69.2	64.8	69.7	63.7
Neutrophil (%)	35.6	34.1	22.4	30.0	33.1	33.5	28.2	33.6	27.0	32.7
Monocyte (%)	1.0	.66	.6	.85	.57	.833	.42	.50	1.75	.14
WBC $10^3/\mu\text{L}$	7.8	6.7	7.5	7.4	7.7	6.6	7.5	7.0	8.3	6.6
RBC $10^6/\mu\text{L}$	6.2	5.8	6.5	6.0	6.1	6.0	6.6	5.6	6.6	5.7
Platelet $10^5/\mu\text{L}$	3.7	3.7	2.7	3.4	3.3	3.8	3.6	3.1	4.1	3.7
Hemoglobin (g/L)	14.3	13.1	14.6	13.3	14.0	13.4	15.0	12.7	14.9	12.6
Hematocrit (%)	42.0	38.5	42.9	39.6	41.5	39.5	44.4	37.2	44.1	37.6

Above data suggest that there were no treatment-related changes in the hematology parameters.

Clinical chemistry:

Three-month sacrifice:

Data on some of the blood chemistry parameters are shown below.

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Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Albumin g/dL	3.8	3.7	3.8	3.7	3.8	3.8	3.6	3.8	4.0	3.6
Alk Phos U/L	70	87.2	101	62.2	83.4	68.0	74.2	62.2	85.2	72.4
ALT U/L	30.4	35.2	33.4	28.6	24.6	32.4	29.0	27.6	29.8	24.4
AST U/L	13.8	15.4	14.2	12.8	12.0	15.8	12.6	10.8	11.6	11.4
Total Bilirubin mg/dL	0.05	0.016	0.06	0.01	0.02	0.012	0.036	0.018	0.07	0.012
CPK U/L	555	277	738	870	788	784	604	475	649	412
Creatinine mg/dL	1.0	1.3	1.0	1.3	1.0	1.3	1.0	1.2	1.0	1.3
Globulin g/dL	2.1	1.9	2.2	2.1	2.0	2.1	2.0	2.1	2.2	2.2
Glucose mg/dL	137	133	139	123	139	139	139	144	140	143
LDH (U/L)	69	62.4	107	70	91.2	98.4	57	74	50.4	47.4
Phosphorus mg/dL	4.8	4.5	5.0	4.6	4.9	4.8	4.6	5.5	5.4	4.5
Potassium mmol/L	4.7	4.8	5.4	4.7	5.0	4.5	5.0	4.8	5.7	4.4
Sodium mmol/L	142	142	145	141	143	143	144	144	146	142
Total protein g/L	5.9	5.7	6.1	5.8	5.8	5.9	5.7	5.9	6.2	5.9
TG (mg/dl)	161.4	67.4	272.4	79.8	138.4	70.6	120.0	79.0	209.4	68.6
Urea Nitrogen mg/dL	18.2	22.8	16.2	23.0	18.6	23.8	16.0	21.0	19.2	22.6
Uric Acid mg/dL	0.4	0.26	0.42	0.32	0.38	0.38*	0.36	0.32	0.38	0.32
Calcium mg/dL	19.4	20.8	21.0	19.2	19.6	18.1	17.6	23.0	20.2	21.4
Cholesterol mg/dL	22.2	55.4	56.6*	86.4	27.2	70.6	26.8	60.6	41.2	59.4

\*Statistically significant

Data suggest that there were no treatment-related changes in the blood chemistry parameters.

One year sacrifice:

Some of the blood chemistry parameters are shown below.