CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-816

PHARMACOLOGY REVIEW(S)

NDA 20-816

JUN 25 1997

THE DIVISION OF ANTI-INFLAMMATORY, ANALGESIC, AND OPHTHALMIC DRUG PRODUCTS

PHARMACOLOGY/TOXICOLOGY REVIEW

INITIAL REVIEW

NDA 20-816

SPONSOR: Alcon Laboratories, Inc.
Post Office Box 6600
Fort Worth, Texas 76134-2099

DRUG: AZOPT™ (Brinzolamide Ophthalmic Suspension) 1%

SUBMISSION: January 26, 1997

DATE RECEIVED: January 28, 1997

DATE ASSIGNED: February 3, 1997

REVIEWER: Almon W. Coulter, Ph.D.

REVIEW COMPLETED: May 31 , 1997

DRUG CATEGORY: Carbonic Anhydrase II Inhibitor

RELATED INDs/NDAs:

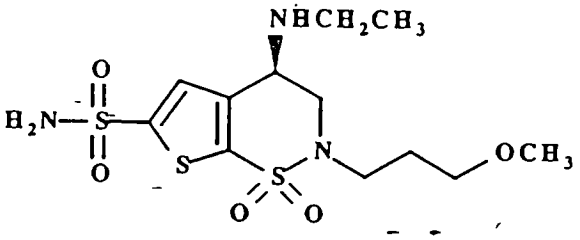
PROPOSED INDICATION:

AZOPT™ Ophthalmic Suspension 1% is indicated in the treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma.

DOSAGE AND ADMINISTRATION:

The recommended starting dose is 1 drop ~0.5 mg (10 mg brinzolamide/mL) per day in the affected eyes. Dosage can be increased to 1 drop tid (1.5 mg/day) if no response is seen after four weeks.

DRUG SUBSTANCE:



Brinzolamide

(R)-(+)-4-Ethylamino-2(3-methoxypropyl)-3,4-dihydro-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide

Molecular Formula: $C_{12}H_{21}N_3O_5S_3$ Molecular Weight: 383.49

Code Nº: ALØ4862, AL-4862, ALO4862
USAN Name: Brinzolamide
CAS Registry Nº: 138890-62-7

FORMULATION:

INGREDIENT	% W/V	mg/mL
Brinzolamide, NF		
Mannitol, USP		
Carbomer 974 P, NF		
Tyloxapol, USP		
Edetate Disodium $2H_2O$, USP		
Benzalkonium Chloride (10% Solution), NF		
Sodium Chloride		
NaOH and/or HCl	Adjust pH	
Purified Water		

PRECLINICAL STUDIES:

7. PACKAGING MATERIAL TESTS

Study 31.	One-Week Topical Ocular Irritation Evaluation in Rabbits of Potential Penetrants From Labels With P-508 or S-246 Label Adhesives TR No. 092:38520:0994	38	5B-7596
Study 32.	Acute Systemic Toxicity in Mice DROP-TAINER®s TR No. 041:38520:0495	39	5B-7982
Study 33.	Elution DROP-TAINER®s TR No. 040:38520:0495	39	5B-7995
Study 34.	Intracutaneous Reactivity Test in Albino Rabbits of DROP-TAINER®s TR No. 042:38520:0495	39	5B-8007
Study 35.	Primary Ocular Irritation in Rabbits DROP-TAINER®s TR No. 043:38520:0495	39	5B-8023
Study 36.	Agar Diffusion Test TR No. 061:38520:0694	39	5B-8048
Study 37.	Elution Test TR No. 062:38520:0694	39	5B-8060
Study 38.	Acute Systemic Toxicity in Mice TR No. 063:38520:0694	39	5B-8072
Study 39.	Intracutaneous Reactivity Test in Albino Rabbits TR No. 064:38520:0694	39	5B-8085
Study 40.	Evaluation of TR No. 038:38560:0589	39	5B-8100
Study 41.	Agar Overlay Test TR No. 044:38520:0690	39	5B-8118
Study 42.	Acute Systemic Toxicity in Mice and Primary Ocular Irritation in Rabbits TR No. 043:38520:0689	39	5B-8144

ABBREVIATIONS USED IN THIS REVIEW

D = day(s) W = week(s) G = group(s) * = $p<0.05$ ** = $p<0.01$
OD = right eye(s) OS = left eye(s) DR = dose related

PHARMACOLOGY:

REPORT 1.
NEUROPHARMACOLOGICAL PROFILE OF AL04862 IN RATS.

Report Nº: TR 421:39600:1192 Vol. 1.15
Compound: AL04862-003, Lot Nº 4035-85-IIA
Formulation: Solution
Route: Intravenous
Strain: Sprague Dawley, 150-250 g males
Number: 14 ♂
Dose Levels: G1 G2 G3 G4
mg/Kg: 0 1.0 10.0 30.0
Control Treatment: 0.9% saline adjusted to pH 3.0 with HCl
Study Site:
Date: July 30, 1992 - January 1, 1993
GLP/QAU Statements: Not present.

SUMMARY

This study was conducted to determine the potential neuropharmacological and potential anticonvulsant activity of AL04862-003. The anticonvulsant activity was evaluated only at 30 mg/Kg in a single group of four rats. The rats were observed for alertness, spontaneous motor activity, ataxia, convulsions, etc. over a 24 hour period. Electric shock (150 mA for 0.2 seconds) was administered to 4 rats five minutes after iv administration of 30 mg/Kg AL04862 . The control received 0.9% saline.

No signs of toxicity were reported over the observation period. The anticonvulsant protection ratio of 2:4 (50%) was established.

REPORT 2.
EFFECT OF AL04862 ON ELECTROLYTE CONCENTRATION AND VOLUME DIURESIS IN RATS.

Report Nº: TR 422:39600:1192 Vol. 1.15
Compound: AL04862-003, lot 4035-85-IIA
Formulation: Solution
Route: Intravenous, at 10 mL/Kg.
Strain: Sprague Dawley, body weight 180-250 g males
Number: 10/group
Dose Levels: G1 G2 G3 G4 G5
mg/Kg: 0 0.3 1.0 3.0 AL04862 5.0 acetazolamide
Positive Standard: Acetazolamide
Control Treatment: 0.9% saline adjusted to pH 3.5 with 0.1 N HCl
Study Site:
Date: January 6, 1992
GLP/QAU Statements: Not indicated.

The purpose of the study was to evaluate the potential effects of AL04862 in urinary volume output, pH, and electrolyte concentrations. Following 18 hours of fasting, the rats were hydrated po with 25 mL/Kg of followed by saline, drug, or acetazolamide administration. Urine was collected four hours later.

No significant change in the urine volume, pH, or electrolyte concentrations were observed at 0.3 mg/Kg. At 1.0 mg/Kg, urine volume, pH (*), Na^+ concentration, and K^+ concentration (*) increased and Cl^- concentration

decreased. At 3.0 mg/Kg, all parameters were significantly increased. At 5.0 mg/Kg acetazolamide, all parameters increased significantly. Clinical signs were not observed in any group during the study.

REPORT 3.
NEUROTOXICITY OF AL04862 IN MICE.

Report Nº: TR 423:39600:1192 Vol. 1.15
Compound: AL04862-003, lot 4035-85-IIA
Formulation: Solution
Route: Oral, gavage at 10 mL/Kg
Diet: *Ad libitum*
Strain: CD-1, 18-28 g body weight
Number: 10 ♂/group
Dose Levels: G1 G2 G3
mg/Kg: 1 10 30
Frequency of Administration: Once
Control Treatment: 0.25% methylcellulose at 10 mL/Kg
Study Site:
Date: July 28, 1992 - October 28, 1992
GLP/QAU Statements: Not indicated.

The purpose of the study was to determine the neurotoxicity of AL04862 administered to mice trained to remain on a rotarod for one minute. The animals were tested again at 30 and 60 minutes after dosing to determine their ability to remain on a rotarod for one minute. The results showed no change in the rotarod performance at 30 and 60 minutes after dosing with 1, 10, or 30 mg/Kg AL04862.

REPORT 4.
PHARMACODYNAMIC ASSAY OF AL04862

Report Nº: TR 424:39600:1192 Vol. 1.15
Compound: AL04862-003, lot 4035-85-IIA
Formulation:
Route:
Strain:
Number: 4 on study
Dose Levels:
Control Treatment: 0.9% saline adjusted to pH 3.0 - 3.5 with 0.1N HCl
Study Site:
Date: January 6, 1993

The purpose of the study was to determine the potential effects of an of AL04862 upon the cardiovascular response

A statistically significant decrease occurred in the cardiovascular response to but the change was not considered to be biologically significant. The other agents had no effect on blood pressure or heart rate, and the

EKG lead II show no change compared to a predose recording. No biological significant effects on O_2 and CO_2 blood gases or pH occurred. However, pCO_2 and pO_2 were increased by 62% and 11%, respectively.

REPORT 5.
EFFECT OF AL04862 ON GASTROINTESTINAL PROPULSION IN MICE.
Report Nº: TR 425:39600:1192 Vol. 1.15

This study, conducted by ..., looked at the potential effect of AL04862-003 in changing the GI time of propulsion of a suspension meal in mice dosed with 1, 10, or 30 mg/Kg AL04862. was suspended in and orally administered at 10 mL/Kg 30 minutes after drug administration. The results showed a decrease in GI motility of 8%, 18%, and 44% for the 1, 10, and 30 mg/Kg AL04862 doses. Only the 44% was significant (*) from the 0.25% methylcellulose control group.

REPORT 6.
EFFECT OF AL04862 ON BARBITURATE SLEEP TIME IN MICE.
Report Nº: TR 426:39600:1192 Vol. 1.15

The ability of oral administered 1, 10, and 30 mg/Kg AL04862-003 to potentiate the sleep time in mice injected intraperitoneally with was evaluated . The results were compared with the 0.25% methylcellulose control. The study was done by

A 57%, 15%, and 35% increase in the sleep time over the control sleep time of 54 minutes was observed for the 1, 10, and 30 mg/Kg AL04862, respectively. These increases were not considered to be biologically significant.

REPORT 7.
CARDIOVASCULAR STUDIES WITH AL04862 IN DOGS.
Report Nº: TR 427:39600:1192 Vol. 1.15

Acute hemodynamic effects of intravenous infusion of AL04862-003 in the open-chest anesthetized dog were evaluated by . Two male and two female anesthetized dogs maintained by artificial respiration received iv fusions of 0, 1.0, or 10.0 mg/Kg AL04862 over 15 minutes. The control treatment was 0.9% saline solution. Effects were measured on arterial BP, heart rate, left ventricular end diastolic pressure, +dP/dt, cardiac output, contractile force, and lead II EKG.

No significant changes were observed on the cardiac and circulatory parameters with the administration of saline or 1.0 mg/Kg AL04862. At 10 mg/Kg, biologically significant increases (>20% change) occurred in cardiac output, +dP/dt, contractile force, and circulatory parameters. Other parameters were not significantly changed. Lead EKG II values showed no gross changes. No significant changes occurred in blood pCO_2, pO_2 and blood pH.

REPORT 8.
THE EFFECT OF AL04862 ON BLOOD GASES IN CONSCIOUS RATS.
Report Nº: TR 428:39600:1192 Vol. 1.15

The potential effects of iv administration of 0 (0.9% saline), 0.3, 1.0, or 3.0 mg/Kg AL04862 on blood pO_2, pCO_2, and pH were determined in rats (10/group) containing an aortic cannula for blood collection. Values were determined at 0, 2, 5, 15, 30, 60, 90, and 120 minutes following administration.

No significant effects were seen at 0.3 and 1.0 mg/Kg. At 3.0 mg/Kg, however, significant (*) increases were reported for pCO_2 at 15 and 90 minutes, and pO_2 was increased significantly ($p \leq 0.05$) at all time points following drug administration. These changes were said to be comparable to the control changes and not biologically significant. Acetazolamide (5.0 mg/Kg iv), the positive control, decreased the pH significantly at 60 minutes and increased the pO_2 significantly (*) at 120 minutes. It did not change the pCO_2.

REPORT 9.
RECEPTOR BINDING PROFILE OF THE CARBONIC ANHYDRASE INHIBITOR, AL04862.
Report Nº: TR 001:39730:0196 Vol. 1.15

Ligand binding was evaluated in a battery of 34 assays conducted by
AL04862 was tested at 1 nM, 100 nM, and 10 µM.

REPORT 10.
LIGAND BINDING PROFILE OF THE CARBONIC ANHYDRASE INHIBITOR AL-8520A.
Report Nº: TR 021:39730:1196 Vol. 1.15

AL-8520A was evaluated at 1 nM, 100 nM, and 10 µM, as in Report 9 above. The report indicated no significant interactions with any of the 34 ligand binding sites evaluated. The study was done by

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REPORT 11.
LIGAND BINDING PROFILE OF THE CARBONIC ANHYDRIDE INHIBITOR AL-7118A.
Report Nº: TR 019:39730:0696 Vol. 1.15

Concentrations of 1 nM, 100 nM, and 10 µM AL-7118A were evaluated by in the above 34 ligand binding assays (see Report 9). No significant interactions were reported.

REPORT 12.
THE IC_{50} OF KEY CARBONIC ANHYDRASE INHIBITORS–NUMBER 96-001.
Report Nº: TR 001:39320:0696 Vol. 1.15

Several carbonic anhydrases developed by Alcon, one of which was AL04862, were evaluated for their ability to inhibit human carbonic anhydrase isozymes I, II, and IV in *in vitro* studies. The average of two measurements for the IC_{50} values for these carbonic anhydrase inhibitors are indicated below.

	IC_{50} ± range, nM		
	CA I	CA II	CA IV
AL04862	1,367	3.19	45
Al07118A	2,183	19.3	908
AL08520A	274	1.28	128
AL04217A (MK-507)	28,032	3.74	32.0
AL04408 (acetazolamide)	657	9.04	33.1

REPORT 13.
***IN VITRO* BINDING (K_i) TO HUMAN CARBONIC ANHYDRASE ISOZYMES I AND II FOR AL04862, AL07118A, AND STANDARDS ACETAZOLAMIDE (AL04408) AND DORZOLAMIDE (AL04217A).**
Report Nº: TA 051:39310:0796 Vol. 1.15

In this study human carbonic anhydrase I and II binding affinities (K_i) for AL04862A, AL04217A (dorzolamide), and AL04408 (acetazolamide) were determined *in vitro*. The human carbonic anhydrase I and II were purified from human erythrocytes. The study was done by

Compound	K_i (nM)	
	HCA-I	HCA-II
AL04862A	32.1 ± 0.99	0.13 ± 0.03*
AL07118A	N.A.	4.49
AL04408 (Acetazolamide)	673 ± 81.8	33.8 ± 4.90
AL04217 (Dorzolamide)	1240 ± 417	0.51 ± 0.09

* Data from TR 017:39310:0496

REPORT 14.
EFFECT OF AL04862A ON INTRAOCULAR PRESSURE IN TWO AGE GROUPS OF RABBITS AFTER A SINGLE TOPICAL OCULAR INSTILLATION.

Report Nº: TR 139:39600:0991 Vol 1.15
Compound: AL04862A
Formulation:

Route: Instillation in the left eye.
Strain: Dutch-belted females, body weight 1.0-2.5 Kg
Number: 9 ♀
Diet: Ralston Purina Certified Rabbit Chow Nº 5325 - 8 oz/day
Dose Levels: 1 mg (25 µL)
Control Treatment: Vehicle administered in the right eye.
Study Site: Alcon Laboratories
Date: August 27, 1991 to October 3, 1991
GLP/QAU Statements: Not indicated.

The purpose of this study was to determine the effects of AL04862A on the intraocular pressure in rabbits. The report contains 2 studies. In the first study, significant decreases ($\alpha = 0.05$) in IOP changes were 18.7%, 20.7%, 9.4%, 6.8%, and 5.4% at 0.5, 1, 2, 3, and 4 hours, respectively. In the second study, a comparison was made between 5 rabbits that had been in house 7 weeks (body weight 1.3-1,6 Kg) and 5 rabbits that had been in house 9 months (weight range 1,8-2.2 Kg). The IOP of AL04862A treated eyes decreased significantly ($\alpha = 0.05$) from the IOP in the contralateral, vehicle treated eyes. These IOP results are indicated below.

	0.5	1	2	3	4	5	6 hours
Young rabbits:	-	13%*	13.3%*	15.5%*	16.2%*	15.9%*	11.4%*
Older rabbits:	7.3%*	17.9%*	12.4%*	11.7%*	7.6%*	10.4%*	10.7%

* Significant at $\alpha = 0.05$

The baseline IOP for the younger rabbits was 26.7 mm Hg and for the older rabbits the IOP was 30.4 mm Hg, a significant ($\alpha = 0.05$) difference.

REPORT 15.
PRELIMINARY TEST OF THE EFFECT OF AL04862A ON INTRAOCULAR PRESSURE IN MONKEYS AFTER A SINGLE TOPICAL OCULAR INSTILLATION

Report Nº: TR 150:39600:0991 Vol. 1.16
Compound: AL04862A suspension
Formulation: AL04862A

Route: Topical in OD.
Strain: Cynomolgus (Macaca fascicularis), body weight ♂ 5.4-8.7 Kg, ♀ 3.9-5.6 Kg
Dose Levels: 0, 600 µg (30 µL) 1x
Number: 5 vehicle control and 9 drug treated animals - animals were mixed sexes (10 ♂, 4 ♀).
Control Treatment: Vehicle instilled in OR
Study Site: Alcon Laboratories
Date: September 19, 1991 to October 11, 1991
GLP/QAU: Not indicated.

This study was conducted to determine the effects of a suspension of AL04862A on IOP in the right eyes made hypertensive with : - the left eyes were untreated. Drug suspension or vehicle were instilled in the eyes after baseline IOP was measured. Additional IOP measurements were then taken at 1, 3, and 6 hours.

RESULTS

EFFECT OF AL04862A ON INTRAOCULAR PRESSURE
(from Vol. 1.16, p. 5A-0267)

		Time (hr)	Mean IOP	Mean % Change		Mean IOP	Mean % Change
AL04862A	OD	0	30.4	0.0	OS	20.6	0.0
		1	22.3	-26.2*		20.4	0.1
		3	20.8	-31.3*		19.1	-5.8
		6	21.8	-27.7*		19.7	-4.0
Control	OD	0	31.2	0.0	OS	18.2	0.0
		1	29.4	-4.8		17.4	-3.8
		3	26.4	-15.8*		17.0	-6.5
		6	29.0	-6.0		18.4	1.3

* Significant at $p<0.001$

REPORT 16.
EFFECTS OF TIMOLOL ON INTRAOCULAR PRESSURE OF CYNOMOGUS MONKEYS.

Report Nº: TR 222:39600:0894 Vol. 1.16
Compound: Timolol
Formulation:
Route: Topical, instilled in the eye

Strain: Cynomolgus (Macaca fascicularis)
Dose Levels: 0, 0.5% Timoptic 50 µg (10 µL) OD and OL
Number: 18
Control Levels: 8 in control and 10 in Timoptic group.
Study Site: Alcon Laboratories
Date: August 29, 1994
GLP/QAU Statements: Not indicated.

The IOP was determined prior to dosing and at 1, 3, and 7 hours after dosing. The right eyes received , producing ocular hypertension . All left eyes were normal and normotensive.

RESULTS

The mean IOP was reduced by 21%, 31%, and 31% in the laser treated right eyes at 1, 3, and 7 hours, respectively, after Timoptic treatment. These reductions differed significantly from the control values at all time points. No significance was produced in the normotensive left eyes or in the vehicle control treated eyes at the three time points.

REPORT 17.
EFFECT OF AL04862A (pH 5, pH 7) ON INTRAOCULAR PRESSURE IN RABBITS AFTER A SINGLE TOPICAL OCULAR INSTILLATION.

Report Nº: TR 174:39600:1091 Vol. 1.16
Compound: AL04862A suspension.
Formulation: Identical to that used in Study 15
Route: Oral, topical. OR treated with two 25 µL of drug solution and OS treated with two 25 µL vehicle.
Strain: Dutch-belted female rabbits, body weight 1.9-2.5 Kg.
Dose Levels: 0 and 1 mg
Number: 7/pH group.
Control Treatment: Vehicle
Study Site: Alcon Laboratories
Date: December 16, 1991
GLP/QAU Statements: Not indicated.

This study evaluated the effect of lowering the IOP in Dutch-belted rabbits after a single topical ocular instillation of Al04862A suspensions of pH 5 and pH 7. Following baseline IOP determinations, the drug or vehicle were instilled and IOP determined at 0.5, 1, 2, 3, 4, and 5 hours.

RESULTS

Significant ($p<0.001$) reduction occurred in the mean percent change of the IOP in the right eyes of the drug treated group at all the designated time points for both pH suspensions. No consistent changes were noted between the pH 5 and the pH 7 group.

REPORT 18.
EFFECT OF AL04862A (pH 7) ON IOP IN MONKEYS AFTER THREE TOPICAL OCULAR INSTILLATIONS.

Report Nº: TR 184:39600:1191 Vol. 1.16

Compound: 2% AL04862A suspension.
Formulation: Identical to that used in Report 15.
Route: Topical, ocular at 30 µL administered 12 hr apart over two days (3 doses)
Strain: Cynomolgus (Macaca fascicularis)
Dose Levels: 0, 600 µg OD (30 µL)
Number: 5 vehicle control and 9 AL04862A group - mixed sex.
Control Treatment: Vehicle
Study Site: Alcon Laboratories
Date: December 16, 1991

This study looked at the effect of a pH 7 suspension of AL0462A on the IOP in laser treated (hypertensive) right eyes of conscious monkeys. The left eyes of the animals were normal and normotensive. Following baseline IOP measurements, 30 µL of the drug suspension or vehicle were instilled in the right eye every 12 hours for three treatments. IOP was determined at 1, 3, 6, 10, and 12 hours following the first instillation, 12 hours after the second instillation, and at 1, 3, and 6 hours following the third instillation.

RESULTS

The mean reduction in IOP of the right eyes of the drug treated group was 19.9% to 28.8% and significant at $p<0.0001$ over the study . There was no significance between the treated eyes and the contralateral eyes in this group. There does not appear to be any significant difference between the pH 5 and pH 7 suspensions in lowering IOP.

REPORT 19.
EFFECT OF VEHICLE AND DROP SIZE ON INTRAOCULAR PRESSURE RESPONSE IN RABBITS TO AL04862.

Report Nº: TR 222:39600:0192 Vol. 1.16
Compound: AL04862A
Formulation:

Route: Topical, ocular
Strain: Dutch Belted rabbits, body weight 2.0 to 3.0 Kg
Dose Levels: 0, 600 µg (30 µL)
Number: 6/group
Control Treatment: 0.5% Carbomer vehicle or .
Study Site: Alcon Laboratories
Date: January 10, 1992 - March 16, 1992
GLP/QAU Statements: Not indicated.

The purpose of this study was to investigate vehicle and drop size of a suspension of AL04862A in reducing IOP. After measuring baseline IOP, the left eyes were topically dosed with one 30 µL of drug suspension. The right eyes were used as controls and dosed with the appropriate vehicle, either 0.5% carbomer . IOP measurements were taken at 0.5, 1, 2, 3, 4, and 5 hours.

RESULTS

Mean % change in IOP values for the carbomer drug suspension were significantly reduced (*) from baseline values for all measurements, except at 0.5 hour. With the drug suspension, IOP values were significantly reduced (*) at 1, 2, 3, and 5 hours. No significant difference was seen in the effect of the two

suspensions in reducing the IOP. Comparing the results with Report 20, no significant differences were found between the four treatments.

REPORT 20.
EFFECT OF VEHICLE ON INTRAOCULAR PRESSURE RESPONSE IN RABBITS TO AL04862.
Report Nº tr 223:39600:0192 Vol. 1.16

This study evaluated the effects of four different AL04862 suspensions in lowering the IOP in Dutch Belted rabbits, following a single topical ocular instillation of 1 mg of drug. The IOP was measured at 0, 0.5, 1, 2, 3, 4, 5, and 6 hours. The four formulations were:

A.
B.
C.
D

The results showed all four formulations produced similar statistically reductions in IOP from baseline values over 6 hours. The 0.5% carbomer formulation produced moderate tearing - the other formulations produced only slight tearing. The study was done at the Alcon Laboratories in January 1992.

REPORT 21.
EFFECT OF AL04862 FORMULATIONS ON INTRAOCULAR PRESSURE IN RABBITS AFTER A SINGLE TOPICAL OCULAR INSTILLATION.
Report TR 263:3900:0392 Vol. 1.16

The purpose of this study was to evaluate the IOP following the instillation of a 2% AL04862 suspension in two different vehicles. Formulations were administered once at 600 µg (30 µL) in the left eye of Dutch Belted rabbits. Both formulations produced a significant lowering of IOP in these animals.

REPORT 22.
COMPARISON OF EFFECT OF AL04862 AND AL04623 SUSPENSION ON INTRAOCULAR PRESSURE IN RABBITS AFTER A SINGLE TOPICAL OCULAR INSTILLATION.
Report TR 258:39600:0392 Vol. 1.16

This study evaluated 2% suspensions of AL04862 (600 µg, 30 µL) and AL04623 (600 µg, 30 µL) in the same vehicle in lowering IOP in Dutch Belted rabbits. IOP measurements were taken at 0, 0.5, 1, 2, 3, 4, and 5 hours after topical ocular instillation of the suspensions in the right eyes - left eyes were dosed with vehicle. Alcon Laboratories directed the study.

Significant (*) reduction occurred in the IOP of both AL04862 and AL04623 suspensions at all time points. The mean IOP in the treated eyes differed significantly from the mean IOP in the contralateral vehicle treated eyes. Mean IOP of AL04862 treated eyes did not differ significantly from the mean IOP of AL04623 treated eyes over the study period.

REPORT 23.
EFFECT OF AL04862 ON INTRAOCULAR PRESSURE IN RABBITS AFTER A SINGLE TOPICAL OCULAR INSTILLATION (DOSE RESPONSE AND VISCOSITY EFFECT).
Report Nº TR 288:39600:0592 Vol. 1.16

Dose response and viscosity effects of 0.5%, 1%, and 2% AL04862 suspensions were evaluated in reducing IOP of the treated left eyes of Dutch-Belted rabbits. Contralateral eyes were treated with matching vehicle, saline, or were left untreated. IOP measurements were recorded at 0 (baseline), 0.5, 1, then hourly up to 6 hours.

The results indicated that > 250 µg instillations of AL04862 suspensions in the carbomer based vehicles produced significant reductions in IOP. Increasing the viscosity of the vehicle with carbomer tended to reduce the onset time for reducing the IOP.

REPORT 24.
COMPARISON OF EFFECT OF 600 µg VS 300 µg AL04862 IN CARBOPOL SUSPENSION ON INTRAOCULAR PRESSURE IN MONKEYS DURING TWO DAYS OF BID DOSING.
Report Nº TR 311:39600:0692 Vol. 1.17

Suspensions of 1% (300 µg) and 2% (600 µg) AL04862 in a vehicle

were administered topically to laser treated eyes of cynomolgus monkeys. The right eyes were treated twice a day at twelve hour intervals for two days. IOP was measured at baseline, 1, 3, 6, and 12 hours after the first and third doses, and 12 hours after the second and fourth doses.

IOP was significantly ($p<0.02$ - 0.001) reduced from IOP values of the contralateral untreated eyes at all measurements in both the 1% and 2% suspensions. No significance occurred between the IOP of the 1% dose and the nontreated contralateral eyes from 3 hours after the first dose through 12 hours after the third dose. The high dose showed no significant difference in the IOP of the treated vs the untreated eyes. Reductions by the second day were 33% with 300 µg AL04862 and 35% with 600 µg AL04862. However, no significant difference was reported between the two concentrations of the AL04862.

REPORT 25.
EFFECT OF 600 µg AL04862 ON THE INTRAOCULAR PRESSURE IN MONKEYS AFTER THREE CONSECUTIVE 10 µL DROPS BID.
Report Nº TA 347:39600:0793 Vol. 1.17

The IOP effects of a 2% suspension of AL04862 were determined following instillation of three 10 µL aliquots 10 minutes apart to the right eyes of cynomolgus monkeys at 0900 and 2100 hours. Following the same schedule, the vehicle was instilled in the right eyes of five additional monkeys IOP was determined at 0, 1, 3, 6, 12 hours after the first dose and at 12, 14, and 16 hours after the second treatment.

The study was done at Alcon Laboratories.

A significant reduction of IOP below the baseline IOP was recorded over the study. The IOP was not statistically different from the IOP of the contralateral, untreated normotensive eyes. The mean IOP in the vehicle treated eyes differed significantly from the values in the contralateral, untreated, normotensive eyes

throughout the evaluation times. The sponsors conclusion was that small consecutive doses of the drug do not affect the maximum reduction or duration of IOP in laser treated monkey eyes.

REPORT 26.
EFFECT OF 300 µg AL04862 VS 300 µg AL04862 ON INTRAOCULAR PRESSURE IN MONKEYS DURING ONE DAY OF BID TOPICAL OCULAR INSTILLATION (CROSSOVER).
Report Nº TR 193:39600:0694 Vol. 1.17

This study compared the IOP effects of AL04862 and AL06218 in hypertensive right eyes of cyanomolgus monkeys. Following baseline IOP determinations, 30 µL of a 1% suspension of each drug was instilled in the right eyes of six animals per group at 0900 and 2100 hours on Day 1. Four weeks later the drugs were reversed in a crossover evaluation of IOP. The left eyes were normotensive and not treated. IOP values were determined at 0, 1, 3, 6, and 12 hours after the first dose and 12 hours after the second treatment.

The 1% AL06218 suspension

. The 1% AL04862 suspension

(the exact formulation was not indicated).

Both of the 1% suspensions significantly reduced the IOP ($p<0.001$), but no significant difference was shown between the two drugs. Slight blinking was reported for both drugs.

REPORT 27.
COMPARISON OF AL04862 AND AL04623 SUSPENSIONS ON INTRAOCULAR PRESSURE IN MONKEYS DURING TWO DAYS OF BID TOPICAL OCULAR INSTILLATION.
Report Nº TR 226:39600:0292 Vol. 1.17

This study compared the IOP of 2% suspensions of AL04862 and AL04623 in treated eyes. Dosage was 600 µg instilled in the right eye, while the left eyes remained untreated. After one week the treatment was reversed in a crossover of the two drugs.

The results indicated no significant difference in the IOP response between the two suspensions. Blinking was reported after instillation of both formulations.

REPORT 28.
DIRECT COMPARISON OF 600 µg CLINICAL FORMULATION AL04862 WITH FORMULATION OF AL05139 FOR EFFECT ON INTRAOCULAR PRESSURE IN MONKEYS.
Report Nº TR 295:39600:0693 Vol. 1.17

IOP response of AL04862 and AL05139 in lowering the IOP in laser treated eyes was determined in this study. Suspensions of 2% of each formulation were instilled in the right eyes made hypertensive

Each monkey received 600 µg in one 30 µL aliquot at 0900 and 2100 hours. The contralateral eyes were normal and remained untreated. The study was done be Alcon Laboratories.

The 2% AL04862 suspension

The 2% AL05139 suspension

The sponsor indicated there was no difference in the IOP between the suspensions of AL04862 and AL05139 with these "optimal" formulations. Both formulations produced significant mean % changes.

REPORT 29.
EFFECT OF 1 MG AL04862 ON INTRAOCULAR PRESSURE IN DUTCH BELTED RABBITS DURING NINE DAYS OF TWICE DAILY TOPICAL OCULAR INSTILLATION.
Report TR 450:39600:1193 Vol. 1.17

A 2% suspension of AL04862 was evaluated for its effect on the IOP of rabbits treated twice a day for eight days and once on Day 9. The left eyes were dosed with about 1 mg (2 drops) - the right eyes were dosed with vehicle. Measurements were taken at 0, 0.5, 1, 2, 3, and 5 hours after the first dose and after the morning dose on Days 3 and 9.

The results showed a significant decrease in the mean IOP on Days 1, 3, and 9. Over time a decrease in the response developed in the treated eyes, with an induced response apparently occurred in the contralateral eyes.

REPORT 30.
EFFECT OF 500 µg AL04862 AND 500 µg AL06218 ON INTRAOCULAR PRESSURE IN DUTCH BELTED RABBITS AFTER A SINGLE INTRAVENOUS INJECTION.
Report Nº TR 123:39600:0394 Vol. 1.17

A 0.5% solution of AL04862 was injected (500 µg in 100 µL) in the lateral ear vein of four Dutch Belted female rabbits (1.8-2.5 Kg). Similarly, four rabbits were injected with 500 µg in 100 µL of a 0.5% solution of the carbonic anhydrase inhibitor AL06218, and four additional animals were injected with vehicle. IOP was measured at baseline and at 0.5, 1, 2, 3, and 4 hours following the injection. Solutions were prepared in sterile physiological saline at pH 4.5-5.0. The pH of the vehicle was 7.0.

AL04862 reduced the IOP by 6.3% at 2 hours ($p<0.04$) and 7.6% at 3 hours ($p<0.04$). No significant reduction was seen with AL06218. IOP reductions from the vehicle were not significant, but decreases of 1.4% to 2.0% were occurred. In general, mean IOP values were not significantly different in the three groups.

REPORT 31.
THE EFFECT OF AL04862 ON OCULAR HEMODYNAMICS SYSTEMIC BLOOD PRESSURE, HEART RATE, AND ACID-BASE BALANCE IN ANESTHETIZED CATS AND NEW ZEALAND ALBINO RABBITS.
Report Nº: TR 093:39600:0294 Vol. 1.17

The purpose of this study was to evaluate the effect of iv 5 mg/Kg AL04862 on intraocular optic nerve head microvascular blood flow in the cat and total and regional intraocular blood flow in the rabbit. Blood flow was measured in the cat with the and with techniques in the rabbit.

In anesthetized, spontaneous breathing cats, the optic nerve head blood flow (ONHBF) was reproducible with topically administered 30 µL of 1% AL04862 for 7 days (mean values were 1.84-1.85 mL/min/100 g), but blood flow was not significantly increased. In the acute dose studies reported in this review, no significant increase (16.5%) occurred in ONHBF over 60 minutes post topical dosing - pH and pCO_2 did not change. With acute iv

dosing, significant increases in arterial CO_2 occurred ($p<0.001$, 67%), with a 46% significant increase ($p \leq 0.05$) in blood flow to the optic nerve head.

In anesthetized rabbits, ocular blood flow following iv AL04862 administration increase. Choroid blood flow was reduced in the mildly ischemic right eyes compared to left eyes. Following iv administration, significant acid-base balance was dose dependent. Increases in blood flow to the optic nerve head and anterior and posterior uveal tissues were also dose dependent following the 2.5 and 5.0 mg/Kg dose.

REPORT 32.
THE IC_{50} RESULTS OF CARBONIC ANHYDRASE INHIBITOR, AL-12353 - NUMBER 96-002.
Report Nº TR 002:39320:1196 Vol. 1.17

The carbonic anhydrase inhibitor (AL-12353) was evaluated in inhibiting human carbonic anhydrase I, II, or IV. The results indicated IC_{50} values of 234, 2.91, and 101 for HCA-I, HCA-II, and HCA-IV, respectively. No data were given comparing AL04862 inhibition of carbonic anhydrase.

PRECLINICAL TOXICOLOGY STUDIES WITH BRINZOLAMIDE:
1. TOPICAL OCULAR STUDIES

STUDY 1.
ONE-DAY TOPICAL EXAGGERATED TOPICAL OCULAR IRRITATION/COMFORT EVALUATION OF AL04862 OPHTHALMIC SUSPENSION.

Report Nº: TR 003:38520:0292 Vol. 1.19
Compound: AL04862
Formulation: 2% suspensions in the following four formulations:
a) Ophthalmic Suspension Vehicle b) Maxidex Vehicle
c) Suspension Vehicle d) Suspension Vehicle
Route: Topical, ocular OS administration
Strain: NZW
Number: 3/group
Dose Levels: 2 Drops OS administered every 30 minutes for a total of ten doses.
Study Site: Alcon Laboratories, Inc., Fort Worth, Texas
Date: March 10, 1992
GLP/QAU: The study was exploratory and non-GLP.

The four 2% AL04862 suspensions and vehicles were tested. Following the first and fifth dose, a comfort evaluation was performed. Prior to the first dose and one hour after the last dose, examination of the test eye was performed.

RESULTS

The results indicated minimal/moderate conjunctival irritation with the four vehicle suspensions and the four 2% AL04862 suspensions. Further development of these suspension formulations are indicated.

STUDY 2.
THREE-MONTH TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862 OPHTHALMIC SUSPENSION IN RABBITS (1 MONTH INTERIM).

Report Nº: TR Nº. 020:38520:0392, protocol Nº N-91-176 Vol. 1.19
Compound: AL04862
Formulation:

	PERCENT (W/V)		
INGREDIENT	VEHICLE	2.0% AL04862	4.0% AL04862
AL04862			
CARBOMER			
SODIUM CHLORIDE, USP			
MANNITOL, USP			
DISODIUM EDETATE, USP			
BENZALKONIUM CHLORIDE, NF			
NaOH/HCl, NF			
WATER FOR INJECTION, USP			

Route: Topical, ocular, 60 µL qid OD administration
Diet: One cup/day Purina Certified Rabbit Chow Nº 5325.
Strain: NZW, 2.5 Kg - 3.1 Kg body weight, 3-5 months old
Number: 3/sex/group
Dose Levels: Group 1: untreated Group 2: Vehicle, 0 mg/day x 34 days
Group 3: 2.0% AL04862, 4.8 mg/day x34 days Group 4: 4.0% AL04862, 9.6 mg/day x34 days
Study Site: Alcon Laboratories, Inc., Fort Worth, TX.
Date: February 4, 1992 to December 12, 1996
GLP/QAU: Both present and signed.

This study was done to determine ocular irritation and systemic toxicity of the vehicle and the two concentrations of AL04862. Included in the study were daily observations, daily examinations, body weight (pre-study, weekly, and at necropsy), biomicroscopic examination (pre-study, W1, W2, W3, W5), indirect ophthalmoscopic examination (pre-study and D34), pachymetry (D 0, D34), serum and hematology D32, plasma and blood drug analysis, necropsy D35, organ weights (liver, kidney, heart, brain, adrenals, gonads, spleen), and histopathology.

RESULTS

- signs: sporadic ocular discharge in OS of treated groups-
- body weight gain: comparable in all groups-
- ocular evaluation: minimal congestion (hyperemia) all groups - all animals remained normal-
- pachymetry: significant increase in OD cornea in G4 D34
- serum chemistry: ALT ♀ ↑ G4 (p=0.0051, 35%) - Amylase ♀ ↑ G2 (*, 24%), G4 (*,35%)-

- hematology: Hct ↓ ♀ G4 (p=0.0047, 8%) - Hb: ↓ ♀ G4 (p=0.16, 8%)-
  MCHC: DR ↑ ♂ G3 (*, 2%), ♂ G4 (*, 4%)
- whole blood concentrations:
  G1 (0.344-0.550 µg/mL), G2(0.116-1.44 µg/mL), G3 (4.43-4.90 µg/mL), G4 (4.35-6.67 µg/mL)-
- plasma concentrations: AL04862 was not detected in plasma of any group-

The results, showed the presence of AL04862 in whole blood of all animals of all groups in this study. These drug levels in the treated animals should not be considered valid, as AL04862 was found in untreated and vehicle control blood samples.

- gross necropsy findings:
  G1 ↑ ♀ 50 cc clear peritoneal fluid
  G2 ↑ ♂ small foci of accessory splenic tissue adjacent to pancreas and focus of cortical nodular hyperplasia right adrenal-
  G3 ↑ ♀ pale kidneys; ↑ ♀ hemorrhagic congested ligament right hip joint-no arthritic changes ligament intact-
  G4 ↑ ♀ diffusely mottled lungs-
- organ weights: adrenal-absolute/relative ↑ ♂ G3 - heart-absolute ↑ ♂ G4-
- histopathology: no changes attributed to the treatment were observed-

The results seen in this one month interim report from the 3-month study did not indicate any remarkable changes in the eyes or in systemic toxicity of any tissue.

STUDY 3.
THREE-MONTH TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862 OPHTHALMIC SUSPENSION IN RABBITS (FINAL).

Report Nº: TR 076:38520:0792 Vol. 1.20
Compound: AL04862
Formulation:

	Percent (W/V)		
Ingredient	**Vehicle**	**2.0% AL04862**	**4.0% AL04862**
AL04862			
Carbamer '			
Sodium Chloride, USP			
Mannitol, USP			
Disodium Edetate, USP			
'			
Benzalkonium Chloride, NF			
Sodium Hydroxide, NF			
Hydrochloric Acid, NF			
Water for Injection, USP			

Route: Topical, ocular
Diet: *Ad libitum*
Strain: NZW, 2.5 to 3.1 Kg body weight D(-1), 3-5 months of age.
Groups: G1 untreated G2 (vehicle) G3 (2.0% AL04862) G4 (4.0% AL04862)
Number: 4/sex/group
Treatment: 2 drops (= 60 µL) qid into the right eye
Study Site: Alcon Laboratories, Inc. Fort Worth, TX
Date: February 4, 1992 - February 23, 1996
GLP/QAU Statements: Both present and signed.

This study evaluated the ocular irritation and systemic toxicity of several concentrations of AL04862 administered to the right eye qid per day of the rabbit for three months. The left eye (OS) served as an untreated control.

All rabbits were observed daily. Body weights were recorded Day -1, weekly for the first four weeks, then biweekly. Biomicroscopic examinations were done Day -1 and W1, 2, 3, 5, 7, 9, 11 and 13. Indirect ophthalmoscopic examinations were done Days -1, 34, and 83. Pachymetry measurements were obtained on D(0), 34, and 90 for both eyes. Blood was collected D88 for clinical chemistry and hematology. Blood was also collected for plasma drug analysis. Necropsy occurred D91, and organ weights for liver, kidneys, heart, brain, adrenals, gonads, and spleen were determined. Histopathology on the eyes, adnexa, and lacrimal tissue from all animals were submitted to for evaluation.

RESULTS AND DISCUSSION

- G1 normal throughout study - G2 (vehicle) some ocular discharge from OD - G3 and G4 developed more OD ocular discharge-
- G3 males with statistically significant ↓ mean body wt (8.5%) D90-
- normal biomicroscopic evaluation - one G4 ♂ with a single occurrence of minimal flare-
- significant ↑ in pachymetry measurements (cornea) in OD of ♀ G4 D34 and in G3 ♂♀ and G4 ♀ D90-
- BUN ↑ G3 and G4 (*, 1.4 and 1.7 times, respectively)-
- platelets significant ↓ and significant ↑ RBC for G3 and G4-
- normal gross pathology-
- ↓ mean absolute ♂ liver wt G3 and G4 - absolute kidney wt ↓ ♂ G2, G3, G4-
- inflammation of heart and kidneys in 2♂ G4 due to *Encephalitozoon cunicule* infection - very slight to moderate corneal inflammation at limbus in one or two eyes of each group -
- whole blood concentrations: (µg/mL ± SD)

	Day 32	Day 88
G3	5.77 ± 0.54 (n=6)	6.53 ± 0.46 (n=8)
G4	6.12 ± 0.60 (n=6)	7.17 ± 0.39 (n=7)

- plasma concentrations:

G3	BQL	BQL
G4	BQL	0.063 ± 0.018

BQL = below quantitation limits

This study did not produce any treatment related significant systemic toxicity. Minimum ocular irritation was reported in the one month interim study (Study 2, TR Nº 020:38520:0392) and at the end of this three month treatment period. There were no significant changes reported in the treated animals. There were several tissues specified for histologic examination that were not examined in all G1 and G4 animals, as determined by audit.

STUDY 4.
ONE-MONTH TOPICAL OCULAR IRRITATION EVALUATION AL04862 GELABLE DROPS IN RABBITS:
Report Nº: TR Nº 093:38520:1293 Vol. 1.20

This study evaluated the topical ocular irritation potential of 1.0% and 3.0% AL04862 Gelable Drops administered to rabbits tid for one month. However, the formulation used in this study is not the marketed formulation; therefore, this study will not be reviewed.

STUDY 5.
SIX-MONTH TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862 OPHTHALMIC SUSPENSION IN RABBITS.

Report Nº: 031:38520:0594 Vol. 1.21
Compound: AL04862
Formulation:

	CONCENTRATION (W/W %)		
Ingredients	**Vehicle**	**2.0% AL04862**	**4.0% AL04862**
AL04862,			
NaCl, USP			
Mannitol, USP			
Edetate Disodium, USP			
Benzalkonium Chloride, NF			
NaOH, NF			
Purified Water, USP			

Route: Topical, ocular
Diet: Purina Certified Rabbit Chow Nº 5325, approximately 1 cup/day.
Dose Groups: G1 (untreated) G2 (vehicle) G3 (2.0% AL04862) G4 (4.0% AL04862)
Treatment: 2 drops (≈ 80 µL) qid in OD only
Strain: NZW, 2.8 to 3.5 Kg body wt on D(0)
Number: 10/sex/group (4/sex/group assigned to 3 month interim sacrifice)
Study Site: Alcon Laboratories, Inc., Fort Worth, TX
Date: December 10, 1992 to January 22, 1997
GLP/QAU Statements: Both present and signed.

This study was designed to evaluate the ocular irritation and systemic toxicity of 2% and 4% AL04862 ophthalmic suspensions administered qid to the right eyes (OD) of rabbits for six months. The left eyes (OS) served as the untreated contralateral control. All rabbits were examined twice/week for toxic signs and observed twice/day for morbidity, moribundity, and general well being. Body weights were recorded Day 0, W 1, 2, 3, and 4, then

biweekly. Biomicroscopic examinations were done D(0) and W 1, 2, 4, 6, 8, and monthly thereafter. Indirect ophthalmoscopic examinations were done Day 0, 90, and 181. Pachymetry measurements were taken Day 0, 90, and 179 for both eyes. Hematology and serum chemistry analyses were done D89 and D180. Blood was taken for drug concentration determinations. Necropsy, organ weight determination, and histopathology were also included in the study.

RESULTS AND DISCUSSION

- some ocular discharge, red and/or swollen eyelids in G2, G3, and G4-
- no significant weight changes were reported-
- no significant changes in body weight of treated compared to controls-
- biomicroscopic evaluation did not reveal significant conjunctival congestion, conjunctival swelling conjunctival discharge, corneal cloudiness, fluorescein staining, lens changes, or neovascularization-
- optic nerve head and retinal and choroidal vessels were within normal limits, when examined by indirect ophthalmoscopy-
- pachymetry measurements:
  D90: significantly larger in both eyes of ♂ G3 and G4 and ♀ OD and ♀ OS in G3, compared to control group -
  ♂ [↑ 8.6% (G3), ↑ 8.9% (G4) in OD D90] - ♀ [↑ 4.2% (G2), ↑ 5.8% (G3), ↑ 8.2% (G4)-]
  D180: significantly larger in OD of ♂ G2, G3, and G4, and in ♀ OD G3 and G4, compared to G1 -
  ♂ OD [↑ 6.5% (G2), ↑ 11.7% (G3), ↑ 11.2% (G4)]-
  ♀ OD [↑ 0.8% (G2), ↑ 6.0% (G3), 4.8% G4)]-
- hematology: MCHC ↑ in ♂♀ G3 (*, 3.1% ♂♀), ♀ G4 (*, 4.3%) D90 only-
- serum chemistry:
  D90: BUN ↑ ♂ (*, 41%) G4 and BUN/creatine ↑ ♂ (*, 34%)-
  D180: globulin ↑ ♂ (*, 12% G2), (*,21% G3), (*,12% G4)-
  albumin/globulin ↓ ♂ (*, 12% G2), (*, 17% G3), (*, 10% G4)-
  CPK ↑ ♂ (*, 1.98x in G2), (*, 1.97x in G4)-
  creatine ↑ ♀ (*, 18% G4)- phosphorus ↑ ♀ (*, 23.8% G4)-
  BUN ↑ ♂ (*, 28.7% G4)- potassium ↓ ♀ (*, 13% G3)-
- whole blood and plasma concentrations:

From Table 1, Vol.1.21, p. 5B-1276

Group	1 Week	2 Weeks	1 Month	3 Months	6 Months
Whole Blood					
Group 1	NS	NS	BQL	BQL	BQL
Group 2	NS	NS	0.17±0.02	0.17±0.04	BQL
Group 3	4.93±0.39	5.07±0.43	5.27±0.37	5.24±0.49	5.41±0.31
Group 4	5.01±0.43	5.09±0.52	5.51±0.33	5.29±0.62	5.21±0.46
Plasma					
Group 1	NS	NS	NA	NA	NA
Group 2	NS	NS	NA	NA	NA
Group 3	NA	NA	NA	BQL	BQL
Group 4	NA	NA	NA	BQL	BQL

BQL = below quantitation limit (whole blood), (plasma)
NS = no sample collected
NA = no analysis done for this group of samples

- gross lesions: lesions that occurred were sporadic and not treatment related-
- organ weights: spleen (organ wt/body wt): 1 ♀ G4 (*, 48%)-
- histopathology: no treatment related lesions in the eyes, adnexa, nasal lacrimal ducts, or other tissues - non-ocular lesions were of random occurrence-

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STUDY 6
THREE-MONTH TOPICAL OCULAR IRRITATION STUDY WITH AL04862 OPHTHALMIC SUSPENSION IN RABBITS.

Report Nº: TR Nº 051:38520:0396 Vol. 1.22
Compound: AL04862, lots PB-054-95 and PB-043-95
Formulation:

(From Table 2, Vol. 1.22, p. 5B-1410)

Ingredient	Suspension Vehicle	1.0% AL04862 Suspension	2.0% AL04862 Suspension
AL04862,			
Sodium Chloride, USP			
Carbomer, 974P, NF			
Tyloxapol, USP			
NaOH/HCl, NF			
Mannitol, USP			
Benzalkonium Chloride, NF			
Purified Water, USP			

Route: Topical, ocular
Diet: One cup of Certified High Fiber Rabbit Chow Nº 5325/day
Groups: G1 (untreated control) G2 (vehicle) G3 (1% AL04862) G4 (2% AL04862)
Strain: NZW, 2.4 to 2.6 Kg body weight on D(0)
Number: 5/sex/group
Study Site: Alcon Laboratories, Inc., Fort Worth, TX
Date: July 26, 1995 to August 1, 1996
GLP/QAU Statements: Both present and signed.

This study evaluated the topical ocular irritation potential of 1% and 2% AL04862 Ophthalmic Suspension administered topically to the eyes tid to rabbits for three months. Animals were observed twice/day, with detailed examinations twice/week. Body weight and eyes were examined biomicroscopically D(0), W1, 2, 3, 6, 7, 10, 11, 12, and at the end of the study. Indirect ophthalmoscopic examinations were done D(0) and W13. Corneal pachymetry measurements were obtained prior to treatment and at three months. At necropsy, eyes and adnexa from all animals were examined grossly and microscopically.

RESULTS AND DISCUSSION

- isolated instances of ocular discharge in OD and/or OS in G2, G3, and G4-
- body weight showed no significant changes from G1-

- biomicroscopic examination:
  - conjunctival congestion: slight ↑ in both eyes in G3 and G4-
  - conjunctival swelling: not observed in any animal-
  - conjunctival discharge:
    OD [1G3♀ D84, 3G4♀ D84, 2G4♂ D91]-
    OS [2G3♀ D84, 2G4♀ D84, 1G2♂ D84, 2G3♂ D84, 3G4♂ D7 and D84-
  - light reflex: not observed in any group-
  - flare: not observed in any group-
  - iritis: 1G4♂ D49 in OD
  - corneal cloudiness: minimal D84 in 3G2♀ (2 OD, 1 OS) and 1G3♂ OS, [D77 1G4♀, 1G4♂ D70-91 OU]-
  - fluorescein staining: (slight)
    OD [2G1♀ D84, 1G2♀ D84, 1G3♀ D84, 1G4♀ D84, 2G1♂ D84, 1G4♂ D84]-
    OS [1G2♂ D84]-
  - lens: no changes reported in any group-
  - neovascularization: not observed in any group-
- indirect ophthalmoscopic examination: reported to be within normal limits at prescreen and at 3 months-
- corneal pachymetry: ↑ for all groups (*, 6% to 8% in G3 and G4)-
- gross observations: slight discharge (OS 1♂G2, OD 1♂G3, OD 1♂G4, OD)-
- histopathology: no treatment related lesions in anterior chamber, cornea, eyelids, Harderian Gland, iris/ciliary body, lacrimal gland, lens, nictitating membrane, optic nerve, retina, or sclera/choroid-

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STUDY 7.
ONE YEAR CHRONIC TOPICAL OCULAR IRRITATION AND SYSTEMIC TOXICITY EVALUATION OF AL04862.

Report Nº: TR 095:38520:0795 Vol. 1.22
Compound: AL04862, assay 99%, no S-enantiomer detected, total impurities = 1.45% for the 14 impurities seen.
Formulation:

Table 2, from p. 5B-1573

INGREDIENT	VEHICLE	1.0% AL04862	2.0% AL04862	4.0% AL04862
AL04862.				
Mannitol, USP				
Sodium Chloride, USP				
Benzalkonium Chloride, NF				
NaOH, NF to adjust pH				
Purified water, USP				

Route: Topical, ocular administration at 80 µL (two drops) for 370 days.
Diet: Primate chow *ad libitum*
Dose Levels: G1 (untreated) G2 (vehicle) G3 1.0% AL04862) G4 (2.0% AL04862) G5 (4% AL04862)
Strain: Cynomolgus, body wt (♂ 3.0-6.0 Kg, ♀ 2.5-3.7 Kg)
Number: 4/sex/group

Study Site: Alcon Laboratories, Inc., Fort Worth, TX
Date: June 23, 1994 to December 6, 1996
GLP/QAU: Both present and signed.

This study was done to determine the potential for producing ocular and systemic toxicity resulting from daily topical administration of AL04862 to monkeys for one year.

The control or test article was administered two drops (80 µL) to the right eye (OD) three times a day. The left eye (OS) served as contralateral controls. Restraining collars were worn by all animals in the study. All animals were observed twice daily for morbidity and examined daily for toxic signs. Body weight was recorded prior to the first treatment and on 15 other days during the study. Biomicroscopic examinations evaluated the conjunctiva, cornea, anterior chamber, light reflex, lens, and iris for both eyes at D(0) and on the same days that body weight was determined. Indirect ophthalmoscopic examinations, corneal pachymetry measurements, specular microscopy and photography, and EKG recordings were done prior to study initiation and at W14, 26/27, and 53. Clinical chemistry and hematology data were collected prior to study initiation and after 3, 6, and 12 months of treatment. Necropsy was done on all animals, and organ weights for the liver, kidneys, heart, adrenals, gonads, brain, and spleen were determined. Histopathology was conducted on the eyes, adnexa, and a full complement of tissues.

RESULTS AND DISCUSSION

- observations: isolated episodes of loose stools, vomiting, and minor injuries to fingers/hands/arm/foot/leg in most groups except G1-
- 1♀G3 (№ X1407) found dead (hanging from cage with a toy and chain around neck-
- body weight: comparable between treated and control-
- biomicroscopic examinations: no significant changes in conjunctival congestion (minimal) - conjunctival swelling (not observed), conjunctival discharge (not observed), light reflex (no changes observed), flare (no instances observed, iritis (not observed)-
- indirect ophthalmic examination: no treatment related effects (all within normal limits)-
- corneal pachymetry: no significant difference between vehicle and drug treated eyes - thickness increased over the 12 months of study-
- EKGs: pretest results on 38 EKGs were said to be normal - all 32 recordings at 3 and 6 months were "of normal variation.." - at 12 months all 31 recording were "of normal variation" except for ♂G3 (#1386) which had first degree and second degree atrioventricular block - The cardiologist stated: "This could be of toxicologic significance."
- hematology: none of the following were DR-
  - eosinophils ↓ ♀ G5 D365(*)-
  - Hct ↓ ♂ G2-5 D92(*)-
  - Hb ↓ ♂ G4 D92(*), G4 D182(**)-
  - lymphocytes ↑ ♂ G4 D364(*)-
  - polysegmented neutrophils ↓ ♂ G4 D364(*)-
  - WBC ↓ ♂ D92 G3 and G5(*)-
- clinical chemistry:
  - ALT ↓ ♀ D183 G3 and G4 (*) and D365 (*)-
  - AST DR-↓♂ D92 (p=0.06221 trend), ↓ ♀ D365 G4 (*)-
  - total bilirubin ↓ ♂ D182 (0.074 trend)-
  - CPK ↓ ♂ G3-5 D364 (*)-
  - creatinine trend ↑ ♀ D365 (p=0.0612)-
  - BUN/creatinine ratio ↑ ♀ D93 G5 (**)-
  - globulin trend ↑ ♂ (p=0.0793)-
  - LDH ↓ ♂ (DR) G4-5 D92 (*), ↓ ♀ G4-5 D365 (**)-
  - potassium ↓ ♂ G4-5 D92 (**)-
  - sodium ↑ ♂ G3,5 D182 (*); ↑ ♀ D183 G4 (*), D365 G4-5 (**)-
  - total protein ↓ ♀ G3D183 (*)-
  - urea nitrogen ↑ ♀ G5 D93 (**), D183 G5 (p=0.017)-
- PK/drug metabolism: BLQ = below limit of quantitation
  - AL04862 systemic blood exposure obtained-
  - AL04930 (O-desmethyl) metabolite systemic blood exposure obtained-
  - AL08520 (N-desethyl) metabolite systemic blood exposure obtained-

- AL05859 (N-desmethoxypropyl) metabolite BLQ of . in blood and in plasma-
- blood steady state for drug and 2 metabolites obtained by D90-
- blood concentrations

BLOOD CONCENTRATIONS OF AL04862
mean (standard deviation) ng/mL

CONCENTRATION	DAY 90	DAY 180	DAY 359
1% AL04862 (Group 3)	12.6(4.6) n=8	11.8(4.4) n=8	10.5(3.2) n=7
2% AL04862 (Group 4)	12.4(3.6) n=8	11.5(2.5) n=8	12.0(3.6) n=8
3% AL04862 (Group 5)	11.5(2.1) n=8	10.9(1.6) n=8	11.2(1.3) n=8

Blood levels of AL04862 were significantly greater for females relative to males in the 1% AL04862 group.

BLOOD CONCENTRATIONS OF AL04930
mean (standard deviation)

CONCENTRATION	DAY 90	DAY 180	DAY 359
1% AL04862 (Group 3)	0.4(0.1) n=8	0.4(0.1) n=8	0.3(0.1) n=7
2% AL04862 (Group 4)	0.5(0.1) n=8	0.4(0.1) n=8	0.4(0.1) n=8
4% AL04862 (Group 5)	0.5(0.1) n=8	0.4(0.1) n=8	0.4(0.1) n=8

Blood levels of AL04930 were significantly greater for females relative to males in the 1% AL04862 group.

BLOOD CONCENTRATIONS OF AL08520
mean (standard deviation)

CONCENTRATION	DAY 90	DAY 180	DAY 359
1% AL04862 (Group 3)	3.3(0.8) N=8	3.5(0.8) N=8	3.3(0.7) N=7
2% AL04862 (Group 4)	6.0(1.0) N=8	5.7(0.8) N=8	5.6(0.8) N=8
4% ALO4862 (Group 5)	8.0(1.4) N=8	7.1(1.3) N=8	7.0(1.2) N=8

Blood levels of AL08520 were significantly greater for males relative to females in the 4% AL04862 group. Dose related increases (*) were seen at the three time points for the treatment groups.

- gross observations: no treatment related lesions were reported in ocular or nonocular tissues-
- histopathology: male(female)

From Summary Incidence Table pp. 5B-1813 to 5B-1826

TISSUE	G3	G4	G5
ADRENAL: capsule, hemorrhage, acute	(1/4)*		
congestion	(1/4)		
cortex, mineralization			1/4
cortex, hypertrophy, focal		(1/4)	
AORTA: adventitia, inflammation, chronic/active	1/4		
BONE MARROW, RIB: acute hemorrhage			1/4

TISSUE	G3	G4	G5
BRACHIAL PLEXUS: acute hemorrhage	1/4	1/4	1/4
BRAIN: cerebellum, meninges, mononuclear cell infiltrate			1/4
cerebellum, neuron, pigment deposit		(1/4)	
cerebrum, meninges, pigment deposit		(1/4)	
cerebrum, neuron, pigment deposit		1/4	1/4
cerebrum, perivascular pigment deposit		1/4	1/4
midbrain, choroid plexus mononuclear cell infiltrate	1/4	(1/4)	
midbrain, gray matter, mononuclear cell infiltrate		(1/4)	
midbrain, gray matter, mineralization	(1/4)		2/4
midbrain, mononuclear cell infiltrate		1/4	
midbrain, perivascular pigment deposition			1/4
pons, meninges, infiltrate, mononuclear cell		(1/4)	
pons, perivascular pigment deposition			1/4
EPIDIDYMIS: ducts, dilatation, unilateral			1/4
ducts, intraluminal cell debris, unilateral			1/4
FATTY TISSUE: cyst	(1/1)	(1/1)	
HEART: epicardium, mononuclear cell infiltrate	(1/4)		
myocardium, degeneration	1/4 (1/4)		
myocardium, mononuclear cell infiltrate	2/4 (1/4)	2/4	
myocardium, inflammation, chronic/active	1/4		
INTESTINE, CECUM: lamina propria, pigment deposition	(1/4)		(1/4)
submucosa, infiltrate, eosinophil			1/4
INTESTINE, COLON: congestion	(1/4)		
lamina propria, pigment deposition		(1/4)	
INTESTINE, RECTUM: lamina propria, pigment deposition	(1/4)	(1/4)	
submucosa, fibrosis			(1/4)
submucosa, mineralization			(1/4)
INTESTINE, DUODENUM: congestion	(1/4)		
lamina propria, pigment deposition			(2/4)
INTESTINE, ILEUM: congestion	(1/4)		
INTESTINE, JEJUNUM: lamina propria, pigment deposition		(1/4)	
KIDNEY: congestion	(1/4)		
cortex, fibrosis		(1/4)	
cortex, mineralization	(1/4)	(1/4)	1/4(1/4)
cortex, tubules, pigment deposit		(1/4)	1/4
medulla, cyst		(1/4)	
papilla, mineralization	1/4 (1/4) (1/4)		1/4
LUNG: bronchiole, inflammation, acute		(1/4)	
bronchus, inflammation, acute		(1/4)	
interstitium, inflammation, chronic/active	1/4	1/4(1/4)	1/4
pleura, fibrosis	(1/4)		
pleura, inflammation, granulomatous		(1/4)	
LIVER: granuloma(s)	(1/4)		
hepatocytes, periportal vacuolization	(1/4)	(1/4)	(1/4)
pigment deposition	(1/4)		(2/4)
LYMPH NODE CERVICAL: congestion	(1/4)		
hemorrhage, acute	(1/4)		
hyperplasia, lymphoid	1/4	1/4	
infiltrate, neutrophil	1/4		
LYMPH NODE, MESENTERIC: hyperplasia, lymphoid	1/4	1/4(1/4)	
pigment deposition		(1/4)	
PANCREAS: serosa, hemorrhage, acute	(1/4)		

TISSUE	G3	G4	G5
PARATHYROID: cyst(s) ectopic thymus	(1/4) 1/4(1/4)	(1/4) (1/4)	(1/4)
PITUITARY: pars intermedia, cyst	(1/4)		
PROSTATE: serosa, hemorrhage, acute	2/4		1/3
SEMINAL VESICLE: serosa, hemorrhage, acute	1/4		1/4
SKELETAL MUSCLE: inflammation, acute myofiber, degeneration		1/4 1/4	
SKIN: dermis, infiltrate, mononuclear cell epidermis, inflammation, suppurative	(1/4) 1/4		(1/4)
SPINAL CORD: meninges, pigment deposit			1/4
SPLEEN: infiltrate, neutrophil			(1/4)
STOMACH: congestion submucosa, granuloma	(1/4)	1/4	
THYROID: ectopic thymus follicles atrophy infiltrate, mononuclear cell	2/4 (1/4) (1/4)	 (2/4) (2/4)	2/4(2/4) (1/4)
URETHRA: infiltrate, mononuclear cell	(1/3)(2/3)	(½)	(½)
URINARY BLADDER: infiltrate, mononuclear cell inflammation, chronic/active muscularis, hemorrhage, acute serosa, hemorrhage, acute			1/4 (1/4) 1/4 2/4

* The bolded lesions occurred in the monkey that died.

The above lesions were graded 1 (minimal), 2 (slight/mild), or 3 (moderate), with most lesions being minimal to slight/mild. No treatment-related lesions were noted in ocular tissues. There were minimal to mild mononuclear cell infiltrates occurring in most all groups in the ciliary body, choroid, sclera, eyelid, ocular muscle, and lacrimal gland. None of the above lesions were reported in the vehicle group. The acute hemorrhage seen in other tissues of G5 were considered to be terminal events associated with euthanasia. Spontaneously occurring lesions in cynomolgus monkeys were said to be mononuclear cell infiltrates, inflammation, neutrophil infiltrates, eosinophil infiltrates, cysts, fibrosis and/or mineralization, duct dilatation (mammary gland, prostate, epididymis), atrophy (thyroid follicle, adrenal cortex), hepatocellular vacuolization, lymph node lymphoid hyperplasia, and skeletal muscle degeneration. Ectopic tissues were congenital, while other lesions were associated with nematodes.

There were no dose related lesions that could be identified in the above table; however, the rather large number of lesions reported in treated groups G3, G4, and G5 might indicate the drug is causing some activity. Clinical chemistry and hematology changes did not appear to be of biological significance.

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2. SYSTEMIC TOXICITY STUDIES

STUDY 8.
ACUTE ORAL TOXICITY STUDY IN RATS WITH AL04862.

Report Nº: TR Nº. 100:38520:0696 Vol. 1.23
Compound: AL04862, lot Nº L-95010757,

Formulation: Suspension
Vehicle: Carboxymethylcellulose 1% viscosity
Route: Oral, gavage at 10 mL/Kg.
Dose Levels: Group: 1 (1000 mg/Kg) Group 2 (2000 mg/Kg)
Strain: Crl:CD®BR, body wt ♂ 216-253, ♀ 160-204; 8-9 weeks old.
Number: 2/sex/group
Study Site:
Date: April 1, 1996 to November 26, 1996
GLP/QAU Statements: Both present and signed.

The animals were observed 1, 2, and 4 hours after dosing, and twice a day for 13 additional days. Gross necropsy was done on all moribund animals, on animals dying during the study, and on all animals at study termination.

RESULTS

- mortality: 1♂G1D6, 2♀G2D4, and 2♂G2D4 euthanized *in extremis*-
- signs: labored breathing, red urine, ↓ activity, tremors, red material around eyes, ↓ defecation, red/yellow stained anogenital region, hunched posture, red material around nose, loss of righting reflex, low carriage, impaired righting reflex-
- body weight of survivors not affected-
- severe hemorrhage on surface of brain of all G2 and one G1 animals-
- focus/foci on glandular stomach-
- estimated 50% mortality occurring between 1000 and 2000 mg/Kg-

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STUDY 9.
ACUTE ORAL TOXICITY STUDY IN MICE WITH AL04862.

Report Nº: TR 101:38520:0696 Vol. 1.23
Compound: AL04862, lot Nº L-950107.
Formulation: Suspension
Vehicle: Carboxymethylcellulose 1% viscosity.
Route: Oral, gavage at 10 mL/Kg.
Strain: Crl:CD-1® (ICR)BR, body wt ♂ 29-36 g, ♀ 20-26 g; 7-8 weeks old.
Dose Levels: Group 1 (1000 mg/Kg) Group 2 (2000 mg/Kg) Group 3 (5000 mg/Kg)
Number: 2/sex/group
Study Site:
Date: April 1, 1996 to November 26, 1996
GLP/QAU: Both present and signed.

The animals were observed 1, 2, and 4 hours after dosing, and twice a day for 13 additional days. Gross necropsy was done on all animals dying on study and on animals at study termination (study Day 15).

RESULTS

- mortality: 1♀G1D5, 1♀G2D7, 1♀G2D9, 2♀G3D5, 1♂G2D9, 1♂G3D6, 1♂G3D14-
- signs: tremors, ↓ defecation and activity, firm areas (ventral abdomen), labored breathing, hunched posture, distended abdomen, pale skin-
- red, mild discoloration in lung-
- body weight loss at D8 in survival animals followed by weight gain-

- distended glandular stomach-
- brain discolored red-
- estimated 50% mortality to be 1406 mg/Kg-

STUDY 10.
TWO WEEK ORAL TOXICITY EVALUATION OF AL04862 IN RATS.

Report Nº: TR Nº 017:38520:0392 Vol. 1.23
Compound: AL04862
Formulation: Solution
Route: Oral, gavage at 5 mL/Kg
Strain: Sprague-Dawley, body weight 121 g - 161 g on D(0)
Dose Levels: Group 1 vehicle control Group 2 20 mg/Kg Group 3 60 mg/Kg Group 4 180 mg/Kg
Number: 10/sex/group
Control Treatment: Water + HCl/NaOH adjust pH to 2 - 3
Study Site: Alcon Laboratories, Inc., Fort Worth, TX.
Date: January 14, 1992 to July 21, 1992
GLP/QAU Statements: Both present and signed.

All animals were observed twice a day and examined 1-2 hours after dosing. Body weights were recorded D(0), 8, 10, and prior to necropsy. Indirect ophthalmoscopic examinations were done D(0) and D14. Serum chemistry and hematology were evaluated during the week of necropsy. Urine was collected D13 from males and D15 from females. Plasma and blood drug levels were determined from the blood collected during W2. Necropsy, organ weights, and histopathology data were collected from all animals. Tissues from the vehicle control group, the high dose group, and the forestomach and any gross lesions seen in the low and mid dose groups were evaluated microscopically.

RESULTS AND DISCUSSION

- red exudate around nose/mouth/eyes/front paws, lethargic, dehydration, ocular discharge, unkempt, rales/gasping, difficulty breathing-
- 1♂G4 sacrificed moribund D7-
- body weight decrease ♂♀ (G3 and G4, *)-
- indirect ophthalmoscopic evaluations indicated the following:
  - 1G1 ♂ early cataract-exhibited blurring of the ocular fundus of OD-
  - 1G1 ♀ with abnormal retina in OD (also seen in prescreen)-
  - 1G3 ♀ with vitreal hemorrhage in OS (said to appear associated with regression of hyaloid artery which occurs in a normal developmental change in young rats-
- serum chemistry significant changes:
  - albumin: ↓ ♂G4 6%; ↓ ♀G4 11.8%-
  - albumin/globulin ratio: ↓ DR ♀G4 21%-
  - AlP: ↑ DR ♂ G4 50%; ↑ DR ♀ G2 35%, G3 46%, G4 55%-
  - Ca: ↑ DR ♂ G3 4.5%, G4 5.5%; ↑ DR G2 4%, G3 5%, G4 5.3%-
  - cholesterol: ↑ DR ♂ G3 34%, G4 40%; ↑ DR ♀ G3 24%, G4 38%
  - BUN/creatine ratio: ↑ ♂ G4 52%; ↑ ♀ G1 27%, G3 30%, G4 26%-
  - globulin: ↑ ♀ G4 13%- • phosphorus: ↑ DR ♂ G3 18%, G4 28%- • potassium: ↓ ♂ G4 11%-
  - sodium: ↑ ♂ 1%-2% (G2, G3, G4)-
  - urea nitrogen: ↑ DR ♂ G3 31%, G4 54%; ↑ ♀ G2 36%, G3 38%, G4 33%-
- hematology significant changes:
  - Hct/Hb/RBCs: ↑ ♀ G2, G3 (not DR)- • lymphocytes: ↑ ♂ G4 6%-

- MCV ↓ DR ♂ G4 4%; ↓ DR ♀ G4 3%-
- MCH ↓ DR ♂ G4 4%-
- monocytes: ↓ ♂ G2 82%, G3 91%-
- polysegmented neutrophils: ↑ ♂ 2.4x-
- RBCs: ↑ ♂ G3 32%

- urinalysis:-
  - ↑ ♂♀ pH, volume-
  - ↓ Na ♀G2 and ♂♀G3 (**) and K ♂♀ (G3 and G4, **)-
- organ weights: brain/heart/kidney/spleen/liver ↓ G4 ♂ (*)-
- gross pathology:
  - G2 1♀ mildly pale kidneys, 1♂ liver moderately congested with accentuated lobular pattern-
  - G3 1♀ with enlarged lymph node, 1♀ with prominent Peyer's patches-
  - G4 1♀ with mottled/dark lungs (this animal sacrificed in extremis D7), 1♀ with moderate bilaterally congested lungs, 1♀ with pinpoint foci of accessory spleen, congested dilated fluid filled uterine horns, and white nodule on right uterine horn-
- histopathology: conducted by

MICROSCOPIC OBSERVATIONS

(From Vol.1.23, 5B-2289 to 5B-2315)

TISSUE	VEHICLE CONTROL ♂	VEHICLE CONTROL ♀	HIGH DOSE ♂	HIGH DOSE ♀
Adnexal glands: subacute inflammation			1/10	1/10
Bone, long: marrow: fatty atrophy	4/10	8/10	8/10	10/10
epiphysis, slight thinning of plate			1/10	2/10
Brain: cerebrum, demyelination			1/10	1/10
Epididymides: aspermia			1/10	
Eyes: posterior synechia			3/20	4/20
Heart: ventricle, inflammation, perivascular, chronic				1/10
Kidneys: renal disease, chronic	1/10	7/10	1/10	7/10
intratubular mineralization, focal		3/10	1/10	8/10
glomerulus, increased cellularity			1/10	
distal convoluted tubules, regenerating nephrosis				1/10
Liver: centrilobular vacuolization				1/10
Lungs: inflammation	1/10	1/10		3/10
focal hemorrhage			3/10	1/10
regeneration bronchiolar epithelium				2/10
hilus, inflammation				1/10
right cranial, right middle lobes, inflammation				1/10
Sternum: bone marrow fatty atrophy	8/10	10/10	8/10	8/10
Stomach: glandular: submucosa, focus of inflammation, acute			1/10	
non-glandular: acanthosis			3/10	1/10
hyperkeratosis			2/10	
squamous epithelial hyperplasia focus			3/10	2/10
squamous papilloma(s)			5/10	6/10
squamous papilloma with inflammation				1/10
epithelium: pustule			1/10	
squamous epithelial hyperplasia focus			1/10	
submucosa, inflammation, subacute, active			1/10	
gastritis, chronic				1/10

TISSUE	VEHICLE CONTROL ♂	VEHICLE CONTROL ♀	HIGH DOSE ♂	HIGH DOSE ♀
Thymus: focal hemorrhage	2/10	1/10	1/10	
congestion		1/10		
medulla, lymphoid hyperplasia				1/9
cortex, atrophy				1/9
Ureter: epithelium, focus, necrosis, mineralization			1/1	
Uterus; hydrometra		1/10		3/10
deciduoma, hemorrhage and necrosis				1/10

Drug related lesions appeared to be centered in the stomach, eye, kidney, lungs, and perhaps the long bone. Lesions in the non-glandular stomach were dose related, with 4/20 lesions in the mid dose and 15/20 in the high dose. The earliest of these lesions were acanthosis and hyperkeratosis, then squamous epithelial hyperplasia, followed by squamous papilloma, and finally severe squamous papillomas. Posterior synechia was seen in the left eye of all but one animal in the high dose. Kidney intratubular mineralization increased in the high dose animals over controls. The one case of aspermia was indicated as marked. Inflammation (pneumonia) was seen in the lungs of three high dose females. The thinning of the zone of proliferating cartilage of the epiphyseal plate of the long bone was reported in three high dose rats.

- plasma and blood concentrations:

Mean AL04862 Whole Blood (µg/mL) and Plasma (ng/mL) Concentrations
(From Vol. 1.23, p. 5B-2466)

GROUP	DAY 15 MALE	DAY 16 FEMALE
WHOLE BLOOD		
Vehicle	7.27±1.99 n=10	4.62±0.88 n=10
20 mg/Kg	7.89±0.82 n=10	7.11±0.66 n=10
60 mg/Kg	7.98±0.73 n=9	6.66±0.76 n=9
180 mg/Kg	10.7±1.1 n=10	6.90±1.02 n=8
PLASMA	BQL	107±117 n=10
20 mg/Kg	32.6±11.9 n=10	76.6±27.8 n=10
60 mg/Kg	61.9±24.4 n=9	62.4±20.5 n=9
180 mg/Kg	483±267 n=10	198±131 n=9

A with the same retention time and the same as AL04862 was detected in blood and serum of these animals. This same was detected in rabbits and shown to be AL04862 by With hemolysis occurring, one should not place high value on the data in the above table.

Oral administration with AL04862 resulted in significant lethargic behavior and apparent dehydration, particularly at the high dose. One animal (high dose) was sacrificed *in extremis* on Day 7. A significant decrease in body weight occurred at 60 and 180 mg/Kg. Other significant changes due to the treatment were

increased BUN, ALP, and cholesterol. Significant decreased occurred with urinary sodium and potassium, and in the mean absolute brain, heart, spleen liver, and kidney weights.

STUDY 11.
FOUR-WEEK RANGE-FINDING ORAL (GAVAGE) TOXICITY STUDY IN RATS WITH AL04862.

Report Nº: TR Nº 059:38520:0496, Protocol Nº N-92-222 Vol. 1.24
Compound: AL04862-03,
Formulation: Solutions
Route: Oral, gavage at 10 mL/Kg
Diet: Purina Rodent Chow 5002 Meal *ad libitum*
Strain: Fischer F344, ≈ 6 weeks old, body weight ♂ 95-130 g, ♀ 89-104 g
Dose Levels: (mg/Kg/day) G1 0 G2 3 G3 10 G4 30 G5 100 G6 300
Control Treatment: pH 3 adjusted deionized water at 10 mL/Kg
Number: 5/sex/group
Study Site:
Date: October 30, 1992 to December 6, 1996
GLP/QAU: Both present and signed.

This study evaluated the subchronic toxicity of AL04862 in rats following daily gavage administration for 28 days. Rats were observed twice daily and at least once daily on weekends and holidays for morbidity and mortality. Body weights were recorded prior to treatment initiation, weekly, and at termination. Food consumption was measured weekly. Serum chemistry, hematological parameters, and urine analysis were conducted on all surviving animals at termination. Necropsy was done on all rats, and microscopic examination was evaluated on 37 collected tissues.

RESULTS

- mortality: all G6 (300 mg/Kg) animals died or were sacrificed moribund-
  These animals had red brains (hypervascularization and/or blood clots), dark material in the stomach mucosa, congestion and necrosis in the glandular stomach, suppurative inflammation of the forestomach, and dark red material in the cecum.
- signs: (seen only in G3-6) discolored/wet inguinal fur - salivation - chromodacryorrhea - discoloration around mouth - rough coat - ptosis - hunched posture, hypoactive - lying on side - cold to touch - labored breathing - emaciated - loss of righting reflex - coma-
- food consumption:
  DR ↓ ♂ G4W1 (* 12%), G5W1 (* 38.5%)-
  DR ↓ ♀ G2W3 (* 5%), G3W3 (* 5%), G4[W1 (* 8.8%),W3 (* 7.5%)],
  G5[W1 (* 32%), W2 (* 15%), W3 (* 8%), W4 (* 9%)]-
- body weight: DR ↓ ♂ G5W1-W4 (16% to 13%)-
  DR ↓ ♀ G3W3 (* 5%), G4W3 (* 5%), G5W1-4 (* 12.4 to 11%)-
- clinical chemistry: significant changes (*) from G1
  - ♂ Na (↑ G3-G5), Cl (↑ G2-G5), AST (↓ G2, G4, G5), P (↑ G5), cholesterol (↑ G4, G5)-
  - ♀ Cl (↑ G3-G5), AlkP (↑ G5), BUN (↑ G5), creatinine (↑ G5), A/G ratio (↓ G5), Ca (↑ G5), P (↑ G5), cholesterol (↑ G5)-
- hematology: (significant changes from G1)
  - ♂ DR G5 [↓RBC (* 9.9%), ↓ Hb (* 6%), ↓ Hct (* 7%), ↑ MCV (* 3%), ↑ MCH (* 3.9%)-
    relative [mature neutrophils ↑ G5 (* 47.4%), lymphocytes ↓ (* 13%)]-
  - ♀ MCH ↓ G2 (* 2%)-
  - anisocytosis, poikilocytosis, and polychromatophilia present in red blood cell morphology-

- urinalysis: ↓ K ♂ G2 (*, 3x), G3 (*, 4.6x), G4 (*, 3.5x), G5 (*, 5.7x)-
  ↓ K ♀ G4 (p=0.051) and G5 (p=0.051)-
  ↓ Na in ♂♀ but not significant-
  ↑ pH, DR but not significant (25%-27% in G5)-
  red stained urine
- organ wts: absolute weights
  - thymus DR ♂ ↓ G2-G5 (* 16% to 50.5%); ♀ ↓ G3 (* 11.8%), G5 (* 8.8%)-
  - spleen ♂ ↓ G4 (* 17.3%) and G5 (* 11.5%)]- • adrenals ♀ DR ↑ G5 (* 29%)-

  relative weights
  - thymus ↓ ♂ DR G3-G5 (* 19%-42%); ♀ ↓ G5 (* 23%)- • kidneys ↓ ♂ G3-G5 (* 8%-20%); ♀ ↑ (* 40%)-
  - spleen ↓ ♂ G4 (* 8%)- • heart ↑ ♀ G5 (* 11%)- • adrenals DR ↑ ♂ G4 (* 25%), G5 (* 50%)-
  - brain ↑ ♀ G5 (* 22%)-
- gross pathology:
  - stomach: mucosa dark material G6 4♂3♀; hypervascularization 1♂G6-
  - brain: red (hypervascularized and/or blood clot G6 4♂3♀-
  - cecum: dark material G6 3♂1♀, red G6 1♂-
  - small intestines: gas filled G6 2♀-
  - eyes: opacity G6 1♀-
- histopathology:
  - thymus: hemorrhage, acute 1♂G5, 1♀G5-
  - stomach, fore: inflammation, suppurative 3♂G6, 4♀G6-
    stomach, fore: serosa, inflammation, suppurative 1♂G6-
    stomach, glandular: congestion 5♂G6, 4♀G6-
    stomach, glandular: necrosis 4♂G6, 5♀G6-
  - kidney: hemorrhage, acute 1♀G6-
    kidney: nephropathy 1♀G1, 2♀G2, 4♀G4, 3♀G5-

Signs were dose related, being observed about 20 hours after drug administration, then subsided within 26 hours in all groups except the high dose. These signs increased in severity with increasing dose. All high dose animals died or were sacrificed *in extremis* during the first week on treatment. Food consumption was significantly reduced in the 30 and 100 mg/Kg groups, resulting in reduction in mean body weight at 100 mg/Kg in both sexes. RBC, Hb, and Hct values were significantly reduced in males dosed at 100 mg/Kg. Clinical chemistry values showing significant increases in both sexes were chlorine, cholesterol, and phosphorus - all were dose related. Other significant changes occurred in Na, AlkP, BUN, AST, creatinine, and calcium. There were also significant increases in urinalysis values for K and Na, and a non-significant increase of 27% in urinary pH at 100 mg/Kg.

Gross and histopathology indicated hypervascularization or blood clots in the brain and congestion and necrosis occurred in the glandular stomach at 300 mg/Kg. Kidney nephropathy was seen in females of G1, G2, G4, and G5, with more animals involved in the treatment groups than in the control. Other findings appeared to be incidental and not related to the drug treatment. Absolute and relative thymus and spleen weights were significantly reduced, while adrenal weights were increased. In general, most of the adverse effects occurred at 300 mg/Kg.

STUDY 12.
FOUR-WEEK ORAL RANGE-FINDING TOXICITY STUDY IN MICE (WITH AL04862).

Report Nº: TR 058:38520:0496 Vol. 1.24
Compound: AL04862-03, lot Nº 4035-85-IIA,
Formulation: Solution

Route: Oral, gavage
Diet: Certified Rodent Chow #5002 *ad libitum*
Strain: CD-1®, body weight ♂ 26-29 g, ♀ 22-25 g
Dose Levels: G1 0 G2 10 G3 30 G4 100 G5 200 G6 300 mg/Kg/day
Number: 5/sex/group
Control Treatment: Deionized water
Study Site:
Date: November 4, 1992 - December 5, 1996
GLP/QAU Statements: Both present with signatures.

The objectives of the study were to evaluate toxicity and establish dosage levels for the 13-week oral toxicity study in mice. The study included observations 2 - 3 times/day, clinical signs, body weight determination pretest and weekly, and weekly food consumption measurement.

RESULTS

- mortality: ♂ 1G2 (sacrificed *in extremis*), 1G5, 5G6; ♀ 2G4, 3G5, 5G6-
- signs: decreased defecation/activity, hunched posture, labored breathing, tremors, eye discoloration-
- body weight: DR ↓ beginning W1 in G4(*,**) and G5(**)-
- food consumption DR ↓ in G3, G4, and G5-

From the results, the study director recommended 10, 30, and 60 mg/Kg/day for the 90-day toxicity study in mice.

STUDY 13.
THIRTEEN-WEEK ORAL TOXICITY STUDY IN MICE WITH AL04862.

Report Nº: TR 126:38520:1294 Vol. 1.25
Compound: AL04862, lot Nº 4495-25
Formulation: Solution
Route: Oral, gavage at 10 mL/Kg
Diet: Certified Rodent Chow #5002 *ad libitum*
Strain: CD-1®, 6 weeks old, body weight ♂29-35 g, ♀ 22-27 g
Dose Levels: G1 0 G2 5 G3 10 G4 20 G5 40 G6 80 mg/Kg/day
Number: 10/sex/group
Control Treatment: 0.5% aqueous methylcellulose
Study Site:
Date: May 25, 1993 - August 1, 1996
GLP/QAU Statements: Both present and signed.

The objective of the study was to evaluate the toxicity of AL04862 in mice treated orally for 13 weeks. The animals were observed 2 to 3 times a day for clinical signs and mortality. Body weights were recorded prior to study and weekly. Food consumption was measured weekly. A complete gross examination was done on all animals. Microscopic evaluation of organs and tissues was also carried out on controls and all groups.

RESULTS

- mortality: 1♂G2, 1♂G4 --not considered drug related-
- clinical signs: decreased defecation or no stools 1♀G3, 1♂G6, 2♀G6 - hunched posture 1♀G3, 1♀G6-stained body 1♂G4, 1♀1♀G6 - labored breathing 1♀G3, 1♂1♀G6 - pale skin 1♀G6-

hair loss 1♀G2, 1♀G5 - penis extended 1G2, 1G5 - subcutaneous mass 1♀G4, 1♀G5-

- body weight: no significant changes from controls-
- food consumption: DR ↓ ♂G5 (*)W1 and G6(**)W1, (**)W2, (**)W6, (**)W10-
  DR ↓ ♀G5 (*)W2 and G6 (*)W1, (**)W2-
- gross observations:
  - eyes: cloudy, mild 1♂G2; 2♀G2, 1♀G3, 1♀G4-
  - kidney: dilation, pelvic 1♂G3, 1♀G3, 1♀G4 - enlarged 1♂G6 - depressed 2♀G6-
  - stomach: glandular, thickened ♂(1G4, 2G5, 1G6); ♀(2G6)-
  - urinary bladder: discolored, trace 1♂G2 - calculus/calculi 1♀G2, 1♀G3, 2♀G4, 1♀G5-
- histopathology:
  - kidney: mineralization ♂(1G4, 1G5, 1G6) - basophilic tubules, cortex ♂(4G5, 2G6), ♀(3G2, 3G4, 2G6)-
    hydronephrosis ♂(1G3, 1G4, 1G6), ♀(1G3, 1G4)-
    nephritis, chronic ♂(1G3, 1G4, 3G5, 1G6)-
    ♀(3G1, 1G2, 7G3, 7G4, 7G5, 8G6)-
    necrosis, moderate 1♀G4-
  - stomach: glandular, mineralization ♂(1G6) - dilatation ♂(2G5) - hyperplasia ♀1G6
  - urinary bladder: hyperplasia ♂(7G2, 10G3, 8G4, 9G5, 10G6) -♀(8G2, 8G3, 5G4, 2G5, 7G6)-
    infiltration, lymphocytic ♂(4G5), ♀(1G2, 1G3)-
    inflammation, subacute ♂(2G2, 4G3, 5G4, 7G5, 7G6) -♀(5G2, 5G3, 2G4, 1G5, 1G6)-
    inflammation, acute ♀(2G2, 2G3, 2G4, 2G5)-

Although food consumption was significantly reduced during the first two weeks of the study at ≥ 40 mg/Kg/day, no significant reduction occurred in body weights. No drug related deaths were reported. Gross observations included a thickening of the glandular stomach, discolored urinary bladder with calculi, and dilated, enlarged, or depressed kidneys. Histopathology revealed extensive hyperplasia and or inflammation in the urinary bladder of both sexes of all dose groups. The subcutaneous masses were urinary bladder nodules of infiltrated lymphocytes. Chronic nephritis was seen in male drug groups ≥ 10 mg/Kg and with increased incidence in all female. Moderate kidney necrosis was reported in one male at 20 mg/Kg, and hydronephrosis appeared only in drug treated animal. Renal cortical basophilia occurred in drug groups dosed at ≥ 5 mg/Kg. These kidney and urinary bladder lesions were said to be common effects of carbonic anhydrase inhibitors in rodents. From these results, the sponsor selected dose levels of 0, 1, 3, and 10 mg/Kg/day for a two year study in mice,

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STUDY 14.
THIRTEEN-WEEK ORAL GAVAGE TOXICITY STUDY IN RATS WITH AL04862.

Report Nº: TR 127:38520:1294 Vol. 1.26
Compound: AL04862-003 (ERM 4554:023; Lot Nº 4035-85-IIA
Formulation: Suspension
Vehicle:
Route: Oral, gavage at 10 mL/Kg
Diet: Purina Certified Rodent Chow 5002 *ad libitum*
Dose Levels: G1 vehicle G2 1 mg/Kg G3 3 mg/Kg G4 10 mg/Kg
Strain: Fischer F344N, ≈ 6 weeks of age, 91 to 126 g body weight
Number: 15/sex/group
Study Site:
Date: April 30, 1993 - October 2, 1996
GLP/QAU Statements: Both present and signed

This study was conducted to evaluate subchronic toxicity of AL04862 when administered orally to rats