Methods: Groups of male rats (5/group) were given oral doses of 100 and 400 mg/kg/day of E 3810 for 7 days. A comparator group was also included which received 400 mg/kg/day of omeprazole in similar fashion. The control group animals received the vehicle (0.5% methylcellulose) in similar fashion. The volumes of administration was not indicated. In order to "increase oral bioavailability", all rats were starved for 19 hrs every day and food was given only for 5 hr/day (11:00 AM to 4:00 PM). This restricted food regimen started from 7 days prior to initiation of the study. Blood samples were collected (time was not specified) on days 1, 3 and 7 of the study from jugular vein/ abdominal acrta for measuring plasma gastrin and TSH levels. Additionally, total cholesterol, HDL-cholesterol, phospholipids, triglyceride, T4 and T3 levels were also monitored in plasma samples obtained on day 7 of the study. At the end of study period all animals were sacrificed, liver and thyroid were weighed. Liver microsomes were obtained and levels of microsomal protein, cytochrome b5, cytochrome P450, CYPP1A1, CYP2B1/2, CYP3A, and activities of aminopyrine N-demethylase, benzphetamine N-demethylase, aniline hydroxylase, p-nitroanisole O-demethylase, UDP-glucuronyl transferase (UDP-GT) and de-iodination of T4 were measured.

Results:

1. **Blood Chemistry**: A dose of 100 mg/kg/day of E 3810 had no significant effect on any of tested parameters. Plasma gastrin level was increased by 440% on day 1 in 400 mg/kg/day E 3810 treated rats by the end of 7 days of treatment increase in gastrin level was only 27% over the control values. Omeprazole (400 mg/kg/day) treatment produced increased gastrin levels (645%-699%) throughout treatment period. E 3810 (400 mg/kg/day) produced transient increases in plasma TSH levels (day 1 = 49%, day 2 = 77% and day 7 = 38%) while omeprazole had no significant effect on plasma TSH levels.

2. **Hepatic Drug Metabolizing Activities**: At 100 mg/kg/day of E 3810, liver cytochrome 450 content was decreased by 15%, while liver microsomal UDP-GT and p-nitroanisole O-demethylase activities were increased by 230% and 30% respectively when compared to control values. At 400 mg/kg/day of E 3810, liver cytochrome 450 content was decreased by 19%, while increases in cytochrome b5 levels (32%) and in liver microsomal UDP-GT (529%), aniline hydroxylase (20%), p-nitroanisole O-demethylase (43%) activities were seen when compared to control values.

At 400 mg/kg/day of omeprazole, increases in liver cytochrome 450 (16%) and cytochrome b5 (54%) content and liver microsomal UDP-GT (345%), aminopyrine N-demethylase (42%), benzphetamine N-demethylase (50%), aniline hydroxylase (46%), p-nitroanisole O-demethylase (87%) activities were seen when compared to control values.

Neither E 3810 nor omeprazole (400 mg/kg/day for 7 days), had any significant effect on in vitro conversion of T4 to T3.

According to sponsor, CY1A1 and CYP2B1/2 isozymes of P450 were induced by the treatment of E 3810 or omeprazole (400 mg/kg/day for 7 days) and the induction of CYP2B1/2 was more marked in the omeprazole treated rats and E 3810 treated rats.

3. Organ Weights: At high dose of E 3810, relative liver and thyroid weights were increased by 30% and 13% respectively, when compared to the control values. In omeprazole treated rats, liver and thyroid weights were increased by 15% and 64% respectively, when compared to the control values.

In this study data clearly indicted that both E 3810 and omeprazole (400 mg/kg/day for 7 days) induces various hepatic drug metabolizing enzymes including UDP-glucuronyl transferase (UDG-GT) activities (E 3810: 529% and omeprazole: 345%). Increased liver weights seen in E 3810 and omeprazole treated rats could be related to induction of hepatic drug metabolizing enzymes. Minor increases seen in thyroid weights in E 3810 treated rats could be related to transient increase in plasma TSH levels.

RABBIT:

Plasma Levels After Administration of E3810 in Rabbits
(870531)

Methods: To study the pharmacokinetic profile of E3810 in rabbits, E3810 was given to female rabbits orally or intraduodenally at 100 mg/kg or intravenously at 50 mg/kg. The plasma levels of E3810 were determined before dosing at 5, 15, 30, 60, 90, 120 and 240 minutes using HPLC-UV system.

Results: The plasma level of E3810 declined quickly to below detectable limits within 2 hours after i.v. dosing. The plasma level of E3810 was very low after oral dosing. The bioavailability was very low (0.36%) following oral administration and much higher (86.44%) following intraduodenal dosing. This information was summarized in Table A-15-1 on page 80 in volume 1.12 and this table is attached below.

Table A-15-1: Mean Pharmacokinetic Parameters of E3810 in Rabbits

Dose/Route (mg/kg)	C_{max} (μg/mL)	T_{max} (min)	k_e (1/hr)	AUC $_{(0-\infty)}$ μg.hr/mL	Systemic availability (%)	Relative bioavailability (po/id %)
50/ iv			1.269±0.42	15.12*	100	
100/po	0.22±0.14	5.00	2.541	0.11±0.05	0.36	0.43
100/po**	0.61±0.58	5.00	4.134	0.24±0.21	NC	0.92
100/id	38.66±6.22	5.00	0.8837±0.19	26.14±7.33	86.44	100

Data are expressed as mean values of 2 - 6 rabbits ± SEM
** (n=2) The mean ± SEM $AUC_{(0-\infty)}$ value is 11.6 ± 3.68 when all 3 animals are included*
*** intravenous E3810 (50mg/kg) administered on previous day*
NC: Not calculated

DOG:

A Study of the Absorption, Metabolism and Excretion Following Oral Administration to the Dog
(CHE726/14)

Methods: To study the pharmacokinetics of E3810, ^{14}C-E3810 was given to fasted dogs by oral gavage at 8 mg/kg (specific radioactivity = 4.44 MBq/mg). The radioactivity in the plasma, urine and feces were determined using liquid scintillation counter.

Results: The maximum plasma level of radioactivity was detected at 30 minutes after dosing (5.8 μg eq./g). The radioactivity was recovered mainly in the feces (53%) and urine (33%). In the non-fasted females, the majority of radioactivity was recovered in feces (63%) and urine (28%).

In Vitro Interaction With Human Liver Cytochrome P-450

Methods: Two major oxidative metabolites (desmethyl LY307640 and LY307640 sulfone) of E3810 were formed via cytochrome P-450 system in animals and human. In this study sponsor tried to identify isoenzyme form(s) of cytochrome P-450 which are responsible for E3810 metabolism in vitro using human liver microsomes and specific inhibitor of various isozymes.

Results: CYP3A form of human cytochrome P-450 catalyzes the conversion of LY307640 (E3810) to LY307640 sulfone while CYP2C19 form is mainly involved in the formation of desmethyl LY307640 (E3810). According to Andersson et al (Br. J. Clin. Pharmacol. 36:521-530, 1993) these two isozymes (CYP3A and CYP2C19) of cytochrome P-450 are also responsible for forming major metabolites of omeprazole.

Sponsor also assessed the effects of LY307640 (E3810) and omeprazole on the metabolism of drug(s) which utilizes CYP3A and/or CYP2C19 form of P-450. CYP2C19 is known to mediate S-Mephentoin 4'-hydroxylation, both LY307640 and omeprazole competitively inhibited hydroxylation reaction with Kiapp of 9.2 ± 1.0 and 4.1 ± 0.4 μM respectively. Kiapp value indicated that LY307640 is less potent inhibitor of CYP2C19 mediated reaction than omeprazole. Inhibition of CYP3A mediated Midazolam 1'-hydroxylation in the presence of LY307640 (E3810) or omeprazole was comparable (Kiapp: LY307640 = 59.4 ± 6.0 μM and omeprazole = 43.6 ± 5.7 μM).

Addendum: (1) the report number is #1 and (2) the testing laboratory is Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.

Pharmacokinetics of E3810 and Its Optical Isomers in Dogs (Report # F-16)

Methods: Male beagle dogs (n=6/group) were given a single dose of E3810 (racemic mixture, 3 mg/kg), R(+)E3810 (1.5 mg/kg) or S(-)E3810 (1.5 mg/kg). The volume of administration was fixed at 0.5 ml/kg. Blood samples were collected from saphenous vein at 5, 15, 30, 35 min, 1, 1.5, 2, 3, 4 and 6 hr after drug administration. The levels of optical isomers of E3810 and their metabolites in dog plasma were monitored by HPLC-UV methods and various pharmacokinetic parameters were calculated.

Results: The plasma levels of unchanged drug E3810 (racemic), R(+)E3810 or S(-)E3810) decreased rapidly. However, S-isomer was eliminated more rapidly than R-isomer since $t_{1/2}$ of S-isomer was shorter (about 14 min) than that seen with R-isomer (about 24 min) and the total clearance from plasma was about 2-fold greater than that of the R-isomer. The volumes of distribution of two isomers were similar. There were no significant chiral conversion in vivo (about 0.8-4.2%). The thioether (M1),

demethylated derivative (M4) and sulfone derivative (M2) as metabolites were seen in plasma (represented as peaks). Similar results were seen when racemic mixture was given to dogs.

Table 3 Comparison of pharmacokinetic parameters of E3810 enantiomers after intravenous administration of R(+)E3810, S(-)E3810 or RS(±)E3810 to male beagle dogs

Table 3-1 Comparison of pharmacokinetic parameters of E3810 enantiomers after intravenous administration of R(+)E3810 or S(-)E3810

Species	Enantiomer	Dose (mg/kg)	t1/2 (min)	Vd (ml/kg)	Cl tot (ml/min/kg)	AUC (μg min/ml)
Beagle dog	R(+)E3810	1.5	24.2	498	14.8	105
	S(-)E3810	1.5	14.6	590	28.3	54
	R/S ratio		1.66	0.84	0.52	1.9

Data shows mean of 6 beagle dogs.

Table 3-2 Comparison of pharmacokinetic parameters of E3810 enantiomers after intravenous administration of RS(±)E3810

Species	Enantiomer (mg/kg)	Dose	t1/2 (min)	Vd (ml/kg)	Cl tot (ml/min/kg)	AUC (μg min/ml)
Beagle dog	3	R(+)E3810	24.0	392	11.8	131
		S(-)E3810	13.4	419	23.0	69
		R/S ratio	1.79	0.94	0.51	1.9

Data shows mean of 6 beagle dogs.

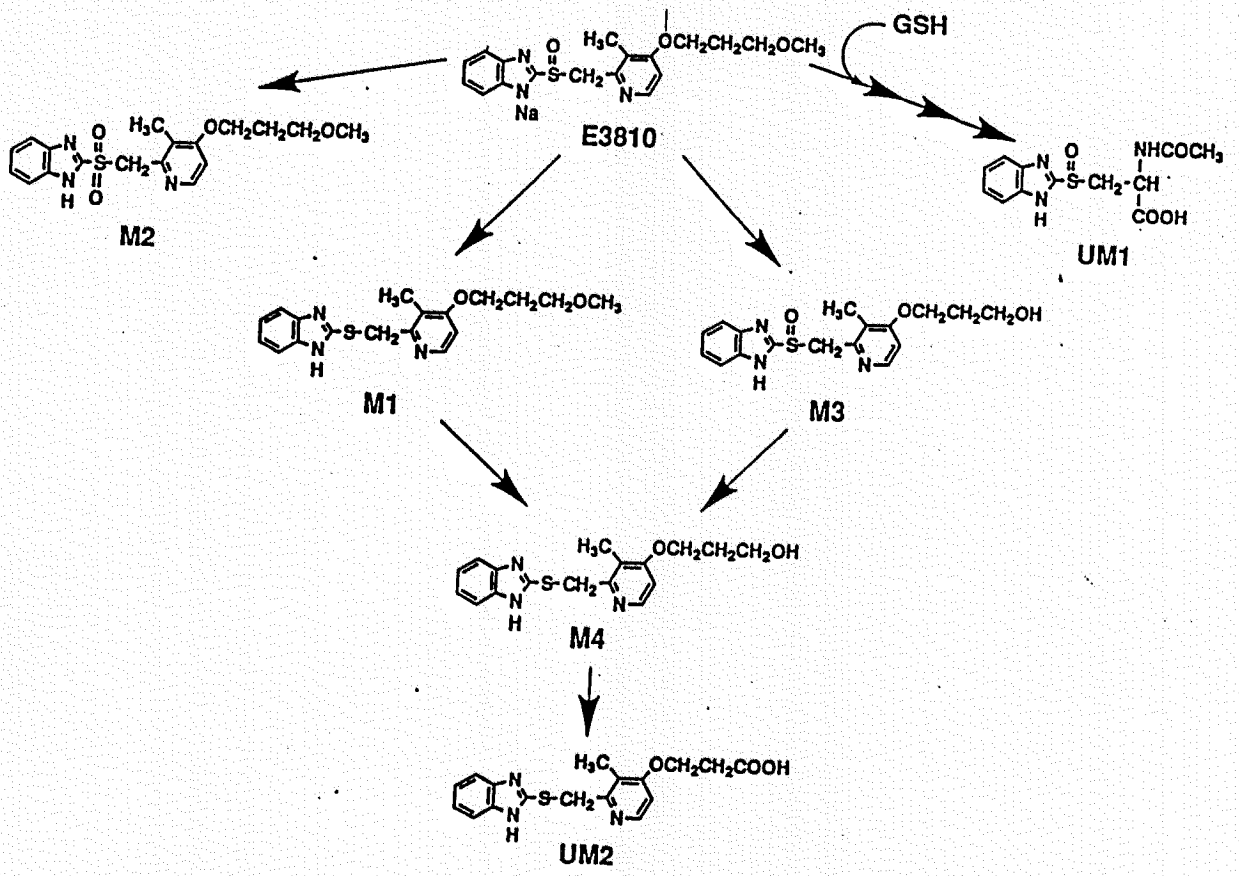


Fig. 1 Proposed metabolic pathway of E3810

Addendum: **(1)** the report numbers are W-19940600, W-19951134 and W19961309, **(2)** in this study, the drugs were given both orally and intravenously and the oral data were not included in this review, **(3)** this study was revised on June 21, 1996 (report # W19961309) and **(4)** the results following both i.v. and oral administrations in the revised report (W19961309) were summarized in Table A-17-1 and this table is attached below.

Table A-17-1: Pharmacokinetic Parameters for R(+)E3810 and S(-)E3810 after Intravenous or Oral Administration of the Corresponding Enantiomers or RS(±)E3810 to Male Beagle Dogs

Administered	R(+)E3810 1.5 mg/kg	S(-)E3810 1.5 mg/kg	RS(±)E3810 3.0 mg/kg	
Measured	R(+)E3810	S(-)E3810	R(+)E3810	S(-)E3810
Intravenous				
$AUC_{(0-\infty)}$ μg min/mL	111.9 ± 9.0	59.9 ± 2.5	140.5 ± 11.2	76.9 ± 8.0
Cl_{tot}, mL/min/kg	13.9 ± 1.2	25.3 ± 1.1	11.0 ± 0.8	20.5 ± 1.9
V_d, mL/kg	483 ± 45	531 ± 52	382 ± 28	395 ± 31
$t_{1/2}$, min	24.1 ± 1.0	14.5 ± 1.1	24.1 ± 0.8	13.4 ± 0.3
Oral				
C_{max}, μg/mL	1.034 ± 0.194	0.346 ± 0.068	1.237 ± 0.205	0.475 ± 0.110
t_{max}, min	25 ± 3	15 ± 0	15 ± 0	15 ± 0
$AUC_{(0-\infty)}$, μg·min/mL	45.7 ± 5.7	9.8 ± 1.4	59.9 ± 5.6	15.4 ± 2.3
Abs · BA, %	42.1 ± 6.4	16.7 ± 2.6	44.3 ± 5.4	20.8 ± 3.6

All values, Mean ± SEM (n=6)
Abs · BA: Absolute Bioavailability

Pharmacokinetics of E3810 in Dogs
(Report # F-10)

Methods: Male beagle dogs were given a single i.v. (1.5 mg/kg) or oral (0.5, 1.5 or 5.0 mg/kg in capsules) dose of E3810 (dissolved in normal saline). In this experiment dogs were fasted for 16 hr prior to drug administration and dogs were treated with 30 ml of 1% sodium bicarbonate via oral catheter at 5 min before and at 10 min after drug administration (sodium bicarbonate treatment was instituted to avoid decomposition of E3810 in the stomach). Blood samples were collected from cephalic vein at 5, 15, 30 45 min, 1, 2, 3, 4, 5 and 6 hr after drug administration to measure plasma drug levels by HPLC methods. Various pharmacokinetic parameters were calculated.

Results: Irrespective of the route of administration drug disappeared from plasma monoexponentially with $t_{½}$ value of 21 - 26 min. AUC values after oral dose increased linearly with dose and oral bioavailability ranged from 62 - 81%.

Table 2 Pharmacokinetic parameters of E3810 following single administration of E3810 to male dogs

Route	Dose	Pharmacokinetic parameters						
	(mg/kg)	C_{max} (μg/ml)	t_{max} (hr)	AUC (μg·hr/ml)	$t_{1/2}$ (hr)	V_d (L/kg)	CL_{tot} (L/hr/kg)	B.A. (%)
i.v.	1.5	4.88 ± 0.13	0.083 ± 0.000	2.63 ± 0.19	0.40 ± 0.04	0.39 ± 0.05	0.58 ± 0.04	
p.o.	0.5	0.70 ± 0.13	0.139 ± 0.056	0.53 ± 0.07	0.43 ± 0.01	0.47 ± 0.15	0.98 ± 0.13	62.2 ± 13.0
	1.5	2.57 ± 0.16	0.194 ± 0.056	2.10 ± 0.24	0.41 ± 0.01	0.42 ± 0.06	0.74 ± 0.10	81.4 ± 13.5
	5	8.45 ± 0.53	0.194 ± 0.056	6.10 ± 0.74	0.35 ± 0.10	0.35 ± 0.06	0.84 ± 0.09	71.3 ± 13.6

All values are shown as mean ± S.E.M. (N=3).
B.A. (%) = AUC (p.o.)/AUC (i.v.) × Dose (i.v.)/Dose (p.o.) × 100

In the above experiment, when secretion of gastric juice was stimulated by pentagastrin (6 μg/kg, i.m. x 2), the plasma level of E3810 was markedly low (data presented graphically) due to decomposition in low gastric pH.

Addendum: The report numbers are W-19961539 and W-19971272 and this is the same study in rats included above (report # F10).

Absorption, Distribution, Metabolism and Excretion Studies of ^{14}C-E3810 in the Dog Following a Single Oral Dose (Report # F-18)

Methods: Male beagle dogs (n=12) were given a single oral (gavage) dose of radioactive ^{14}C-E3810 (10 mg/kg). Blood samples were collected from jugular vein at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 24, 48 and 72 hour after drug administration. Urine samples were collected during 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hr after dosing and feces samples were collected at 24 hr intervals for up to 168 hr after drug administration. At 0.5 hr, 24 hr, 7 days and 28 days after drug administration 3 dogs/time point were sacrificed to determine tissue distribution. Radioactivity in each sample was measured by LSC method. Additionally, profile of radioactivity was further examined by [redacted]

Results: In plasma as well as in whole blood T_{max} was 0.25 hr. About 64.5% and 29.0% of the administered radioactivity were excreted in feces and urine respectively during 168 hr after drug administration and most of excretion occurred during the first 24 hr after dosing. Radioactivity was distributed throughout the body (T_{max} was 0.5 hr in all tissues except in thyroid gland and lens in which T_{max} was 24 hr). Levels of radioactivity in bile, gall bladder, liver, urinary bladder, small intestine, stomach and kidney were significantly higher than that seen in plasma. At 28 days after the drug administration, radioactivity was still detected in melanin containing parts of the eye.

TABLE 6a

Mean concentrations of radioactivity in tissues following oral administration of ^{14}C-E3810 (10 mg/kg) to dogs (n=3/time point)

Results are expressed as μg equivalents E3810 (sodium salt)/g

Tissue	Sacrifice time							
	0.5 hours		24 hours		7 days		28 days	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Adrenal glands	5.89	0.90	0.519	0.166	0.255	0.054	ND	
Aorta	5.17	0.43	0.318	0.108	0.145	0.028	ND	
Bile	2420	730	38.1	14.9	1.15	0.47	ND	
Bone marrow	2.51	0.52	ND	-	ND	-	ND	
Brain (remaining)	1.70	0.35	0.322	0.109	0.253	0.035	ND	
Cerebellum	1.77	0.40	0.326	0.136	0.264	0.052	ND	
Cerebrum	1.63	0.37	0.329	0.122	0.299	0.030	0.068	-
C.S.F.	0.518	0.107	ND		ND	-	ND	
Eye								
Aqueous humour	ND	-	ND		ND	-	ND	
Choroid/sclera	9.27	2.91	3.40	1.39	4.52	1.76	2.15	2.19
Ciliary body	62.7	9.2	27.3	12.2	23.9	3.6	15.0	13.9
Cornea	0.830	0.219	0.170	-	ND	-	ND	
Iris	29.5	5.6	23.7	8.8	12.6	2.0	7.10	6.37
Lens	0.081	0.035	0.649	0.850	0.052	-	ND	
Optic nerve	3.13	0.69	ND		ND	-	ND	
Vitreous humour	ND		ND		ND	-	ND	
Fat	2.58	0.35	0.097	0.021	ND	-	ND	
Gall bladder	330	166	8.42	8.89	0.538	-	0.094	-
Heart	4.96	0.83	0.314	0.097	0.263	0.024	0.054	-
Kidney (cortex)	25.7	5.8	0.978	0.362	0.461	0.061	ND	
Kidney (medulla)	33.2	5.0	0.612	0.194	0.250	0.037	ND	
Liver	50.3	3.6	2.26	0.87	1.03	0.09	0.156	0.006
Lungs	5.57	0.96	0.464	0.167	0.191	0.021	ND	
Lymph nodes	4.95	1.27	0.373	0.156	0.088	-	ND	
Muscle	4.12	0.96	0.298	0.132	0.253	0.025	0.096	0.008
Pancreas	6.65	1.21	0.385	0.147	0.230	0.073	ND	
Pituitary	5.98	1.17	0.430	-	ND	-	ND	
Prostate	6.25	1.44	0.684	0.335	0.363	0.112	ND	
Sciatic nerve	3.40	0.40	0.211	0.079	0.090	-	ND	
Skin (non-pigmented)	2.85	0.41	0.330	0.038	0.445	0.128	0.178	0.036
Skin (pigmented)	2.98	0.70	0.412	0.072	0.396	0.171	0.128	0.055
Spinal cord	1.42	0.39	0.229	0.050	0.122	-	0.057	-
Spleen	3.95	1.09	0.325	0.129	0.182	0.026	ND	
Submaxillary gland	6.52	1.11	0.538	0.275	ND	-	ND	
Testes	4.11	0.67	0.388	0.123	0.171	0.028	ND	
Thymus	3.88	0.62	0.314	0.149	ND	-	ND	
Thyroid gland	7.02	0.89	30.0	3.3	5.58	0.76	1.25	0.20
Urinary bladder	25.1	27.9	0.719	0.130	0.182	-	ND	
Stomach								
Body mucosa	7.41	4.38	1.56	1.47	ND	-	ND	
Pyloric mucosa	11.7	9.1	1.91	1.60	ND	-	ND	
Body muscle	9.33	7.54	0.542	0.251	0.178	0.007	ND	
Pyloric muscle	10.8	8.2	0.565	0.280	0.225	0.037	ND	
Body tissue	27.2	13.3	4.28	0.98	0.181	0.050	ND	
Small intestine	15.4	1.7	1.05	0.51	0.187	0.015	ND	
Whole-blood	6.09	0.94	0.190	0.065	ND	-	ND	
Plasma	8.86	1.78	0.162	0.059	ND	-	ND	

ND No radioactivity detected
SD Standard deviation

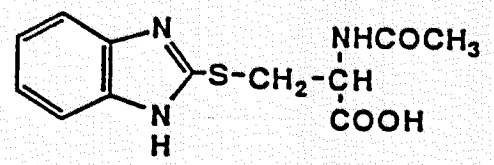
In plasma, unchanged drug, E3810-sulfone, E3810-thioether and E3810-demethylated thioether peaks (by this layer chromatography) were seen.

Addendum: The report number is W-19940655 / W19961540.

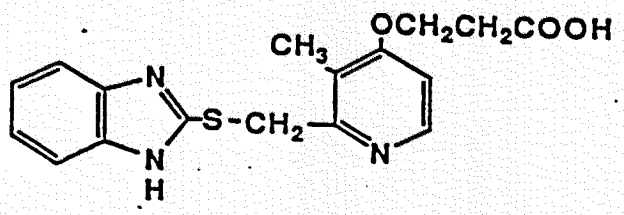
Pharmacokinetics of ^{14}C-E3810 After Oral Administration to Dogs (Report # F-19)

Methods: This is not a new study, rather sample (urinary, fecal and biliary) collected in an earlier study (HRC/ESI/02/921621) were re-analyzed by [redacted] methods for the presence of M5 (mercapturic acid conjugate) and M6 (carboxylic acid derivative) metabolites.

Results: During the first 4 hour after drug administration, about 6% and 76% of urinary radioactivity were excreted as M5 and M6 respectively. From 4 hr post-dose, no M5 metabolite was seen in urine while M6 was still present. In feces (0-24 hr), about 24% and 76 of the fecal radioactivity were represented by M5 and M6 metabolites respectively. The metabolites in the bile collected from gallbladder at 15 min after drug administration were M5 and M6. M5 and M6 represented 24% and 70% of the biliary radioactivity respectively.



Mercapturic acid conjugate (M-5, Lot No. T911617-55-2)



Carboxylic acid derivative (M-6, Lot No. T88090-4-2C2)

Absorption: In the rat, plasma levels of unchanged E3810 were determined after intravenous, intraduodenal and oral administrations. Blood samples were collected 5 min, 15 min 20, min, 1 hour, (only for oral administation and 1.5 and 2 hours after the i.v. or intraduodenal administration. Radioactivity in the portal vein was measured by way of TLC and scintillator after intraduodenal administration of ^{14}C-E3810. In the dog, plasma levels of E3810 were determined 5, 15, 30, 45 minutes, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours after intravenous and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours oral administration. E3810 was measured by HPLC.

Rat: Half-life of elimination of E3810 in plasma was 12 minutes and distribution volume was 0.5 L/kg after intravenous administration (50 mg/kg). Absorption after intraduodenal administration (50 mg/kg) was quick with Tmax being 5 minutes and Cmax being 17 ug/ml. Absolute bioavailability of intraduodenal administration (50 mg/kg) was 19%. Peak plasma concentration of unchanged E3810 reached in 5 minutes follwoing oral adminstration at dose levels of 10, 30 and 100 mg/kg. Plasma level of E3810 was less than half of the Cmax (0.044 ug/ml) 30 minutes after oral dosing. The relative bioavailability of E3810 given orally was 0.2% at the dose of 10 mg/kg. Tmax of radioactivity following intraduodenal administration of 20 mg/kg was 15 minutes. In the portal vein cannulated rat, 73.8% of an intraduodenal dose was found in the protal blood flow.

Dog: T1/2 of 25 minutes and Vd of 0.77 L/kg were obtained following intravenous (50 mg/kg) administration. E3810 was absorbed rapidly following oral administraion with peak concentration (8.2 ug/ml) reaching at 15-30 minutes. Elimination T 1/2 was 24 minutes. The bioavailability of E 3810 administerd orally (100 mg/kg) in the low-pH conditions was 3.6%. However, the availability of E3810 orally adminstered during the high-pH conditions was 76%. The pharmacokinetics of E3810 are linear over the dose-range examined (1-30 mg/kg), and no accumulation or induction after repeated dosing (13 weeks) were observed.

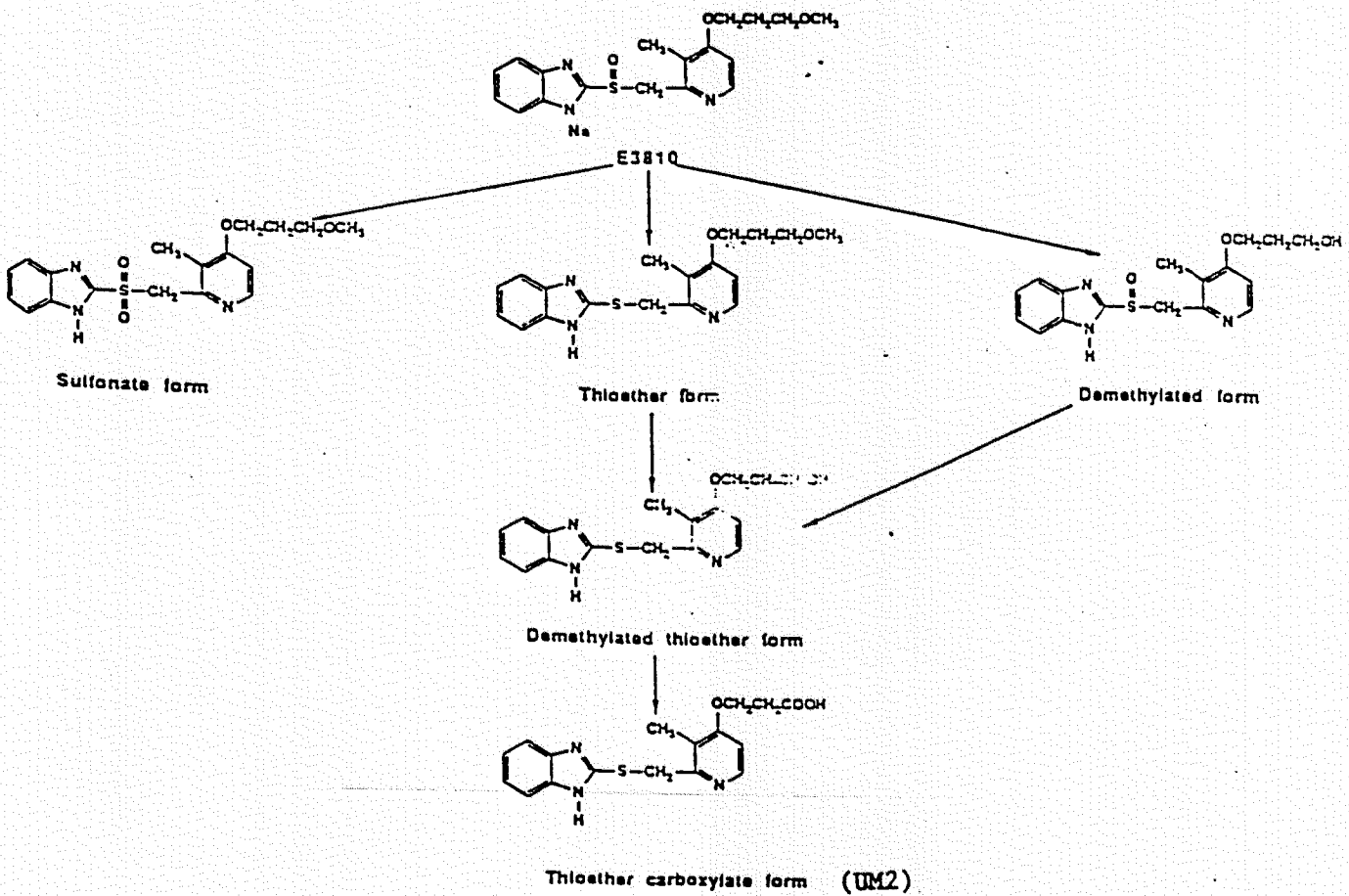
Distribution: Tissue distribution of radioactivity after the intraduodenal and oral administrations of ^{14}C-E3810 to rats and dogs, respectively, were studied. Tissue samples were obtained at 15 minutes, 2 hours, 24 hours and 9 days after intraduodenal administration in rats and at 30 minutes and 8 days after oral administration in dogs. Radioactivity was measured by scintillator.

Rat: Except for the intestine, which was exposed to ^{14}C-E3810 (20 mg/kg, i.d.) directly, the highest level of radioactivity was found 15 minutes after administration in the kidneys (11x the blood level of 5 ug E3810 eq./ml). Lower levels were seen in the various parts of the stomach (6.8x), the liver (6.7x), the bladder (1.5x), thyroid gland (2x) and plasma.

Dog: The highest level of radioactivity were observed in bile (74 x the blood level of 12.4 ug E3810 eq./ml) and gallbladder (33x) at 30 minutes after oral administration of a single 10 mg/kg dose of ^{14}C-E3810 to dogs. Lower levels were seen in ciliary body (7.2x), renal medulla (2.8x), liver (5.6x), gastric or pyloric mucosa (4.3-3.4x) and small intestine (2.3x). Radioactivity in thyroid was low (10.6 ug E3810 eq./g). At 8 days after dosing, the levels of radioactivity in most tissues have declined except in thyroid (11.8 vs 0.1 ug E3810 eq./ml in blood). In an intracellular study, radioactivity was found to be localized in the microsomal fraction of gastric mucosal cell homogenate. Similar pattern of distribution was reported following intravenous administration.

Metabolism: Unchanged form and metabolites in plasma, urine, feces and bile after oral (20 mg/kg for rats and 10 mg/kg for dogs) or intravenous administration (5 mg/kg for dogs) of ^{14}C-E3810 were analysed by

The proposed metabolic pathway pf E3810 is shown below:



It was oxidized at the position of sulfoxide to the sulfone-E3810 or reduced to the thioether-E3810. It was also demethylated. The thioether-E3810 and demethylated-E3810 may be converted to the demethylated thioether-E3810 which is then oxidized to the thioether carboxylic acid-E3810 (UM-2). Other unidentified polar metabolites i.e. UM-1, -3, -4 & -5 were present. In plasma, ten percent of radioactivity was in UM-1 form and 12 % of radioactivity was in unchaged form 15 minutes after oral administration in rats. In dogs, unchanged E3810, thioether-E3810 and polar metabolites represented 19, 23 and 36 % of radioactivity 30 minutes after oral administration of 10 mg/kg. UM-2 was the main metabolite in the urine and feces of rats and dogs.

Excretion: Radioactivity was measured in the urine and feces following intraduodenal and oral administrations of ^{14}C-E3810 to rats and dogs, respectively. Urninary and fecal excretion of radioactivity were measured 1, 2, 3, 4, 5, 6, 7 and 8 days for rats and 0.33, 1, 2, 3, 4, 5, 6 and 7 days for dogs after administration.

Rat: Thirty-six % of the intraduodenal administration of a single 20 mg/kg dose of radioactivie E3810 was excreted in the urine and 43% was excreted in the feces within 24 hours after dosing. The excretion was mainly in the bile following intraduodenal administration (61 % was excreted in 24 hours). Thus, a part of the radioactivity excreted into the bile was entered hepatic recirculation.

Dog: Within 24 hrs after oral administration (10 mg/kg), 36% and 38% of the administered radioactivity were excreted in the urine and the feces, respectively. During the 7 days after the oral adminstration, 41% and 55% of the administered radioactivity were excreted in the urine and the feces, respectively. Urinary and fecal excretions of radiaoctivity following a singel 5 mg/kg intravenous dose of radioactive E3810 were 39 and 41%, respectively within 24 hours and 44 and 58, respectively in 7 days. Oral absorption was estimated to be 93% based on the ratio of urinary excretion of total radiocitivity according to sponsor.

Plasma Protein Binding of E3810 *Ex Vivo* and *In Vitro*
(Report #W-19961533)

Methods: The protein binding of E3810 was studied in the rat, dog and human blood. The blood samples were obtained from health male volunteers, male Beagle dogs and male rats and mixed with solutions of E3810 at a final concentration of E3810 of 0.2, 1.0 and 5.0 µg/ml (*in vitro*). For *ex vivo* study, ^{14}C-E3810 was given to rats intraduodenally at 20 mg/kg or intravenously at 5 mg/kg or to dogs orally at 10 mg/kg or intravenously at 5 mg/kg. The blood samples were collected up to 24 hours. The bound fraction was separated from unbound fraction using ultracentrifugation and the radioactivity was determined using liquid scintillation counter.

Results: The *in vitro* study indicated that E3810 was highly bound to the human plasma protein (96.2-98.6%). In dog plasma, the protein binding was 91.7-93.3%. In rat plasma, the protein binding was 91.4-92.1%. The *ex vivo* study indicated that the protein binding of E3810 was 70.5-83.6% in rats, 76.4-83.8% in dogs.

Plasma Protein Binding of Optical Isomers of E3810 *In Vitro*
(Report #W-19961245)

Methods: The protein binding of E3810 was studied in the rat, dog and human blood. The blood samples were obtained from health male volunteers, Beagle dogs and SD rats and mixed with solutions of R(+)E3810, S(-)E3810 and the racemic mixture of the 2 enantiomers at 2 µg/ml (R(+)E3810), 10 µg/ml (S(-)E3810) and 20 µg/ml (mixture). The bound fraction was separated from unbound fraction using ultracentrifugation and the plasma concentrations of the isomers were determined using

Results: The isomers and racemic mixture were highly bound to the human plasma protein (97.1-97.6%). In dog plasma, the protein binding of two isomers and racemic mixture was 90.4-93.4%. In rat plasma, the protein binding was 92.2-93% for R(+)E3810, 82.4-84.4% for S(-)E3810.

Distribution of ^{14}C-E3810 to Blood Cells
(Report #W-19961224)

Methods: The distribution of radioactivity into blood cells was investigated in the blood obtained from male rats and dog and humans. ^{14}C-E3810 was incubated with 2 ml of blood at a concentration of 10.98 µg/50 µl for 5, 30 and 60 minutes. The blood cells were then separated by centrifugation and the radioactivity was determined using

Results: The ratio of radioactivity in erythrocyte to plasma was 0.51, 0.44 and 0.16 in rats, dogs and humans after 5 minutes incubation. The ratio in rats was increased to 1.7 after 1 hour incubation. However, the ratio in dogs and human was not changed over time.

To compare the similarities and differences between mice, rats, dogs and humans, the pharmacokinetic parameters of rabeprazole are summarized in the following table.

Pharmacokinetics of rabeprazole in mice, rats, dogs and humans

Species/dose	C_{max}, µg/ml	$AUC_{0-\infty}$, µg.h/ml	$t_{1/2}$, hr	Vd, l/kg	Cl,l/hr/kg	F (%)
Mouse:						
Oral						
100 mg/kg, male	9.36	1.43				
100 mg/kg, female	4.75	1.38				
Rat:						
I.V.						
20 mg/kg, male	----	2.46	0.1	1.02	8.1	----
20 mg/kg, female	----	3.21	0.09	0.67	6.2	----
Oral						
20 mg/kg, male	1.1	0.52	0.1	----	38.4	21.1
20 mg/kg, female	0.89	0.34	0.17	----	59.7	10.6
I.D.						
20 mg/kg, male	4.56	0.9	0.11	1.17	22.2	36.6
20 mg/kg, female	16.6	2.91	0.07	0.37	6.9	90.7
Dog:						
I.V.						
1.5 mg/kg, male	4.88	2.63	0.4	0.39	0.58	
Oral						
1.5 mg/kg, male	2.57	2.1	0.41	0.42	0.74	79.8
Human:						
Oral						
20 mg	0.2-0.44	0.88	0.7-1.5	0.34	0.23	52

For mouse, AUC = $AUC_{0-3\ hr}$

In general, the plasma concentration of E3810 was increased with dose. Its oral bioavailability was variable since E3810 is unstable in the gastric juice. The oral bioavailability can be improved by pretreatment with sodium bicarbonate buffer or delivering directly into the duodenum or using the enteric coated tablets. For example, in rats, the bioavailability following oral dose of E3810 was ~11-21% while the bioavailability following intraduodenal administration of E3810 was ~37-91%. In humans, the oral bioavailability of enteric coated tablets of E3810 was 52%. After oral administration, C_{max} was reached within 5 minutes in mice and rats and ~8-11 minutes in dogs. E3810 was quickly declined following i.v. and oral administrations with half life of ~6-10 minutes in rats, 24 minutes in dogs and 42-90 minutes in humans. E3810 was oxidized at the position of sulfoxide to the sulfone-E3810 (M2) or reduced to the thioether-E3810 (M1). It was demethylated to desmethyl-E3810 (M3). The metabolism of E3810 to its sulfone metabolite (M2) is mediated by CYP3A form of human cytochrome P450 enzyme while the conversion of E3810 to the desmethyl metabolite (M3) is mediated by CYP2C19 form. Thioether-E3810 (M1) is the major metabolite identified in the human plasma. Very low level of M2 was also detected in the human plasma. Both M1 and M3 were detected in the mouse, rat and dog plasmas. The carboxylic acid derivative (M6) was the major metabolite identified in the mouse, rat and dog urine. M6 was also a major metabolite in the dog feces and bile. Mercapturic acid (M5) was also found in the urine of the rat and dog. The desmethyl metabolite (M3) is pharmacologically active with similar potency to the parent compound. The tissue distribution studies indicated that the highest radioactivity was detected in the thyroid followed by the liver, gastric mucosa, bone marrow and pituitary gland in rats. The highest radioactivity was detected in the bile followed by eye, liver, stomach and kidney in dogs. The volume of distribution was similar in dogs (~0.4 l/kg) and in humans (0.34 l/kg). In rats the volume of

distribution ranged from 0.37 l/kg to 1.17 l/kg. The total clearance was 0.23 l/hr/kg in humans, ~0.6-0.7 l/hr/kg in dogs and ~6-60 l/hr/kg in rats. E3810 was highly bound to plasma protein in humans (96.2-98.6%) and rats and dogs (91.4-93.3%). The major route of excretion was by urine and feces in mice, rats and dogs. The radioactivity was recovered in the feces (42-50% in mice, 46-53% in rats and 53% in dogs) and urine (20-23% in mice, 39-44% in rats and 33% in dogs). In humans, ~90% of the drug was excreted in the urine, mainly as mercapturic acid and carboxylic acid.

TOXICITY:

ACUTE TOXICITY:

Acute toxicity studies (Study Numbers: 881222, 881212 & 871246):

Date of study: March 15, 1988 for mouse and rat studies, Dec. 1 for dog study.

Lot no: 87092101

Species/ strain	no/sex/dose	Route of administration	Dose range (mg/kg)	Highest non lethal dose (mg/Kg)	LD50 (mg/kg)	Toxic signs
Mouse/ICR	5	p.o.	629-2400(M) 629-3000(F)	629(M) 786(F)	1206 (M) 1012 (F)	(1)
Rat/Sprague-Dawley	5	p.o.	819-2000(M) 655-2000(F)	1280(M) 819 (F)	1447 (M) 1322 (F)	(2)
Dog/Beagle	1	p.o.	80-2000	2000	>2000	(3)
Mouse/ICR	5	i.v.	131-320 (M) 164-320 (F)	164(M) 205(F)	220 (M) 237 (F)	(4)
Rat/Sprague-Dawley	5	i.v.	98-192 (M) 98-300 (F)	123 (M) 98 (F)	157 (M) 152 (F)	(5)

Observation period: 14 days.

E3810 was dissolved in water for the oral route and in physiological saline for the i.v. route.

Time to death: The death occurred within 24 hours after dosing by oral administration and within 5 minutes after intravenous administration in mice. In rats, most deaths occurred between 30 minutes and 24 hours after oral administration and within 15 minutes after i.v. administration.

(1) & (2): Lateral or prone position, labored breathing, hypoactivity and/or convulsion.