CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-565

PHARMACOLOGY REVIEW

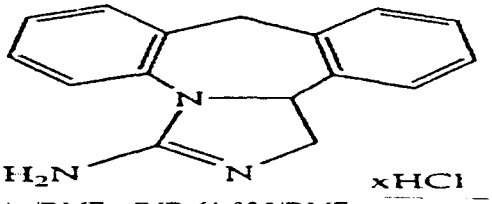
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **NDA 21-565**
Review number: 000
Sequence number/date/type of submission: 000/December 19, 2002/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Allergan Inc., 2525 Dupont Drive, P. O. Box 19534, Irvine, CA 92623-9534
Manufacturer for drug substance: Boehringer Ingelheim Pharma KG, D-55216 Ingelheim am Rhein, Germany

Reviewer name: Zhou Chen, Ph.D.
Division name: Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #: HFD-550
Review completion date: September 30, 2003

Drug:

Trade name: RELESTAT
Generic name (list alphabetically): **Epinastine HCl ophthalmic solution 0.05%**
Code name: AGN 198027-A, WAL 801 CL
Chemical name: 3-Amino-9, 13b-dihydro-1H-dibenz[c,f]imidazo[1,5-a]azepine hydrochloride
CAS registry number: 108929-04-0, 80012-43-7
Mole file number: Not indicated
Molecular formula/molecular weight: $C_{16}H_{15}N_3 \bullet HCl$, MW: 285. [redacted]
Structure:



Relevant INDs/NDAs/DMFs: IND 61,025/DMFs [redacted] and [redacted]

Drug class: H_1 histamine receptor antagonist

Indication: Prevention of [redacted] allergic conjunctivitis

Clinical formulation:

Component	Concentration (% w/v)	Concentration (mg/ml)
Epinastine HCl	0.05	0.5
Benzalkonium chloride	0.01	0.1
Edetate disodium, USP	[redacted]	
Monobasic sodium phosphate	[redacted]	
Sodium chloride	[redacted]	
Sodium hydroxide	[redacted]	
[redacted] HCl and/or [redacted] NaOH	[redacted]	
Purified water	[redacted]	

Route of administration: Topical, ocular

Proposed use: 1 drop (30-35 µl), bid (For a 50 kg adult, total dose can reach 0.07 mg/patient/day or 0.0014 mg/kg, 0.0518 mg/m^2)

Executive Summary

I. Recommendations

A. Recommendation on Approvability

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.

B. Recommendation for Nonclinical Studies

No recommendation is necessary.

C. Recommendations on Labeling

Modifications of labeling are recommended in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy section.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Epinastine is an H_1-receptor antagonist. In pharmacological studies, epinastine showed strong antihistaminic and antiserotonin activities, but the drug had no histamine H_2 antagonistic action and no anticholinergic effects. Epinastine also showed an α-adrenolytic effect.

Epinastine was rapidly absorbed following ocular administration in rabbits and monkeys with very low systemic exposure. High concentrations of radioactivity were measured in the surface tissues (conjunctiva, cornea and sclera) and in pigmented tissues (iris and ciliary body). In *in vitro* studies, epinastine reversibly bound to bovine ocular melanin. In studies conducted in rats and monkeys, the bioavailability of the drug after oral administration was low. Epinastine was distributed throughout peripheral body tissues, but did not cross the blood brain barrier. Epinastine and its metabolites passed the placental barrier in rat study. The radioactivity was also observed in the milk of lactational rats. The *in vitro* serum protein-binding rate was about 60% in rats and 40% in humans. Conversion of epinastine to metabolites was similar across animal species tested. Epinastine and metabolites were excreted in urine and feces across animal species. Biliary excretion and entero-hepatic circulation were observed.

The sponsor conducted several single dose systemic toxicity studies in mice, rats, and dogs, and repeated dose systemic toxicity studies with duration up to 1 year in rats and monkeys. In both single dose and repeated dose toxicity studies, no specific target organ of toxicity was established. There was a great safety margin between the toxic doses and proposed human daily ocular dose, suggesting that toxicity observed in the systemic toxicity studies would not present a safety concern in clinical human application at the

proposed human daily ophthalmic dose (0.07 mg/day). The following table shows safety margins between the proposed human ophthalmic dose and the 1-year rat and monkey toxicity study data.

Key findings, systemic exposure and comparative dose of epinastine HCl in animal studies vs. human dose

Species/ treatment duration	Key findings	Dose (mg/kg/day)	Animal/human ratio	Dose (mg/m^2/day)	Animal/human ratio	Cmax (ng/ml)	Animal/human ratio
Rat/1 year	No effect	10 (NOAEL)	7000	60	1160	2.36	56
	↓body weight gain, ↑salivary gland weight	100	70000	600	11600	104	2500
Monkey/1 year	No effect	8 (NOAEL)	5170	96	1850	114.14	2700
	↑salivation, emesis and diarrhea, ↓body weight gain and food consumption	60	42857	720	13900	1790	43000
Human		1.4 µg/kg/day*		0.0518		0.042	

*Human (50 kg) dose was based on 35 µl of 0.05% epinastine HCl instilled in both eyes twice daily.

Seventeen genotoxicity studies were submitted in the original NDA submission and two more in a supplement. Epinastine HCl was negative in *in vivo* genotoxicity studies, including the mouse micronucleus assay and chromosome aberration assay in Chinese hamster ovary cells. Epinastine was also negative in a cell transformation assay using Syrian hamster embryo cells, a point mutation assay in V79/HGPRT mammalian cells, and an *in vivo/in vitro* unscheduled DNA synthesis assay using rat primary hepatocytes. Epinastine was negative in Ames tests. Positive results were seen in two *in vitro* chromosomal aberration studies conducted in 1980s with human peripheral lymphocytes and with V79 cells, respectively. In two Ames tests and two *in vitro* chromosomal aberration assays using human lymphocytes conducted in 2001 to 2003, newly synthesized batches of epinastine did not induce a genotoxic response.

Carcinogenicity studies were conducted in mice and rats because epinastine HCl was originally developed as an oral tablet for chronic administration. The dose selection was not concurred by the agency. The high dose of 40 mg/kg/day was selected by the sponsor because it was 200-fold anticipated human oral dose, which was one of the acceptable criteria for dose selection according to European guidelines when studies were conducted (1986-1988). Epinastine was not carcinogenic at doses up to 40 mg/kg.

Epinastine HCl was not considered as teratogenic in rabbits and rats. However, at 75 mg/kg, total resorption was noted in 3 rabbits and abortion in one rabbit. A decrease in fertility index was seen in HD females (120 mg/kg) in the combined fertility/embryo-fetal development/prenatal and postnatal development study in rats. In the same study, a decrease in pup body weight gain during lactation was observed following an oral dose (120 mg/kg) to female rats from pre-mating to the end of lactation. No drug-related abnormal findings were noted regarding F_0 reproductive parameters, F_1 behavioral parameters, and F_1 fertility parameters in prenatal and postnatal toxicity study.

Several ocular toxicity studies with duration ranging from 1 month to 6 months were conducted in Himalayan rabbits, New Zealand white (NZW) rabbits, and cynomolgus monkeys. Epinastine HCl eye drops (0.1-1%) were weakly irritating to rabbit eyes in two studies. In other ocular toxicity studies in rabbits and monkeys, the drug was well

tolerated. Based on nonclinical data, epinastine HCl ophthalmic solution presented no safety concerns regarding its clinical application.

The following table summarizes safety margins between animal doses using data from 6-month rabbit and monkey studies and the proposed human daily ophthalmic dose. At the highest concentration, 0.5% epinastine, daily AUC exposure in the rabbit and monkey was 8.7-fold human exposure (one drop of 0.05% epinastine in both eyes, bid).

Systemic exposure to epinastine HCl in rabbits and monkeys after 6-month ocular treatment

	Treatment	Dosing frequency	Cmax (ng/ml)	Animal/human	AUC (ng-hr/ml)	Animal/human
Human	0.05%	bid	0.042		0.7	
Rabbit	0.5%	tid	3.25	77	6.1	8.7
Monkey	0.5%	tid	1.51	[redacted]	6.1	8.7

B. Pharmacologic Activity

Epinastine is an H_1-receptor antagonist. In *in vitro* studies, epinastine had strong antihistaminic and antiserotonin activities, but the drug had no histamine H_2 antagonistic action and no anticholinergic effects. Epinastine also showed an α-adrenolytic effect. In *in vivo* pharmacology studies, epinastine inhibited histamine- and serotonin-induced bronchospasm in guinea pigs, histamine-induced urtica in rats and dogs, serotonin-induced edema in rats, allergen-mediated eosinophilia in the lung, and LTB_4-induced leukocyte accumulation in skin chambers in guinea pigs. Epinastine showed an inhibitory effect on histamine-induced increase in microvascular permeability in rat conjunctivae. In several studies with a passive ocular anaphylaxis (POA) model in rats, epinastine eye drops showed marked antiallergic activity in a dose-dependent manner.

C. Nonclinical Safety Issues Relevant to Clinical Use

Summarized from nonclinical studies, epinastine HCl ophthalmic solution was generally well tolerated. No toxicologically significant side effects were noted. There are no nonclinical safety concerns relevant to clinical use.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(See memo attached)

C. cc: list:
NDA 21-565/Division File
NDA 21-565/Original NDA
HFD-550/CSO/Rodriguez
HFD-550/MO/Chambers
HFD-590/AD Pharm/Hastings
HFD-550/TL Pharm/YangJ

APPEARS THIS WAY
ON ORIGINAL

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Studies reviewed:

U83-0047: Pharmacology of the antihistamine WAL 801 CL. Vol. 11, Page 062
U86-0298: *In vitro* biochemical comparison of WAL 801, astemizole, and terfenadine. Vol. 12, Page 291
U86-0408: WAL 801 CL in comparison with other antihistamines. Vol. 12, Page 306
U86-0653: WAL 801 CL: No preclinical evidence of active anxiolytic or antidepressant properties. Vol. 12, Page 468
U92-0664: Effects of epinastine hydrochloride on rabbit nasal mucosal blood flow. Vol. 13, Page 266
U93-0075: Antiallergic activity: Passive ocular anaphylaxis (POA) in rats. Vol. 13, Page 318
U93-0861: Antiallergic activity: Passive ocular anaphylaxis (POA) in rats: duration of effect. Vol. 13, Page 342
U94-0251: Report concerning the protective effects on histamine-induced local eye irritation. Vol. 13, Page 377
U94-0326: Accumulation of granulocytes in the lung and skin of guinea pigs: inhibition by the anti-H_1, antiallergic agent epinastine. Vol. 13, Page 404
U94-0333: Histamine release into the conjunctivae after antigen challenge in rats. Vol. 14, Page 001
U95-0011: WAL 801 (0.1%, 0.3%, 1%, 3%, w/v) ophthalmic solution/Effect on intraocular pressure in normotensive albino rabbits. Vol. 14, Page 040
U95-0232: Effect of oral WAL 801 CL (epinastine) in a model of neurally mediated bronchospasm [Challenge of BDE rats with N^6-2-(4-aminophenyl) ethyladenosine). Vol. 14, Page 072
U95-0547: Effects of WAL 801 on the mediator release from human skin mast cells, human peripheral basophils and human eosinophils. Vol. 14, Page 111
U96-2204: Anti-inflammation activity: Effects in inflammatory cell accumulation and pathological findings in the conjunctivae of normal rats. Vol. 14, Page 146
U96-2205: Microvascular permeability in the conjunctivae elicited by histamine, compound 48/80 and PAF in rats. Vol. 14, Page 167
U96-2206: Anti-inflammatory activity: Effects in inflammatory cell accumulation and pathological findings in the conjunctivae of normal and allergic rats. Vol. 14, Page 239

Studies NOT reviewed:

This report summarized several studies addressing the pharmacologic characteristics of WAL 801.

The first study was to determine WAL 801's bronchodilator effects on histamine-induced bronchospasm in guinea pigs. The bronchoconstriction (tidal volume reduction) was induced by iv injection of 0.1% histamine (5-10 μg/kg). Tidal volume was determined with a body plethysmograph. WAL 801 was given intravenously, as inhalant, and orally 2 min (iv and ih) or 60 min (po) before the bronchospasm induction. Two other histamine antagonists, ketotifen and promethazine, were used as references. The results showed that in all dosing routes, WAL 801 dose-dependently inhibited histamine-induced bronchospasm. The ED_{50} (the dose necessary to produce a 50% reduction in bronchospasm) values are summarized in the table below. The maximum effect of WAL 801 that appeared at about 60 min after iv or inhalation dosing was later than the reference compounds, but lasted longer.

ED_{50} values of WAL 801 on histamine-induced bronchospasm

Treatment	WAL 801	Ketotifen	Promethazine
Intravenous (μg/kg)	0.5-6	0.1-1	0.6-3
ED_{50} (μg/kg)	0.88	0.22	1.10
Inhalation (% for 30 s)	0.003-0.03	0.003-0.03	
ED_{50} (% for 30 s)	0.0056	0.0058	
Oral (μg/kg)	10-100	10-100	300-3000
ED_{50} (μg/kg)	47	19	940

WAL 801's bronchodilator effect on serotonin-induced bronchospasm was investigated in male rats. The bronchoconstriction was induced by iv injection of serotonin (100 μg/kg), and was measured by the "overflow" technique using a bronchospasm transducer. WAL 801 (10-300 μg/kg) was given intravenously 1 min before the bronchospasm induction. Two other histamine antagonists, ketotifen and promethazine, were used as references. The results showed that WAL 801 dose-dependently inhibited serotonin-induced bronchospasm with the ED_{50} better than the reference compounds (see table below).

ED_{50} values of WAL 801 on serotonin-induced bronchospasm (μg/kg)

Treatment	WAL 801	Ketotifen	Promethazine
Intravenous (μg/kg)	10-300	30-300	30-300
ED_{50}	25	67	68

In a study with acetylcholine-induced bronchospasm in guinea pigs, WAL 801 showed no significant effect.

In a histamine-induced urtica study, male rats were treated with histamine (ic) in sheared skin (5 μg for iv test or 10 μg for po test) to induce urtica. WAL 801 was administered intravenously (1 min before histamine dosing) or orally (60 min before histamine). Animals were terminated 20 min after histamine injection and the size of the area with urtica in the skin was determined. Results showed that WAL 801 had a strong action against histamine-induced urtica in rats. The ED_{50} values are summarized in the table below.

ED_{50} values of WAL 801 on histamine-induced urtica (mg/kg)

Treatment	WAL 801	Ketotifen	Promethazine	Cyproheptadine	Clemastine	Mepyramine
Intravenous (mg/kg)	0.002-0.008	0.1-1.0	0.25-1.0	0.1-0.4	0.5-2.0	1.25-5.0
ED_{50}	0.0038	0.32	0.40	0.15	0.60	1.50
Oral (mg/kg)	1-10	0.1-1.0	1-6			
ED_{50}	2.1	0.32	2.5			

A similar histamine-induced urtica study was performed in dogs. Histamine (2 μg) was injected (ic) to the sheared ventral side of the animals to induce urtica. Animals were examined 20 min later for the size of the area with urtica in the skin. WAL 801 and a reference compound (mepyramine, a histamine receptor antagonist) were administered intravenously (1 min before histamine dosing) or orally (60 min before histamine). Results showed that WAL 801 had a strong action against histamine-induced urtica in dogs. The ED_{50} values are summarized in the table below.

ED_{50} values of WAL 801 on histamine-induced urtica (mg/kg)

Treatment	WAL 801	Mepyramine
Intravenous (mg/kg)	0.01-0.10	
ED_{50}	0.056	
Oral (mg/kg)	0.03-0.30	5-20
ED_{50}	0.21	6.8

In a study to investigate WAL 801's effect on serotonin-induced edema in rat's paw, serotonin (2 μg/0.1 ml) was administered by subplantar injection into the right rear paw of fasted rats. The degree of inflammatory swelling was determined by measuring the dorsoplantar thickness before and 30 min after serotonin injection. WAL 801 and reference compounds (see table below) were administered intravenously (shortly before serotonin dosing) or orally (60 min before serotonin dosing). Results showed that WAL 801 inhibited serotonin-induced edema through both iv and po routes. In iv route, WAL 801 was more potent than ketotifen and promethazine. Methysergide, a pure antiserotonin agent, had the best effect.

ED_{35} values of WAL 801 on serotonin-induced rat paw edema (mg/kg)

Treatment	WAL 801	Ketotifen	Promethazine	Cyproheptadine	Clemastine	Methysergide
Intravenous (mg/kg)	0.03-0.3	0.3-3	1-4	0.1-0.8	2.0	
ED_{35}	0.05	1.20	1.70	0.15	↓17%	
Oral (mg/kg)	2.5-10	1.25-20	3-30			0.05-0.2
ED_{35}	4.4	5.6	9.0			0.045

In vitro experiments to measure the drug's antihistaminic and antiserotonin activities were also conducted. Strips of isolated guinea pig rectum, tracheal chain and lungs (for antihistaminic activity), and isolated rat stomach fundus (for anti-$5HT_1$ activity) and aortic strips (for anti-$5HT_2$ activity) were prepared and contraction was induced by histamine or serotonin (10^{-8} to 10^{-6} mM). ED_{50} values of histamine and serotonin were calculated from cumulative contraction curves. The shift of the ED_{50} values by WAL 801 and other reference compounds was then determined and pA_2 values were calculated. The results, summarized in the table below, showed that WAL 801 had a distinct antihistaminic effect, which was about 10 times stronger than that of diphenhydramine and about 1/3 of the effect of ketotifen. The antiserotonin effect of WAL 801 was weaker than that of ketanserin ($5HT_2$) and slightly weaker than ketotifen ($5HT_1$ and $5HT_2$). The sponsor also indicated that WAL 801 had no anticholinergic effect but no data were provided.

Antihistaminic and antiserotonin activities of WAL 801 (pA2 ± SD)

Antihistaminic activity	WAL 801	Ketotifen	Diphenhydramine	Ketanserin
Lung strip	9.1 ± 0.1	9.5 ± 0.1	7.6± 0.3	
Rectum	8.5± 0.3	9.0± 0.15	7.8± 0.1	
Antiserotonin activity				
Tracheal chain	7.8± 0.4	Ineffective		In effective
Fundus strip	5.9± 0.1	6.3± 0.01		
Aorta strip	7.8 ± 0.2	8.4 ± 0.1		9.3± 0.1

In an *in vitro* study to determine the drug's effect on histamine H_2 antagonism with isolated guinea pig atrium, WAL 801 showed no histamine H_2 antagonistic action. Such an effect was found in the case of cimetidine.

A study to measure WAL 801's action against anaphylactic reactions was conducted with experimentally induced asthma in rats. Rats were sensitized by iv injection of high-titer IgE antiserum against egg albumin. The passive anaphylaxis of the lungs was induced by antigen provocation (egg albumin 25 mg/kg, iv). WAL 801 and other reference compounds were administered intravenously 1 min before the asthma provocation. Results showed that WAL 801 was better than ketotifen and promethazine in the inhibition of anaphylactic broncho constriction. At 0.2 mg/kg iv, up to a 77% inhibition was reached. The ED_{50} values are summarized in the table below.

ED_{50} values of WAL 801 on inhibition of anaphylactic broncho constriction in rats (mg/kg)

Treatment	WAL 801	Ketotifen	Promethazine	Cyproheptadine	Clemastine	Methysergide
Intravenous (mg/kg)	0.03-0.2	0.1-1.0	0.03-1.0	0.005-0.02	0.3-2.0	5-40
ED_{50}	0.052	0.24	0.2	0.008	1.1	0.0035

A passive cutaneous anaphylaxis (PCA) test with WAL 801 was conducted in rats. Twenty-four hr after local sensitization of tissue mast cells through ic injection of antiserum, a hypersensitivity reaction at the particular part of the skin was induced by iv injection of the specific antigen with [redacted]. Animals were killed 20 min later and the diameters of the PCA reaction were measured. WAL 801 and reference compounds (see table below) were administered intravenously (1 min before antigen administration) or orally (60 min before antigen administration). WAL 801 showed a dose-dependent inhibition of PCA in rats following both iv (64% inhibition at 0.15 mg/kg) and po (86% inhibition at 10 mg/kg) administrations. The ED_{50} values are summarized in the table below.

ED_{50} values of WAL 801 on inhibition of PCA in rats (mg/kg)

Treatment	WAL 801	Ketotifen	Promethazine	Cyproheptadine	Mepyramine	Cromoglycate
Intravenous (mg/kg)	0.05-0.15	0.1-1.0	1-10	0.1-0.4	2.5-7.5	0.7-1.4
ED_{50}	0.13	0.26	6.0	0.15	4.5	0.95
Oral (mg/kg)	4-10	5-30	3-30			
ED_{50}	6.3	12	8.6			

A study to measure WAL 801's action against anaphylactoid dextran reaction in the rat paw was conducted. Dextran induced a degranulation of mast cells and the release of mediator substances (histamine and serotonin) that subsequently led to the development of edema. To induce the reaction, 100 µg of dextran in 0.1 ml physiological saline solution was injected into the right rear paw (subplantar). The degree of inflammatory swelling was determined by measuring the dorsoplantar thickness in 0.01 mm at 30 min after dextran injection. WAL 801 and other reference compounds were administered intravenously shortly before dextran injection or orally 60 min prior to dextran injection. Results showed that WAL 801 was better than

ketotifen and promethazine in the inhibition of anaphylactoid dextran reaction. At 0.2 mg/kg iv, a 67% inhibition was reached. After oral administration, WAL 801 showed similar effects as ketotifen and promethazine. The ED_{50} values are summarized in the table below.

ED_{50} values of WAL 801 on inhibition of anaphylactoid dextran reaction in rats (mg/kg)

Treatment	WAL 801	Ketotifen	Promethazine	Cromoglycate	Sm 857 NA
Intravenous (mg/kg)	0.05-0.2	0.1-1.0	0.3-3.0	2.5-10	
ED_{50}	0.058	0.24	0.38	2.8	
Oral (mg/kg)	1.25-5	0.5-10	1-10		1-10
ED_{50}	2	1.4	2.6		4

A passive peritoneal anaphylaxis study was performed to measure the effect of WAL 801 on the reaction of mast cell *in vivo*. Male rats were passively sensitized by ip injection of 0.3-1 ml anti-egg albumin antiserum containing IgE. The antigen provocation was induced 2 hr later by ip injection of egg albumin. Animals were killed 3 to 5 min after antigen provocation and the peritoneal liquid was collected. The amount of histamine was determined fluorometrically in duplicate assays with a spectrofluorometer. WAL 801 (0.0003 to 0.1 mg/kg) and the reference compound, Sm 857 NA (0.5 and 1.0 mg/kg), were administered intravenously 1 min before antigen provocation. Results showed no significant inhibition of histamine release by WAL 801. The positive control compound, Sn 857 NA, showed significant inhibition.

An *in vitro* study was conducted to evaluate WAL 801's effect on anaphylactic histamine release from mast cells. Male rats were passively sensitized by ip injection of 0.5-1 ml anti-egg albumin antiserum containing IgE. Animals were killed 24 hr later and the peritoneal liquid was collected. Sensitized cells were extracted from the fluid. Cells were incubated for 15 min at 37 °C with egg albumin (6 µg) and WAL 801 or reference compounds. The release of histamine was determined spectrofluorometrically. Results showed WAL 801, as other references, inhibited histamine release from mast cells *in vitro* (see table below). The EC_{50} for WAL 801 was 98 µg/ml, which was higher than the effective antihistaminic concentrations. In addition, the sponsor indicated in a figure that the cell protective range overlapped the cytotoxic range in which histamine was released. No detailed study information was provided.

EC_{50} values of WAL 801 on inhibition of *in vitro* histamine release (µg/ml)

Treatment	WAL 801	Ketotifen	Cromoglycate
concentration (µg/kg)	30-300	10-100	0.3-30
EC_{50}	98	39	2.2

WAL 801's effect on platelet aggregation was studies in rats. Animals (3/group) were treated orally with WAL 801 at 5 and 10 mg/kg. One hr later, blood samples were collected. Platelet aggregation, induced by collagen, was measured photometrically in platelet-rich plasma (PRP). Results showed that at 5 mg/kg and 10 mg/kg doses, the inhibition of platelet aggregation was 42% and 100%, respectively.

In a study to investigate WAL 801's effects on carrageenan-induced inflammation in rat paw, WAL 801 at 30 mg/kg (po) showed no effects at 3 hr after dosing.

Cardiovascular effects of WAL 801 were studies in both anesthetized and conscious dogs. In anesthetized dogs, WAL 801 solution was injected intravenously (0.01, 0.03 or 0.3 mg/kg) to 4 or 5 dogs. Heart rate, left ventricular pressure, aortic pressure, femoral artery pressure, and femoral artery flow and resistance were measured. Results showed a slight increase

in aortic pressure and femoral pressure in animals at 0.3 mg/kg. These changes might not be significant since the increase was not great (see table below)

Effects of WAL 801 in the anesthetized dogs

	Aortic pressure (mmHg)					
	Systolic			Diastolic		
Dose (mg/kg, iv)	Before	After	Change (%)	Before	after	Change (%)
0.01	139± 4	145± 4	4± 2	110± 3	113± 2	2± 2
0.03	130± 7	141± 6	9± 3	90± 6	109± 5	12± 3
0.3	140± 6	152± 4	9± 3	100± 5	121± 3	13± 4
	Femoral artery pressure (mmHg)					
	Systolic			Diastolic		
Dose (mg/kg, iv)	Before	After	Change (%)	Before	after	Change (%)
0.01	165± 7	169± 4	3± 2	112± 4	115± 2	3± 2
0.03	155± 9	172± 9	12± 3	100± 7	111± 5	12± 3
0.3	170± 8	183± 5	8± 4	110± 5	126± 5	15± 5

Three conscious dogs were treated orally with WAL 801 at 3 mg/kg. Dogs were previously implanted with pressure gauges allowing continuous monitoring of left ventricle pressure and the rate of the pressure rise. In addition, heart rate and respiratory rate were also measured. Results showed that WAL 801 at 3 mg/kg had no effects on left ventricular pressure, the force of cardiac contraction and heart rate. A 20% decrease in respiratory rate (from 32/min to 25/min) was observed. The sponsor indicated that this was due to a distinct reaction (↓48%) in one animal.

In an *in vitro* study to investigate WAL 801's chronotropic and inotropic effects in the isolated guinea pig atrium, WAL 801 was added to the organ bath cumulatively at concentrations of 10^{-6}, 10^{-5} and 10^{-4} g/ml. Concentrations of 10^{-6} and 10^{-5} g/ml produced weak positive inotropic effects. The highest concentration caused biphasic effects: an initial increase (44%) followed by a decrease (25%). In the mid and high concentrations, a decrease in pulse rate was noted.

Effects of WAL 801 on the force of cardiac contraction and pulse rate in isolated guinea pig atria

	Change in force of cardiac contraction		Change in pulse rate	
Concentration (g/ml)	mg	%	mg	%
10^{-6}	75± 25	8±3	4±2.4	3± 2.0
10^{-5}	250± 42.8	23±4.7	-14±3.6	-13± 3.8
10^{-4}	458 ± 27.1 and -317± 101.4	44± 7.2 and -25± 8.2	-67±5.3	-57± 4.6

WAL 801's effects on α-adrenolysis were investigated. The seminal vesicles of rats were removed and placed in an organ bath. Contractions were induced by 10^{-7} g/ml of epinephrine. WAL 801 ($2 \times 10^{-9} - 2 \times 10^{-8}$ g/ml) or phentolamine ($1.6 \times 10^{-9} - 1 \times 10^{-8}$ g/ml) was used in this study, and the inhibition of epinephrine-induced contractions was measured. Results showed that WAL 801 inhibited epinephrine-induced contraction with an EC_{50} of 5×10^{-9} g/ml, which was 3 times less potent than phentolamine ($EC_{50} = 1.8 \times 10^{-9}$ g/ml).

WAL 801's effects on blood glucose and lactate were investigated. Blood samples were collected from male albino rats at 0.5, 1, 2, 4 and 6 hr after oral administration of WAL 801 (10 and 30 mg/kg) or PSS. No biologically relevant changes in blood glucose were noted. A decrease in blood lactate levels was noted (see table below).

Effects of WAL 801 in blood lactate in rats (mmol/l, mean ± SEM)

		Hrs after dosing				
Dose (mg/kg, po)	Control	0.5	1	2	4	6

10	2.7± 0.05	2.49± 0.05	2.30± 0.06	2.29± 0.05	2.27± 0.08	2.37± 0.06
30	2.7± 0.05	2.36± 0.12	2.40± 0.11	1.72± 0.03	2.10± 0.09	2.62± 0.10

The drug's effects on diuresis were determined in rats. Rats were loaded with water (5 ml/100 g) and treated with WAL 801 at 10 and 40 mg/kg (gavage). Urine samples were collected for up to 5 hr after WAL 801 dosing. Urine volume, sodium, potassium and chloride excretions were determined. Results showed that at 40 mg/kg dose, the chloride excretion was reduced by 31% (37.3 ± 5.92 μgEq/100 g BW vs. control's 53.8 ± 4.06 μgEq/100 g BW).

WAL 801's effects on nocturnal motility in female mice (10/group) were investigated by measuring the interruptions of horizontal photoelectric barrier beam. WAL 801 at the doses up to 40 mg/kg (po by gavage) showed no effects on nocturnal motility.

The interaction of WAL 801 and alcohol in female mice was investigated. Mice (10/group) were treated with (po) WAL 801 and reference compounds at 2.5, 10 or 40 mg/kg. Thirty min later, mice were intraperitoneally injected with ethanol (3.7 g/kg). Ethanol abolished postural reflex in mice. In this study, the duration of the lateral and dorsal position was determined. Results (see table below) showed that WAL 801, as well as promethazine and ketotifen, prolonged the duration of the alcohol-induced abolition of postural reflex at 10 and/or 40 mg/kg.

Effects of WAL 801 on the duration of alcohol-induced postural reflex abolition in mice (mean ± SEM)

	min			%		
Dose (mg/kg)	WAL 801	Ketotifen	Promethazine	WAL 801	Ketotifen	Promethazine
0	10± 2.4	18.2± 4.1	15.3± 4.6	100	100	100
2.5	13.2± 2.0		12.6± 3.5	132± 37		82± 34
10	16.8± 3.2	20.6± 4.0	24.1± 4.5	168± 52	113± 34	158± 56
40	23.7± 4.5	27.2± 5.5	37.0± 4.6	237± 73	149± 45	242± 79

The interaction of WAL 801 and hexobarbital in mice was investigated. Mice (10/group) were treated with (po) WAL 801 and reference compounds at 2.5, 10 or 40 mg/kg. Thirty min later, mice were intraperitoneally injected with hexobarbital sodium (100 g/kg). Hexobarbital abolished postural reflex in mice. In this study, the duration of the lateral and dorsal position was determined. Results showed that WAL 801 did not significantly change the duration of the hexobarbital-induced abolition of postural reflexes. However the reference compounds, promethazine (10 and 40 mg/kg) and ketotifen (40 mg/kg), prolonged the duration of the hexobarbital-induced abolition of postural reflexes.

To clarify a possible sedative side effect of WAL 801, the effect of WAL 801 on sleep and waking behavior was studied in cats. Cats (5 or 6/group) with permanently implanted electrodes for EEG recording were treated with WAL 801 (po by gavage) at 1, 3, 10 or 20 mg/kg. The test began at 7:30 and 8 am. EEG was continuously recorded from 7:30 am to 3:30 pm. Results indicated that WAL 801 had no effect on sleep and waking behavior.

WAL 801's anticonvulsive effects were investigated in mice (10/group). Mice were treated with WAL 801 (po by gavage) at 40 mg/kg or phenobarbital (as a reference) at 6.25, 12 or 40 mg/kg. Pentetrazol was injected (ip) 1 hr later to induce clonicotonic convulsions. The number of animals that did not exhibit convulsions was recorded. Results showed that WAL 801

had no effects on pentetrazol-induced convulsions, while phenobarbital showed a dose-dependent inhibition.

An apomorphine climbing test was performed on male mice to determine the dopamine-antagonistic and neuroleptic effects of WAL 801. Mice (10/group) were treated orally (by gavage) with WAL 801 (10, 40 and 100 mg/kg) and reference compounds (ketotifen 40 mg/kg and promethazine 100 mg/kg). Seventy-five min later, 1.25 mg/kg of apomorphine was subcutaneously injected to mice. Animals were then put individually in glass cylinders. Inside the cylinders, there were wire mesh cylinders. The typical apomorphine climbing behavior was evaluated. Results showed that no neuroleptic effect was noted after administration of any of the three compounds. The neuroleptic agent, haloperidol, showed positive effects with an oral ED_{50} of 0.23 mg/kg.

WAL 801's respiratory effect was investigated in dogs. Dogs were intubated with an endotracheal tube, and connected to a volumometer which measured the expiratory volume. A breathing bag filled with oxygen was connected to the volumometer into which the animals rebreathed. WAL 801 was given to dogs (iv) at 0.3 mg/kg and breathing test was carried out, during which the CO_2 concentration in the O_2-filled breathing bag increased from 5% to 10%. End-expiratory CO_2 concentration (Vol CO_2), arterial CO_2 partial pressure (PA CO_2), the respiratory minute volume, respiratory rate (RR), and heart rate were determined. Results showed that WAL 801 (0.3 mg/kg, iv) had no inhibitory effects on these parameters. The stimulating effects of CO_2 on the respiration were not affected by the drug.

U86-0298: *In vitro* biochemical comparison of WAL 801, astemizole, and terfenadine. Vol. 12, Page 291

The purpose of this *in vitro* study was to compare biochemical profiles of WAL 801 with two other marketed antihistamines, astemizole, and terfenadine using various receptor binding and synaptosomal uptake tests. The affinity of these three compounds to different receptors is summarized in the table below. WAL 801 showed a high affinity to H_1-receptor but not H_2 receptor. WAL 801 also showed high affinity to α_1, α_2 and $5HT_2$ receptors.

Receptor binding tests for WAL 801, astemizole, and terfenadine (Ki, nM)

Receptor	WAL 801	Astemizole	Terfenadine
H_1	7.8	13.8	61.3
H_2	3826	2984	> 10000
α_1	13.2	126	3734
α_2	33.3	6667	> 10000
$5HT_1$	2885	32746	16373
$5HT_2$	19.5	27.9	1414
β_1	44000	> 10000	>> 10000
β_2	19330	> 10000	>> 10000

The table below summarizes IC_{50} values of the three compounds against histamine in guinea pig brain (central H_1-receptors) and ileum (peripheral H_1 receptors) tissues. No differences were found between the bindings to the CNS and peripheral H_1-receptors.

CNS and peripheral receptor binding test for WAL 801, astemizole, and terfenadine (IC_{50}, nM)

H_1 receptor location	WAL 801	Astemizole	Terfenadine
Guinea pig brain	5.4	19.3	253
Guinea pig ileum	12.0	22.3	215

The table below summarizes IC_{50} values of WAL 801 and its two enantiomers [(-)-rotatory isomer WAL 1190 and (+)-rotatory isomer 1191] in different receptor binding tests. WAL 801 was clearly different from its enantiomers.

Receptor binding tests for WAL 801 and two enantiomers (IC_{50}, nM)

Receptor	WAL 801	(-)-WAL 1190	(+)-WAL 1190
H_1	9.8	15.8	5.3
H_2	4030	1750	2550
α_1	23	350	12
α_2	60	1400	27
$5HT_1$	3700	5100	4000
$5HT_2$	45.5	66	260

The table below summarizes IC_{50} and Ki values of the three compounds in norepinephrine (NE) and 5HT uptake tests. The inhibition of the uptake was weak.

Inhibition of NE and 5HT uptake by synaptosomes (IC_{50} and Ki, nM)

H_1 receptor location	WAL 801		Astemizole		Terfenadine	
	IC_{50}	Ki	IC_{50}	Ki	IC_{50}	Ki
NE	> 10000	> 1000	5100	4250	5100	4250
5HT	8500	8430	4700	4660	>> 10000	>> 10000

U86-0408: WAL 801 CL in comparison with other antihistamines. Vol. 12, Page 306

The purpose of this study was to compare the antihistaminic and CNS effects of WAL 801 with other antihistamines. The first test in this report was histamine-induced spasm of the isolated guinea pig ileum. Ileum strips were suspended in 20-ml organ bath. Spasms were induced by histamine (0.1 µg/ml). WAL 801 and reference compounds (astemizole, terfenadine, cyproheptadine and mepyramine) were added to the bath at various concentrations (see table below) 2 min before the addition of histamine. Results (see table below) showed that WAL 801 had spasmolytic activity on the isolated guinea pig ileum induced by histamine. The potency was similar to classic antihistamines, cyproheptadine and mepyramine.

EC_{50} values of WAL 801 on inhibition of *in vitro* histamine-induced ileum spasms (ng/ml)

Treatment	WAL 801	Astemizole	Terfenadine	Cyproheptadine	Mepyramine
Concentration (ng/ml)	1-10	50-200	200-800	1-5	1-8
EC_{50}	2.6	100	370	2.0	2.3

The second test was a histamine wheal test in male rats. Animals were shaved and Evans Blue (25 mg/kg) was injected (iv). Skin wheals were induced by ic injection of histamine (5 µg for iv assay and 10 µg for po assay) into the shaved skin. WAL 801 and reference compounds (astemizole and terfenadine) were administered by iv (shortly before histamine injection) or po (60 min before histamine injection). Animals were terminated 20 min after histamine injection and the area of the wheal was determined. Results are summarized in the table below. WAL 801 was more potent after iv dosing comparing to other reference compounds.

ED_{50} values of WAL 801 on inhibition of histamine-induced skin wheal (mg/ml)

Treatment	WAL 801	Astemizole	Terfenadine
iv injection			
Concentration (mg/ml)	0.002-0.008	0.1-1.0	0.1-1.0
ED_{50}	0.0039	0.39	0.26
po			
Concentration (mg/ml)	1-10	0.3-3	1-10
ED_{50}	2.3	0.36	3.6

The sponsor also investigated the effect of WAL 801 on the sleep-wakefulness patterns in cats to clarify any possible sedative side effect of the drug. No dose-dependent significant changes of the sleep parameters were noted. This test was reviewed under Study # U83-0047.

U86-0653: WAL 801 CL: No preclinical evidence of active anxiolytic or antidepressant properties. Vol. 12, Page 468

The purpose of this study was to determine whether WAL 801 had active psychotropic properties. The active anxiolytic properties of WAL 801 were evaluated in a reward-punishment conflict test. Male rats were trained to press a lever 20 times for a food reward (FR 20) and to cease this activity as soon as a light signal indicated that even though a food reward could be obtained at each individual press of the button; however, at the same time an electrical punishment stimulus was administered over the floor grating of the test chamber at each press of the button. One round consisted of 4 alternating FR conflict periods lasting 3 min each. WAL 801 (10 and 40 mg/kg) and diazepam (15 mg/kg) were administered orally. The test began 1 hr after administration of the substance. The results showed that diazepam demonstrated its anti-emotional property in this test by activating the lever-pressing inhibition in the conflict without having a stronger effect on the food-rewarded FR behavior. WAL 801 did not show any anti-conflict effect after 10 mg/kg and 40 mg/kg, but the food-rewarded activity began to decrease in this dosage range. Therefore, no grounds that WAL 801 could act anxiolytically were revealed in this experiment.

The active antidepressant properties were evaluated in the chick call test. The call frequency of isolated male chicks was recorded for 2 hr over microphones in the soundproofed test chambers. Antidepressants activated this behavior especially in the second hr of the test, when the placebo control animals had already begun to stop their calling. WAL 801 (1 and 10 mg/kg) was administered intraperitoneally. Control animals and treated animals were tested in parallel with each dosage. The results showed that WAL 801 did not induce any activation of the contact call that exceeded the control group. On the contrary, at 10 mg/kg, WAL 801 diminished the call frequency. Consequently, no evidence of active antidepressant properties of WAL 801 was revealed in this experiment.

U92-0664: Effects of epinastine hydrochloride on rabbit nasal mucosal blood flow. Vol. 13, Page 266

The purpose of this study was to determine the potency of α-blocking activity and the effect of epinastine on the hemodynamics in rabbit nasal mucosa and isolated rat and guinea pig blood vessel. In the hemodynamics assay in rabbit nasal mucosa, rabbits were treated with epinastine by intra-arterial administration. The effect of epinastine on the nasal mucosal blood flow was continually examined using the organ-reflectance spectrophotometry by two parameters: nasal mucosal hemoglobin (Hb) and oxygen saturation of Hb (SO_2). Results showed

that epinastine slightly decreased the basal mucosal Hb and SO_2 but the effect was reversed within a min. The sponsor also indicated the Hb and SO_2 were decreased by intra-arterial administration of norepinephrine (3 μg). These changes, regarded as decreased blood flow, were completely inhibited by phentolamine (an α-receptor antagonist, 500 μg/kg, iv). The norepinephrine-induced decrease in blood flow was also inhibited by epinastine (iv, 0.1-3 mg/kg) in a dose-dependent manner with an ED_{50} value of 1.8 mg/kg, iv. The sponsor indicated that after iv injection of epinastine at 10 mg/kg, two of five rabbits died.
[**Reviewer's comments**: The sponsor indicated that the drug was given by intra-arterial administration in the Method section. However, in the Results section, the route was written as intravenous. In addition, the methods for the tests with NA were not provided.]

In the tests with isolated blood vessels, guinea pig thoracic aorta and rat tail artery strips were prepared. The effects of epinastine and other α-blockers on the contraction induced by norepinephrine, histamine and transmural electrical stimulation were examined. Results are summarized in the table below. It could be concluded that epinastine was a weak α-antagonist, and the hemodynamics of nasal mucosa was not affected by epinastine.

Effects of epinastine, prazosin and phentolamine on the contractile response induced by norepinephrine, histamine, or electrical stimulation (mean ± SE)

pA2	Isolated guinea pig aorta			Isolated rat tail artery	
	Epinastine	Prazosin	Phentolamine	Epinastine	Prazosin
Norepinephrine	6.84± 0.09	9.21± 0.13	8.21± 0.31	7.44± 0.18	9.89± 0.18
Histamine	8.11± 0.22				
EC_{50} (M)	Isolated rat tail artery, electrical stimulation				
	Epinastine	Prazosin	Phentolamine		
	$1.85\pm 0.88 \times 10^{-7}$	$5.36\pm 0.44 \times 10^{-10}$	$2.10\pm 0.52 \times 10^{-8}$		

U93-0075: Antiallergic activity: Passive ocular anaphylaxis (POA) in rats. Vol. 13, Page 326

The purpose of this study was to evaluate the antiallergic effect of WAL 801 eye drops using a passive ocular anaphylaxis (POA) model in rats. At the beginning of the study, 10 μl of antiserum was injected into the lower lid of both eyes of each animal. Animals were then treated topically with 10 μl of vehicle, mepyramine (an H_1-receptor antagonist, 0.1%), or WAL 801 (0.1, 0.3 and 0.5%) 72 hr after the sensitization and repeated 10 min later. Five min later, animals were treated with [redacted] and 12.5 mg ovalbumin (1 ml/kg) intravenously. Thirty min after the antigen challenge, animals were terminated, and the lower and upper eyelids were removed and processed. The Evans blue dye extravasation was compared, and percentage inhibition was calculated. Results are summarized in the table below. WAL 801 eye drops (0.1, 0.3 and 0.5%) showed marked antiallergic activity in a dose-dependent manner.

Passive ocular anaphylaxis in rats by WAL 801 (mean ± SE)

Treatment	Control	Mepyramine (0.1%)	WAL 801 (0.1%)	WAL 801 (0.3%)	WAL 801 (0.5%)
Evans blue stain (μg)	21.786± 1.165	14.825± 1.8332	16.141± 1.9094	6.988± 1.0721	2.402± 0.5764
% inhibition		32	26	68	89

U93-0861: Antiallergic activity: Passive ocular anaphylaxis (POA) in rats: duration of effect. Vol. 13, Page 342

The purpose of this study was to evaluate the duration of the antiallergic effect of WAL 801 eye drops using a passive ocular anaphylaxis (POA) model in rats. At the beginning of the study, 10 µl of antiserum was injected into the lower lid of both eyes of each animal. Animals were then treated topically with 10 µl of vehicle, ketotifen, levocabastine (an H_1-antagonist), or WAL 801 (0.3%) 72 hr after the sensitization and repeated 10 min later. Five min later, animals were treated with [redacted] and 12.5 mg ovalbumin (1 ml/kg) intravenously. Thirty min after the antigen challenge, the animals were terminated. [**Reviewer's comments**: This statement is not right. The animals should be terminated 5 min, 2, 4 and 6 hr after challenge to obtain duration information. In the Results section, the sponsor indicated that the effect of treatment was observed prior to challenge. It should be prior to sacrifice.] The lower and upper eyelids were removed and processed. The [redacted] extravasation were compared, and percentage inhibition was calculated. Results are summarized in the table below. WAL 801 eye drops (0.3%) showed marked antiallergic activity that lasted for 4 hr.

Passive ocular anaphylaxis in rats by WAL 801 (mean ± SE)

	[redacted] stain (µg)				% inhibition		
Time	Control	WAL 801	Ketotifen	levocabastine	WAL 801	Ketotifen	levocabastine
5 min	25.87± 1.26	13.14± 0.88			49		
2 hr	30.33± 2.82	23.38± 1.55	15.77± 1.64	12.80± 1.37	23	48	30
4 hr	39.16± 1.38	27.28± 2.97	23.38± 2.19	24.51± 1.18	30	40	40
6 hr	24.11± 1.14	27.34± 2.52	16.39± 1.32	14.58± 1.62	-13	32	37

U94-0251: Report concerning the protective effects on histamine-induced local eye irritation. Vol. 13, Page 377

The purpose of this study was to determine the efficacy and the schedule of topical administration of WAL 801 against local inflammatory response to the instillation of histamine into the guinea pig eye. Histamine (1 mg in 10 µl) was instilled into the conjunctival sac of both eyes of normal guinea pigs. One eye was treated with WAL 801 (1 mg/ml) five minutes before challenge and the other eye was exposed to the solvent. Conjunctival hyperemia, conjunctival edema and eyelid edema were evaluated, and scoring photos of both eyes were taken 10 min and 1 and 2 hr after challenge. The sponsor also evaluated the time-dependent protective effect of WAL 801. In that case both eyes were treated 4, 6 and 12 hours before challenge, and a parallel control group was treated with placebo for each time evaluated. Results, summarized in the tables below, showed that topical WAL 801 effectively suppressed histamine-induced inflammatory reactions in the guinea pig eyes. The protective effects of WAL 801 lasted for at least 6 hours.

Clinical evaluation of histamine-induced conjunctivitis in NAIVE guinea pigs (Mean ± SD, n=6)

	10 min after challenge		1 hour after challenge		2 hours after challenge	
	Right eye (WAL 801)	Left eye (Control)	Right eye (WAL 801)	Left eye (Control)	Right eye (WAL 801)	Left eye (Control)
Conjunctival hyperemia	0.83 ± 0.61	2.57 ± 0.48	0.46 ± 0.45	1.86 ± 0.77		
	0.93 ± 0.21	2.37 ± 0.57			0.30 ± 0.33	1.23 ± 0.53
Mean (n=12)	0.88 ± 0.44	2.47 ± 0.51				
Conjunctival edema	0.23 ± 0.23	2.80 ± 0.31	0.26 ± 0.39	2.20 ± 0.77		
	0.37 ± 0.20	2.63 ± 0.51			0.10 ± 0.17	0.97 ± 0.26
Mean (n=12)	0.30 ± 0.22	2.72 ± 0.41				
Eyelid edema	0.27 ± 0.24	2.83 ± 0.32	0.16 ± 0.20	2.16 ± 0.90		
	0.20 ± 0.18	2.70 ± 0.50			0.10 ± 0.11	1.23 ± 0.23
Mean (n =12)	0.23 ± 0.21	2.77 ± 0.41				

Clinical evaluation of histamine-induced conjunctivitis in NAIVE guinea pigs 10 min after challenge (Mean ± SD, n=3)

Dosing time	4 hr before challenge		6 hr before challenge		12 hr before challenge	
	WAL 801	Control	WAL 801	Control	WAL 801	Control
Conjunctival hyperemia	0.73 ± 0.30	2.90± 0.17	1.03± 0.15	2.83± 0.30	1.40± 1.00	1.46± 0.65
Conjunctival edema	0.26 ± 0.05	2.53± 0.25	0.60± 0.30	2.56± 0.32	1.73± 0.83	1.93± 0.85
Eyelid edema	0.20 ±0.10	2.36 ± 0.30	0.66 ± 0.35	2.43 ± 0.55	1.63 ± 1.10	1.70 ± 1.10

U94-0326: Accumulation of granulocytes in the lung and skin of guinea pigs: inhibition by the anti-H_1, antiallergic agent epinastine. Vol. 13, Page 404

The purpose of this study was to investigate whether the antiallergic/H_1-antagonistic drug epinastine had an inhibitory role on inflammatory granulocyte infiltration in respiratory or dermal tissue. The sponsor developed a

The results showed that Epinastine as well as the reference compounds, betamethasone and SC 41930, demonstrated a dose-dependent inhibition of granulocyte accumulation. The rank order of oral activity was epinastine > betamethasone > SC 41930 for bronchial eosinophilia (see table below).

Bronchoalveolar eosinophilia following passive lung anaphylaxis in guinea pigs

Compound	Dose (mg/kg, po)	% inhibition (mean ± SE)	ED_{50}
Epinastine	5.0	26.0± 13.4	6.0
	6.0	58.5± 12.6	
	7.5	75.3± 5.7	
	10	66.3± 10.5	
SC41930	20.0	24.1± 14.8	38.3
	30.0	40.3± 11.8	
	60.0	67.9± 20.2	
Betamethasone	10.0	31.5± 11.1	15.7
	20.0	61.7± 8.3	
	30.0	73.8± 10.2	

[redacted] results showed that fixation of skin chambers to animal skin filled with 30 ng of the highly chemotactic agent, LTB_4, provoked a remarkable accumulation of leukocytes in the chamber fluid 5 hr later, with nearly no cells in the control fluid. As expected, the specific LTB_4 antagonist was able to inhibit this response (ED_{50} = 13.5 mg/kg) and so did betamethasone (ED_{50} = 5.8 mg/kg). Epinastine caused a dose-dependent inhibition with an ED_{50} value of 12 mg/kg (see table below).

Transdermal chemotaxis of guinea pig leukocytes induced by LTB_4

Compound	Dose (mg/kg, po)	% inhibition (mean ± SE)	ED_{50}
Epinastine	1.0	20.8± 9.0	12.0
	3.0	42.0± 4.9	
	10.0	48.2± 2.7	
	20.0	51.4± 3.3	
SC41930	5.0	8.8± 11.4	13.5
	10.0	46.8± 5.5	
	20.0	60.2± 4.5	
	33.0	77.9± 6.0	
Betamethasone	1.5	17.9± 12.0	5.8
	2.5	34.9± 5.5	
	5.0	42.6± 10.6	
	10.0	64.6± 8.1	

In conclusion, epinastine demonstrated inhibitory activities on allergen-mediated eosinophilia in the lung and LTB_4-induced leukocyte accumulation in skin chambers in guinea pigs.

U94-0333: Histamine release into the conjunctivae after antigen challenge in rats. Vol. 14, Page 001

The purpose of this study was to evaluate the effect of WAL 801 eye drops on histamine release into the conjunctivae after an antigen challenge in an experimental model of passive ocular anaphylaxis in male Wistar rats. At the beginning of the study, 10 µl of antiserum was injected into the lower lid of both eyes of each animal. Animals were then treated topically with 10 µl of placebo, ketotifen (0.05, 0.1, 0.3 and 0.5%), sodium cromoglycate (1%, 2% and 4%), or WAL 801 (0.05, 0.1, 0.3 and 0.5%) 72 hr after the sensitization and repeated 10 min later. Five

min after the second dosing, animals were terminated, and the conjunctivae were removed and placed individually in 1 ml of 0.9% physiological saline at 37°C. The challenge was performed *in vitro* by adding antigen solution, 100 µl of ovalbumin (0.4 mg/ml). The reaction was stopped 15 min later using 1 ml of 0.4 N perchloric acid. The amount of histamine released was measured with a fluorometer. Results are summarized in the table below. WAL 801 eye drops at all concentrations showed an inhibitory effect, with the highest inhibition at 0.5%. Ketotifen produced a 23% inhibition only at 0.5%. Cromoglycate group showed no inhibitory effect.

Histamine release into the conjunctivae after antigen challenge in rats

Treatment	% inhibition	Treatment	% inhibition	Treatment	% inhibition
WAL 801 0.05%	20	Ketotifen 0.05%	7	Sodium cromoglycate 1%	-22
WAL 801 0.1%	16	Ketotifen 0.1%	-4	Sodium cromoglycate 2%	1
WAL 801 0.3%	22	Ketotifen 0.3%	-6	Sodium cromoglycate 4%	-33
WAL 801 0.5%	35	Ketotifen 0.5%	23		

U95-0011: WAL 801 (0.1%, 0.3%, 1%, 3%, w/v) ophthalmic solution/Effect on intraocular pressure in normotensive albino rabbits. Vol. 14, Page 040

The purpose of this study was to investigate the effect of WAL 801 of different concentrations (0.1-3%) on intraocular pressure (IOP) in rabbits. New Zealand white rabbits (3/sex/group) were treated topically (50 µl) with a single dose of balanced salt solution, WAL 801 (0.1%, 0.3%, 1%, or 3%) or a reference Isoglaucon (clonidine 0.25%) in the right eye. The IOP was measured using a floating tip tonometer before the instillation and at 0.5, 1, 2, 3, 4, 5 and 6 hr after the instillation. Maximum decreases in IOP for each group are summarized in the table below. When the areas under the curve (AUC) of the IOP were compared, only 3% WAL 801 showed statistically significant reduction in IOP. No difference was noted between WAL 801 at all concentrations and clonidine 0.25%.

Maximum mean IOP decrease and AUC in rabbits treated with WAL 801 (mean ± SD)

Treatment	IOP change (mmHg)	Time of the change	AUC (mmHg-min)
Balanced salt solution	-0.48± 2.55	0.5 hr	234 ± 970
WAL 801 0.1%	-1.83± 3.48	6 hr	-228 ± 824
WAL 801 0.3%	-2.42 ± 3.62	1 hr	-226± 559
WAL 801 1%	-2.48± 2.49	1 hr	-383± 781
WAL 801 3%	-6.67± 2.14	1 hr	-1151± 962
Isoglaucon	-2.68± 1.78	2 hr	-620.5± 285.87

U95-0232: Effect of oral WAL 801 CL (epinastine) in a model of neurally mediated bronchospasm [Challenge of BDE rats with N^6-2-(4-aminophenyl) ethyladenosine]. Vol. 14, Page 072

The purpose of this study was to compare WAL 801 and ketotifen for the ability to block bronchospasm induced by APNEA [N^6-2-(4-aminophenyl) ethyladenosine, an adenosine A_3 receptor agonist] in BDE rats. Surgery (trachea, jugular vein and carotid artery cannulation) was performed for the measurement and calculation of tracheal airflow, tidal volume, breathing rate, pulmonary resistance, dynamic compliance, blood pressure and heart rate. Animals (10-11/group) were treated with WAL 801 (0.03 to 10 mg/kg) or ketotifen (0.1 to 10 mg/kg) by oral gavage. Two hr later, 0.2 µmol/ml/kg APNEA was injected intravenously to induce bronchospasm. Air flow, esophageal pressure and blood pressure were recorded before and after APNEA challenge for at least 5 min. The results showed that both ketotifen and epinastine

inhibited APNEA-induced bronchospasm in a dose-dependent manner. However, epinastine was more potent with lower ED_{50} values (see table below) in the inhibition of APNEA-induced increase in total lung resistance (R_L), decrease in dynamic compliance (Cdyn) and tidal volume (TV).

ED_{50} values of epinastine and ketotifen in the inhibition of APNEA-induced bronchospasm (mg/kg)

	APNEA-induced ↑ in lung resistance	APNEA-induced ↓ dynamic compliance	APNEA-induced ↓ tidal volume
Epinastine	0.47	1.88	0.69
Ketotifen	4.55	12.28	2.23

U95-0547: Effects of WAL 801 on the mediator release from human skin mast cells, human peripheral basophils and human eosinophils. Vol. 14, Page 111

Human peripheral basophils, eosinophils and human skin mast cells were isolated and preincubated with WAL 801 (10^{-3} to 10^{2} µM) for 15 min at 37 °C. Cells were then activated with different secretagogues (30 min, 37 °C) resulting in exocytosis and production of newly generated mediators. The secretagogues used are listed in the table below. In one experiment, basophils were primed with IL3 and IL5 for 10 min before anti-IgE activation. After the activation, histamine release was measured in both supernatants and cell pellets using an automated fluorometric method. Leukotriene C_4 (LTC_4) and eosinophil cationic protein (ECP) contents were measured using RIA kits.

Different secretagogues for isolated human peripheral basophils, eosinophils and human skin mast cells

Cells	Basophils	Eosinophils	Skin mast cells
Anti-IgE antibodies	0.01%		0.1%
Calcium ionophore A23187	1 µM	1 µM	1 µM
Calcium ionophore ionomycin	1 µM	1 µM	
C5a	1 µg/ml	10 µg/ml	
Phorbol ester TPA	1 µM	1 µM	
Concanavalin A (Con A)	1 µg/ml		
FMLP	1 µM		

For basophils, preincubation of basophils with a wide concentration range of WAL 801 (1 nM to 10 µM) did not inhibit IgE-, A23187-, ionomycin-, and TPA (a PKC activator)-induced histamine release from basophils. At higher concentrations (>10 µM), WAL 801 induced inhibition of these activation pathways (20.8 ± 0.5%, 10.9 ± 1.6%, 14.9 ± 0.7%, and 20%, respectively). Similar to these observations, cell priming with IL-3 or IL-5 led to strong histamine release, and WAL 801 suppressed this pathway only at the highest concentration (100 µM, 14.8 ± 2.7% for IL-3 priming and 23.7 ± 2.6% for IL-5 priming). LTC_4 was the main arachidonic acid metabolite generated in human basophils. *In vivo,* histamine and LTC_4 induce different types of inflammatory reactions, namely the acute and the late phase reaction, respectively. Preincubation of basophils with WAL 801 and further stimulation with anti-IgE antibodies revealed no significant changes at lower concentrations but a dose-dependent inhibition at concentrations higher than 100 nM. WAL 801 had no effect on C5a- and FMLP-(chemotactic tripeptide) induced histamine release. At > 1 µM, WAL 801 dose-dependently inhibited ConA-induced histamine release (up to 24.1 ± 6.8% at 100 µM).

For eosinophils, WAL 801 did not alter A23187-, ionomycin-, C5a- and TPA-induced histamine release even at the highest concentration (100 µM).

For human skin mast cells, WAL 801 strongly reduced IgE-mediated histamine release in a dose-dependent fashion with a maximum of 86.0±14.0 % inhibition compared to control. Histamine release from skin mast cells due to A23817 was also inhibited in a concentration-dependent fashion.

In conclusion, the pharmacologically and clinically relevant concentrations of WAL 801 appeared to be inactive on mediator release from both human basophils and human eosinophils induced by different secretagogues. The sponsor indicated that the inhibition at higher concentrations might be nonspecific suppression by interaction with surface membrane structures. However, WAL 801 showed inhibitory effect on human skin mast cells.

U96-2204: Anti-inflammation activity: Effects in inflammatory cell accumulation and pathological findings in the conjunctivae of normal rats. Vol. 14, Page 146

The purpose of the study was to evaluate the effect of WAL 801 eye drops, dissolved in sterilized 0.9% NaCl physiological solution, on inflammatory cell accumulation and pathological findings in normal male Wistar rat conjunctivae. Animals (10/group) were topically treated with WAL 801 placebo, WAL 801 NaCl placebo, or WAL 801 (dissolved in NaCl placebo 0.01%, 0.05%, 0.1, 0.3, and 0.5%) in each eye (10 µl). Ten min after the treatment, ovalbumin (200 mg plus 8 ml saline solution, 1 ml/kg, iv) was administered to all animals. Two hr later, animals were terminated. The conjunctivae were extracted and processed for cell counting (neutrophils, lymphocytes, eosinophils, and macrophages) and vascularization evaluation in three zones of the test area (upper tarsal, mid tarsal and fornix). The results showed that topical treatment to the eye with WAL 801 CL demonstrated practically no alterations in the cell count and vascularization except for the number of lymphocytes (0.5% only) and neutrophils that were decreased when compared to the control groups. It seemed that WAL 801 inhibited neutrophil accumulation. [**Reviewer's Comments**: The sponsor did not provide the unit in cell counting. No pretreatment data were provided. It is hard to draw a conclusion from the results.]

Neutrophil and lymphocyte changes in the conjunctivae of rats treated with WAL 801 (mean ± SE)

	Neutrophils			Lymphocytes		
Treatment	Upper tarsal	Mid tarsal	Fornix	Upper tarsal	Mid tarsal	Fornix
WAL 801 placebo	1.63± 0.28	1.81± 0.19	1.60± 0.17	1.01± 0.12	1.12± 0.20	0.79± 0.15
WAL 801 NaCl placebo	1.68± 0.22	1.72± 0.20	1.50± 0.12	0.48± 0.06	0.74± 0.11	0.38± 0.07
WAL 801 0.01%	1.45± 0.17	1.22± 0.20	1.47± 0.20	0.44± 0.06	0.61± 0.07	0.37± 0.05
WAL 801 0.05%	1.02± 0.15	0.90± 0.15	0.94± 0.14	0.65± 0.07	0.69± 0.08	0.62± 0.07
WAL 801 0.1%	0.91± 0.06	0.93± 0.13	0.69± 0.11	0.36± 0.08	0.72± 0.12	0.53± 0.07
WAL 801 0.3%	0.89± 0.09	0.85± 0.16	0.67± 0.12	0.38± 0.05	0.45± 0.06	0.35± 0.08
WAL 801 0.5%	0.52± 0.07	0.56± 0.12	0.54± 0.04	0.16± 0.06	0.26± 0.06	0.24± 0.06

U96-2205: Microvascular permeability in the conjunctivae elicited by histamine, compound 48/80 and PAF in rats. Vol. 14, Page 167

The purpose of this study was to evaluate the effect of WAL 801 eye drops on the increases in microvascular permeability in the conjunctivae elicited by histamine, Compound 48/80 and PAF in male Wistar rats. Ketotifen and sodium cromoglycate were also included in this study. The substances WAL 801 (0.01-0.5%), ketotifen (0.003-0.5%), or sodium cromoglycate (1-10%) were applied topically twice with a 10-min interval in between. Five min

after the second administration, the inducing agents (histamine 100 µg/10 µl/eye, compound 48/80 33.3 µg/10 µl/eye, and PAF 20 ng/10 µl/eye) were injected into the conjunctivae. Immediately following the injection of inducing agents, an [redacted] mg/ml/kg) was given intravenously. Thirty min later animals were sacrificed and the conjunctivae were removed and processed. Dye leakage was examined for increased permeability. The results (see tables below) showed that both WAL 801 and ketotifen inhibited vascular leakage induced by histamine, 48/80 and PAF. Sodium cromoglycate's inhibitory effect on the vascular permeability was slight.

Inhibitory effect of WAL 801 on vascular permeability in rat conjunctivae (% inhibition)

Concentrations	0.01%	0.03%	0.05%	0.1%	0.3%	0.5%	ED_{50}
Histamine (100 µg/eye)	7	6	48	57	73	81	0.1115%
Compound 48/80 (33.3 µg/eye)			15	29	52	62	0.2822%
PAF (20 ng/eye)			-4	-4	30	35	0.8245%

Inhibitory effect of sodium cromoglycate on vascular permeability in rat conjunctivae (% inhibition)

Concentrations	1%	2%	4%
Histamine (100 µg/eye)	14	15	14
Compound 48/80 (33.3 µg/eye)	20	27	20
PAF (20 ng/eye)	4	29	9

Inhibitory effect of ketotifen on vascular permeability in rat conjunctivae (% inhibition)

Concentrations	0.003%	0.005%	0.01%	0.03%	0.05%	0.1%	0.3%	0.5%	ED_{50}
Histamine (100 µg/eye)	49	43	41	74	75	77			0.0067%
Compound 48/80 (33.3 µg/eye)			18	27	27	78	80	82	0.0692%
PAF (20 ng/eye)			8	5	-16	24	47	66	0.3341%

U96-2206: Anti-inflammatory activity: Effects in inflammatory cell accumulation and pathological findings in the conjunctivae of normal and allergic rats. Vol. 14, Page 239

The purpose of the study was to evaluate the effect of WAL 801 eye drops, compared with ketotifen and sodium cromoglycate, on inflammatory cell accumulation and pathological findings in normal and allergic rat conjunctivae. The allergy model used was the passive ocular anaphylaxis (POA) in the rat. For the allergic animals, rats (10/group) were treated with antiserum solution (10 µl, injected into the lower lid of both eyes of each animal). Seventy-two hr later animals were topically treated with WAL 801 (0.05%, 0.1, 0.3, and 0.5%), ketotifen (0.05%, 0.1, 0.3, and 0.5%), sodium cromoglycate (1%, 2% and 4%) or placebo in each eye (10 µl). Two topical administrations to the eye were made with a 10-min interval in between. Five min after the second administration, the challenge was carried out with an antigen solution (25 mg ovalbumin/ml/kg, iv). Two hr later, the animals were terminated. The conjunctivae were extracted and processed for histological examination. The parameters evaluated included: global assessment, % mast cell degranulation, cell counts (neutrophil, lymphocyte, eosinophil and mononuclear cells) from the following conjunctivae areas: upper tarsal plate, mid tarsal plate and upper fornix.

For the normal rats, animals were topically treated with WAL 801 (0.05%, 0.1, 0.3, and 0.5%), ketotifen (0.05%, 0.1, 0.3, and 0.5%), sodium cromoglycate (1%, 2% and 4%) or placebo in each eye (10 µl) similar to in allergic animals. Two hr later, the animals were terminated. The examination was the same as in the allergic animals.

Results showed that in allergic animals, the mast cell degranulation was significantly inhibited by WAL 801 at all concentrations (35.3-37.5%). Similar effects were noted with ketotifen (↓31.4-50.4%) and sodium cromoglycate (↓51.8-61.2%).

Also in the allergic animal models, WAL 801's effect on neutrophils was not consistent. The drug lowered eosinophil counts at all concentrations. Only at 0.5%, WAL 801 decreased the lymphocyte count and vascularization. WAL 801 increased macrophage count but it seemed that lower concentrations (0.05, 0.1 and 0.3%) had more increases. Regarding the global index of the inflammatory response in the allergic animals, the inflammatory response was inhibited at all concentrations.

Ketotifen and sodium cromoglycate produced a decrease in most of the cell counts, vascularization, and the global index of the inflammatory response in the allergic animals.

For normal rats, WAL 801, ketotifen and sodium cromoglycate produced no biologically relevant changes.

In conclusion, in the POA rats model, WAL 801, ketotifen, and sodium cromoglycate applied topically to the eye showed a great activity in inhibiting the inflammatory and allergic response in the conjunctivae. The three treatments to the eyes of normal rats showed no substantial variations in the conjunctival tissue.

Pharmacology summary and conclusions:

Epinastine is an H_1-receptor antagonist. In *in vitro* studies, epinastine had strong antihistaminic and antiserotonin activities, but had no histamine H_2 antagonistic action and no anticholinergic effects. Epinastine also showed an α-adrenolytic effect. In *in vivo* pharmacology studies, epinastine inhibited histamine- and serotonin-induced bronchospasm in guinea pigs, histamine-induced urtica in rats and dogs, serotonin-induced edema in rats, allergen-mediated eosinophilia in the lung and LTB_4-induced leukocyte accumulation in skin chambers in guinea pigs. Epinastine showed an inhibitory effect on histamine-induced increase in microvascular permeability in rat conjunctivae. In several studies with a passive ocular anaphylaxis (POA) model in rats, epinastine eye drops showed marked antiallergic activity in a dose-dependent manner.

II. SAFETY PHARMACOLOGY:

GP1997/097/PH2: General pharmacology: The effects of epinastine, 0.375, 0.75, 1.5 and 3 mg/kg iv on QT-time and other ECG parameters as well as on arterial blood pressure, heart rate, and cardiac contractility in anesthetized pigs in comparison with terfenadine. Vol. 10, Page 330
GP1997/099/PH2: General pharmacology: The effects of 0.01, 0.1 and 1 µmol/l epinastine and terfenadine on action potential configuration in isolated single ventricular myocytes from guinea pig hearts, investigated by means of the whole cell current clamp technique. Vol. 10, Page 363
U92-0667: Effects of [redacted], metabolite and decomposition of epinastine hydrochloride, on central nervous system and general pharmacology of optical isomers of epinastine hydrochloride. Vol. 13, Page 282

GP1997/097/PH2: General pharmacology: The effects of epinastine, 0.375, 0.75, 1.5 and 3 mg/kg iv on QT-time and other ECG parameters as well as on arterial blood pressure, heart rate, and cardiac contractility in anesthetized pigs in comparison with terfenadine. Vol. 10, Page 330

The purpose of this study was to analyze effects of epinastine on the ECG parameters as well as BP, HR and cardiac contractility in male pigs and to compare those effects with terfenadine. Terfenadine is an H_1 receptor antagonist known to increase the risk of adverse cardiac effects in patients. Pigs (5/group) were anesthetized with nitrous oxide and pentobarbital. Both drugs were cumulatively administered (iv) at 0.375, 0.75, 1.5 and 3 mg/kg. Three pigs were treated four times with saline (0.1 ml/kg) and five pigs with glycofurol, the respective solvents of epinastine and terfenadine. Arterial blood pressure was measured in the left femoral artery by means of a saline filled catheter attached to an external pressure transducer. A cathetertip pressure transducer was introduced into the left ventricle and left ventricular pressure was recorded. The maximal rate of rise of left ventricular pressure development (LV-dP/dt) was calculated. Subcutaneous needle electrodes were used to record ECG. All parameters were continuously monitored.

Following the administration of saline and glycofurol, there were no biologically relevant changes observed.

Epinastine at 3 mg/kg iv caused a significant increase in QT time by maximally 63.6 ± 17.0 ms. Arterial blood pressure, heart rate and LV-dP/dtmax were lowered in a dose-dependent manner by maximally -16.3 ± 4.2/-13.5 ± 3.5 mmHg, -24.2 ± 6.4 /min, and -715.1 ± 125.1 mmHg/s, respectively.

Terfenadine, 0.375, 0.75, 1.5, and 3 mg/kg iv caused a significant increase in QT time by maximally 161.3 ± 47.8 ms, whereas QRS duration was not changed. Arterial blood pressure, heart rate and LV-dP/dtmax were significantly lowered by maximally -52.2 ± 8.4/-42.5 ± 8.1 mmHg, -49.2 ± 11.7 beats/min, and -1041.5 ± 349.4 mmHg/s, respectively.

Control values of QT time versus the corresponding RR interval showed a linear relationship with the following equation of the regression line: QT = 103.2 + 0.53*RR[ms], r = 0.962, which was similar to that of epinastine: QT = 116.9 + 0.52*RR[ms], r = 0.974. After treatment with terfenadine the equation was: QT = -218.7 + 1.95*RR[ms], r = 0.914. The regression line of terfenadine differed significantly from the control and epinastine regression lines ($p < 0.001$, see figure below).

In conclusion: epinastine did not change QT time in anesthetized pigs except for a rate dependent prolongation following the decrease in heart rate. In this respect the drug differed markedly from terfenadine which induced a pronounced heart rate-independent prolongation of QT time. Furthermore, epinastine caused only minor changes in arterial blood pressure, heart rate and cardiac contractility. These might be attributed to spontaneous changes during longer lasting anesthesia. The sponsor indicated that the α-adrenolytic activity of epinastine might also contribute to the observed hemodynamic effects. In contrast to epinastine, terfenadine induced a pronounced hypotension and a decrease in heart rate and cardiac contractility.

GP1997/099/PH2: General pharmacology: The effects of 0.01, 0.1 and 1 µmol/l epinastine and terfenadine on action potential configuration in isolated single ventricular myocytes from guinea pig hearts, investigated by means of the whole cell current clamp technique. Vol. 10, Page 363

The purpose of this study was to investigate effects of epinastine (0.01, 0.1 and 1 µM) on action potential configuration in isolated single guinea pig ventricular myocytes in comparison with terfenadine (0.01, 0.1 and 1 µM) using the whole cell current clamp method. It has been reported that the histamine H_1-receptor antagonists, terfenadine and astemizole, can induce the syndrome of torsades de pointes, i.e. QT interval prolongation and life-threatening ventricular tachycardia. These two drugs were found to prolong cardiac repolarization in *in vitro* studies due to blockade of one or more of the cardiac potassium channels that determine the duration of the action potential.

Ventricular myocytes were isolated from guinea pigs of either sex. A small aliquot of isolated myocytes was placed in a perfusion chamber mounted on the stage of an inverted microscope [redacted]. The myocytes were stimulated through the patch-clamp electrode every 3 s with 10 ms supramaximal rectangular impulses. Action potentials (AP) were recorded using a patch clamp amplifier at a sampling rate of 500 Hz. Myocytes were treated with test drugs for 5 min after an equilibration period of about 5 min. Resting membrane potential (RMP), maximum upstroke velocity of the action potential (Vmax), plateau potential (PP), and action potential duration at 90% repolarization (APD90%) were evaluated.

Epinastine at concentrations up to 1 µM, did not induce any change in action potential configuration, especially no prolongation of the action potential duration. In contrast to epinastine,

terfenadine caused a marked prolongation of the action potential duration (↑ADP90% by +55.0 ± 10.8 ms at 1 μM).

In conclusion, epinastine had no electrophysiologic effects on action potential configuration *in vitro*. The drug differed markedly from the histamine H_1-receptor antagonist terfenadine that induced a prolongation of action potential duration.

U92-0667: Effects of [redacted], metabolite and decomposition of epinastine hydrochloride, on central nervous system and general pharmacology of optical isomers of epinastine hydrochloride. Vol. 13, Page 282

This report summarized the effects of [redacted], the main metabolite and decomposition of WAL 801, and (+)- and (-)-WAL 801on CNS, respiratory and cardiovascular and peripheral systems.

To determine the effect of [redacted] on EEG in gallamine-immobilized cats, pairs of electrodes were fixed symmetrically with respect to the median on the frontal, parietal and occipital regions, respectively. Vehicle and [redacted] (0.1, 1 and 10 mg/kg) were injected (iv) cumulatively and the effects were observed for 60 min. The results showed that at 10 mg/kg, [redacted] induced multiple and high-amplitude abnormal waves in all animals.

To determine [redacted] effect on spinal reflex in cats, the spinal cords of animals were severed at T_{13}-L_1 and a laminectomy was performed. A bipolar electrode was attached to ventral root of L_7 or S_1 for monitoring the reflex potential (VRR). The other electrode was attached to the dorsal root at the same level for electrical stimulation (0.1 msec, 0.2 Hz). The average of 10 potentials were calculated. Vehicle and [redacted] 0.1, 1 and 10 mg/kg) were injected (iv) cumulatively and the effects were observed for 60 min. Monosynaptic reflex (MSR) and polysynaptic reflex (PSR) were quantified by estimating the peak reflex potentials and the area between reflex potentials and basal potentials, respectively. Results showed that at 10 mg/kg, two of four animals died and the spinal reflex was affected in the remaining two animals. MRS was transiently increased in one animal. In the other animal, [redacted] induced a prolonged decrease in MSR. Regarding PSR, [redacted] caused a 60% increase in one animal, and no changes occurred in the other animal.

To determine [redacted] effect on muscle strength, male mice (5/group) were treated orally with distilled water or [redacted] at 0.3, 3 and 30 mg/kg. Animals were forced to hang on horizontal wire by their forelimb, and were examined whether they could secure themselves by holding the hind limbs on the wire within 5 sec. The effects were examined every hr for 4 hr. Results showed [redacted] had no effects on muscle force of mice.

The effects of (+)- or (-)-WAL 801 on respiration, blood pressure, heart rate and blood flow were determined in pentobarbital-anesthetized dogs. Animals were treated with (+)- or (-)-WAL 801 at 0.01, 0.1, 1 or 10 mg/kg (iv). The respiratory and cardiovascular parameters were continually recorded for 60 min. Results showed that both (+)- and (-)-WAL 801 induced tachycardia (↑18.7-47.6%) at ≥ 1 mg/kg and hypotension (↓29-39%) at 10 mg/kg. The respiratory rate was increased by (+)-WAL 801 at ≥ 0.1 mg/kg (↑7-22/min) and by (-)-WAL 801 at 10 mg/kg (↑16/min). The blood flow, ECG and central venous pressure were not affected.

The infiltration anesthetic effect of (+)- and (-)-WAL 801 was investigated in male guinea pigs. The back of each animal was shaved and the rapid contractile responses of skin to the stimulation by a needle were evaluated. (+)- or (-)-WAL 801 (0.01, 0.03, 0.1 and 0.3%) was injected (0.1 ml, ic). The skin contraction at the injection loci was tested at 10, 20, 30, 60, 90 and 120 min after administration. Results showed that (+)- and (-)-WAL 801 (0.1% and 0.3%, ic, 0.1 ml) reduced the reflex contraction of skin with the duration up to 90 to 120 min.

The α-blocking activity was investigated by measuring the contraction in vas deferens of male guinea pigs. The preparations of vas deferens were mounted in a 20-ml organ bath. Tonic contraction was induced by addition of norepinephrine (NE, 10^{-5} M). (+)- and (-)-WAL 801 (10^{-8} M to 10^{-4} M) were tested for the inhibition of NE-induced contraction. The results showed that (+)-WAL 801 significantly inhibited the contraction at concentrations ≥ 10^{-6} M, while (-)-WAL 801 significantly inhibited the contraction at concentrations ≥ 10^{-5} M.

Safety pharmacology summary:

Epinastine had no effects on nocturnal motility in mice, behavior changes during the apomorphine climbing test in mice, and sleep and waking behavior in cats. The drug had no evidence of active anxiolytic or antidepressant properties. [redacted], the main metabolite and decomposition of WAL 801, caused abnormal EEG in cats at a high dose of 10 mg/kg, iv. Epinastine did not change QT time in anesthetized pigs except for a rate dependent prolongation following the decrease in heart rate. Epinastine caused slight decreases in arterial blood pressure, heart rate and cardiac contractility. Epinastine had no electrophysiologic effect on action potential configuration in an *in vitro* test.

III. PHARMACOKINETICS/TOXICOKINETICS:

Studies reviewed:

Absorption:
PK-01-035: Toxicokinetic analysis for Study No. TX00032 entitled "Epinastine: a 6-month ocular toxicity study in rabbits". Vol. 15, Page 105
PK-01-039: Toxicokinetic analysis for Study No. TX00033 entitled "Epinastine: a 6-month ocular toxicity study in monkeys". Vol. 15, Page 129
U86-0750: Biochemical investigations with WAL 801 CL-^{14}C in rhesus monkeys (absorption, distribution, metabolism, excretion). Vol. 16, Page 162
U90-0690: Plasma concentrations during a 2-year cancerogenicity study in the rat. Vol. 16, Page 384
U91-0017: Plasma concentrations of WAL 801 CL in parallel to a carcinogenicity study over 18 months in mice (administration in the diet). Vol. 17, Page 001
U91-0144: Plasma concentrations and renal excretion of WAL 801 CL during a 52-week intragastric toxicity study in rhesus monkeys. Vol. 17, Page 021
U91-0332: Plasma concentrations and renal excretion of WAL 801 CL during a 1-year toxicity study in the rat administration with the feed. Vol. 17, Page 082
U92-0409: Pharmacokinetic studies on ^{14}C-epinastine hydrochloride (WAL 801 CL) (I)-blood level, tissue distribution and urinary and fecal excretion after single and repeated administration in rats. Vol. 17, Page 128

U92-0410: Pharmacokinetic studies on ^{14}C-epinastine hydrochloride (WAL 801 CL) (II)-dose-linearity, placental transfer, biliary excretion, entero-hepatic circulation, transfer into milk, protein binding and metabolism after single administration in rats. Vol. 17, Page 175
U94-0295: Absorption, distribution and excretion of ^{14}C-WAL 801 CL after ophthalmic administration in rabbits. Vol. 17, Page 252
U96-0004: WAL 801 CL (0.3%; w/v) ophthalmic solution: Determination of pharmacokinetic parameters and bioavailability after: --a single instillation into the conjunctival sac of pigmented rabbit eyes, --a single intravenous administration in pigmented rabbits. Vol. 17, Page 379
U97-2105: Epinastine (WAL 801 CL): 4-week local tolerance study of WAL 801 CL eye drops by instillation into the conjunctival sac of rabbits: Concentrations in aqueous humor and plasma on day 28. Vol. 18, Page 001

Distribution:
PK-01-051: *In vitro* binding of ^{14}C-epinastine to ocular bovine melanin. Vol. 15, Page 182
PK-01-077: ^{14}C-epinastine hydrochloride ocular tissue distribution studies in cynomolgus monkeys following a single ophthalmic administration. Vol. 15, Page 200
PK-02-008: ^{14}C-epinastine hydrochloride ocular tissue distribution studies in cynomolgus monkeys following repeated ophthalmic administration. Vol. 15, Page 307
U84-0606: Whole animal autoradiographic studies with [^{14}C]WAL 801 CL in rats. Vol. 16, Page 106
U86-0216: Placental passage of WAL 801 CL in rats. Vol. 16, Page 124
U90-0608: Concentrations of ^{14}C activity in blood, plasma and bone marrow of mice and Chinese hamsters after oral administration of single doses of 250 mg/kg and 350 mg/kg WAL 801 CL-^{14}C respectively. Vol. 16, Page 359
U95-0065: ^{14}C-WAL 801 CL (0.3%; w/v) ophthalmic solution: Distribution in the ocular structures after three instillations into the eyes of pigmented rabbits. Vol. 17, Page 294
U96-0003: WAL 801 CL (0.3%; w/v) ophthalmic solution: Penetration into the aqueous humor after single instillation into the eye of pigmented rabbits. Vol. 17, Page 322

Metabolism:
PK-02-048: Metabolite profiles in ocular tissues of cynomolgus monkey following repeated ocular administrations of ^{14}C-epinastine hydrochloride. Vol. 16, Page 001
U92-0666: Plasma level of [redacted], a metabolite of WAL 801 in man and enantiomers of WAL 801 in rabbits. Vol. 17, Page 219
U98-0244: Structure elucidation of human hepatic microsomal metabolite of epinastine. Vol. 18, Page 034

Excretion:
U84-0546: Biochemical investigations with WAL 801 CL-^{14}C in rats (absorption, distribution, metabolism, excretion). Vol. 16, Page 058
U86-1014: Total excretion and metabolic pattern in rats, mice, dogs, monkeys and pigs after peroral administration of WAL 801 CL-^{14}C. Vol. 16, Page 234

Studies NOT reviewed:

[redacted]

PK-01-035: Toxicokinetic analysis for Study No. TX00032 entitled "Epinastine: a 6-month ocular toxicity study in rabbits". Vol. 15, Page 105

Key study findings: Following ocular administration, epinastine was absorbed into the systemic circulation in rabbits. The systemic exposure increased proportionally with dose.

Document #: PK-01-035
Study #: TX00032
Conducting laboratory and location: Allergan, 2525 Dupont Drive, Irvine, CA 92612
Method:
Dosing:

Animal: New Zealand white rabbits
Route: Ocular, topical
Dosage: 0.05%, 0.1% and 0.5% epinastine HCl, left eye only, tid x 175 days

Drug: Epinastine HCl ophthalmic solution
Study design:

Group	Treatment	Formulation #	Lot #	Dosing regimen
1	Placebo			Left eye only, one drop (35 µl) tid x 175 days
2	0.05% epinastine HCl	9343X	11765	
3	0.1% epinastine HCl	9347X	11766	
4	0.5% epinastine HCl	9346X	11767	

This TK assay was part of the 6-month ocular toxicity study (Study TX00032) in rabbits. Animals were topically (ocular) dosed with 0% (placebo), 0.05%, 0.1%, or 0.5% epinastine HCl ophthalmic solutions for 6 months. Each rabbit received one drop (35 µl) of solution three times daily to the left eye. After 26 and 175 days of dosing, blood samples were collected from rabbits (4/sex/group/timepoint) at 0 (pre 3rd dose), and at 0.5, 1, 2, 3, 4, 6, and 8 hr post 3rd dose. Epinastine concentrations in plasma were measured using a validated [redacted] method with a quantitation range of [redacted] ng/ml.

Results:

Results are summarized in the table below. Following ocular administration for up to 6 months, epinastine was absorbed into the systemic circulation in rabbits. The systemic exposure increased proportionally with increasing dose.

Plasma TK parameters in rabbits treated with epinastine HCl ophthalmic solution for 6 months

Treatment	Cmax (ng/ml)	Tmax (hr)	AUC_{0-t} (ng-hr/ml)
Day 26	Mean ± SD		Mean ± SE
0.05% epinastine HCl	0.79± 0.46	0.5	1.13± 0.12
0.1% epinastine HCl	1.25± 0.70	0.5	2.04± 0.18
0.5% epinastine HCl	5.17± 3.78	0.5	7.79± 0.85
Day 175			
0.05% epinastine HCl	0.56 ± 0.30	0.5	1.08± 0.11
0.1% epinastine HCl	1.00 ± 0.49	0.5	1.70± 0.14
0.5% epinastine HCl	3.25 ± 1.09	0.5	6.10± 0.37

PK-01-039: Toxicokinetic analysis for Study No. TX00033 entitled "Epinastine: a 6-month ocular toxicity study in monkeys". Vol. 15, Page 129

Key study findings: Following ocular administration, epinastine was absorbed into the systemic circulation in monkeys. The systemic exposure increased proportionally with dose.

Document #: PK-01-039
Study #: TX00033
Conducting laboratory and location:
Method:
Dosing:

Animal: Cynomolgus monkeys
Route: Ocular, topical (left eye only)
Dosage: 0.05%, 0.1% and 0.5% epinastine HCl, left eye only, tid x 174 days
Drug: Epinastine HCl ophthalmic solution

Study design:

Group	Treatment	Formulation #	Lot #	Dosing regimen
1	Placebo	9342X		Left eye only, one drop (35 µl) tid x 174 days
2	0.05% epinastine HCl	9343X	R12070	
3	0.1% epinastine HCl	9347X	R12070	
4	0.5% epinastine HCl	9346X	R12070	

This TK assay was part of the 6-month ocular toxicity study (Study TX00032) in monkeys. Animals were topically dosed with 0% (placebo), 0.05%, 0.1%, and 0.5% epinastine HCl ophthalmic solutions for 6 months. Each monkey received one drop (35 µl) of solution three times daily to the left eye. After 28 and 174 days of dosing, blood samples were collected from monkeys (4/sex/group/timepoint) at 0 (pre 3rd dose), and at 0.5, 1, 2, 3, 4, 6, and 8 hr post 3rd dose. Epinastine concentrations in plasma were measured using a validated , method with a quantitation range of ng/ml (LOQ = ng/ml).

Results:

Results are summarized in the table below. Following ocular administration for up to 6 months, epinastine was absorbed into the systemic circulation in monkeys. The systemic exposure increased proportionally with increasing dose. No big differences were found between male and female animals, and no accumulation was noted.

Plasma TK parameters in monkeys treated with epinastine HCl ophthalmic solution for 6 months (mean ± SD)

Treatment	Cmax (ng/ml)	Tmax (hr)	AUC_{0-t} (ng-hr/ml)
Day 28			
0.05% epinastine HCl	0.170± 0.035	0.563± 0.678	0.441± 0.213
0.1% epinastine HCl	0.325± 0.106	0.313± 0.458	1.00± 0.51
0.5% epinastine HCl	1.86± 0.61	0.813± 0.799	7.52± 1.90
Day 174			
0.05% epinastine HCl	0.148± 0.091	1.00± 0.89	0.486± 0.345
0.1% epinastine HCl	0.231± 0.058	0.938± 1.270	0.849± 0.381
0.5% epinastine HCl	1.51± 0.70	0.813± 0.530	6.06± 2.30

U86-0750: Biochemical investigations with WAL 801 CL-^{14}C in rhesus monkeys (absorption, distribution, metabolism, excretion). Vol. 16, Page 162

Key study findings: The bioavailability of WAL 801 CL was low (20-42%) after oral dosing in monkeys. The drug was eliminated mainly through urine (60%). One metabolite, WAL 1097-N-glucuronide, was identified in urine and was considered as a main metabolite component. There were 3 to 5 smaller polar metabolites.

Report #: U86-0750
Study #: Not indicated
Conducting laboratory and location: Department of Biochemistry, Boehringer Ingelheim KG, D-6507 Ingelheim
Date of study initiation: April 1985
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Rhesus monkeys
N: 2/sex/group
Age/weight: 5-13 kg
Route: Intravenous and oral
Dosage: 1 mg/kg for iv and 8 mg/kg for oral, single dose
Drug: ^{14}C-WAL 801 CL (Batch 2, purity: [redacted], 100 µCi/mg)
Methods: [redacted]

The purpose of this study was to determine the PK profiles of epinastine in monkeys following a single oral or iv dose of ^{14}C-WAL 801 CL. Blood samples were collected prior to dosing, and at 2, 5, 10, 15, 30 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 30, 48 and 72 hr (iv) or 15, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hr (po) after drug administration. Urine and feces samples were collected in 24-hr fractions for 96 hr. The metabolite pattern of the drug in urine was determined using [redacted] methods.

Results:

Plasma PK parameters: Results are summarized in the table below. The median half-life value of plasma WAL 801 CL after iv dosing was 2.8 hr. The bioavailability after oral dosing was 20-42%.

Plasma PK parameters in monkeys treated with ^{14}C-WAL 801 CL (median)

	WAL 801 CL			Radioactivity concentration		
	Cmax (ng/ml)	Tmax (hr)	$AUC_{0-\infty}$ (ng-hr/ml)	Cmax (ng-eq/ml)	Tmax (hr)	$AUC_{0-\infty}$ (ng-eq hr/ml)

IV (1 mg/kg)			572			4140
PO (8 mg/kg)	279	3	1470	3530	3.5	30536

Excretion: After a single iv or oral dose of ^{14}C-epinastine hydrochloride to monkeys, the major portion of radioactivity was excreted within 48 hr. About 60% of radioactivity was eliminated in urine (see table below).

Cumulative renal and fecal excretion of ^{14}C-WAL 801 CL in rats (median number of % dose)

	iv		po	
Time (hr)	Urine	Feces	Urine	Feces
0-24	52.92	9.93	49.37	1.12
0-48	57.64	22.00	53.77	25.46
0-72	59.02	22.53	55.16	29.29
0-96	59.97	22.69	56.06	29.66
Total	81.12		85.72	

Metabolism: The parent compound was the main component in monkey urine. A glucuronide of WAL 1097 was isolated and was considered as a main component of the polar metabolites. There were 3 to 5 smaller polar metabolites in the [redacted] radiochromatogram, which were slightly higher than the background.

In summary, monkeys were treated with ^{14}C-WAL 801 CL by iv or oral route. The bioavailability of WAL 801 CL was low (20-42%) after oral administration. The drug was eliminated mainly through urine (60%). One metabolite, WAL 1097-N-glucuronide, was identified in urine and was considered as a main metabolite component. There were 3 to 5 smaller polar metabolites.

U90-0690: Plasma concentrations during a 2-year cancerogenicity study in the rat. Vol. 16, Page 384

Key study findings: WAL 801 CL was detected in all plasma samples. There was not a uniform trend.

Report #: U90-0690
Study #: F87
Conducting laboratory and location: Department of Biochemistry, and Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim
Date of study initiation: September 22, 1986
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Rats/Chbb:THOM SPF
N: 20/sex
Age/weight: 42±2 days old, males: 175.2 g, females: 149.1 g
Route: Oral (in feed)
Dosage: 40 mg/kg/day for 2 years (The dosage was the same as used in the HD group in the carcinogenicity study.)
Drug: WAL 801 CL (Batch X)
Methods: [redacted]

This was a satellite assay of a carcinogenicity study. The purpose of this assay was to determine the plasma absorption of WAL 801 CL under the conditions of the carcinogenicity study. Animals were treated with WAL 801 CL orally (in feed) at 40 mg/kg/day for 2 years. Blood samples were collected from 12 animals/sex in weeks 1, 24, 48 and 64 at 1 hr (7am) and 7 hr (1pm) after the start of the light phase.

Results:

Results are summarized in the table below. WAL 801 CL was detected in all plasma samples. It seemed that the values in different weeks were different. There was not a uniform trend. In weeks 24 and 64, the drug concentrations between males and females were different.

Mean plasma concentrations of WAL 801 CL (ng/ml) in pooled plasma samples (3 rats/sample)

Time of sampling	Sex	Week 1	Week 24	Week 48	Week 64
7am	♂	3.52	16.42	11.45	5.68
	♀	4.18	12.12	10.05	20.71
1pm	♂	2.32	12.32	10.21	3.92
	♀	2.93	6.87	7.16	15.43

U91-0017: Plasma concentrations of WAL 801 CL in parallel to a carcinogenicity study over 18 months in mice (administration in the diet). Vol. 17, Page 001

Key study findings: WAL 801 CL was detected in all plasma samples. Steady state was reached within week 1. Plasma concentrations of epinastine in male animals were higher than in females in all weeks listed.

Report #: U91-0017
Study #: F81
Conducting laboratory and location: Department of Biochemistry, and Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim
Study period: August 1986 to April 1988
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Mice/Chbi:NMRI
N: 90/sex
Age/weight: 42±2 days old, males: 30.7 g, females: 26.4 g
Route: Oral (in feed)
Dosage: 40 mg/kg/day x 78 weeks or 546 days (The dosage was the same as used in the HD group in the carcinogenicity study.)
Drug: WAL 801 CL (Batch IX)
Methods:

This was a satellite assay of a carcinogenicity study. The purpose of this assay was to determine the plasma absorption of WAL 801 CL under the conditions of the carcinogenicity study. Animals were treated with WAL 801 CL orally (in feed) at 40 mg/kg/day for 78 weeks. Blood samples were collected from 15 animals/sex in weeks 1, 24, 48, 64 and 78 at 4 hr (10am) after the start of the light phase. Plasma aliquots from 3 mice were pooled for one determination.

Results:

Results are summarized in the table below. WAL 801 CL was detected in all plasma samples. No accumulation was seen between Week 1 and other weeks, suggesting the attainment of the steady state within Week 1. Plasma concentrations of epinastine in male animals were higher than in females in all weeks listed.

Mean plasma concentrations of WAL 801 CL (ng/ml, mean ± SD) in pooled plasma samples (3 mice/sample)

Sex	Week 1	Week 24	Week 48	Week 64	Week 78	Mean
♂	4.72± 1.32	4.36± 1.42	4.76± 1.16	3.93± 0.96	5.92± 2.64	4.74± 1.62
♀	3.24± 1.46	2.86± 1.04	3.36± 0.39	2.84± 0.65	3.52± 1.69	3.16± 1.04

U91-0144: Plasma concentrations and renal excretion of WAL 801 CL during a 52-week intragastric toxicity study in rhesus monkeys. Vol. 17, Page 021

Key study findings: WAL 801 CL was absorbed in a dose-dependent manner after oral administration to monkeys. The urinary excretion was also in a dose-dependent manner.

Document #: U91-0144
Study #: F60
Conducting laboratory and location: Department of Biochemistry, and Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim
Date of study initiation: 1/20/1986
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Rhesus monkeys
N: 4/sex/group (1/sex in control group for blank samples)
Age/weight: Age: unknown; weight: ♂: 4.5 kg, ♀: 4.0 kg
Route: Oral by gavage
Dosage: 1, 8 or 60 mg/kg, qd x 52 weeks
Drug: WAL 801 CL (Batch VIII)
Methods:

The purpose of this TK assay was to determine the absorption of the drug, the steady state values, and urinary excretion during the study period. Blood samples were collected at 0 (prior to the dosing), 3 and 6 hr after administration in Weeks 1, 24 and 51. Urine samples (24-hr) were collected in Weeks 2, 25 and 47.

Results:

Plasma concentrations: Results (mean plasma concentrations from Weeks 24 and 51) are summarized in the table below. WAL 801 CL was detected in almost all plasma samples with the exception of several low dose samples collected prior to the dosing (< LOQ of [redacted] g/ml). The concentrations increased steeply as the doses increased. Generally speaking, in Weeks 24 and 51, the plasma concentrations were higher than in Week 1, indicating that the steady state was not reached in Week 1. No significant differences were noted between males and females.

Mean plasma concentrations (Weeks 24 and 51) of WAL 801 CL in monkeys (ng/ml, mean ± SD)

Dosage (mg/kg)	1	8	60
Prior to dosing	0.23	2.15± 0.88	240.99± 134.54
3 hr after dosing	12.8± 3.52	114.14± 22.16	1187.13± 395.62
6 hr after dosing	4.47± 2.01	44.15± 12.73	1789.58± 739.414

Urine excretion: Results are summarized in the table below. The excretion increased in a dose-dependent manner.

Mean urinary excretion of WAL 801 CL in monkeys

mg/24 hr	Week 2	Week 25	Week 47	Mean± SD (week 25 and 47)
1 mg/kg	0.30	0.31	0.44	0.38± 0.15
8 mg/kg	3.22	3.97	5.43	4.7± 1.53
60 mg/kg	26.22	54.09	46.06	50.1± 19.71
% dose/24 hr				
1 mg/kg	6.97	6.13	7.68	6.9± 2.42
8 mg/kg	8.34	9.06	11.61	10.3± 3.35
60 mg/kg	9.87	17.95	14.27	16.1± 4.45

U91-0332: Plasma concentrations and renal excretion of WAL 801 CL during a 1-year toxicity study in the rat administration with the feed. Vol. 17, Page 082

Key study findings: WAL 801 CL was absorbed in a dose-dependent manner after oral administration to rats. The plasma drug concentrations and renal excretion increased with rising doses super-proportionally. Higher values were noted in male rats.

Document #: U91-0332
Study #: F33
Conducting laboratory and location: Department of Biochemistry, and Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim
Date of study initiation: August 1, 1985
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Rats/Chbb:THOM
N: 5/sex/group
Age/weight: ♂: 59-day old, 256.4 g, ♀: 63-day old, 187.0 g
Route: Oral (by feed)
Dosage: 2, 10 or 100 mg/kg, qd x 52 weeks
Drug: WAL 801 CL (Batch #s: VI, VII and VIII)
Methods:

This TK assay was part of a one-year toxicity study using satellite animals. The purpose of this TK assay was to determine the absorption of the drug, the steady state values, and urinary excretion during the study period. Blood samples were collected at 7am and 11am (1 hr and 5 hr after the start of the light phase) in Weeks 5, 13, 26, 39 and 52. Samples of 24-hr urine were collected in Weeks 4, 12, 25, 38 and 51.

Results:

Plasma concentrations: Plasma drug concentrations at 2 mg/kg were below the LOQ level. No data about the LOQ were provided by the sponsor. Results from Weeks 25, 39 and 52

are summarized in the table below. No data from Weeks 5 and 13 were provided. Plasma drug concentrations increased more steeply with rising doses. The concentrations in male animals seemed higher than in female rats.

Mean plasma concentrations of WAL 801 CL in rats (ng/ml)

7am	Week	25	39	52	mean
Male	10 mg/kg	2.37	2.21	4.06	2.88
	100 mg/kg	87.00	91.75	246.25	141.67
female	10 mg/kg	1.97	1.35	2.20	1.84
	100 mg/kg	49.03	41.40	77.78	56.07
11am					
Male	10 mg/kg	1.69	1.87	2.81	2.19
	100 mg/kg	32.75	59.84	171.73	88.11
female	10 mg/kg	2.14	1.07	2.09	1.82
	100 mg/kg	35.79	30.14	69.87	45.27

Urine excretion: Results are summarized in the table below. The excretion increased in a dose-dependent manner. Only a small portion of the drug was renally excreted as parent compound. Male rats excreted more drug than female rats.

Mean urinary excretion of WAL 801 CL in rats

µg/24 hr	Dose (mg/kg)	Week 4	Week 12	Week 25	Week 38	Week 51	Mean
Male	2	3.84	2.77	3.14	3.71	3.53	3.38
	10	21.4	13.84	14.09	15.50	24.31	17.83
	100	620.5	513.81	569.86	860.01	1572.84	827.40
Female	2	1.98	2.18	2.40	2.21	1.70	2.09
	10	13.47	14.22	12.02	11.73	13.40	12.97
	100	272.23	284.38	342.61	232.45	411.64	310.58
% dose							
Male	2	0.59	0.33	0.33	0.36	0.31	
	10	0.63	0.32	0.30	0.30	0.45	
	100	1.88	1.27	1.24	1.72	2.99	
Female	2	0.45	0.43	0.44	0.38	0.28	
	10	0.61	0.55	0.44	0.40	0.44	
	100	1.31	1.24	1.38	0.87	1.47	

In conclusion, WAL 801 CL was absorbed in a dose-dependent manner after oral administration to rats. Plasma drug concentrations and renal excretion increased with rising doses super-proportionally. Higher values were noted in male rats.

U92-0409: Pharmacokinetic studies on ^{14}C-epinastine hydrochloride (WAL 801 CL) (I)-blood level, tissue distribution and urinary and fecal excretion after single and repeated administration in rats. Vol. 17, Page 128

Key study findings: ^{14}C-epinastine was rapidly absorbed after oral administration with bioavailability of 52-63%. Radioactivity was mainly distributed in the GI tracts, liver and kidneys. Radioactivity was removed mainly in feces. Urinary excretion was low. About 96% of the total dose was eliminated from the body 168 hr after the final dose.

Document #: U92-0409
Study #: Not indicated
Conducting laboratory and location: Kawanishi Pharma Research Institute, Department of Biochemistry, Nippon Boehringer Ingelheim Co. Ltd., 103 Takada Yato Kawanishi, Hyogo/Japan 666-01

Date of study initiation: Not indicated
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Rats/Sprague-Dawley
N: Not indicated (3-5/sex/time point)
Age/weight: 7-week old, 160-203 g for males and 100-162 g for females for single dose study (fasted rats); 182-247 g for males and 133-152 g for females for repeated dose study (fed rats)
Route: Intravenous and oral (by gavage)
Dosage: 1 mg/kg for iv and 5 mg/kg for oral, single dose
5 mg/kg/day oral for 14 days
Drug: ^{14}C-WAL 801 CL (Batch #: 8, 7100 kBq/mg, radiochemical purity: —, dissolved in saline (0.5 mg/ml) for iv dosing and in water (1 mg/ml) for oral administration
Methods: —

The purpose of this study was to determine PK profiles of epinastine in rats following a single oral or iv dose, and repeated oral administration of ^{14}C-epinastine hydrochloride.

Blood samples were collected from 3 to 5 animals each at 2, 15, 30 min and 1, 2, 3, 4, 6, 8, 10, 12 24, 48 and 72 hr (iv) or 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hr (po) after the single dose administration. In the repeated oral administration group, blood samples were collected at 0.5 and 4 hr after the 1st administration, immediately after each administration on days 2, 4, 6, 8, 10, 12 and 14, 30 min after each dosing on days 5 and 9, and at 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120 and 168 hr after the last dose.

For the measurement of tissue distribution of radioactivity and whole body autoradiography, animals were terminated at 0.5, 3, 10 and 48 hr after a single oral administration, and at 3, 10, 48 and 96 hr after the repeated oral dosing.

Urine and feces samples were collected in 24-hr fractions for 96 hr after a single administration. For repeated administration to rats, samples were collected every 24 hr during the treatment and up to 120 hr after the last dose, and from 120 hr to 168 hr.

Results:

Blood PK parameters: Results are summarized in the table below. After single oral administration, there was a second peak (— ng-eq/ml) at 3 to 4 hr after the dosing. In the repeated treatment group, the mean blood level at 24 hr after the 1st dose was 28.4 ng-eq/ml, and at 24 hr after the last dose was 176.0 ng-eq/ml. The ratio was 6.20.

Blood PK parameters in rats treated with ^{14}C-WAL 801 CL (mean ± SD)

		Cmax (ng-eq/ml)	Tmax (hr)	$AUC_{0-72\ hr}$ (ng-eq hr/ml)	T1/2 (hr)	Bioavailability (%)
IV (1 mg/kg)	M			1208.7± 108.4	56.8 ± 3.5	
	F			1028.8± 86.0	78.7± 17.8	
PO (5 mg/kg) Single dose	M	382.5± 82.2	0.5± 0.2	3796.0± 478.3	45.7± 2.0	62.8
	F	401.4± 42.9	0.3± 0.1	2652.6± 243.7	64.3± 10.1	51.6
PO (5 mg/kg) repeated dose		(mean Cmax) 117.8	3.2	2971.3	62.3	

Distribution: Tissue distribution data are summarized in the table below. Radioactivity levels in most tissues reached the maximum at 0.5 or 3 hr after single oral administration. In repeated group, high levels of radioactivity were observed from 3 hr to 10 hr after the last dose. In both single and repeated dose groups, high levels of radioactivity were noted in the GI tracts, liver, kidney, mesenteric gland, pancreas and adrenal gland. The levels were low in the thymus, skin, blood, muscle, eye, and brain. The radioactivity disappeared from tissues except the liver considerably at 48 hr in the single dose group.

Tissue concentrations of radioactivity after oral administration of ^{14}C-WAL 801 CL in rats (ng-eq/g or ml, mean ± SD)

3 hr after dosing	Single dose, Males	Single dose, Females	Repeated dose, Males	Repeated dose, Females
Blood	321.5± 83.5	212.5± 69.5	214.5± 7.5	215.5± 5.5
Plasma	347.0± 44.0	220.0± 39.0	218.0± 9.0	224.0± 5.5
Whole brain	74± 16	68.0± 13.0	107.5± 6.0	115.0± 9.0
Eye	185± 8.5	159.0± 53.5	103.0± 6.0	105.5± 13.0
Thymus	650.5± 88.5	610.0± 36.5	476.0± 101.0	585.5± 82.0
Heart	1402.5± 88.5	946.5± 365	451.0± 49.5	476.5± 58.0
Lung	1462± 308	1034.0± 359.5	913.0± 350.5	980.0± 222.0
Liver	7984.5± 1662.5	4852.5± 2054	2728.0± 806.0	2122.5± 527.5
Kidney	3833± 1520	2105.0± 791.5	1243.0± 228.0	929.0± 145.5
Adrenal gland	2577.5± 776	2101.0± 721.0	887.5± 199.5	1004.5± 57.5
Pancreas	4068.5± 550.5	3006.0± 871.0	2693.0± 703.5	1759.0± 749.5
Skin	391± 40.5	339.0± 84.0	294.5± 45.5	289.5± 41.0
Large intestine	9344.0± 4059.0	8468.5± 1016.0	26716.0± 7279.0	18843.5± 896.0
Cecum	11412.5± 7401.0	10946.0± 3855.5	19798.0± 4927.5	32589.0± 9983.5
Small intestine	67755.0± 12215.5	43662.0± 10937.0	21128.5± 1095.5	25014.0± 6383.5
Duodenum	17610.0± 7581.5	6705.0± 3044.0	4204.0± 751.5	4907.5± 1943.0
Stomach	3353.0± 286.0	4267.5± 1838.5	2594.5± 218.5	4381.5± 653.5
Mesenteric gland	4863.0± 277.0	3984.0± 283.5	1825.5± 337.0	1613.0± 340.0
Bone marrow	980.5± 221	638.0± 321.0	623.5± 59.5	592.5± 46.5

Excretion: Results for the single dose group are summarized in the table below. The major portion of the radioactivity was excreted within 24 hr. About 77% of radioactivity was eliminated in feces. For the repeated dose group, within 168 hr after the last dose, 7.9% and 88.1% of total dose were excreted in urine and feces, respectively.

Cumulative renal and fecal excretion of ^{14}C-WAL 801 CL in rats (% of dose, mean ± SD)

	Male			Female		
Time (hr)	Urine	Feces	Total	Urine	Feces	Total
0-24	20.98± 6.89	72.89± 9.68	93.87± 5.02	18.88± 4.13	74.15± 4.65	93.02± 1.98
0-48	21.29± 6.91	76.37± 7.02	97.66± 2.63	19.13± 4.21	77.01± 5.15	96.14± 0.95
0-72	21.43± 6.87	76.67± 6.76	98.10± 2.50	19.19± 4.20	77.13± 5.16	96.33± 0.96
0-96	21.52± 6.89	77.94± 4.79	99.45± 2.69	19.24± 4.19	77.27± 5.22	96.51± 1.04

In summary, ^{14}C-epinastine was rapidly absorbed after oral administration with bioavailability of 52-63%. Radioactivity was mainly distributed in the GI tracts, liver and kidneys. Radioactivity was removed main in feces. Urinary excretion was low. Approximately 96% of the total dose was eliminated from the body 168 hr after the final dose.

U92-0410: Pharmacokinetic studies on ^{14}C-epinastine hydrochloride (WAL 801 CL) (II)-dose-linearity, placental transfer, biliary excretion, entero-hepatic circulation, transfer into milk, protein binding and metabolism after single administration in rats. Vol. 17, Page 175

Key study findings: The Cmax and AUC increased proportionally with dose. Urinary and fecal excretion was constant and independent of dose. WAL 801 CL and its metabolites passed the placenta to a low extent. Biliary excretion was about 20%. In the entero-hepatic circulation study, 13% of the dose was re-excreted in bile. The radioactivity was transferred into the stomach of pups via milk. Plasma protein binding rate was 66-67% *in vitro*, and 63-90% *in vivo*. Slightly higher binding (85-90%) was observed at 10 and 48 hr after oral administration. About 60% of radioactivity in urine after oral administration was unchanged WAL 801.

Document #: U92-0410
Study #: Not indicated
Conducting laboratory and location: Kawanishi Pharma Research Institute, Department of Biochemistry, Nippon Boehringer Ingelheim Co. Ltd., 103 Takada Yato Kawanishi, Hyogo/Japan 666-01
Date of study initiation: Not indicated
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Rats/Sprague-Dawley
Age/weight: 7-week old, 160-203 g for males and 240-335 g for females (Female rats on the 12th day and 18th day of pregnancy and those on the 14th day of delivery were used for the experiments of placental transfer and transfer into milk, respectively, in a fed condition. In other experiments, 7-week-old fasted male rats were used.)
Route: Oral (by gavage)
Dosage: 5 mg/kg (in the dose-linearity study: 5, 10 and 30 mg/kg)
Drug: ^{14}C-WAL 801 CL (Batch #: 8, 7100 kBq/mg, radiochemical purity: [redacted], dissolved in water
Methods: [redacted]

The purpose of this study was to determine the dose-linearity, placental transfer, biliary excretion, entero-hepatic circulation, transfer into milk, protein binding and metabolism after single administration of ^{14}C-epinastine hydrochloride.

Blood samples were collected from 5 animals/time point at 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hr after the administration.

Urine and feces samples were collected from 3 rats/dose in 24-hr fractions for 72 hr after the single administration, and then from 72 hr to 96 or 144 hr thereafter.

Animals designated for the measurements of placental transfer and whole body autoradiography were sacrificed at 0.5, 3, 10 and 48 hr (3 rats/time point) after oral administration.

For biliary excretion assay, bile samples were collected every 30 min up to 4 hr and every 1 hr from 4 hr to 10 hr, then from 10 hr to 24 hr and from 24 hr to 48 hr after oral administration. In the entero-hepatic circulation experiment, a pooled bile fraction, which was collected from 3 animals in a period of 0 to 4 hr after oral administration, was administered into the duodenum of other rats at a volume of 3 ml/kg. Bile samples were collected every 30 min up to 4 hr and every 1 hr from 4 hr to 8 hr, then from 8 hr to 24 hr and from 24 hr to 48 hr.

For the measurement of transfer into milk, pups were suckled (two at each time point) at 1, 2, 4, 6, 8, 24 and 48 hr after dosing to female rats on the 14th day of delivery, and the radioactivity of the stomach contents in pups was measured.

In the *in vitro* protein binding assay with the [redacted] method, ^{14}C-WAL 801 CL was added to male rat plasma (3/group) at final concentrations of 25, 250 and 2500 ng/ml, and plasma samples were incubated at 37°C for 30 min. Radioactivity in the filtrate containing unbound drugs was measured. In the *in vivo* protein binding assay, blood samples were collected at 0.5, 3, 10, and 48 hr (3 rats/time point) after oral administration of ^{14}C-WAL 801 CL.

The metabolites were separated and determined in the 10-hr bile and 24-hr urine using [redacted]

Results:

Dose-linearity: Blood PK parameters and urinary and fecal excretion data are summarized in the tables below. Blood concentrations increased with dose proportionally. Most of the radioactivity was eliminated from the body within 24 hr after dosing.

Blood PK parameters in rats treated with ^{14}C-WAL 801 at 5, 10 and 30 mg/kg (mean ± SD)

Dose (mg/kg)	5	10	30
Cmax (ng-eq/ml)	382.5± 82.2	862.4± 121.7	2524.2± 514.4
Tmax (hr)	0.5± 0.2	0.5± 0	0.6± 0.3
T1/2 (hr)	45.7± 2.0	55.8± 3.6	49.7± 3.1
AUC_{0-72} (ng-eq hr/ml)	3796.0± 478.3	10489.7± 644.5	36637± 2793.2

Cumulative renal and fecal excretion of ^{14}C-WAL 801 CL in rats (% of dose, mean ± SD)

Dose	5 mg/kg			10 mg/kg			30 mg/kg		
Time	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
0-24 hr	21.0±6.9	72.9±9.7	93.9± 5.0	32.9±3.2	61.9±4.8	94.7±2.0	34.9±7.1	58.1±8.5	93.0±2.3
0-48 hr	21.3± 6.9	76.4±7.0	97.7±2.6	33.3±3.2	63.7±5.0	97.0±1.8	35.8±7.7	60.2±8.4	95.9±1.7
0-72 hr	21.4± 6.9	76.7±6.8	98.1± 2.5	33.4±3.3	64.0±5.0	97.4±1.8	36.1±8.0	60.4±8.4	96.6±1.3
0-96 hr	21.5± 6.9	77.9±4.8	99.5±2.7						
0-144 hr				33.6±3.4	64.3±4.9	97.9±1.6	36.7±8.5	60.7±8.4	97.5±0.8

Placental transfer and whole body autoradiography: The tissue distribution data related to the placental transfer on days 12 and 18 of pregnancy are summarized in the table below. The radioactivity levels were found in the ovary, uterus, placenta, fetus and amniotic fluid. The level in the fetus reached the maximum at 3 hr. The concentration in the fetal liver was high among the fetal tissues on the 18th day of pregnancy; however, the radioactivity in the fetal liver decreased nearly to the background level at 48 hr. The distribution of the radioactivity in other tissues was similar to that in the non-pregnant rats.

Tissue concentrations of radioactivity after oral administration of ^{14}C-WAL 801 CL in pregnant rats (ng-eq/g or ml, mean ± SD)

	Pregnant day 12		Pregnant day 18	
	3 hr	48 hr	3 hr	48 hr
Blood	409.5± 62.0	22.5± 0.5	407.5± 99.5	20.5± 0.5
Plasma	405.0± 35.5	23.0± 2.0	427.5± 112.0	23.0± 1.0
Ovary	2083.5± 444.5	48.0± 3.0	2772.5± 933.5	47.5± 4.0
Uterus	3365.5± 181.5	42.0± 5.5	3127.5± 989.5	40.0± 12.0
Fetus	256.5± 123.5	31.0± 2.5	235.5± 40.0	34.5± 1.0

Amniotic fluid	45.5± 9.5	3.0± 1.5	36.0± 5.0	6.5± 0.5
Placenta	1166.0± 155.0	56.0± 10.0	1134.0± 454.0	49.0± 3.0
Carcass	1956.0± 272.5	17.0± 2.0	2413.0± 275.5	21.0± 2.0
Fetal liver			372.5± 15.5	50.5± 16.5

Biliary excretion and entero-hepatic circulation: The cumulative biliary excretion after single oral administration reached a plateau level of 19.5% of the dose within 48 hr after administration. The biliary excretion after duodenal administration of the bile, which was obtained from other rats used for the biliary excretion study, was 13.0% of the intra-duodenal dose within 48 hr.

Transfer into milk: The time-course of radioactivity in the stomach contents of pups after oral administration of ^{14}C-WAL 801 CL to dams was similar to the blood profiles in the dams. Radioactivity in the stomach contents was the largest (—. ng-eq) when the pups were suckled during 1.5 and 2 hr after administration. Then the radioactivity decreased in a similar way to that in the blood of dams. The values at 24 hr and 48 hr were — and — ng-eq, respectively.

Protein binding: The *in vitro* assay (see table below) showed that the plasma protein binding of ^{14}C-WAL 801 CL in a range of 25 - 2500 ng/ml was constant, 66 - 67%.

***In vitro* rat plasma protein binding of ^{14}C-WAL 801 Cl (mean ± SD)**

Concentration (ng-eq/ml)	% bound
25	66.5 ± 0.8
250	67.2 ± 1.9
2500	65.9 ± 0.6

In the *in vivo* assay, protein binding at 0.5 hr after administration was about 63%, which was similar to that *in vitro* (see table below). Afterwards, the binding increased as the concentrations decreased, that was, about 74%, 90% and 85% at 3, 10 and 48 hr, respectively.

***In vivo* rat plasma protein binding of ^{14}C-WAL 801 Cl (mean ± SD)**

Time (hr)	Concentration (ng-eq/ml)	% bound
0.5	513.5± 172.0	62.6± 2.6
3	347.0± 44.0	73.9± 4.6
10	151.5± 19.0	89.5± 0.7
48	27.0± 2.5	84.9± 6.4

Metabolism: The proportion of unchanged WAL 801 CL and its metabolites to the total radioactivity in 0-24 hr urine and 0-10 hr bile after single oral administration is summarized in the table below. About 60% of the radioactivity in urine were unchanged drug. In bile, most of the radioactive components were polar compounds, which remained at the origin on TLC assay. The proportion of unchanged drug was less than 10%.

Metabolic pattern of radioactivity in 0-24 hr urine and 0-10 hr bile (% of total radioactivity)

	Urine		Bile	
	WAL 801	Others	WAL 801	Others
Before hydrolyzed	59.2	23.2	10.0	82.8
Hydrolyzed with β-glucuronidase	55.1	30.3	5.2	90.0
Hydrolyzed with arylsulfatase	58.1	28.1	4.2	73.9

In summary, in the dose-linearity assay, Cmax and AUC increased proportionally with dose and the half-lives were constant. Urinary and fecal excretion was constant and independent

of dose. WAL 801 CL and its metabolites passed the placenta to a low extent. Biliary excretion was about 20%. In the entero-hepatic circulation study, 13% of the dose was re-excreted in bile. The radioactivity was transferred into the stomach of pups via milk. Plasma protein binding rate was 66-67% *in vitro* at concentrations ranging 25-2500 ng/ml, and 63-90% *in vivo*. The *in vivo* protein binding was increased with decreased plasma WAL801 CL concentration. Slightly higher binding (85-90%) was observed at 10 and 48 hr after oral administration. About 60% of radioactivity in urine after oral administration was unchanged WAL 801.

U94-0295: Absorption, distribution and excretion of ^{14}C-WAL 801 CL after ophthalmic administration in rabbits. Vol. 17, Page 252

Key study findings: Radioactivity was rapidly absorbed after both oral and ocular administration. The main route of excretion was fecal excretion. High concentrations of radioactivity were noted in cornea and conjunctiva. The elimination in lens was slow.

Document #: U94-0295
Study #: BCA9402
Conducting laboratory and location: Kawanishi Pharma Research Institute, Dept. of Biochemistry, Nippon Boehringer Ingelheim Co. Ltd., 103 Takada Yato Kawanishi, Hyogo/Japan 666-01
Date of study initiation: Not indicated
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Male Japanese white rabbits
Age/weight: 1.6-2.0 kg
Route: Ocular, topical and oral (by gavage)
Dosage: 5 mg/kg for oral and 20 µl both eyes (200 µg/animal), single dose
Drug: ^{14}C-WAL 801 CL (Batch #: 9, 601 MBq/mg, radiochemical purity: , dissolved in water (1 mg/ml) for oral dosing and in PSS (5 mg/ml, 0.5%) for ocular dosing
Methods: , X-ray film, and

The purpose of this study was to determine the absorption, ocular distribution and excretion of ^{14}C-epinastine hydrochloride after a single ocular topical administration or oral administration.

Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 48 hr (3 animals/time point) after administration.

Urine and feces samples were collected in 24-hr fractions for 72 hr after the single administration, and from 72 hr to 120 hr. Expired air was also collected.

For the examination of semimicro autoradiography of the eye and radioactivity concentrations in the eye, animals were terminated at 0.25, 0.5, 1, 4, 8 and 24 hr after dosing. The eyeballs were removed and processed for examination.

Results:

Blood and plasma PK parameters after oral and ocular administration are summarized in the table below.

Blood and plasma PK parameters in rabbits treated with ^{14}C-WAL 801 orally or topically

(mean ± SD)	Cmax (ng-eq/ml)	Tmax (hr)	T1/2 (hr)	$AUC_{0-\infty}$ (ng-eq hr/ml)
Oral (5 mg/kg), blood	187.23± 85.36	0.5	57.75± 7.41	2252.36± 674.13
Plasma	308.77± 135.04	0.5	36.41± 11.89	2607.35± 479.8
Ocular (200 µg/animal), blood	26.82± 15.68	0.25	62.09± 8.25	124.40± 48.33
Plasma	19.91± 10.56	0.25	46.61± 11.63	129.62± 55.73

The urinary, fecal and expirational excretion of radioactivity is summarized in the table below. The main excretion route after both oral and ophthalmic administration was fecal excretion.

Cumulative renal, fecal and expirational excretion of ^{14}C-WAL 801 CL in rabbits (% of dose, mean ± SD)

	Oral				Ophthalmic			
Time	Urine	Feces	Expired air	Total	Urine	Feces	Expired air	Total
0-24 hr	4.6±1.1	72.6±8.1	0.21±0.03	77.4±7.4	23.5±8.2	41.5±7.4	0.44±0.11	65.4±3.4
0-48 hr	5.7± 0.6	85.5±1.7	0.25±0.04	91.4±1.4	25.9±7.5	60.8±8.8	0.55±0.07	87.3±4.1
0-72 hr	6.2±0.6	88.0±1.9	0.7±0.05	94.5±1.3	26.5±7.6	67.4±8.1	0.60±0.06	94.5±0.7
0-120 hr	6.6±0.9	90.1±1.3	0.8±0.06	97.0±1.4	26.8±7.4	70.2±7.8	0.62±0.05	97.6±1.1

Results of semimicro autoradiography of the eye showed that high levels of radioactivity were located in cornea, conjunctiva, anterior sclera. Middle and low levels were noted in ciliary body, iris, aqueous humor, choroid and extraocular muscle.

The concentrations of radioactivity in the eye are summarized in the table below. High levels of radioactivity were observed in the cornea, conjunctiva, iris, ciliary body and sclera. Elimination in the lens (Cmax = [redacted]-eq/g) was slow.

Tissue concentrations of radioactivity after ocular administration of ^{14}C-WAL 801 CL in rabbits (ng-eq/g or ml, mean ± SD)

	15 min	1 hr	4 hr	24 hr
Blood	29.66±9.06	12.50±3.74	2.81±0.87	0.66±0.17
Plasma	21.90±6.71	14.15±2.97	4.64±1.47	0.68±0.24
Aqueous humor	59.18±12.03	52.43±10.00	48.17±6.32	2.53±0.51
Conjunctiva	7010.24±2071.09	2199.35±766.60	279.08±87.10	30.04±1.86
Cornea	8509.89±1676.92	4397.40±276.11	2096.46±13.66	143.74±27.43
Iris	312.83±51.80	454.85±78.85	356.43±30.33	25.03±4.30
Ciliary body	193.81±64.91	175.86±58.06	141.88±50.45	9.71±3.51
Lens	8.62±2.98	9.99±3.06	16.31±4.46	17.21±3.58
Vitreous body	0.35±0.12	0.48±0.17	0.29±0.10	0.09±0.02
Sclera	124.63±45.66	140.68±56.38	91.72±30.92	3.09±1.61
Retina	47.14±19.61	21.69±5.47	8.60±2.69	1.20±0.18
Choroid	135.17±51.76	87.90±23.22	46.28±17.65	2.06±0.37
Optic nerve	16.32±5.61	8.64±1.51	5.58±1.57	0.54±0.17

In summary, after oral and ocular administration of ^{14}C-epinastine HCl, radioactivity was rapidly absorbed. The main route of excretion was fecal excretion after both oral and ocular administration. High concentrations of radioactivity were noted in the cornea and conjunctiva following topical ocular application. Elimination in lens was slow.

U96-0004: WAL 801 CL (0.3%; w/v) ophthalmic solution: Determination of pharmacokinetic parameters and bioavailability after: --a single instillation into the conjunctival sac of pigmented rabbit eyes, --a single intravenous administration in pigmented rabbits. Vol. 17, Page 379

Key study findings: After iv administration, WAL 801 was eliminated very rapidly from the plasma. After ocular administration, the systemic exposure was low with bioavailability of 53%.

Document #: U96-0004
Study #: 08993
Conducting laboratory and location: [redacted]

Date of study initiation: 12/27/1993
GLP compliance: Yes
QA report: Yes (X) No ( )
Species/strain: Rabbits/Fauve de Bourgogne
Age/weight: 4-month old, 2-2.5 kg
N: 6 (3/sex)
Route: Ocular, topical and intravenous injection
Dosage: 50 µl, right eye only, single dose (150 µg/animal) for ocular instillation
0.15 mg/ml, iv, single dose (150 µg/animal) for iv injection
Drug: WAL 801 CL (0.3%) eye drop solution (Batch #: F 3048)
Methods: [redacted]
Study design:

Group	Animal number	Day 1	Day 7
1	3 males	Ocular instillation of WAL 801	Intravenous injection of WAL 801
2	3 females	Intravenous injection of WAL 801	Ocular instillation of WAL 801

The purpose of this study was to determine PK parameters of epinastine hydrochloride 0.3% in rabbit blood after a single ocular topical administrations of epinastine hydrochloride (0.3%) into the right eye, and after a single iv injection of the same amount of WAL 801 0.3% in the same rabbits. Blood samples were collected from 3 animals/time point at 5 min, 10 min, and 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hr after each iv injection, and at 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hr after each ocular instillation.

Results:

PK parameters are summarized in the table below. No differences were noted between male and female animals. After iv administration, WAL 801 was eliminated very rapidly from the plasma. After ocular administration, Cmax was [redacted] and the bioavailability was 53%.

Mean PK parameters in plasma after a single dose of iv or topical administration in rabbits

Intravenous injection	Topical ocular administration		
$AUC_{0-8\ hr}$ (ng-hr/ml)	Tmax (min)	Cmax (ng/ml)	$AUC_{0-8\ hr}$ (ng-hr/ml)
114.0±27.0	1.3±0.4	4.6±1.2	60±14

In summary, rabbits were treated topically or intravenously with WAL 801 eye drops at the same dose (150 µg/animal). No differences were noted between male and female animals. After iv administration, WAL 801 was eliminated very rapidly from the plasma.

U97-2105: Epinastine (WAL 801 CL): 4-week local tolerance study of WAL 801 CL eye drops by instillation into the conjunctival sac of rabbits: Concentrations in aqueous humor and plasma on day 28. Vol. 18, Page 001

Key study findings: High exposure to the drug was noted in the treated eyes. The systemic absorption of WAL 801 CL was low.

Document #: U97-2105
Study #: BB2A13
Conducting laboratory and location: Department of Pharmacokinetics and Drug Metabolism, Boehringer Ingelheim KG, D-55216 Ingelheim/Rhein, Germany
Date of study initiation: 12/15/1992
GLP compliance: Yes
QA report: Yes (X) No ( )
Species/strain: Rabbits/Himalayan
Age/weight: 5-month old, 2-2.7 kg for males and 1.9-2.6 kg for females
N: 2/sex/dose group
Route: Ocular, topical, right eye only (The left eye remained untreated.)
Dosage: 50 µl, 6 times/day (at 45-min intervals) x 28 days
Drug: WAL 801 CL (0%, 0.1%, 0.3% and 0.5%) eye drops (Batch #s: F 3047, F 3048 and F 3049)
Methods: [redacted]

The purpose of this study was to determine the exposure of epinastine hydrochloride in rabbit plasma and aqueous humor at the end of a 4-week local tolerance study. Blood and aqueous humor samples were collected from 2 animals/sex/dose group at 30 min after the last ocular instillation on day 28.

Results:

Results are summarized in the table below. WAL 801 CL was seen in all aqueous humor samples from the treated eyes, and in plasma from MD and HD groups. The drug concentrations were below the limit of quantitation ([redacted] ng/ml) in aqueous humor from untreated eyes, and in LD animal plasma.

Plasma and aqueous humor concentrations of WAL 801 in rabbits (geometric mean)

Treatment	Aqueous humor (ng/ml), right eye	Aqueous humor (ng/ml), left eye	Plasma (ng/ml)
0.1%	69.4	BLQ	BLQ
0.3%	1125.2	BLQ	1.9
0.5%	1074.9	BLQ	3.2

In summary, in rabbits treated topically with WAL 801 CL eye drops for 4 weeks, high exposure to the drug was noted in the treated eyes. The systemic absorption of WAL 801 CL was low.

Distribution:

PK-01-051: *In vitro* binding of ^{14}C-epinastine to ocular bovine melanin. Vol. 15, Page 182

Key study findings: Epinastine reversibly bound to bovine ocular melanin *in vitro*. The mean percentage of ^{14}C-epinastine bound to melanin up to 24 hr ranged from 48.1% to 90.3% over the concentration range of 0.1 to 30 µM with saturable bindings at high concentrations.

Report #: PK-01-051
Study #: PK-01-P002
Conducting laboratory/location: Allergan, 2525 Dupont Drive, Irvine, CA 92612
Date of study initiation: 3/5/2001
GLP: No
QA report: Yes ( ) No (X)

Drug: ^{14}C-epinastine (Lot #: CFQ12116, 55 mCi/mmol, 191 µCi/mg)
Epinastine (Lot #: R12070)
Methods: The binding of ^{14}C-epinastine to melanin was determined *in vitro* by [redacted]. The melanin suspensions (1 mg/ml in phosphate buffer) spiked with ^{14}C-epinastine over the concentration range of 0.1 to 30 µM were incubated at 37°C for 0, 2, 4, 6, and 24 hr. Radioactivity was measured in the supernatant (free concentration) and melanin suspension by [redacted]. The reversibility of binding was examined by repetitive extraction of the incubated melanin suspension with phosphate buffer.

Results:

The table below summarizes the percent bound of epinastine to melanin suspensions at various drug concentrations. The mean values ranged from 48.1 to 90.3%. The binding appeared to reach equilibrium after 2 hr of incubation. Differences in binding among different drug concentrations were found, indicating saturable binding. After seven wash cycles, quantitative recovery of the radioactivity was removed from the melanin pellets of the 24-hr 10 and 30 µM incubations, indicating that binding to melanin was reversible.

Percent of ^{14}C-epinastine bound to bovine melanin (%, mean ± SD)

^{14}C-epinastine (µM)	0 hr	2 hr	4 hr	6 hr	24 hr
0.1	85.8± 1.5	89.2± 1.5	88.9± 1.5	90.3± 1.1	89.7± 0.8
0.3	8.35± 1.1	88.0± 1.7	88.5± 1.3	88.1± 1.2	87.1± 0.8
1	79.3± 0.8	84.8± 0.7	85.2± 1.2	85.0± 1.1	83.3± 1.3
3	74.4± 1.9	79.6± 1.7	77.8± 7.2	77.8± 7.2	78.9± 1.5
10	61.8± 2.2	69.6± 2.4	71.2± 2.0	71.2± 2.0	68.2± 2.7
30	48.1± 2.7	59.6± 1.0	61.1± 1.8	61.1± 1.8	57.6± 0.7

PK-01-077: ^{14}C-epinastine hydrochloride ocular tissue distribution studies in cynomolgus monkeys following a single ophthalmic administration. Vol. 15, Page 200

Key study findings: Following a single 35 µl ocular administration of ^{14}C-epinastine HCl to the monkey (both eyes), high radioactivity was measured in the surface tissues (eyelids, bulbar conjunctiva, cornea and sclera). The radioactivity in most intraocular tissue was low but was high in pigmented tissues (iris and ciliary body), suggesting melanin binding of the drug. After a single unilateral administration, very low concentrations of radioactivity were detected in the untreated eye, suggesting that the radioactivity could reach the untreated eye through systemic circulation. The plasma radioactivity concentrations were very low.

Report Nº: PK-01-077
Study Nº: PK-00-P001
Conducting laboratory/location: [redacted]

Study initiation: 2/21/2001
GLP: Yes
QA report: Yes (X) No ( )
Drug: ^{14}C-epinastine (Batch #: NPE/ALG083/8, 55 mCi/mmol, 191 µCi/mg, radiochemical purity: [redacted]
Epinastine HCl (Batch #: R12328, purity: [redacted]
Methods: [redacted] for radioactivity concentrations (LOQ: [redacted]

Dosing
Species/strain: Male cynomolgus monkeys, 2 to 3 years old, 2.70-3.04 kg
N: 7
Frequency: Single dose
Route: Ocular topical
Volume: 35 µl, 0.05% ^{14}C-Epinastine HCl, both eyes for 6 animals (Phase A) and one eye (right eye only) for the other 1 animal (Phase B)
Observations and times: Animals in Phase A were terminated at 0.5, 1, 2, 4, 8 and 24 hr after dosing (1 animal/time point). The Phase B animal was terminated at 1 hr after dosing. Ocular tissues and blood samples were collected.

Results:

Phase A: Results are summarized in the table below. After bilateral ocular administration, radioactivity was similar in the left and right eyes. High concentrations of radioactivity were noted in the eyelids, conjunctiva, cornea and sclera. In the intraocular tissues, high radioactivity concentrations were noted in the iris and ciliary body, suggesting melanin binding of the drug. Concentrations in the plasma were very low.

Ocular distribution of ^{14}C-epinastine radioactivity (bilateral administration)

	Cmax (ng-eq/g or ml)			Tmax (hr)	
Tissue	Left eye	Right eye	Mean (both eyes)	Left eye	Right eye
Eyelids	1135	1954	1545	1	2
Lower bulbar conjunctiva	322.7	535.5	429.1	0.5	1
Upper bulbar conjunctiva	415.7	588.5	502.1	0.5	0.5
Cornea	278.9	429.3	354.1	0.5	2
Sclera	121.7	92.2	107.0	1	2
Aqueous humor	1.545	2.626	2.086	4	4
Iris	1138	1013	1076	24	24
Ciliary body	121.3	48.9	85.1	24	24
Lens	1.97	1.98	1.978	24	4
Choroid and retina	28.17	18.43	23.3	24	2
Vitreous humor	0.888	0.222	0.555	0.5	1
Optic nerve head	6.934	5.545	6.194	2	2
Lacrimal glands	29.32	38.65	33.99	0.5	2
Plasma	0.44			2	