1. A-05610 (azelastine HCl): Testing the acute toxicity after single intravenous administration in rats. Vol. 21, Page 001.

Report Nº: A-05610/3000909753
Study Nº: 909753
Compound: Azelastine-HCl (Batch #: 035119, purity = 100.4%)
Control: Water
Route: Intravenous
Animal: [redacted] rats, 7-week old, 148-218 g for males and 8-week old, 135-165 g for females
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraße2
D-33790 Halle/Westfalen
Study Initiation: November 29, 1995
GLP/QAU: Yes
Study Design:

Group	Dose (mg/kg), iv, single dose	Dosing volume (ml/kg)	N (sex/group)
1	Vehicle control	2.15	2
2	3.16	2.15	5
3	14.7	2.15	5
4	21.5	2.15	5
5	31.6	2.15	5

The purpose of this study was to determine the acute toxicity of azelastine following a single iv injection in rats. The animals were observed for 14 days following dosing. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily on weekdays, and once daily on weekends and holidays
Clinical observations	Continually observed for the 1st 4-6 hr following dosing, and daily thereafter
Body weights	Weekly
Gross pathology	At the end of the observation period, a gross pathology examination was conducted in all animals.

Results:

Mortality: Deaths occurred in the animals at the doses of 14.7 mg/kg (1 male), 21.5 mg/kg (1 male and 2 females) and 31.6 mg/kg (5 males and 4 females) within 2-20 min following dosing. The calculated LD_{50} values for males and females were 22.0 mg/kg and 24.4 mg/kg, respectively.

Clinical signs: Clinical signs are summarized in the table below. At 14.7 mg/kg, the signs appeared within 2 min after dosing and lasted for up to 1 day. At 21.5 and 31.6 mg/kg, the signs appeared immediately after dosing and lasted for up to 2 days (sunken sides: up to 4 days).

Clinical signs in rats treated with azelastine HCl

	Males with clinical signs					Females with clinical signs				
N	2	5	5	5	5	2	5	5	5	5
Dose (mg/kg)	0	3.16	14.7	21.5	31.6	0	3.16	14.7	21.5	31.6
Stilted gait				3				1	2	1
Clonic convulsion, moderate			3	1	1			4		1
Clonic convulsion, severe			2	4	1			1	5	4
Decrease of muscle tone			2	4	5			1	2	5
Loss of righting reflex, lateral position			1	3	5			1	2	5
Loss of righting reflex, dorsal position			1	3	5			1	2	4
Loss of pinna reflex			1	1	5				2	4
Loss of pain reflex			1	1	5			1	2	4
Loss of corneal reflex			2	1	5			1	2	4
Salivation			1	4					2	1
Dyspnea					5				1	5
Sunken sides				3				3	3	1
Strenuous respiration								1		

Body weights: No treatment-related differences were noted.

Necropsy: No alternations were detected.

In summary, rats were treated with a single iv dose of azelastine HCl at 3.16, 14.7, 21.5 and 31.6 mg/kg. Mortalities were noted at the doses of 14.7 mg/kg or higher within 20 min of dosing. At the same doses, clinical signs including clonic convulsion, decrease of muscle tone and loss of reflex were observed. No alternations were detected in necropsy examination. NOEL was determined as 3.16 mg/kg. The minimum lethal dose was 14.7 mg/kg in males and 21.5 mg/kg in females.

2. D-20383 (R(-) azelastine HCl): Testing the acute toxicity after single intravenous administration in rats. Vol. 21, Page 026.

Report Nº: D-20383/3000909764
Study Nº: 909764
Compound: R(-) Azelastine-HCl (Batch #: Hi x 3268, purity > 99.5%)
Control: Water
Route: Intravenous
Animal: [redacted] rats, 7-week old, 161-206 g for males and 8-week old, 139-170 g for females
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe2
D-33790 Halle/Westfalen
Study Initiation: November 29, 1995
GLP/QAU: Yes
Study Design:

Group	Dose (mg/kg), iv, single dose	Dosing volume (ml/kg)	N (sex/group)
1	Vehicle control	2.15	2
2	3.16	2.15	5
3	14.7	2.15	5
4	21.5	2.15	5
5	31.6	2.15	5

The purpose of this study was to determine the acute toxicity of R(-) azelastine HCl following a single iv injection in rats. The animals were observed for 14 days following dosing. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily on weekdays, and once daily on weekends and holidays
Clinical observations	Continually observed for the 1st 4-6 hr following dosing, and daily thereafter
Body weights	Weekly
Gross pathology	At the end of the observation period, a gross pathology examination was conducted in all animals.

Results:

Mortality: Deaths occurred in the animals at the doses of 21.5 mg/kg (2 males) and 31.6 mg/kg (5 males and 5 females) within 3-25 min following dosing. The calculated LD_{50} values for males and females were 22.9 mg/kg and 26.9 mg/kg, respectively.

Clinical signs: Clinical signs were observed at the doses of 14.7 mg/kg and higher (see table below) between 1 min and 5 hr after dosing. Stilted gait and sunken sides were seen for up to 4 days. The severity increased with dosage.

Clinical signs in rats treated with R(-) azelastine HCl

	Males with clinical signs					Females with clinical signs				
N	2	5	5	5	5	2	5	5	5	5
Dose (mg/kg)	0	3.16	14.7	21.5	31.6	0	3.16	14.7	21.5	31.6
Stilted gait								1	4	
Clonic convulsion, moderate			3	1				4		
Clonic convulsion, severe			2	4	5			1	5	5
Decrease of muscle tone								1		5
Loss of righting reflex, lateral position				3	5			1		5
Loss of righting reflex, dorsal position				2	5					5
Loss of pinna reflex				2	5					5
Loss of pain reflex				2	5					5
Loss of corneal reflex				2	5			1		5
Salivation			1	3				1	2	
Dyspnea				1	3			1		1
Sunken sides				2	2			4	5	
Strenuous respiration				1				1		
Piloerection				1						
Vocalization				3	1					1

Body weights: No treatment-related differences were noted.

Necropsy: No alternations were detected.

In summary, rats were treated with a single iv dose of R(-) azelastine HCl at 3.16, 14.7, 21.5 and 31.6 mg/kg. Mortalities were noted at the doses of 21.5 mg/kg (2 males) and 31.6 mg/kg (all animals) within 25 min of dosing. Clinical signs including clonic convulsion, decrease of muscle tone and loss of reflex were observed in animals treated at the doses of 14.7 mg/kg or higher. No alternations were detected in necropsy

examination. NOEL was determined as 3.16 mg/kg. The minimum lethal dose was 21.5 mg/kg in males and 31.6 mg/kg in females.

3. D-20382 (S(+) azelastine HCl): Testing the acute toxicity after single intravenous administration in rats. Vol. 21, Page 050.

Report Nº: D-20382/3000909775
Study Nº: 909775
Compound: S(+) Azelastine-HCl (Batch #: Hi x 3267, purity > 99.5%)
Control: Water
Route: Intravenous
Animal: [redacted] rats, 7-week old, 151-203 g for males and 8-week old, 139-165 g for females
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe2
D-33790 Halle/Westfalen
Study Initiation: November 30, 1995
GLP/QAU: Yes
Study Design:

Group	Dose (mg/kg), iv, single dose	Dosing volume (ml/kg)	N (sex/group)
1	Vehicle control	2.15	2
2	3.16	2.15	5
3	14.7	2.15	5
4	21.5	2.15	5
5	31.6	2.15	5

The purpose of this study was to determine the acute toxicity of S(+) azelastine HCl following a single iv injection in rats. The animals were observed for 14 days following dosing. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily on weekdays, and once daily on weekends and holidays
Clinical observations	Continually observed for the 1st 4-6 hr following dosing, and daily thereafter
Body weights	Weekly
Gross pathology	At the end of the observation period, a gross pathology examination was conducted in all animals.

Results:

Mortality: Deaths occurred in the animals at the doses of 21.5 mg/kg (3 males and 1 female) and 31.6 mg/kg (5 males and 5 females) within 5-18 min following dosing. The calculated LD_{50} values for males and females were 21.4 mg/kg and 24.5 mg/kg, respectively.

Clinical signs: Clinical signs were observed at the doses of 14.7 mg/kg and higher (see table below). These findings were detected almost immediately after dosing and lasted for up to 6 hr (sunken sides for 3 days).

Clinical signs in rats treated with S(+) azelastine HCl

	Males with clinical signs					Females with clinical signs				
N	2	5	5	5	5	2	5	5	5	5
Dose (mg/kg)	0	3.16	14.7	21.5	31.6	0	3.16	14.7	21.5	31.6
Stilted gait				1					1	
Clonic convulsion, moderate			4	1				2	3	
Clonic convulsion, severe			1	4	5			3	2	5
Decrease of muscle tone				3	5				1	5
Loss of righting reflex, lateral position				3	5				1	5
Loss of righting reflex, dorsal position				3	5				1	5
Loss of pinna reflex				3	5			1	1	5
Loss of pain reflex				3	5			1	1	5
Loss of corneal reflex				3	5			1	1	5
Salivation				4	1			1	2	
Dyspnea				1	1			1	1	3
Sunken sides			3	2				5	4	
Strenuous respiration								2		
Vocalization			1		1					1

Body weights: No treatment-related differences were noted.

Necropsy: No treatment-related alternations were detected.

In summary, rats were treated with a single iv dose of S(+) azelastine HCl at 3.16, 14.7, 21.5 and 31.6 mg/kg. Mortalities were noted at the doses of 21.5 mg/kg (3 males and 1 female) and 31.6 mg/kg (all animals) within 18 min after dosing. Clinical signs including clonic convulsion, decrease of muscle tone and loss of reflex were observed in animals treated at the doses of 14.7 mg/kg or higher. No alternations were detected in necropsy examination. NOEL was determined as 3.16 mg/kg. The minimum lethal dose was 21.5 mg/kg in males and females.

4. R(-) azelastine-HCl (D-20382): 4-week oral toxicity study after repeated administration in rats. Vol. 21, Page 075.

Report Nº: D-20382/3000913634
Study Nº: 913634
Compound: R(-) azelastine-HCl (Batch #: Cha x 214, purity = 98.96%)
Control: Drinking water
Route: Oral (gavage)
Animal: Wistar rats, 6-week old, 180-234 g for males and 7-week old, 139-174 g for females
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe2
D-33790 Halle/Westfalen

Study Design:

Group	Dose (mg/kg), qd x 4 weeks	Dosing volume (ml/kg)	N (sex/group)
1	Control	2.15	10
2	10	2.15	10
3	31.6	2.15	10
4	100/82.5*	2.15	10

* On Day 8 the high dose was reduced to 82.5 mg/kg.

GLP/QAU: Yes
Study Initiation: March 18, 1997

The purpose of this study was to determine the toxicity of R(-) azelastine in rats following oral (gavage) administrations for 4 weeks. 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	Weekly
Reflexes	Pain, pinna and corneal reflexes were tested once a week.
Eye, hearing and dental examinations	Prior to the first dosing and in Week 4
Clinical pathology	Week 4
Urinalysis	Week 4
Ophthalmoscopic examinations	Groups 1 and 4, prior to the first dosing and in Week 4
Gross pathology	At the end of the study (Week 5), all animals were euthanized. A complete gross pathology examination was conducted in all animals including intercurrently deceased animals.
Organ weights	The following organs from each surviving animal were weighed: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus.
Histopathologic examinations	All fixed organs and tissues of all animals in Groups 1 and 4, as well as the liver , kidneys, lungs, lymph nodes, testes, epididymides, ovaries, and uteri of Groups 2 and 3 animals were examined microscopically (see Addendum, Page 53).

Results:

Mortality: In the animals treated with the drug at 100/82.5 mg/kg, one male died on Day 11 and one female died on Day 10. No mortalities were noted in the other groups.

Clinical observations: Clinical signs are summarized in the table below. All signs lasted for only a few min in Group 2 animals. In mid and high dose animals, the saliva was occasionally reddish discolored.

Clinical signs observed in rats treated with R(-) azelastine HCl (animals affected)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Salivation		1 (0.1)	10 (6)	10 (12.5)		2 (0.2)	7 (5.0)	10 (13.5)
Clonic convulsion			10 (7)	10 (17.6)		1 (0.1)	10 (12.3)	10 (20.9)
Hypokinesia			7 (3.3)	6 (2.9)			2 (0.4)	7 (2.6)
Hyperkinesia			2 (0.5)	4 (0.9)			4 (0.4)	3 (0.8)
Vocalization by touching			1 (0.1)	2 (0.7)			2 (0.9)	1 (0.2)
Coordination disturb			6 (1.6)	7 (4.2)			3 (0.6)	8 (5.3)
Sunken sides				10 (8.7)			10 (3.7)	10 (18.7)
Piloerection				4 (2.4)				9 (9.4)
Face pale								6 (3.9)
Emaciated								4 (2.8)
Stilted gait								7 (5.3)

* () mean number of animal days with clinical signs

Body weight: Decreased body weight gain was noted in animals in high dose group (see table below).

Body weight changes in rats treated with R(-) azelastine HCl (g)

Group	Dose	Day 1		Day 28		% of control		Body wt. gain		% of control	
	(mg/kg)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	Control	208±13.5	156±6.6	319±31.4	189±12.3			111	33		
2	10	207±11.2	153±7.9	300±33.1	183±14.9	94	96.8	93	30	83.8	90.9
3	31.6	210±10.9	156±7.9	318±29.3	188±10.4	99.7	99.5	108	32	97.3	97.0
4	100/82.5	200±16.3	154±10.4	246±22.2	167±8.4	77.1	88.4	46	13	41.4	39.4

Food consumption: At 100/82.5 mg/kg, a decrease in food consumption was noted throughout the treatment period in males (16.2 g/day vs. control's 23.5 g/day) and females (10.8 g/day vs. control's 15.4 g/day).

Reflex testing, eye, hearing and dental examinations: No abnormal findings were observed.

Ophthalmoscopy: No treatment-related changes were noted.

Hematology: No toxicologically significant changes were noted.

Clinical chemistry: Several very slight changes in clinical chemistry test were noted in Groups 3 and 4 animals (see table below). These changes might not be toxicologically significant.

Clinical chemistry changes in the rats treated with R(-) azelastine HCl

	Males				Females			
Group	1	2	3	4	1	2	3	4
ALT (u/l)	29.1±4.55	29.9±5.05	32.9±4.86	38.8±6.48	29.4±5.3	33.5±3.0	34.8±5.3	43.0±0.6
ALP (u/l)	382.6±43.9	372.0±38.2	414.4±57.6	474.7±62.6	269.4±31.6	256.5±39.5	283.2±37.0	382.0±0.9
Total protein (g/l)	62.6±1.6	61.9±2.8	60.8±1.4	56.8±2.2	62.2±1.9	61.9±.6	59.4±2.5	55.8±.8
Triglyceride (mmol/l)	2.3±0.5	1.8±0.7	2.1±0.5	1.5±0.4	1.5±0.2	1.5±.3	1.3±0.3	1.2±0.2
P (mmol/l)	3.1±0.3	3.1±0.1	3.5±0.3	3.6±.2	2.7±0.3	2.9±0.4	3.0±0.2	3.2±0.3
Albumin (g/l)	33.7±1.0	33.7±.4	32.5±0.7	30.9±.3	36.0±1.1	34.8±.2	33.4±1.9	31.3±.3

Urinalysis: No treatment-related differences were noted.

Gross necropsy: Small uteri were noted in 5 of 10 high dose females. In the same dose group, dilated and tightly filled stomachs were noted in 1/10 male and 5/10 female animals.

Organ weights: Liver weights were slightly increased in mid and high dose female animals (see table below).

Liver weight changes in rats treated with R(-) azelastine HCl for 4 weeks

Group ♀	Control	Low	Mid	High
Liver (g)	7.5±0.7	7.3±0.7	8.5±0.6	8.0±1.1
Relative (%)	4.1±0.3	4.1±0.3	4.7±0.2	4.9±0.5

Histopathological examinations: The positive findings obtained from the histopathological test are summarized in the table below. At 100/82.5 mg/kg, the vaginal mucosa in 9/10 females was on proestrus cycle stage. The toxicological significance of these changes, together with male testis changes, was not determined.

Histopathological findings in rats treated with R(-) azelastine HCl

	Males				Females			
Group	1	2	3	4	1	2	3	4
Liver, N	10	10	10	10	10	10	10	10
Hepatocyte vacuolization, minimal				2				
Single cell necrosis, moderate				1*				
Lungs, N	10	10	10	10	10	10	10	10
Alveolar histiocytosis, minimal				6				7
Slight				1				1
Testes, N	10	10	10	10				
Tubular atrophy, slight				1				
Moderate				2				
Uterus, N					10	10	10	10
Atrophy, minimal								2
Slight								5
Moderate								3

* Intercurrently dead male

In summary, rats were treated orally with R(-) azelastine HCl for 4 weeks at the doses of 10, 31.6 and 100/82.5 mg/kg. In low dose animals, salivation (3 rats) and slight clonic convulsion (1 rat) were the only clinical findings. In high dose animals, mortalities were noted in two females, and body weight gain and food consumption were decreased. Clonic convulsion, coordination disturbance, salivation and hypokinesia were seen in both male and female animals at mid and high doses. Post-mortem examination showed minimal vacuolization of hepatocytes (2 males), single cell necrosis (1 male), histiocytosis in the lungs (7 males and 8 females). NOAEL was considered below 10 mg/kg in both males and females.

S(+) azelastine-HCl (D-20382): 4-week oral toxicity study after repeated administration in rats. Vol. 22, Page 001.

Report Nº: D-20382/3000913623
Study Nº: 913623
Compound: S(+) azelastine-HCl (Batch #: Cha x 210, purity = 98.91%)
Control: Drinking water
Route: Oral (gavage)
Animal: Wistar rats, 6-7-week old, 187-257 g for males and 141-173 g for females
Study Site: ASTA Medica AG
Institute of Toxicology
Kantstraβe2, D-33790 Halle/Westfalen

Study Design:

Group	Dose (mg/kg), qd x 4 weeks	Dosing volume (ml/kg)	N (sex/group)
1	Control	2.15	10
2	10	2.15	10
3	31.6	2.15	10
4	100/82.5*	2.15	10

* On Day 8 the high dose was reduced to 82.5 mg/kg.

Study Initiation: February 18, 1997
GLP/QAU: Yes

The purpose of this study was to determine the toxicity of azelastine in rats by gavage administrations for 4 weeks. 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	Weekly
Reflexes	Pain, pinna and corneal reflexes were tested before dosing once a week.
Eye, hearing and dental examinations	Prior to the first dosing and in Week 4
Clinical pathology	Week 4
Urinalysis	Week 4
Ophthalmoscopic examinations	Groups 1 and 4, prior to the first dosing and in Week 4
Gross pathology	At the end of the study (Week 5), all animals were euthanized. A complete gross pathology examination was conducted in all animals including intercurrently deceased animals.
Organ weights	The following organs from each surviving animal were weighed: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus.
Histopathologic examinations	All fixed organs and tissues of all animals in Groups 1 and 4, as well as the liver and macroscopically changed organs and tissues of Groups 2 and 3 animals were examined microscopically (see Addendum, Page 53).

Results:

Clinical observations: Two females at 100 mg/kg died on Days 7 and 10, respectively. The dose in this group was reduced to 82.5 mg/kg to prevent further mortality. Clinical signs are summarized in the table below. Salivation was noted shortly after dosing and lasted for 5 min, 8-36 min, and 3 hr in low, mid and high dose groups, respectively. In high dose animals, the saliva was occasionally reddish discolored.

Clinical signs observed in rats treated with S(+) azelastine HCl (animals affected)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Salivation			6 (2.5)	10 (17.2)		1 (0.1)	7 (2.8)	10 (15)
Clonic convulsion				8 (5.6)				10 (13)
Hypokinesia				1 (0.4)				9 (0.9)
Vocalization by touching				1 (0.3)				2 (1.9)
Coordination disturb								4 (0.4)
Sunken sides								5 (5.6)

* () mean number of animal days with clinical signs

Body weight: Decreased body weight gain was noted in animals in high dose and mid dose groups (see table below).

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

Group	Dose	Day 1		Day 25		% of control		Body wt. gain		% of control	
	(mg/kg)	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	Control	223±13	165±7.5	307±19.8	198±11.6	100	100	84	33	100	100
2	10	230±14.4	162±6.3	321±34.1	195±9.6	100	98.5	91	33	100	100
3	31.6	226±11.7	159±7.2	303±29.6	184±10.8	98.7	92.9	77	25	**91.6**	**75.6**
4	100/82.5	219±18.5	159±9.2	284±29.3	184±15.0	92.5	92.9	65	25	**77.4**	**75.6**

Food consumption: A slight decrease in food consumption was observed in male and female animals in high dose group at the beginning of the treatment period (see table below).

Daily food consumption in rats treated with S(+) azelastine HCl (g)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Days 1-8	21.0±1.0	21.7±2.4	20.5±.6	**18.7±2.2**	15.1±0.9	14.2±.2	14.4±1.0	**10.1±2.5**
Days 8-15	21.1±1.3	21.8±2.7	20.3±1.8	**19.7±.7**	15.3±.3	14.4±1.1	14.2±0.9	**11.9±4.4**
Days 15-22	21.0±1.5	21.8±2.9	20.2±.9	**19.4±.6**	15.1±1.1	14.6±1.1	14.2±.0	16.2±2.5
Days 22-25	21.4±.8	22.4±2.9	20.9±.1	20.2±1.8	15.4±1.7	15.0±1.6	14.2±1.2	17.6±2.1

Reflex testing, eye, hearing and dental examinations: No abnormal findings were observed.

Ophthalmoscopy: No treatment-related changes were noted.

Hematology: No toxicologically significant changes were noted.

Clinical chemistry: In Group 4 females, slight increases in ALT (48.9 u/l vs. control's 30.7 u/l), inorganic phosphorus (3.65 mmol/l vs. control's 2.66 mmol/l) and ALP (352.5 u/l vs. control's 248.7 u/l) levels, and a decrease in albumin (35 g/l vs. control's 39.2 g/l) were noted. A slight increase in ALT was also noted in male rats in high dose group (42.3 u/l vs. control's 32.6 u/l).

Urinalysis: No treatment-related differences were noted.

Gross necropsy: No toxicologically significant changes were noted.

Organ weights: Liver weights were increased in high dose female animals (see table below).

Liver weight changes in rats treated with S(+) azelastine HCl for 4 weeks

Group ♀	Control	Low	Mid	High
Liver (g)	7.5±0.6	7.6±0.5	7.3±0.5	8.7±1.3
Relative (%)	3.9±0.3	4.0±0.2	4.1±0.3	4.8±0.6

Histopathological examinations: One male rat and one female rat in high dose group showed slight or minimal vacuolization of predominantly centrolobular hepatocytes.

In summary, rats were treated orally with S(+) azelastine HCl for 4 weeks at the doses of 10, 31.6 and 100/82.5 mg/kg. In low and mid dose animals, salivation up to 30 min was the only clinical sign noted. Body weight gain decrease was noted in mid and high dose animals. In high dose animals, mortalities were noted in two females. Clonic convulsion, salivation and hypokinesia were seen in both male and female animals. A slight increase in ALT, ALP levels and liver weight, and a decrease in albumin levels were noted in

females. A slight increase in ALT levels were also noted in males. Histopathological examination showed minimal to slight vacuolization of predominantly centrolobular hepatocytes. 10 mg/kg was determined as NOAEL.

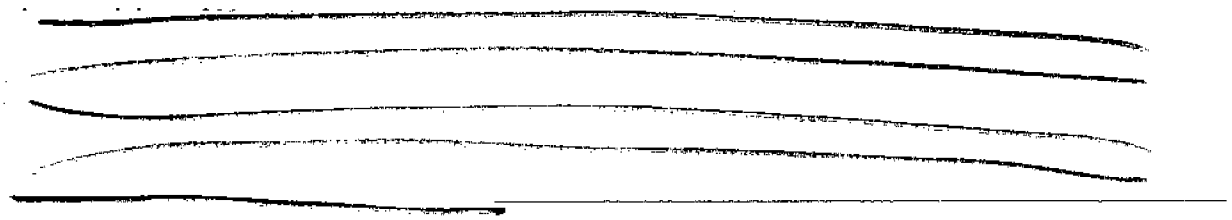
Addendum: Histopathology inventory for NDA 21-127

Study Nº	**910912**	**913623**	**913634**
Duration	**26 weeks**	**4 weeks**	**4 weeks**
Species	**Dogs**	**Rat**	**Rat**
Adrenals		+	+
Aorta		+	+
Axillary lymph node		+	+
Bone marrow smear			
Bone (femur/tibia)		+	+
Brain		+	+
Cecum		+	+
Cervix		+	+
Colon		+	+
Duodenum		+	+
Epididymis		+	+
Esophagus		+	+
Eye	+	+	+
Fallopian tube			
Gall bladder			
Gross lesions		+	+
Harderian gland		+	+
Heart		+	+
Hypophysis			
Ileum		+	+
Injection site			
Jejunum		+	+
Kidneys	+	+	+
Knee joint		+	+
Lachrymal gland	+		
Larynx			
Liver	+	+	+
Lungs	+	+	+
Lymph node, bronchial			
Lymph node, cervical			
Lymph node, mandibular			
Lymph node, mesenteric		+	+
Mammary gland		+	+
Nasal cavity			
Optic nerves		+	+
Ovaries	+	+	+
Pancreas		+	+
Parathyroids		+	+
Peripheral nerve		+	+
Pharynx			
Pituitary		+	+
Prostate		+	+
Rectum		+	+
Salivary gland		+	+
Sciatic nerve			
Seminal vesicles		+	+
Skeletal muscle		+	+
Skin		+	+
Spinal cord		+	+
Spleen		+	+
Sternum		+	+
Stomach		+	+
Testes	+	+	+
Thymus		+	+
Thyroid		+	+

Study Nº	910912	913623	913634
Duration	26 weeks	4 weeks	4 weeks
Species	Dogs	Rat	Rat
Tongue		+	+
Trachea		+	+
Urinary bladder		+	+
Uterus		+	+
Vagina		+	+

Draft

Labeling



SUMMARY:

Azelastine HCl has been approved as a nasal spray formulation under NDA 20-114. Many nonclinical studies were submitted with the NDA submissions and were reviewed by Dr. Misoon Chun (HFD-570). Please refer to pharmacology review for NDA 20-114 for further information regarding these pharmacology, pharmacokinetics, and toxicology studies (including carcinogenicity, reproduction and genotoxicity studies).

Pharmacology:

Azelastine is an H_1 receptor antagonist. The pharmacology studies indicated that azelastine HCl at the proposed concentration (0.05%) was effective on allergic conjunctivitis in different animal models. At concentrations of 0.01 to 0.2%, azelastine demonstrated inhibitory effects (28-72% inhibition) on histamine-induced allergic responses in rabbit eyes. In a guinea pig model of ocular allergy, azelastine HCl eye drops (0.001% to 0.1%) significantly inhibited histamine-stimulated conjunctival vascular permeability with ED_{50} of 0.005%. In a study using rat conjunctival anaphylaxy model, azelastine HCl ophthalmic solution 0.1% and 0.05% showed antiallergic effect evidenced by inhibition of Evans blue extravasation by 88.3% and 77.4%, respectively.

ADME:

In rat and rabbit PK studies with a single ocular instillation of ^{14}C-azelastine HCl solution, the external ocular tissues (eyelid and cornea) showed higher Cmax and AUC levels than internal ocular tissues. The ocular Cmax and AUC values in sensitized rats (with inflamed eye) were higher than in normal rats, indicating therapeutically beneficial effects. The untreated eyes had very low radioactivity concentrations, indicating a low contralateral distribution of ^{14}C-azelastine. In pigmented rabbits, relatively high concentrations of ^{14}C-azelastine were noted in iris-ciliary body and choroid with pigment epithelium, indicating melanin binding of the drug. Plasma drug concentrations were very low relative to the ocular tissues. Terminal half life could not be determined. Forty-eight hr after dosing, about 82% and 97% of the radioactivity was recovered in the urine and feces in rabbits and rats, and fecal elimination was predominant.

The TK data obtained from the 26-week toxicity study in dogs showed that there were no measurable azelastine HCl concentrations in plasma (0.5 hr after dosing) and aqueous humor (1 day after the last day of treatment).

Toxicology:

OCULAR IRRITATION TESTS AFTER SINGLE APPLICATION

The results obtained from 3 single dose ocular toxicity studies are summarized in the table below. In 2 non-GLP studies, slight irritation was noted.

Summary of single dose ocular irritation studies

Species/strain	Sex and age	N	Concentration	Volume	Vehicle	Observations
Rabbit/white Russian	♂ and ♀, 6-7 months	6	0.5%	0.1 ml one eye		Slight, transient redness (1), minimal discharge (2), mean Draize index = 0.42
Rabbit		6	0.05%	One eye		Small corneal lesions of small area (3), conjunctival redness (1), slight circumcorneal injection (1), mean Draize index = 7.33
Rabbit		6	0.1%	One eye		Conjunctival redness (6), slight circumcorneal injection (6), slight corneal lesions, mean draize index = 14.5

(): number of animals exhibiting toxic signs

OCULAR IRRITATION TESTS AFTER REPEATED APPLICATION

The ocular irritation studies with repeated application are summarized in the table below. According to the results, azelastine HCl in different formulations was not ocular irritant. No systemic toxic effects were observed.

Summary of ocular irritation studies with repeated application

Species /strain	Sex	N	Concen-tration	Volume	Dose	Vehicle	Observations
Rabbit/ white Russian	♀	3	0.05%	0.1 ml	Qd x 5 days, right eye		No adverse effects were noted. Mean Draize index = 0
Rabbit/ white Russian	♂ and ♀	3	0.05%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival redness (1) at 24 hr after the 1st instillation and 1 hr after the 2nd instillation, mean Draize index = 0.13
Rabbit/ white Russian	♂ and ♀	3	0.05%	0.1 ml	Qd x 5 days, right eye		No adverse effects were noted. Mean Draize index = 0
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		No adverse effects were noted. Mean Draize index = 0
Rabbit/ white Russian	♂,	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival redness was noted in one animal 1 hr after the 2nd dosing, and in another animal at 24 hr after the 2nd and 1 hr after the 3rd instillations. Mean Draize index = 0.2
Rabbit/ white Russian	♂	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival redness was noted in one animal during the 1st 4 days of treatment. The same animal had ocular discharge at 1 hr after the 1st dosing. Mean Draize index = 0.47
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival redness (1) at 24 hr after the 2nd dosing, mean Draize index = 0.07
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival hyperemia (2), slight hypersecretion (3), mean Draize index = 0.67

Species /strain	Sex	N	Concen-tration	Volume	Dose	Vehicle	Observations
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		No adverse effects were noted. Mean Draize index = 0
Rabbit/ white Russian	♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival hyperemia (1) at 1 hr after the 2nd instillation, mean Draize index = 0.07
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight conjunctival hyperemia (1) at 1 hr after the 3rd instillation, mean Draize index = 0.07
Rabbit/ JW-NIBS	♀	4 per group	0.01% and 0.1%	3 drops	16 times per day x 5 days left eye		0.01%: negative results. 0.1%: positive findings in the fluorescein instillation test (2/4). Similar changes were also noted in the comparative control (sodium cromoglicate 2%) group (2/4)
Rabbit/ white Russian	♂	3	0.05%	0.1 ml	Qd x 5 days, right eye		No irritation effects were noted. However, azelastine showed a surface anaesthetic effect on the cornea.
Rabbit/ white Russian	♂	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight hyperemia (1) at 1 hr after the 1st dosing, mean Draize index = 0.07, decreased corneal sensitivity (3)
Rabbit/ white Russian	♂ and ♀	3	0	0.1 ml	Qd x 5 days, right eye		Slight discharge (1) after each application, mean Draize index = 0.4
Rabbit/ white Russian	♂ and ♀	3	0.1%	0.1 ml	Qd x 5 days, right eye		Slight hyperemia (1) at 1 hr after the 2nd dosing, mean Draize index = 0.07, slight decreased corneal sensitivity (2)
Rabbit/ white Russian	♂	3	0.05%	0.1 ml	Qd x 5 days, right eye		Slight hyperemia (1) at 24 hr after the 4th dosing and 1 hr after the 5th instillation, mean Draize index = 0.13
Rabbit/ white Russian	♂	3	0	0.1 ml	Qd x 5 days, right eye		No irritation effects were noted.
Rabbit/ white Russian	♂ and ♀	5/sex	0.1%	0.1 ml	5 times per day x 5 days, right eye		Slight hyperemia was noted in all treated eyes at different observation times. The mean irritation index was 0.8 in males and 0.64 in females.
Rabbit/ white Russian	♂ and ♀	2/sex	0.2%	0.1 ml	5 times per day x 5 days, right eye		Diffuse hyperemia in conjunctiva (1) at 1 hr after the 1st instillation, mean irritation index = 0.1
Rabbit/ white Russian	♂ and ♀	5/sex	0.1%	0.1 ml	5 times per day x 5 days, right eye		Slight hyperemia (3♂, 4♀) at different observation times. The mean irritation index was 0.23 in males and 0.29 in females.
Rabbit/ white Russian	♂ and ♀	5/sex	0.05%	0.1 ml	5 times per day x 5 days, one- eye		Slight hyperemia (2♀) at 5 min after 5th application on Days 2 and 4, respectively. The mean irritation index was 0 in males and 0.06 in females.

(): number of animals exhibiting toxic signs

SPECIAL TOXICOLOGY

Several special toxicological studies are summarized in the table below. In these studies, azelastine HCl showed no effects on IOP changes and corneal reepithelization process. However, azelastine HCl appeared to have local anaesthetic effect evidenced by decreasing corneal sensitivity.

Summary of special toxicity studies

Species/ strain	Sex	N	Concen -tration	Volume	Dosing regimen	Vehicle	Observations
Rabbit/ [redacted]	♂	8	1%	50 µl	18 times at 20 min interval, left eye	[redacted]	No IOP alterations
Rabbit/New Zealand		4 per group	0.1%		14 times at 4 hr interval, one eye		No effect on the corneal reepitheliazation process
Rabbit/ white Himalayan	♂ and ♀	3 per group	0.01%, 0.05%, 0.1% 0.5%	0.1 ml	Single dose, right eye		0.01%: negative results. 0.05% and 0.1%: decreased corneal sensitivity. 0.5%: sensitivity was not measurable for 30 to 65 min.
Rabbit/ [redacted]		8 per group	0.1% 0.02%	50 µl	Single dose, one eye		0.1%: anaesthetic effect longer than with antazoline and benoxinate, and higher than antazoline. 0.02%: slightly decreased sensitivity
Guinea pig/Dunkin-Hartley	♂	4 per group	0.2%				Azelastine 0.2% in olive oil developed no sensitizing capacity.

SUBACUTE AND CHRONIC OCULAR TOXICOLOGY

Several subacute and chronic ocular toxicity studies are summarized in the following table. No systemic toxicity was noted.

Summary of subacute and chronic toxicity studies

Species/ strain	Sex	N	Concen -tration	Volume	Dosing regimen	Vehicle	Observations
Rabbit [redacted]	♂	8 per group	0.1% 0.05%	50 µl	Qid x 4 weeks, right eye	[redacted]	Mean Draize index: Azelastine 0.1% in PSS: 1.7-3.2 Azelastine 0.1% in spray: 3.1-3.3 Azelastine 0.05% in PSS: 0.8-4.2 Azelastine 0.05% in spray: 1.7-3.3 PSS: 0.7-3.3 No other abnormalities in IOP, corneal sensitivity and histological examinations were observed.
Dog/Brack-Beagle	♀	5	0.1%	0.1 ml	5 times daily x 14 days, right eye		Slight conjunctival redness was noted in all animals at different times during the treatment period. Mean Draize index = 1.0.
Dog/Beagle [redacted]	♂ and ♀	4-6/sex/ group	0.05%	50 µl	Bid, qid or 8 times daily x 26 weeks, right eye		Transient and moderate ocular discharge was noted in 1/6 ♂ and 2/6 ♀ in 8-application/day group. NOEL: 4 times per day

FURTHER INFORMATION

The studies summarized below compared the toxicity profiles of azelastine HCl racemate and two enantiomers. The study results indicated that there were not enantioslective properties in therapeutic doses.

Summary of toxicity studies

Test substance	Species/ strain	Sex	N	Dosage	Lowest toxic dose	Lowest lethal dose (mg/kg)	LD_{50} (mg/kg)	NOAEL (mg/kg)	Observations
Rac-azelastine HCl	Rat [redacted]	♂ and ♀	5/sex/ group	3.16-31.6 mg/kg, iv, single dose	14.7 mg/kg	14.7 ♂ 21.5 ♀	23.1	3.16	Similar clinical signs were observed in all 3 studies at ≥ 14.7 mg/kg: stilted gait, clonic convulsion, decreased muscle tone, loss of righting reflex, loss of pinna reflex, loss of pain reflex, loss of corneal reflex, salivation, dyspnea, sunken sides.
R(-)-azelastine HCl					14.7 mg/kg	21.5 ♂ 31.6 ♀	24.4	3.16	
S(+)-azelastine HCl					14.7 mg/kg	21.5 ♂ 21.5 ♀	22.7	3.16	
R(-)-azelastine HCl	Rat [redacted]	♂ and ♀	10/sex/ group	10, 31.6 and 100/82.5 mg/kg, po by gavage, qd x 4 weeks	31.6 mg/kg	100		<10	Both enantiomers had similar toxicity profiles in CNS (coordination disturbances and convulsion). However, R(-)-azelastine HCl at high dose showed slight atrophy in uterus, cycle arrest in proestrus and muitifocal tubular atrophy.
S(+)-azelastine HCl					31.6 mg/kg	100		10	

EVALUATION:

Azelastine HCl is an H_1-receptor antagonist. Pharmacological studies indicated that azelastine HCl at the proposed concentration (0.05%) was effective on allergic conjunctivitis in different animal models.

Azelastine HCl in a nasal spray formulation was approved in November 1996 (NDA 20-114) for the treatment of seasonal allergic rhinitis. The recommended dose is 2 sprays per nostril twice daily (up to 1.1 mg/mg). The proposed clinical dose of azelastine HCl ophthalmic solution is 0.1 mg/patient/day or 0.002 mg/kg for a 50 kg adult, which is much lower than the approved nasal spray dose. From the systemic toxicity standpoint, the drug is safe.

Based on the results obtained from numerous ocular irritation studies, it could be concluded that as an ocular formulation, azelastine HCl ophthalmic solution was well tolerated. Decreased corneal sensitivity was observed in several studies at the concentrations >0.01%. However, no toxicity impact was noted in any ocular studies including a 26-week study. Hence, the slight decrease in corneal sensitivity should not preclude the clinical use of azelastine HCl ophthalmic solution 0.05%.

RECOMMENDATION:

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy section.

/S/ 1-4/2000
Zhou Chen, Ph.D.

Concurred by: /S/ 4Jan00
Andrea Weir, Ph.D.

cc:
NDA 21-127/Division File
NDA 21-127/Original NDA
HFD-550/CSO/Rodriguez
HFD-550/MO/Boyd Chambers
HFD-550/TL Pharm/Weir
HFD-550/Pharm/ChenZ
HFD-345

1 page(s) of revised draft labeling has been redacted from this portion of the review.