CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-127

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

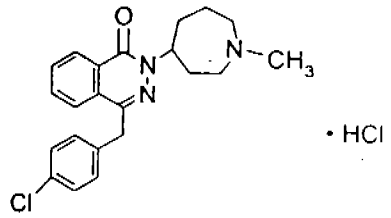
KEY WORDS: Azelastine Hydrochloride Ophthalmic Solution, 0.05%, allergic conjunctivitis, histamine receptor antagonist

Reviewer Name: Zhou Chen, Ph.D.
Division Name: DAAODP, HFD-550
Review Completion Date: November 30, 1999

IND/NDA number: **NDA 21-127**
Serial number/date/type of submission: 000/August 2, 1999/commerical
Information to sponsor: Yes (X), No ( )
Sponsor: ASTA Medica, Inc.
890 East Street
Tewksbury, MA 01876

Drug:

Generic Name: **Azelastine Hydrochloride Ophthalmic Solution 0.05%**
Code Name: A5610
Trade Name: To be determined
Chemical name: (±)-1-(2H)-phthalazinone, 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-, monohydrochloride
CAS Registry Number: 79307-93-0
Molecular Formular: $C_{22}H_{24}ClN_3O$ HCl, MW: 418.37



Proposed Dose: One drop (30 µl) in the affected eye, bid (Total dose could be 0.06 mg/patient/day or 0.0012 mg/kg for a 50 kg adult)

Relevant INDs/NDAs/DMFs: [redacted]

Drug Class: H_1-receptor antagonist

Clinical Formulation:

Component	Quantity (%, w/v)
Azelastine hydrochloride	0.05
Benzalkonium chloride	0.0125
Disodium edetate dihydrate	[redacted]
Hydroxypropyl methylcellulose	
Sorbitol solution (70%)	
Sodium hydroxide	
Water for injection	

Table of Content

NDA 21-127, Azelastine Hydrochloride Ophthalmic Solution 0.05%

Indication: Allergic conjunctivitis
Route of administration: Ocular, Topical

Proposed clinical protocol or use:

Azelastine is being developed by the sponsor for the prevention and relief of the signs and symptoms of allergic conjunctivities. The recommended dose is one drop instilled into each affected eye two times per day at an interval of 8-10 hr.

Previous clinical experience:

Two phase 1 clinical studies, four phase 2 clinical studies and twelve phase 3 clinical studies have been finished by the sponsor in the United States or in the European countries. Among these studies, several studies were conducted in children (age 4-12 years old) for the pediatric indication. The longest study duration was 8 weeks. The drug appeared effective and well tolerated. Adverse events reported in these studies included application site reaction, headache, taste perversion. No serious adverse events were reported.

Introduction and drug history:

Azelastine hydrochloride is an H_1-receptor antagonist. The drug was initially developed as an oral formulation for the treatment of allergic rhinitis and bronchial asthma and was approved in many countries not including the United States. Subsequently, azelastine hydrochloride was formulated as a nasal spray and marketed in the United States and many other countries for the treatment of allergic rhinitis at a recommended dose of 2 sprays (containing 0.274 mg of azelastine hydrochloride) per nostril twice daily. The submitted ocular formulation has been approved for use in adults in 22 countries since January 1998.

NONCLINICAL STUDIES:

Pharmacology:

1. Ocular antihistaminic activity in rabbit. Study #: 88-014(F), Vol. 16, Page 001.

The purpose of this study was to determine the ocular antihistaminic activity of azelastine HCl at different concentrations. The drug was dissolved in physiological saline solution (PSS) at the concentrations of 0.1, 0.2, 0.5, 1 and 2 mg/ml. Male rabbits were repeatedly treated with the drug solutions via ocular instillation into right eyes (50 µl, 4 times at a 20-min interval). Twenty min after the last dose, the treated eyes were challenged with 50 µl of histamine solution (20 mg/ml). The lesions in the iris and conjunctiva were evaluated using Draize's method before and 30 min after the challenge. The results showed (see table below) that even at a concentration as low as 0.01%, histamine-induced ocular response was partially inhibited.

Inhibitory effect of azelastine solution on histamine-induced ocular response

Azelastine concentration (%)	0.01	0.02	0.05	0.1	0.2
Inhibition (%)	42.02	27.97	33.97	51.98	72.03

2. Comparative ocular antihistaminic activity. Study #: 88-015(F), Vol. 16, Page 023.

The purpose of this study was to determine the ocular antihistaminic activity of azelastine HCl solution. The drug was dissolved in PSS at the concentration of 1 mg/ml (0.1%). Male rabbits were repeatedly treated with the drug via ocular instillation into right eyes (50 µl, 4 times at a 20-min interval). Twenty min after the last dose, the treated eyes were challenged with 50 µl of histamine solution (20 mg/ml). The lesions in the iris and conjunctiva were evaluated using Draize's method before and 30 min after the challenge. For the purpose of comparation, PSS, chlorphenyramine 0.1% (an H_1-receptor antagonist) and Pheramin N 0.1% (an H_1-receptor antagonist) solutions were also included in this study. The results showed that at concentration of 1 mg/ml, azelastine HCl inhibited histamine-induced ocular response by 50.6% relative to PSS control. The inhibition with chlorphenyramine and Pheramin N was greater (90.7% and 72.1%, respectively).

3. Evaluation of azelastine hydrochloride in an ocular antiallergy model. Study #: PH-95-E3, Vol. 16, Page 043.

The purpose of this study was to determine the effect of azelastine HCl on histamine-stimulated vascular permeability. Male guinea pigs were treated intravenously with Evans Blue dye (1 ml, 1 mg/ml). Fifteen min later, 20 µl of vehicle or azelastine HCl at concentrations of 0.001, 0.01 and 0.1% was applied topically onto 1 eye of each animal. Thirty min after topical application, animals were challenged subjunctivally with histamine (300 ng). The animals were sacrificed 30 min later and the area and intensity of extravasated blue dye were measured. The results indicated that azelastine HCl significantly and dose-dependently inhibited histamine-induced vascular permeability with an ED_{50} of 0.005%.

4. Report on anti-allergic formulations administered by conjunctival topical route, Vol. 16, Page 051.

The purpose of this study was to determine the efficacy of azelastine HCl solution 0.1% and 0.05% in a rat model of allergic conjunctivitis. Male Wistar rats were immunized with egg albumin via ip injection. Fourteen days later, the allergic conjunctivitis was induced by instillation of albumin by ocular topical route. Evans blue, an indicator for the allergic reaction, was intravenously injected immediately before the induction. Azelastine HCl, [redacted] 2% (a mast cell stabilizer) and diphenhydramine 0.1% or 0.2% (an H_1-receptor antagonist) were instilled into the right conjunctiva 15 min before, immediately before and 15 min after the allergic challenge. The animals were sacrificed 30 min after the antigenic stimulation, and the ocular tissues were prepared. Evans blue extracted from the ocular tissues was measured with a spectrophotometer. The results (see table below) indicated that azelastine HCl 0.1% and 0.05% inhibited the vasoactive response in the rat conjunctival anaphylaxy model.

Percent inhibition of vasoactive response in the rat allergic conjunctivitis model (%)

Treatment	Azelastine 0.1%	Azelastine 0.05%	Cromoglycate 2%	Diphenhydramine 0.1%	Diphenhydramine 0.2%
Inhibition	88.3	77.4	47.9	75.6	76.9

Pharmacokinetics:

1. Pharmacokinetics of azelastine after a single instillation of [^{14}C]azelastine eyedrops (0.1%; w/v) into the right conjunctival sac of albino rats, with and without an experimentally induced allergy. Vol. 20, Page 039.

Report Nº: A-05610/7095050151
Compound: [Phenyl-U-^{14}C]Azelastine-HCl (Batch #: A-5610 eyedrops 10/02/95, purity = 96.2-97.6%, radioactivity: 0.2140 μCi/μl, 1 μCi/μg), The formulation was the same as the clinical formulation
Dose: 10 μl, single dose
Route: Topical, ocular (right eye only)
Animal: Male Wistar rats, 7-8-week old, 263-370 g
Study Site: [redacted]
Study Initiation: March 13, 1995
GLP/QAU: Yes

The ocular absorption, distribution and excretion of ^{14}C-azelastine were investigated in this study in rats (sensitized or non-sensitized) after a single ocular instillation. In the sensitized rats, allergy was induced by ip injection of egg albumin solution followed by (14-day later) allergic challenge with a single instillation of 30% egg albumin solution (10 μl, right eye). Radioactivity was measured with a liquid scintillation counter. The sample collecting schedule is listed in the table below.

Sample collecting schedule

	Number of animals		Time of sacrifice	Sampling				
Group	sensitized	normal	(hr after dosing)	Blood	Plasma	Ocular tissues*	Urine	Feces
1	5	5	0.5	+	+	+		
2	5	5	1	+	+	+		
3	5	5	2	+	+	+		
4	5	5	4	+	+	+		
5	5	5	6	+	+	+		
6	5	5	12	+	+	+	+	+
7	5	5	24	+	+	+	+	+
8	5	5	48	+	+	+	+	+
9**	5	5	24	+	+	+	+	+

* The left eyes were sampled only at 0.5 hr after instillation. Ocular tissues included eyelid, cornea, aqueous humor, iris-ciliary body, lens, vitreous, chorio-retina, sclera.
** Group 9 animals were not dosed.

Results:

General information: After instillation, visual inspection showed no toxicity and intolerability.

Ocular distribution: The PK parameters are summarized in the following table. The external tissues showed higher Cmax and AUC levels than internal tissues, and the Cmax and AUC values in sensitized animals (with inflamed eye) were higher than in normal rats, indicating therapeutically beneficial effects. The external tissues of the left (untreated) eyes had very low radioactivity concentrations at 0.5 hr after dosing, indicating a low contralateral distribution of ^{14}C-azelastine.

PK parameters in rats treated with ^{14}C-azelastine HCl

	Sensitized animals				Non-sensitized animals			
	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)	Left eye at 0.5 hr (ng-eq/g)	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)	Left eye at 0.5 hr (ng-eq/g)
Eyelid	0.5	15196	66553	48.57	0.5	7709	36605	27.74
Cornea	0.5	46933	22956	52.57	0.5	20168	14836	9.34
Aqueous humor	1	142	349	0	1	103	361	0
Iris-ciliary body	0.5	4976	4229	0	0.5	1633	3403	0
Lens	0.5	87	844	7.91	0.5	47	632	9.74
Chorio-retina	0.5	1203	1799	47.67	0.5	369	1440	44.9
Sclera	0.5	4930	3207	52.22	0.5	2878	3281	28.16
Vitreous	0.5	45	407	10.34	0.5	28	446	8.68

Systemic availability: The blood and plasma concentrations of radioactivity are listed in the table below. After ocular instillation, radioactivity appeared rapidly in the circulation. There was no difference between sensitized and non-sensitized animals. The plasma concentrations were lower than those in the ocular tissues, indicating possibly rapid distribution from plasma to peripheral body tissues.

Blood and plasma concentrations of azelastine HCl (ng-eq/ml)

	Sensitized animals			Non-sensitized animals		
	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)
Blood						
Plasma						

Excretion of radioactivity in urine and feces: Radioactivity excretion is summarized in the table below. 98% of applied dose was found with 48 hr after dosing. Fecal elimination was predominant.

Radioactivity excretion in urine and feces after ocular instillation of ^{14}C-azelastine in rats (% of dose)

	Sensitized animals		Non-sensitized animals	
Time (hr)	Urine	Feces	Urine	Feces
0-24	1.857	68.709	0.972	68.442
24-48	0.227	27.113	0.382	28.164
Total	2.084	95.822	1.354	96.606

In summary, azelastine was rapidly distributed in the ocular tissues with higher radioactivity concentrations in the eyelid and cornea, and in the inflamed eyes. Plasma

concentrations were very low relative to the ocular tissues. 98% of radioactivity was recovered in the urine and feces and fecal elimination was predominant.

2. Pharmacokinetics of azelastine after a single instillation of [^{14}C]azelastine eyedrops (0.1%; w/v) into the right conjunctival sac of pigmented rabbits. Vol. 20, Page 132.

Report Nº: A-05610/7095060152
Compound: [Phenyl-U-^{14}C]Azelastine-HCl eye drops (Batch #: A-5610 eyedrops 10/02/95, purity = 96.2-97.6%, radioactivity: 0.2137 µCi/µl, 1 µCi/µg), The formulation was the same as the clinical formulation
Dose: 25 µl/25 µg, single dose
Route: Topical, ocular (right eye only)
Animal: Male [redacted] rabbits (pigmented), 4-month old, 2.3-2.9 kg
Study Site: [redacted]
Study Initiation: February 18, 1995
GLP/QAU: Yes

The ocular absorption, distribution and excretion of ^{14}C-azelastine were investigated in this study in pigmented rabbits after a single ocular instillation. Radioactivity was measured with a liquid scintillation counter. The sample collecting schedule is listed in the table below.

Sample collecting schedule

Group	Number of animals	Time of sacrifice (hr after dosing)	Sampling			
			Blood	Ocular tissues*	Urine	Feces
1	5	0.5	+	+		
2	5	1	+	+		
3	5	2	+	+		
4	5	4	+	+		
5	5	6	+	+		
6	5	12	+	+	+	+
7	5	24	+	+	+	+
8	5	48	+	+	+	+
9**	5	24	+	+	+	+

* Both left and right eyes were sampled. Ocular tissues included tears, palpebral conjunctiva, bulbar conjunctiva, nictitating membrane, cornea, aqueous humor, iris-ciliary body, lens, vitreous, choriod, retina, sclera, harderian gland.
** Group 9 animals were not dosed.

Results:

General information: After instillation, visual inspection showed no toxicity and intolerability.

Ocular distribution: The PK parameters are summarized in the following table. The external tissues showed higher Cmax levels than internal tissues. However, iris-ciliary body showed relatively high concentrations, suggesting that the drug may bind to pigment.

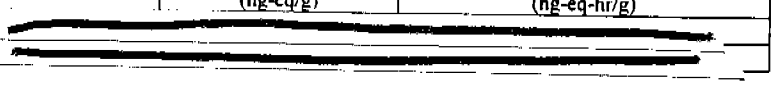
PK parameters in pigmented rabbits treated with ^{14}C-azelastine HCl

	Right eyes (treated)			Left eyes (untreated)			Left eye Cmax/right eye Cmax (%)
	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)	
Tears	0.5	8299	10032	2	14	312	0.17
Palpebral conjunctiva	0.5	3614	5426	1	6	22	0.17
Bulbar conjunctiva	0.5	1909	5919	48	6	173	0.31
Nictitating membrane	0.5	3703	25641	1	6	63	0.16
Cornea	0.5	5911	18923	2	0.44	5	0.01
Aqueous humor	0.5	50	85				000
Iris-ciliary body	48	1604	64811	6	129	4553	8.04
Lens	1	15	250	0.5	0.25	0.34	1.67
Vitreous	0.5	2	8	48	0.22	5	11.0
Retina	2	22	450	4	22	465	100
Choroid	6	345	14085	6	230	7568	66.67
Sclera	0.5	1185	22036	0.5	20	42	1.69
Harderian gland	0.5	51	168	1	13	56	25.49

Systemic availability: The blood and plasma concentrations of radioactivity is listed in the table below. After ocular instillation, radioactivity appeared rapidly in the circulation. The plasma concentrations were lower than those in the ocular tissues, suggesting rapid distribution from plasma to peripheral body tissues. Forty-eight hr after dosing, no radioactivity could be detected in plasma.

Blood and plasma concentrations of azelastine HCl (ng-eq/ml)

	Tmax (hr)	Cmax (ng-eq/g)	AUC_{0-48} (ng-eq-hr/g)
Blood			
Plasma			



Excretion of radioactivity in urine and feces: Radioactivity excretion is summarized in the table below. 82.3% of applied dose was found with 48 hr after dosing. Fecal elimination was predominant.

Radioactivity excretion in urine and feces after ocular instillation of ^{14}C-azelastine in rabbits (% of dose)

Time (hr)	Urine	Feces
0-24	17.251	55.114
24-48	1.385	8.514
Total	18.636	63.628

In summary, azelastine was rapidly distributed in the ocular tissues with higher radioactivity concentrations in the external ocular tissues. However, relatively high concentrations were noted in iris-ciliary body, indicating possible melanin binding of the

drug. Plasma drug concentrations were very low relative to the ocular tissues. 82.3% of radioactivity was recovered in the urine and feces and fecal elimination was predominant.

3. Azelastine eye drops: plasma concentration monitoring to the 26-week toxicity study after repeated application to the eye of the dog and subsequent 6-week recovery period (Study #: 910912). Vol. 20, Page 211.

Report Nº: A-05610/7097013156
Study Nº: B910912
Compound: Azelastine HCl 0.05% (Batch #: 44603, purity = 99.7%), The formulation was the same as the clinical formulation
Dose: 50 µl
Route: Topical, ocular (right eye only)
Animal: [redacted] beagle dogs 6-7-month old, 9.1-16.0 kg for males and 8.5-11.9 kg for females
Study Site: ASTA Medica AG, Biological Research Biochemistry
Weismullerstr 45
D-60314 Frankfurt, Germany
Study Initiation: March 18, 1996
GLP/QAU: Yes

The purpose of this study was to determine the TK parameters of azelastine in dogs after multiple ocular administrations of azelastine eye drops for 26 weeks. This was a part of the 26-week toxicity study. The day of the first dosing was designated as day 1. The study design is listed in the table below. [redacted] S assay was used in determining azelastine's concentrations. The lower limit of quantification (LOQ) was [redacted] with a limit of detection (LOD) of [redacted]

Study design

Group	N/sex	Dosing Frequency/day	Dosing interval (hr)	Sampling	
				Blood	Aqueous humor
1*	6	8	1	Day 1 and Week 26: once from each animal	During necropsy, aqueous humor was collected from each eye of each animal one day after the last day of treatment.
2	4	2	7	Day 1 and Week 26: predose, 0.5 hr after the first dose and 0.5 hr after the last administration	
3	4	4	2.5		
4	6	8	1		

* vehicle control

Results:

Except for 1 control plasma sample in which a concentration of 9.48 ng/ml was received, there were no measurable azelastine concentrations in any of the plasma and aqueous humor samples. These results suggested that following administration of azelastine eye drops into the conjunctival sac of the dog eyes, azelastine HCl was below the quantifiable concentrations in either plasma (0.5 hr after dosing) or aqueous humor (1 day after the last day of dosing).

Toxicology:

General comments: In several eye irritation studies using Draize scale, the irritation index was graded by the sponsor according to a modified method (see below) by Gilman et.al. (J. Toxicol.-Cut. & Ocular Toxicol., 2, 107-111, 1983).

Index ranges	Gradation
0-10	non-irritant
11-25	slightly irritant
26-56	moderately irritant
57-110	severely irritant

OCULAR IRRITTION TESTS AFTER SINGLE APPLICATION

1. **Report on the study of azelastine in aqueous solution for irritating effects on the mucous membrane following single application to the eye of the rabbit. Vol. 16, Page 069.**

Report Nº: ASTA-TX-17-80/81
Compound: Azelastine-HCl 0.5% (Batch #: 1660, dissolved in water for injection)
Route: Topical, ocular (one eye only)
Dose: 100 µl, single dose
Animal: White Russian rabbits, 6-7-month old, 2.0-2.5 kg, N = 6
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: December 2, 1980
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following a single ocular administration. Ocular irritation was observed using Draize scale at 1, 4, 8, 24, 48 and 72 hr after dosing. Systemic clinical signs were also observed.

Results:

No systemic toxic effects were noted.

No abnormal findings were noted in the iris and cornea. In the conjunctival examination, 1 animal showed transient, slight hyperemia at 1 hr after dosing. Four and eight hr after dosing, 1 and 2 animals showed hypersecretion, respectively. Twenty-four hr after dosing, no abnormal findings were observed. The mean ocular irritation index observed within 24 hr was 0.42, which was within the range of non-irritation.

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following a single ocular instillation in this study.

2. Compared acute ocular tolerance. Vol. 16, Page 086.

Report Nº: A 5610/7100000006
Study Nº: 87-067(F)
Compound: Azelastine-HCl 0.05% (Batch #: 076007, dissolved in PSS)
Route: Topical, ocular (one eye only)
Dose: Single dose
Animal: Rabbits, N = 6/group. No further information was provided.
Study Site: [redacted]
Study Initiation: Not provided (Study period: 1987)
GLP/QAU: No

The purpose of this study was to determine the ocular tolerance of azelastine solution 0.05% and compare the ocular tolerance with [redacted] a mast cell stabilizer) in rabbits following a single ocular administration. Ocular irritation was observed using Draize scale before and after dosing (No detailed information of the study method was provided).

Results:

The ocular lesion scores based on the Darize scale are summarized in the table below. The average indices for azelastine and [redacted] were 7.33 and 11.33, respectively.

Ocular lesion grade in rabbits treated with azelastine or [redacted]

	Azelastine 0.05%						[redacted]					
Rabbit	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Cornea: Opacity-degree of density	0	0	1	0	1	1	1	1	1	1	1	1
Area of cornea involved	0	0	1	0	1	1	1	1	1	1	1	1
Total score	**0**	**0**	**5**	**0**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Iris: folds, congestion, swelling, injection						1	0	1	0	1	1	1
Total score	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**5**	**0**	**5**	**5**	**5**
Conjunctiva:												
Redness					1	0	0	1	1	1	1	1
Chemosis										1		1
Discharge											1	1
Total score	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**2**	**2**	**4**	**4**	**6**
Total score (cornea+iris+conjunctiva)	**0**	**0**	**5**	**0**	**7**	**10**	**5**	**12**	**7**	**14**	**14**	**16**

In summary, azelastine HCl solution 0.05% produced several slight ocular changes. However, the final scores were located within the non-irritation range.

3. Compared acute ocular tolerance. Vol. 16, Page 099.

Report Nº: A 5610/7100000007
Study Nº: 87-065(F)
Compound: Azelastine-HCl 0.1% (Batch #: 076007, dissolved in PSS)
Route: Topical, ocular (one eye only)
Dose: Single dose
Animal: Rabbits, N = 6/group. No further information was provided.
Study Site: [redacted]

Study Initiation: Not provided (Study period: 1987)
GLP/QAU: No

The purpose of this study was to determine the ocular tolerance of azelastine solution 0.1% and compare the ocular tolerance with pheramin N (0.005% diphenhydramine HCl, an H_1 receptor antagonist) and chlorphenamine meleate 0.1% (an H_1 receptor antagonist) in rabbits following a single ocular administration. Ocular irritation was observed using Draize scale before and after dosing (No detailed information of the study method was provided).

Results:

The ocular lesion scores based on the Darize scale are summarized in the table below. The final grade for each individual animal treated with azelastine 0.1% was 12 or 17 (mean = 14.5), indicating slight irritation. The mean indices for diphenhydramine HCl and chlorphenamine meleate 0.1% were 6 and 0.7, respectively.

Ocular lesion grade in rabbits treated with azelastine

	Azelastine 0.05%					
Rabbit	**1**	**2**	**3**	**4**	**5**	**6**
Cornea: Opacity-degree of density	1	1	1	1	1	1
Area of cornea involved	1	1	1	2	2	2
Total score	**5**	**5**	**5**	**10**	**10**	**10**
Iris: folds, congestion, swelling, injection	1	1	1	1	1	1
Total score	**5**	**5**	**5**	**5**	**5**	**5**
Conjunctiva:						
Redness	1	1	1	1	1	1
Chemosis						
Discharge						
Total score	**2**	**2**	**2**	**2**	**2**	**2**
Total score (cornea+iris+conjunctiva)	**12**	**12**	**12**	**17**	**17**	**17**

In summary, azelastine HCl solution 0.1% produced slight ocular irritation.

OCULAR IRRITATION TESTS AFTER REPEATED APPLICATION

1. Azelastine eye drops 0.05%, Batch # 11/3: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 149.

Project Nº: 856473
Compound: Azelastine-HCl 0.05% (Batch #: A5610-L/VI Ch.-B. 11/3). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Female white Russian rabbits, 6-7-month old, 2.3-2.8 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritating properties of azelastine solution 0.05% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application in treated eyes

Results:

No treatment-related abnormal ocular findings were detected. No systemic abnormal findings were noted.

In summary, azelastine HCl solution 0.05% showed no ocular irritant effects in rabbits following daily ocular instillation for 5 days in this study.

2. Azelastine eye drops 0.05%, Batch # 11/5: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 187.

Project Nº: 856484

Compound: Azelastine-HCl 0.05% (Batch #: A 5610-L/VI Ch.-B 11/5). [redacted]

Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 6-8-month old, 2.3-2.7 kg, 2 males and 1 female
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: Feburary 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritating properties of azelastine solution 0.05% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application in treated eyes

Results:

The only positive finding was slight conjunctival hyperemia (Draize score = 1) in one male rabbit seen only at 24 hr after the first instillation and 1 hr after the 2nd dosing. No ocular toxic effects were observed in other ocular examinations. No systemic abnormal findings were noted. The final mean Draize index was 0.13, which was within non-irritation range.

In summary, azelastine HCl solution 0.05% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

3. Azelastine eye drops 0.05%, Batch # 11/6: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 225.

Project Nº: 856495
Compound: Azelastine-HCl 0.05% (Batch #: A 5610-L/V Ch.-B 11/6). [redacted]

Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days

Animal: White Russian rabbits, 6-month old, 2.5-2.6 kg, 2 females and 1 male
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritating properties of azelastine solution 0.05% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in the treated eye
Fluorescein examination	24 hr after the 5th application in the treated eye

Results:

No treatment-related abnormal ocular findings were detected. No systemic abnormal findings were noted either.

In summary, azelastine HCl solution 0.05% showed no ocular irritant effects in rabbits following daily ocular instillation for 5 days in this study.

4. Azelastine eye drops 0.1%, Batch # 11/35: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 263.

Project Nº: 856506
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/35). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 7-8 months old, 2.5-3.0 kg, 2 females and 1 male
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritating properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in the treated eye
Fluorescein examination	24 hr after the 5th application in the treated eye

Results:

No treatment-related abnormal ocular findings were detected. No systemic abnormal findings were noted either.

In summary, azelastine HCl solution 0.1% showed no ocular irritant effects in rabbits following daily ocular instillation for 5 days in this study.

5. Azelastine eye drops 0.1%, Batch # 11/36: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 301.

Project Nº: 856517
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/36). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 6-9 months old, 2.4 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in the treated eye
Fluorescein examination	24 hr after the 5th application in the treated eye

Results:

Short-lasting, slight conjunctival hyperemia (Draize score = 1) was observed in one animal at 1 hr after the 2nd instillation, and in another animal at 24 hr after the 2nd dosing and 1 hr after the 3rd dosing. The final mean Draize index was 0.2, which was within the non-irritation range. No adverse effects were noted in the iris and cornea. No abnormal findings were noted in other ophthalmic and systemic examinations.

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

6. Azelastine eye drops 0.1%, Batch # 11/37: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 16, Page 339.

Project Nº: 856517
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/37). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 7 months old, 2.5 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152, D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in the treated eye
Fluorescein examination	24 hr after the 5th application in the treated eye

Results:

Slight conjunctival hyperemia (Draize score = 1) was observed in one animal during the first 4 days of instillation. The same animal also showed slight discharge (Draize score = 1) at 1 hr after the first instillation. The final mean Draize index was 0.47, which was within the non-irritation range. No adverse effects were noted in the iris and cornea. No abnormal findings were noted in other ophthalmic and systemic examinations.

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

7. Azelastine eye drops 0.1%, Batch # 11/38: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 17, Page 001.

Project Nº: 856530
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/38). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 6-month old, 2.4-2.8 kg, 2 males and 1 female
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: Not indicated (Study Period: 1987)
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application

Results:

The only positive finding was slight hyperemia (Draize score = 1) in the female rabbit seen only at 24 hr after the 2nd instillation. No ocular toxic effects were observed in

other ocular examinations. Mean Draize index was 0.07. No systemic abnormal findings were noted.

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

8. Azelastine eye drops 0.1%, Batch # 11/40: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 17, Page 039.

Project N°: 856541
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/40). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 6-month old, 2.2-2.6 kg, 2 males and 1 female
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: Not indicated (Study Period: 1987)
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application

Results:

One male rabbit was sacrificed on Day 2 due to broken vertebral column. No ocular or systemic toxicity effects were noted in this study.

In summary, azelastine HCl solution 0.1% showed no ocular irritant effects in rabbits following daily ocular instillation for 5 days in this study.

9. Azelastine eye drops 0.1%, Batch # 11/41: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 17, Page 077.

Project Nº: 856552
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/41). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 6-month old, 2.0-2.3 kg, 2 males and 1 female
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application

Results:

Slight conjunctival hyperemia and slight hypersecretion were observed (see table below). The severity (mean Draize index = 0.67) was still located in the non-irritant range. No abnormal findings were noted in other ophthalmic and systemic examinations.

Conjunctival redness and discharge observations in rabbits treated with azelastine solution 0.1%

Draize score (1-3)	Day 1		Day 2		Day 3		Day 4		Day 5		Days 6-8
Hr after dosing	1	24	1	24	1	24	1	24	1	24	
Redness											
Male 1	1	0	0	1	1	0	1	0	0	0	0
Female	0	0	0	0	0	0	0	0	0	0	0
Male 2	1	0	0	0	1	0	0	0	0	0	0
Discharge											
Male 1	0	0	0	0	1	0	0	0	0	0	0
Female	1	0	1	0	0	0	0	0	0	0	0
Male 2	0	0	1	0	0	0	0	0	0	0	0

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

10. Azelastine eye drops 0.1%, Batch # 11/42: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 17, Page 115.

Project Nº: 856563
Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/42). [redacted]
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Female white Russian rabbits, 4-month old, 2.10-2.45 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application

Results:

Slight conjunctival hyperemia (Draize score = 1) was observed in one animal at 1 hr after the 2nd instillation. No adverse effects were noted in the iris and cornea. Mean Draize index was 0.07. No abnormal findings were noted in other ophthalmic and systemic examinations.

In summary, azelastine HCl solution 0.1% was not irritating to the ocular mucous membrane in rabbits following daily ocular instillation for 5 days in this study.

11. Azelastine eye drops 0.1%, Batch # 11/43: Testing the mucous membrane irritancy after repeated application to the rabbit eye for 5 days. Vol. 17, Page 153.

Project Nº: 856574

Compound: Azelastine-HCl 0.1% (Batch #: A 5610-L/V Ch.-B 11/43). [redacted]

Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 4-month old, 2.1-2.2 kg, one male and two females
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 17, 1987
GLP/QAU: Yes

The purpose of this study was to determine the mucosal irritant properties of azelastine solution 0.1% in rabbits following ocular administration (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic clinical signs were also observed.
Fundus camera examination	After the 4th instillation in treated eyes
Fluorescein examination	24 hr after the 5th application

Results:

The only abnormal finding was slight conjunctival hyperemia (Draize score = 1) observed in the male animal at 1 hr after the 3rd instillation. No adverse effects were noted in the iris and cornea. Mean Draize index was 0.07. No abnormal findings were noted in other ophthalmic and systemic examinations.

In summary, azelastine HCl solution 0.1% was not irritant in rabbits following daily ocular instillation for 5 days in this study.

12. Ocular mucosal irritation test of AST-ED in rabbits. Vol. 17, Page 191.

Report Nº: A-05610/7100000011
Compound: Azelastine eye drops 0.1% and 0.01% (Batch #: K770700). [redacted]

Control: [redacted]
Route: Topical, ocular
Dose: 3 drops/dose x 16 times (at 30 min intervals)
Animal: Female [redacted] rabbits, 7-month old, 2.1-2.2 kg, N = 4/group

Study Site: [redacted]
Study Initiation: July 1, 1987
GLP/QAU: No
Study Design:

Group	1		2		3	
Eye	Left	Right	Left	Right	Left	Right
Treatment	Azelastine 0.1%	Vehicle	Azelastine 0.01%	PSS	Intal	Untreated
Dosing regimen	3 drops/dose/30 min x 16 per day x 5 days					
N	4/group					

The purpose of this study was to determine the acute ocular toxic potential of azelastine solution in rabbits following ocular administration (16 times per day) for 5 days. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Ocular irritation was observed using Draize scale at the time of grouping and 30 min after the final dosing. Fluorescein examination was also performed during the observation.
Necropsy	30 min after the final dose, all survivors were sacrificed and necropsy was performed on the eyeball and eye appendages.
Histopathology	Eyeball and appendages

Results:

The only abnormal finding was fluorescein positive foci in 2 animals each in 0.1% azelastine group and in Intal control group. No other remarkable findings were observed. Post-mortem examinations showed no positive findings.

In summary, rabbits were treated with azelastine HCl solution 0.1% or 0.01% 16 times daily for 5 days. No remarkable findings were noted at 0.01%. Animals treated at 0.1% showed corneal changes (fluorescein positive foci in 2/4 animals). Similar changes were also noted in the comparative control [redacted] group.

13. Azelastine eye drops (Prof. Schreier), Sample A (0.05% azelastine HCl solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 17, Page 273.

Report Nº: A-05610/3000877004
Study Nº: 877004
Compound: Azelastine-HCl eye drops 0.05% (Sample A). The drug was prepared with sodium phosphate buffer solution.
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 8-month old, 2.30-2.61 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14

Study Initiation: March 26, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular irritation potential of 0.05% azelastine HCl solution in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy and slit lamp biomicroscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

Results:

Clinical observations: There were no systemic toxic effects. No signs of ocular irritation in iris, cornea and conjunctiva were noted. The irritation index was 0.

Ophthalmic and slit lamp biomicroscopical examinations: No treatment-related abnormal findings were observed.

Aesthesiometrical examination: Decreased corneal sensitivity was noted in all animals. On Day 1, immediately after dosing, the corneal sensitivity was reduced (the length of filament was 0.5-1.0 cm vs. control's 2.0 cm). The values returned to normal 44 to 90 min later. On Days 3 and 5, the reduced sensitivity was not as pronounced as on day 1 (filament length was 1.0-1.5 vs. control's 1.5-2.0), and lasted for about 20 min.

In summary, azelastine HCl 0.05% in sodium phosphate buffer solution showed no ocular irritant effects in rabbits in this study. However, a surface anaesthetic effect was noted on the cornea.

14. Azelastine eye drops (Prof. Schreier), Sample B (0.1% azelastine HCl solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 17, Page 320.

Report Nº: A-05610/3000877252
Study Nº: 877252
Compound: Azelastine-HCl eye drops 0.1% (Sample B). The drug was prepared with sodium phosphate buffer solution.
Route: Topical, ocular (Left eye only. Right eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 8-9-month old, 2.33-2.67 kg, N = 3
Study Site: ASTA Medica AG

Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: March 26, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular irritation potential of 0.1% azelastine HCl solution in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy and slit lamp biomicroscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

Results:

Clinical observations: There were no systemic toxic effects. One animal showed slight hyperemia in the conjunctiva 1 hr after the first dosing. This finding was not seen again during the study. The irritation index was 0.07, which was within the non-irritant range.

Ophthalmic and slit lamp biomicroscopical examinations: No treatment-related abnormal findings were observed.

Aesthesiometrical examination: Decreased corneal sensitivity was noted in all animals. The corneal sensitivity was not measurable for up to 7 to 17 min. The detailed study results are summarized in the table below.

Corneal surface anaesthetic effect of azelastine solution 0.1% (length of filament, cm)

Time	Animal # 2251						Animal # 2211						Animal # 2235					
(min)	Day 1		Day 3		Day 5		Day 1		Day 3		Day 5		Day 1		Day 3		Day 5	
	r	l	r	l	r	l	r	l	r	l	r	l	r	l	r	l	r	l
0	2.0	2.0	2.0	2.0	1.5	1.5	1.5	2.0	1.5	2.0	1.0	1.5	2.0	2.0	1.5	1.5	1.5	1.5
2			2.0	0.5	1.5	nm					1.0	nm					2.0	0.5
3	2.0	nm							1.5	nm					2.0	0.5		
4							1.0	0.5					2.0	Nm				
7	1.5	0.5	2.0	0.5	1.5	nm					1.0	nm					1.5	0.5
8							1.5	0.5	2.0	nm			2.0	Nm	2.0	0.5		
12	1.5	0.5	2.0	0.5	1.5	0.5					1.5	nm					1.5	0.5
13							1.5	1.0	1.5	0.5					1.5	1.0		
14													2.0	0.5				
17			2.0	0.5	1.5	0.5					1.0	0.5					1.5	0.5
18	1.5	0.5					1.5	1.5	1.5	1.0					1.5	1.0		
19													2.0	0.5				
22			2.0	1.0	1.5	0.5					1.5	0.5					1.5	1.0
23	2.0	0.5					1.5	1.5	1.5	1.0					2.0	1.0		

Time	Animal # 2251						Animal # 2211						Animal # 2235					
(min)	Day 1		Day 3		Day 5		Day 1		Day 3		Day 5		Day 1		Day 3		Day 5	
	r	l	r	l	r	l	r	l	r	l	r	l	r	l	r	l	r	l
24													2.0	0.5				
32			2.0	1.0	1.5	1.0					1.0	1.0					1.5	1.5
33	1.5	0.5					1.0	2.0	2.0	1.0					1.5	1.5		
34													1.5	1.0				
42			2.0	1.5	1.5	1.5					1.0	1.0					2.0	1.5
43	2.0	1.0							1.5	1.0					1.0	1.0		
44													2.0	1.5				
52			2.0	1.5	1.5	1.5					1.0	1.5					2.0	1.5
53	1.5	1.5							1.5	1.5					2.0	1.5		
54													1.5	1.5				
63	2.0	2.0	2.0	1.5					1.5	1.5								
64													2.0	2.0				
72			2.0	2.0														

Nm: not measurable

In summary, azelastine HCl 0.1% in sodium phosphate buffer solution was not irritating to the ocular mucous membrane in rabbits in this study. However, a surface anaesthetic effect was noted on the cornea.

15. Azelastine eye drops (Prof. Schreier), Sample C (10% 2-hydroxypropyl-β-cyclodextrin solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 17, Page 367.

Report Nº: A-05610/3000877263
Study Nº: 877263
Compound: 10% 2-hydroxypropyl-β-cyclodextrin solution
Route: Topical, ocular (Left eye only. Right eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 1 male: 8-month old, 2.87 kg; 2 females: 8 and 9 months old, 2.74 and 2.93 kg
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: March 26, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular toxicity of 10% 2-hydroxypropyl-β-cyclodextrin solution, which was used as a solvent in a series of ocular irritation studies, in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes

Parameter	Procedure
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

Results:

Clinical observations: There were no systemic toxic effects. One animal showed slight discharge after each application. This finding was not seen again 72 hr after the last dose. The irritation index was 0.4, which was within the non-irritant range.

Ophthalmic and slit lamp biomicroscopical examinations: No treatment-related abnormal findings were observed.

Aesthesiometrical examination: No treatment-related effects were noted.

In summary, 10% 2-hydroxypropyl-β-cyclodextrin solution showed no ocular irritant effects and no corneal anaesthetic effects in rabbits in this study.

16. Azelastine eye drops (Prof. Schreier), Sample D (0.1% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 17, Page 414.

Report Nº: A-05610/3000877274
Study Nº: 877274
Compound: 0.1% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 9-month old, 1 male: 2.46 kg, 2 females: 2.25 and 2.79 kg
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: March 23, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular toxicity of 0.1% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy and slit lamp biomicroscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes

Parameter	Procedure
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

Results:

Clinical observations: There were no systemic toxic effects. One animal showed slight hyperemia in the conjunctiva 1 hr after the second dosing. This finding was not seen again during this study. The irritation index was 0.07, which was within the non-irritant range.

Ophthalmic and slit lamp biomicroscopical examinations: No treatment-related abnormal findings were observed.

Aesthesiometrical examination: A slight decrease in corneal sensitivity was noted in 2 animals on day 3 (see table below).

Corneal surface anaesthetic effect of azelastine solution 0.1% (length of filament, cm)

Time	Animal # 2285		Animal # 2318	
(min)	Right eye (treated)	Left eye (untreated)	Right eye (treated)	Left eye (untreated)
0	3.0	2.5	2.0	2.0
2	1.5	2.5	0.5	1.0
7	1.5	2.0	0.5	1.5
12	2.0	2.0	1.5	2.0
17	2.5	2.0	1.0	1.5
27	2.5	2.0	1.0	1.5
37			1.5	1.0

In summary, 0.1% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution showed no ocular irritant effects. However, a slight decrease in corneal sensitivity was noted.

17. Azelastine eye drops (Prof. Schreier), Sample E (0.05% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 17, Page 461.

Report Nº: A-05610/3000877285
Study Nº: 877285
Compound: 0.05% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 9-month old, 2.52-2.78 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: March 23, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular toxicity of 0.05% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy and slit lamp biomicroscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

Results:

Clinical observations: There were no systemic toxic effects. No signs of ocular irritation in iris and cornea were noted. One animal showed slight hyperemia in the conjunctiva at 24 hr after the 4th application and 1 hr after the 5th dosing. The irritation index was 0.11, which was within the non-irritant range.

Ophthalmic and slit lamp biomicroscopical examinations: No treatment-related abnormal findings were observed.

Aesthesiometrical examination: No surface anaesthetic effect of azelastine on cornea was noted.

In summary, 0.05% azelastine HCl in 10% 2-hydroxypropyl-β-cyclodextrin solution was not irritating to the ocular mucous membrane and had no corneal anasethetic effects.

18. Azelastine eye drops (Prof. Schreier), Sample F (buffer solution): Testing the irritation after repeated application to the eye of the rabbit for 5 days. Vol. 18, Page 001.

Report Nº: A-05610/3000877296
Study Nº: 877296
Compound: Phosphate buffer solution
Route: Topical, ocular (Right eye only. Left eyes served as untreated controls.)
Dose: 100 µl, qd x 5 days
Animal: Male white Russian rabbits, 9-month old, 2.42-2.71 kg, N = 3
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: April 23, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular toxicity of buffer solution, which was used as a solvent in a series of ocular irritation studies, in rabbits following ocular administrations (qd) for 5 days. This was followed by a 3-day observation period. The first day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	The signs of ocular irritation in the treated eyes were observed using Draize scale at 1 and 24 hr after each application during the 5-day treatment-period, thereafter once daily for 3 days. Systemic toxic effects were also observed.
Indirect ophthalmoscopy and slit lamp biomicroscopy	Prior to the first application and on Day 4 of application (70 min after dosing), both eyes
Corneal surface sensitivity (aesthesiometrical examination)	Prior to as well as after the first, third and fifth applications with an aesthesiometer, both eyes

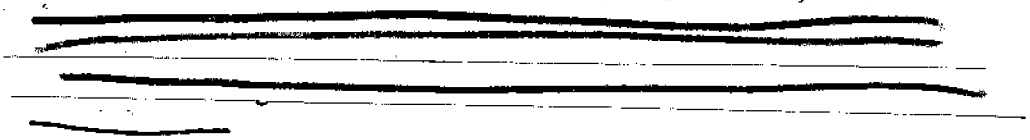
Results:

No systemic and ocular toxic effects were observed. The irritation index was 0. Ophthalmoscopical, slit lamp biomicroscopical and aesthesiometric examinations showed no treatment-related abnormal eye findings.

In summary, phosphate buffer solution showed no ocular toxicity in rabbits in this study.

19. Azelastine nasal spray, Form 35: Testing the local tolerance after 5 days application (5 times daily) to the eye of the rabbit. Vol. 18, Page 048.

Report Nº: A-05610/3000889615
Study Nº: 889615
Compound: Azelastine nasal spray 0.1% (Batch #: 17/35, purity > 99.5%). The



Control:
Route: Topical, ocular (right eye only)
Dose: 100 µl, 5 times per day (at 90 min intervals) x 5 days
Animal: White Russian rabbits, 6-11 months old, 2.13-2.89 kg, N = 5/sex
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe 2
D-4802 Halle-Kunsebeck
Study Initiation: June 29, 1992
GLP/QAU: Yes

The purpose of this study was to determine the ocular tolerance of 0.1% azelastine nasal spray in rabbits following ocular administrations (5 times a day) for 5 days. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Daily
Ocular examinations	
Irritant effect	Signs of irritation on the cornea, iris and conjunctiva were assessed quantitatively and qualitatively using Draize scale prior to the first application and daily prior to the first application and 5 min after the second and fifth applications. After the end of the treatment period, the animals were observed for 3 more days.
Schirmer tear test	Prior to the first application, on Day 5 (at least 60 min after the second application)
Fluorescein staining	Prior to the first application and on Day 4 (at least 30 min after the fifth application).

Results:

Clinical observations: No adverse clinical signs were observed.

Ophthalmoscopy:

Irritant effect: Slight hyperemia of the conjunctiva was noted in treated eyes at different times (see table bellow). The mean irritation index was 0.6 in males and 0.4 in females, which suggested that the drug be non-irritant in this test. Three days after the treatment period, no signs were noted.

Draize grade on conjunctival redness 5 min after the 2nd and 5th applications

Animal	Day 1		Day 2		Day 3		Day 4		Day 5	
Males	2nd	5th	2nd	5th	2nd	5th	2nd	5th	2nd	5th
1	1	1	0	1	1	0	0	0	1	1
2	0	0	1	0	0	0	1	0	1	0
3	0	0	0	0	0	1	0	1	0	0
4	0	0	0	1	1	0	0	0	0	1
5	0	1	0	1	1	1	0	1	0	0
Females	2nd	5th	2nd	5th	2nd	5th	2nd	5th	2nd	5th
1	0	0	0	1	1	0	0	1	0	0
2	0	1	1	1	0	1	0	0	0	0
3	0	1	0	0	0	1	0	1	1	1
4	0	0	0	1	0	0	0	0	0	0
5	0	0	0	0	0	1	0	1	1	0

No changes were noted in control (left) eyes.

Schirmer tear test and fluorescein staining: No drug-related differences were noted.

In summary, rabbits were topically treated with 0.1% azelastine nasal spray (5 times per day) for 5 days. In all animals slight conjunctival hyperemia was noted. The ocular irritation index was 0.8 in males and 0.64 in females. No other drug-related changes were noted. In conclusion, the drug was not irritating to the ocular mucous membrane when applied topically to the rabbit eye for 5 days.

20. Azelastine 0.2% nasal spray: Testing the mucous membrane irritancy after repeated application to the rabbit for 5 days. Vol. 16, Page 113.

Report Nº: CWH-TX-173-85/86
Study Nº: 889615

Compound: Azelastine nasal spray 0.1% (Batch #: A5610-L/III, Ch.-B: 11/2). The [redacted]

Control: Untreated (left eye)
Route: Topical, ocular (right eye only)
Dose: 100 µl, qd x 5 days
Animal: White Russian rabbits, 5 months old, 2.1-2.2 kg, N = 2/sex
Study Site: ASTA Werke AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 10, 1986
GLP/QAU: Yes

The purpose of this study was to determine the ocular irritating properties of 0.2% azelastine nasal spray in rabbits following ocular administrations (once daily) for 5 days. Signs of irritation on the cornea, iris and conjunctiva were assessed using the Draize scale before and 1 hr after application during the treatment period, and thereafter once daily for 3 days.

Results:

Clinical observations: No adverse clinical signs were observed.

Ocular irritation assessment: No abnormal findings were noted in the cornea and iris. One female showed diffuse hyperemia in conjunctiva 1 hr after the first application. The mean irritation index was 0.1, which was within the non-irritation range.

In summary, rabbits were topically treated with 0.2% azelastine nasal spray (once daily) for 5 days. Diffuse conjunctival hyperemia was noted in 1 female 1 hr after the first dosing. The mean ocular irritation index was 0.1. No other drug-related changes were noted. In conclusion, the drug was not irritant to the ocular mucous membrane when applied topically to the eye for 5 days.

21. Azelastine eye drops (0.1%): Testing the local tolerance after 5 days application (5 times daily) to the eye of the rabbit. Vol. 18, Page 117.

Report Nº: A-05610/3000889648
Study Nº: 889648
Compound: Azelastine-HCl 0.1% (Batch #: 13/68, purity > 99.5%, the formulation was the same as the clinical formulation except for the concentration)
Control: PSS (left eye)
Route: Topical, ocular (right eye only)
Dose: 100 µl, 5 times per day (at 90 min intervals) x 5 days

Animal: White Russian rabbits, 9-10 months old, 2.47-2.99 kg, N = 5/sex
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe 2
D-33790 Halle/Westfalen
Study Initiation: July 12, 1993
GLP/QAU: Yes

The purpose of this study was to determine the ocular tolerance of 0.1% azelastine HCl eye drops in rabbits following ocular administrations (5 times a day) for 5 days. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	**Procedure**
Clinical observations	Daily
Ocular examinations	
Irritant effect	Signs of irritation on the cornea, iris and conjunctiva were assessed quantitatively and qualitatively using Draize scale prior to the first application and daily prior to the first application and 5 min after the second and fifth applications. After the end of the treatment period, the animals were observed for 3 more days.
Schirmer tear test	Prior to the first application, on Day 5 (at least 60 min after the second application)
Ophthalmoscopical investigation and slit lamp biomicroscopy	Prior to the first application and on Day 5 (at least 30 min after the 5th application)
Fluorescein staining	Prior to the first application and on Day 4 (at least 30 min after the fifth application).

Results:

Clinical observations: No adverse clinical signs were observed.

Ophthalmoscopy:

Irritant effect: Slight conjunctival hyperemia was noted in most animals at different times (see table bellow). The mean irritation index was 0.23 for males and 0.29 for females, which suggested that the drug be non-irritant in this test. No irritation was noted in any animals during the 3-day post-treatment observation.

Draize grade on conjunctival redness 5 min after the 2nd and 5th applications

Animal	Day 1		Day 2		Day 3		Day 4		Day 5	
Males	2nd	5th	2nd	5th	2nd	5th	2nd	5th	2nd	5th
1	0	0	0	0	0	0	1	1	0	0
2	0	0	0	0	0	0	0	0	0	1
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	1	1	0	1	0	0
5	0	0	1	1	0	0	0	0	0	0
Females	2nd	5th	2nd	5th	2nd	5th	2nd	5th	2nd	5th
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	1	0	0	0	1
3	0	0	0	1	0	0	0	1	0	0
4	0	0	0	0	0	1	0	1	0	1
5	0	0	0	0	0	1	0	0	1	0

No changes were noted in control (left) eyes.

Schirmer tear test, ophthalmoscopy, slit lamp biomicroscopy and fluorescein staining: No drug-related differences were noted.

In summary, rabbits were topically treated with 0.1% azelastine HCl eye drops (5 times per day) for 5 days. In most animals slight conjunctival hyperemia was noted. The ocular irritation index was 0.23 for males and 0.29 for females. No other drug-related changes were noted. In conclusion, azelastine HCl 0.1% eye drops was not irritant to the ocular mucous membrane in the rabbits treated topically for 5 days in this study.

22. Azelastine eye drops 0.05% (stored for at least 12 months at 40 °C): Testing the local tolerance after 5 days application (5 times daily) to the eye of the rabbit. Vol. 18, Page 187.

Report Nº: A-05610/3000911608
Study Nº: 911608
Compound: Azelastine-HCl 0.05% (Batch #s: 43601, 44603 and 44604, purity > 98.91%, the formulation was the same as the clinical formulation. The drug was stored for at least 18 months at 40 °C. The drug was stable throughout the study period.)
Control: PSS (left eye for females and right eye for males)
Route: Topical, ocular (right eye for females and left eye for males)
Dose: 100 µl, 5 times per day (at 90 min intervals) x 5 days
Animal: White Russian rabbits, 7 months old, 2.21-3.12 kg for males and 2.44-2.91 kg for females, N = 5/sex
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe 2
D-33790 Halle/Westfalen
Study Initiation: April 22, 1996
GLP/QAU: Yes

The purpose of this study was to determine the ocular tolerance of 0.05% azelastine HCl eye drops (stored for at least 12 months at 40 °C) in rabbits following ocular administrations (5 times a day) for 5 days. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Clinical observations	Daily
Ocular examinations	
Irritant effect	Signs of irritation on the cornea, iris and conjunctiva were assessed quantitatively and qualitatively using Draize scale prior to the first application and daily prior to the first application and 5 min after the second and fifth applications. After the end of the treatment period, the animals were observed for 3 more days.
Schirmer tear test	Prior to the first application, on Day 5 (at least 60 min after the second application)
Ophthalmoscopical investigation and slit lamp biomicroscopy	Prior to the first application and on Day 5 (at least 30 min after the 5th application)
Fluorescein staining	Prior to the first application and on Day 4 (at least 30 min after the fifth application).

Results:

Clinical observations: No adverse clinical signs were observed.

Ophthalmoscopy:

Irritant effect: Slight conjunctival hyperemia was noted once each in 2 females' treated eyes and was considered incidental finding. No irritation was noted in any control eyes.

Schirmer tear test, ophthalmoscopy, slit lamp biomicroscopy and fluorescein staining: No drug-related differences were noted.

In summary, rabbits were topically treated with 0.05% azelastine HCl eye drops (stored for at least 12 months at 40 °C) for 5 days. Slight conjunctival hyperemia was noted twice in 2 treated eyes in females. The ocular irritation index for females was 0.06. No other drug-related changes were noted.

SPECIAL TOXICOLOGY

1. Acute effect on intraocular pressure (IOP) in rabbit. Vol. 18, Page 258.

Report Nº: A-05610/7100000003
Study Nº: 88-153(F)
Compound: Azelastine-HCl 1% in PSS solution
Route: Topical, ocular (left eye only, the right eye was untreated.)
Dose: 50 µl, from 8am to 2 pm at 20-min intervals on Day 3
Control: PSS on Day 2
Animal: [redacted] rabbits, 1.5-2.5 kg, N = 8
Study Site: [redacted]
Study Initiation: November 25, 1987
GLP/QAU: No

The purpose of this study was to determine the effect of azelastine HCl on intraocular pressure in rabbits following ocular administrations (at 20 min interval) for 6 hr. IOP was measured with a pneumatonographer at 7:30 am and 2:30 pm on Days 1, 2 and 3. On Day 1, the animals were not treated. On Day 2 and Day 3, the animals (left eyes) were treated with PSS and 1% azelastine solution, respectively.

Results: No IOP alteration was observed between treated and untreated eyes, and between the control and treated eyes.

2. Study of effect of LC-5505 at 0.1% on the corneal reepithelization process in rabbits. Vol. 18, Page 289.

Report Nº: A-05610/7100000001
Study Nº: 88-018(F)
Compound: Azelastine HCl 0.1% (Batch #: 076007) in PSS solution
Route: Topical, ocular (one eye per animal)
Control: PSS, colircusi dexamethasone (0.1%)
Animal: New Zealand white rabbits, N = 4/group
Study Site: [redacted]
Study Initiation: Not provided
GLP/QAU: No

The purpose of this study was to determine the effects of azelastine on corneal reepithelialization process in rabbits following ocular administrations. Ocular drug treatment was carried out at 0, 4, 8, 12, 20, 24, 28, 32, 36, 44, 48, 52, 56 and 60 hr following corneal de-epithelialization. Corneal lesions were photographed and evaluated at 0, 5.5, 11.25, 23, 29.5, 35.25, 47, 53.75 and 58.5 hr following de-epithelialization. In some animals, corneal lesions disappeared before the scheduled treatment or evaluation.

Results:

No biologically significant differences were noted. The time for thorough healing of corneal lesions was 65.5 hr, 66.5 hr and 72.5 hr in the eyes treated with azelastine, dexamethasone and PSS, respectively. The corneal reepithelialization process was not affected by 0.1% azelastine solution.

3. Examination of the surface anaesthetic action of azelastine-HCl on the cornea of albino rabbit. Vol. 18, Page 305.

Report Nº: A-05610/3000864808
Study Nº: 864808
Compound: Azelastine-HCl 0.5%, 0.1%, 0.05% and 0.01% (Batch #: PAX6145) in Aqua ad iniectabilia solution
Route: Topical, ocular (right eye only)
Dose: 100 µl, single dose
Animal: White Himalayan rabbits, 5-12 months old, about 2 kg, N = 3/group
Study Site: ASTA Pharma AG
Study Initiation: Not provided
GLP/QAU: No

The purpose of this study was to determine azelastine's local anaesthetic activity in rabbits following ocular administration. Corneal sensitivity was measured using a [redacted] aesthesiometer prior to the treatment, and after dosing at different intervals for up to 114 min.

Results:

At a concentration of 0.5%, no corneal sensitivity was measurable for 30 to 65 min after dosing. At 0.1% and 0.05%, decreased sensitivity was seen for up to 40 min (the length of filament was 0.5-1.5 cm vs. control's 2.0-3.5 cm). No significant reduction in corneal sensitivity was noted in animals treated with azelastine HCl at 0.01%. In conclusion, rabbit corneal sensitivity was reduced following topical ocular application of azelastine HCl solution at the concentrations $\geq$ 0.05%.

4. LC-5505: Determination of its anaesthetic activity and comparation with benoxinate and antazoline. Vol. 18, Page 271.

Report Nº: A-05610/7100000002
Study Nº: 88-169(F)
Compound: Azelastine-HCl 0.1% or 0.02% (Batch #: 076007) in PSS solution
Route: Topical, ocular (one eye per animal)
Dose: 50 µl, single dose
Control: PSS, benoxinate (0.4 or 0.12 mg/ml), antazoline (5 mg/ml)
Animal: [redacted] rabbits, about 2 kg, N = 8/group
Study Site: [redacted]
Study Initiation: Not provided
GLP/QAU: No

The purpose of this study was to determine whether azelastine had local anaesthetic activity in rabbits following ocular administration. The drug was compared with benoxinate (an anaesthetic agent) and antazoline (an H_1-receptor antagonist with local anaesthetic activity as unwanted side effect). Corneal sensitivity was measured using [redacted] aesthesiometer prior to the treatment, and at 0.5, 1, 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42 and 46 min after dosing. For azelastine treated animals, extra measurements were performed at 50, 60, 70, 80, 90 and 100 min after dosing.

Results: The data of corneal sensitivity are summarized in the table below. The anaesthetic effect of azelastine 0.1% was higher than that of antazoline, and lasted longer than antazoline and benoxinate. Azelastine 0.02% also showed decreased sensitivity compared to the control animals.

Pressure necessary for blinking in rabbits' cornea (g/mm^2)

Treatment	Control	Azelastine 0.1%	Azelastine 0.02%	Antazoline	Benoxinate 0.4 mg/ml	Benoxinate 0.12 mg/ml
Time (min)						
-5	1.05±0.11	1.27±0.13	1.15±0.10	1.01±0.12	1.14±0.12	1.20±0.18
0.5	1.12±0.06	0.69±0.72	1.18±0.16	1.01±0.12	8.50±3.36	2.76±1.19
1	1.13±0.06	1.14±0.93	1.30±0.27	1.02±0.12	21.53±16.86	4.58±3.44
2	1.10±0.06	2.14±1.49	1.49±0.40	1.09±0.14	43.75±10.83	7.08±3.90
6	1.06±0.12	21.81±21.90	2.10±0.52	1.65±0.47	42.23±13.80	8.42±4.43
10	1.09±0.13	31.25±10.83	4.09±.98	2.23±0.63	31.53±18.61	4.95±2.15
14	1.04±0.18	27.21±8.94	4.69±1.93	1.99±0.51	18.58±13.90	3.17±1.45

Treatment	Control	Azelastine 0.1%	Azelastine 0.02%	Antazoline	Benoxinate 0.4 mg/ml	Benoxinate 0.12 mg/ml
Time (min)						
18	1.04±0.17	25.38±9.90	4.39±1.47	1.70±0.44	7.81±3.95	2.22±0.69
22	1.07±0.12	19.82±4.29	3.86±1.46	1.40±0.28	5.17±3.35	1.64±0.49
26	1.06±0.13	16.88±5.40	3.73±.44	1.32±0.22	2.77±1.55	1.36±0.30
30	1.06±0.11	13.53±2.41	2.95±0.77	1.33±0.24	2.18±0.97	1.22±0.26
34	1.07±0.12	13.74±1.67	3.17±1.35	1.10±0.13	1.57±0.41	1.24±0.19
38	1.05±0.11	12.13±2.04	2.58±0.90	1.09±0.14	1.56±0.66	1.17±0.16
42	1.05±0.07	11.57±1.73	2.53±0.72	1.06±0.13	1.20±0.14	1.14±0.12
46	1.05±0.11	10.29±2.22	2.14±0.56	1.04±0.09	1.15±0.12	1.17±0.16
50		8.21±1.95	1.96±.63			
60		5.08±1.29	1.53±0.51			
70		2.81±0.60	1.43±0.44			
80		2.34±0.60	1.23±0.14			
90		1.71±0.25	1.18±0.08			
100		1.39±0.23	1.12±0.04			

In summary, azelastine solution 0.1% showed an anaesthetic effect in rabbit cornea, which was higher and lasted longer than that produced by antazoline, and longer than benoxinate. Azelastine 0.02% also showed a slight decrease in corneal sensitivity.

5. Study on the sensitizing capacity of LC-5505 by ocular application. Vol. 20, Page 001.

Report Nº: A 5610/7100000009
Study Nº: 89-428
Compound: Azelastine-HCl (Batch #: 106010)
Animal: Male Dunkin-Hartley albino guinea pigs, mean weight = 270 g
Study Site: [redacted]

Study Initiation: November 6, 1989
GLP/QAU: No
Study design (n=4 in each group)

		Induction phase			Challenging phase	
Test material	Group	Sensitizing agent	Vehicle	Route	Challenging agent, 25 µl	Time
Oxazolone (positive control)	1	Oxazolone	[redacted]	Transcutaneous, qd x 5 (abdominal wall) 0.4 ml	Oxazolone (2%)	Day 11
	2				0.9% saline solution	Day 11
	3			Intraplantar	Oxazolone (2%)	Day 7
	4				0.9% saline solution	Day 7
	5				0.9% saline solution	Day 7
	6				Oxazolone (2%)	Day 7
	7				0.9% saline solution	Day 7
Azelastine	8	Azelastine		Transcutaneous, qd x 5 (abdominal wall) 0.4 ml	Azelastine (0.2%)	Day 11
	9				0.9% saline solution	Day 11
	10			Intraplantar	Azelastine (0.2%)	Day 7
	11				0.9% saline solution	Day 7
	12				Azelastine (0.2%)	Day 7
	13				0.9% saline solution	Day 7

The purpose of this study was to determine the sensitizing capacity of azelastine after topical ocular application in guinea pigs. In a preliminary study to determine the ocular tolerance of the drug, it was found that the drug in olive oil at the concentrations of 2% and 4% was not well tolerated. At the concentration of 0.2%, the drug caused only

very slight signs of irritation. Hence, this concentration was chosen in the study. The study consisted of induction phase and challenge phase. The first day of induction phase was designated as Day 1. The vehicle used in the challenge phase was olive oil.

The evaluation criteria were based on the conjunctivitis and/or blepharitis, palpebral edema and/or chemosis, exudation and keratitis and close of the eye. Evaluation was conducted at 1, 2, 18, 24, 48, 72 hr and 5 and 6 days after the challenge.

Results:

The results are summarized in the table below. Lesions including conjunctival disorder, chemosis, eyelids redness with the tendency to keep eyes closed were observed in the positive control animals, indicative of the sensitizing capacity of [redacted] In one azelastine group (Group 12), a slight eyelids redness was detected during the first hr after the challenge. Since this finding was mild and transient, it was not considered an immunologic reaction.

Mean values of ocular lesion in guinea pigs

	Groups							
Time	1	2	3	4-5	6	7-11	12	13
1 hr	2	0	1	0	1	0	0.5	0
2 hr	2	0	1	0	1.25	0		
18 hr	2	0	2.5	0	2.5	0		
24 hr	1.5	0	2.25	0	2.5	0		
48 hr	1	0	2	0	1.5	0		
72 hr	0.5	0	2	0	0.5	0		
5 day	0	0	0.75	0				
6 day	0	0	0.5	0				

1 = mild and transient, 2 = intermediate, 3 = severe

In summary, sensitizing capacity of azelastine was evaluated in guinea pigs. Positive responses were only observed in animals treated with [redacted], a positive control compound. Azelastine at 0.2% concentration in olive oil developed no sensitizing capacity in guinea pigs.

SUBACUTE AND CHRONIC OCULAR TOXICOLOGY

1. Study on the toxic effects caused by the topical ocular application of two preparations of LC-10010 during four weeks in rabbit. Vol. 18, Page 337.

Report Nº: A-05610/3000868072
Study Nº: 88-426(F)
Compound: Azelastine-HCl ophthalmic solution 0.05% (Batch#: 12/9) and 0.1% (Batch #: 13/48). The formulation was composed of azelastine, [redacted]
Route: Topical, ocular (right eye only)
Dose: 50 µl, qid (at 5 or 6 hr intervals) x 4 weeks
Control: PSS, azelastine 0.1% and 0.05% in PSS solution

Animal: [redacted] rabbits, 3-4 month-old, 2.5-4 kg, N = 8/group
Study Site: [redacted]

Study Initiation: October 7, 1988
GLP/QAU: Yes

The purpose of this study was to determine the toxicity of azelastine HCl in rabbits following ocular administrations (qid) for 4 weeks. Eight animals were included in each of the 5 study groups. Six of the 8 animals were used for toxicity observations, and the 2 remaining animals were used for IOP and corneal sensitivity measurements. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Body weights	Weekly
Clinical observations	Draize test, slit lamp biomicroscopy, and fluorescein examinations were performed weekly.
Ocular fundus examination	Two days before the initiation of the treatment, and one day after the end of the treatment
Esthesiometry	Two days prior to the study initiation, and weekly thereafter
Tonometry	Day 27
Histopathologic examinations	Only eyes and the annexes were examined microscopically.

Results:

Body weights: No treatment-related abnormal findings were noted.

Ocular observations: Slight corneal opacity was observed in all groups including PSS control animals. The degree and area were both graded 1. Slight conjunctival hyperemia was noted in 1 animal each in Groups 4 and 5. These changes were not considered azelastine-related. In addition, the average ocular lesion indexes, summarized in the table below, were located in the non irritant range.

Average ocular lesion indexes in rabbits treated with azelastine (n = 6)

Group	Treatment	Day 7	Day 14	Day 21	Day 28
1	Azelastine 0.1% in PSS	1.7±1.0	3.2±1.0	1.7±1.0	1.7±1.0
2	Azelastine 0.1% ophthalmic solution	3.1±1.0	3.3±1.0	3.3±1.0	3.3±1.5
3	Azelastine 0.05% in PSS	4.2±0.8	1.7±1.0	0.8±0.8	4.2±0.8
4	Azelastine 0.05% ophthalmic solution	2.8±1.2	1.7±1.0	3.3±1.0	3.3±1.0
5	PSS	0.7±0.3	2.5±1.0	1.7±1.0	3.3±.0

Tonometry: No treatment-related IOP changes were noted.

Esthesiometry: There was a time-related tendency to corneal sensitivity decrease in both eyes in all 5 groups. There were no significant differences between treated and untreated animal groups, and between treated and untreated eyes. The decrease could be due to the handling and measuring procedures performed in this study.

Histopathology: No lesions attributed to the treatment with azelastine were observed.

In summary, rabbits were treated topically with azelastine (0.1% and 0.05%) with different formulations for 4 weeks. No toxic effects attributed to the treatment with azelastine were observed.

2. Azelastine-HCl solution (0.1%): Testing the local tolerance after 14 days application (5 times daily) to the eye of the Beagle dog (irritancy, Schirmer tear test, ophthalmoscopical investigation, slit lamp biomicroscopy, aesthesiometry, fluorescein staining). Vol. 19, Page 001.

Report Nº: A-5610/3000876374
Study Nº: 876734
Compound: Azelastine-HCl 0.1% in physiological saline solution (Batch #: 079051, purity = 100.3%)
Control: PSS (left eye)
Route: Topical, ocular (right eye only)
Dose: 100 µl, 5 times per day (at 90 min intervals) x 14 days (tid on weekends)
Animal: Female Brack-Beagle dogs, 3-5 years old, 9.1-16.6 kg, N = 5
Study Site: ASTA Medica AG
Toxikologie Degussa-Asta
Artur-Ladebeck-Str. 128-152
D-4800 Bielefeld 14
Study Initiation: February 27, 1990
GLP/QAU: Yes

The purpose of this study was to determine the ocular tolerance of 0.1% azelastine HCl in PSS in dogs following ocular administrations (5 times a day) for 14 days. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily on weekdays, and once daily on weekends and holidays
Clinical observations	Daily
Body weights	Weekly
Food consumption	Daily
Reflexes	Pain, pupil, patellar and corneal reflexes were tested once a week prior to application.
Ocular examinations	
Irritant effect	Signs of irritation on the cornea, iris and conjunctiva were assessed quantitatively and qualitatively using Draize scale prior to the first application and on Days 1, 3, 8 and 14 prior to the first application and 5 min after the second application. Further assessment was done on Days 15 and 16.
Schirmer tear test	Prior to the first application, on Day 8 (at least 60 min after the second application), and on Day 15
Ophthalmoscopical investigation and slit lamp biomicroscopy	Prior to the first application and on Day 14
Corneal surface sensitivity (aesthesiometrical examination)	Prior to the first application and on Days 2, 9 and 16
Fluorescein staining	Prior to the first application and on Day 15.

Results:

Mortality: No mortality occurred.

Clinical observations: No adverse clinical symptoms were observed.

Body weight: Decreased body weights were noted during the treatment period. The sponsor indicated that the decrease was due to stress.

Body weight changes in dogs treated with azelastine HCl (g)

Pre-treatment	Day 4	Day 10
13620±2863	13180 ± 3077	12960±2813

Food consumption: Food consumption was increased during treatment Days 8-15 (27.5 g/kg/day vs. pretest's 21.7 g/kg/day). The sponsor indicated that this was a stress-induced effect due to frequent handling for applications and investigations.

Reflex testing: No abnormal findings were observed.

Ophthalmoscopy:

Irritant effect: Slight injection of scleric blood vessels was noted in all animals at different times (see table bellow). The mean irritation index was 1.0 for each animal.

Draize grade on conjunctival redness before application and 5 min after the 2nd application

Animal	Day 1		Day 3		Day 8		Day 14		Day 15	
	Before	5 min	Before	5 min	Before	5 min	Before	5 min	Before	5 min
1	0	0	0	0	1	1	1	1	0	0
2	0	1	1	1	1	1	1	1	0	0
3	0	1	1	1	1	1	1	1	0	0
4	0	0	1	1	1	1	0	0	1	0
5	0	1	0	1	0	0	0	0	0	0

No changes were noted in control (left) eyes.

Schirmer tear test, ophthalmoscopy, slit lamp biomicroscopy, corneal surface sensitivity and fluorescein staining: No drug-related differences were noted.

In summary, dogs were topically treated with 0.1% azelastine HCl solution (5 times per day) for 14 days. In all animals slight injection of scleric blood vessels was noted. The ocular irritation index was 1.0. No other drug-related changes were noted. In conclusion, the drug was not irritant in this 14-day study.

3. Azelastine eye drops: 26-week toxicity study after repeated application to the eye of the dog and subsequent 6-week recovery period. Vol. 19, Page 064.

Report Nº: A-05610/3000910912
Study Nº: 910912
Compound: Azelastine-HCl 0.05% eye drops (Batch #: 44603, purity = 99.7%) The formulation was the same as the clinical formulation.
Route: Topical, ocular (right eye only, the left eye remained untreated)
Dose: 50 µl, 2, 4 or 8 times daily for 26 weeks

Animal: Beagle dogs/HSD, 6-7 months old, 9.1-16.6 kg for males and 8.5-11.9 kg for females

Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraße 2
D-33790 Halle/Westfalen

Study Initiation: March 18, 1996

GLP/QAU: Yes

Study design

		N/sex		Dosing Frequency/day	Dosing interval (hr)
Group	Treatment	Main	Recovery		
1	Vehicle control	4	2	8	1
2	Azelastine eye drops 0.05%	4	0	2	7
3		4	0	4	2.5
4		4	2	8	1

The purpose of this study was to determine the ocular and systemic toxicity of 0.05% azelastine eye drops in dogs following ocular administrations (up to 8 times a day) for 26 weeks followed by a 6-week recovery period. The day of the first dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Daily
Body weights	Weekly
Food consumption	Daily
Reflexes	Pain, pupil, patellar and corneal reflexes were tested prior to the first application, in the first week of treatment, and once every 3 weeks thereafter.
Heart rate	Prior to the first application, in the first week of treatment, and once every 3 weeks thereafter
ECG	Prior to the initiation of the treatment, and in Weeks 13 and 25
Hearing and dental examinations	Prior to the first application, and in Weeks 26 and 32 (recovery animals)
Clinical pathology	Blood samples were collected before the treatment, and in Weeks 13 and 26 for hematology and clinical chemistry tests. Urine samples were collected in Weeks 27 and 33.
Ocular examinations	
Irritant effect	Prior to the initiation of the treatment, and once weekly during the treatment period (prior to the first application and within 30 min after the last application)
Slit lamp biomicroscopy	Prior to the initiation of the treatment, and in Weeks 1-6, 8, 12, 16, 20, 24 and 26 of the treatment period and in weeks 29 and 32 of the recovery period
Ophthalmoscopical investigation	Prior to the initiation of the treatment, and in Weeks 13 and 26 of the treatment period and in week 32 of the recovery period
Gross pathology	At the end of the study, all animals were euthanized. A full gross pathology examination was conducted in all animals.
Organ weights	The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroids.
Histopathologic examinations	The organs and tissues examined microscopically for all animals are listed in addendum, Page 53. The eyes and their adnexae were only examined in main control and high dose animals.
TK	Refer to TK section

Results:

Mortality: No mortality occurred.

Clinical observations: The only treatment-related clinical sign was eye secretion in 1/6 male (Weeks 1, 2 and 5) and 2/6 female (Weeks 5, 11 and 12, and Weeks 15 and 16) Group 4 animals. No other abnormal findings were considered treatment-related.

Body weight: No drug-related changes in body weights were noted.

Food consumption: No treatment-related differences in food consumption were observed.

Reflex testing: Testing of pain, pupil, corneal and patellar reflexes as well as hearing and dentition did not reveal any abnormal findings due to the treatment with azelastine.

ECG, body temperature and heart rate: No drug-related changes were observed.

Ophthalmoscopy: With the exception of eye secretion in 3 Group 4 animals, no treatment-related abnormal findings in ophthalmic examinations were observed.

Hematology, clinical chemistry and urinalysis: No drug-related changes were noted.

Gross necropsy: No treatment-related abnormal findings were noted.

Organ weights: A decrease in spleen weight (males) and pituitary weight (females) were noted in treated dogs (see table below). The toxicological significance was not determined. In recovery animals, no differences were noted.

Spleen and pituitary weights in dogs treated with azelastine (g)

N = 4	Spleen in males				Pituitary in females			
Group	1	2	3	4	1	2	3	4
Weight	42.6±13.4	35.3±10.0	36.0±5.3	33.5±5.1	0.080±0.007	0.073±0.008	0.070±0.005	0.068±0.009

Histopathology: No treatment-related changes were noted.

In summary, dogs were topically treated with azelastine eye drops 0.05% 2 to 8 times daily for 26 weeks. The only treatment-related finding was moderate ocular secretion noted in 3 of 12 animals receiving 8 applications per day. The mean irritation score was <1, which was within the non-irritant gradation. Hence, 8 applications per day was considered NOAEL by the sponsor.

FURTHER INFORMATION

Azelastine is a racemate. The following studies were conducted to determine whether there were stereospecific differences in the toxicological profile of the two enantiomers.