**Pentagastrin-stimulated Gastric Acid Secretion 21 Hours after Test Drug Administration (mEq/2 hours, mean ± SE)**

| Day | E3810 | Omeprazole |
|---|---|---|
| 0 (control) | 17.53±2.40 | 17.53±2.40 |
| 2 (drug treatment) | 13.75±2.53 | 8.22±2.45 |
| 4 (drug treatment) | 11.87±3.66 | 5.98±1.39 |
| 6 (drug treatment) | 11.13±2.28 | 6.47±1.57 |
| 8 (recovery) | 12.56±1.91 | 6.68±1.32 |
| 9 (recovery) | 15.61±2.55 | 12.53±2.27 |
| 10 (recovery) | 16.28±3.36 | 16.51±2.02 |
| 12 (recovery) | 18.84±2.66 | 20.80±3.11 |

Effect in three ulcerogenic Sprague-Dawley rat models (cold-restraint stress-induced erosion, Shay gastric ulcer, and cysteamine-induced duodenal ulcer models) (Report W-890384): Pretreatment with both E3810 and omeprazole, at oral dose of 10 mg/kg reduced the mean erosion index in cold-restraint stress model. The effect of omeprazole on ulcer scores in Shay gastric ulcer model was less pronounced at the two lower doses (10 and 30 mg/kg) when compared to E 3810, particulary for the 10 mg dose. However, at 100 mg/kg omeprazole produced lower ulcer score than E3810 did. In the cysteamine-induced duodenal ulcer model, both compounds (1, 3 and 10 mg/kg for E3810 and 3 and 10 mg/kg for omeprazole) were administered subcutaneously ten minute before subcutaneous injection of cysteamine HCl (500 mg/kg). The rats were sacrificed 18 hours later. Following 1, 3 and 10 mg/kg of E 3810, the cysteamine-induced ulcer index was decreased an average of 41, 69 and 74%, respectively. With omeprazole, the ulcer index was decreased 35 and 54 % following 3 and 10 mg/kg, respectively.

Inhibition of gastric secretion in pylorus-ligated rats: Their effect on the total volume of gastric secretions, total acid output and total pepsin secretion over a four-hour period is shown below:

| Treatment | Total Volume (ml) | Acid Output (uEq/hour) | Pepsin Produced (mg/hour) |
|---|---|---|---|
| Control | 6.6 ± 0.7 | 155.9 ± 21.9 | 122.3 ± 13.7 |
| E3810 | | | |
| 1 mg/kg | 5.3 ± 0.7 | 117 ± 21 | 103.4 ± 17.2 |
| 3 mg/kg | 4.6 ± 0.6 | 69.2 ± 9.8** | 91.6 ± 11.2 |
| 10 mg/kg | 3.8 ± 0.8* | 62.3 ± 5.5** | 82.6 ± 5.4* |
| 30 mg/kg | 2.1 ± 0.2** | 15.2 ± 2.0** | 29.3 ± 4.1** |
| Omeprazole | | | |
| 10 mg/kg | 4.1 ± 0.5* | 39.8 ± 3.5** | 73.1 ± 9.5** |

* = $p < 0.05$
** = $p < 0.01$

A comparison between equal doses of E3810 and omeprazole indicates that omeprazole may be a slightly more potent inhibitior in this system.

## Secondary Activity

1. **Effects of E3810 on Gross Behavior and Pentylenetetrazole-induced Convulsions in Mice**: In mice, E3810 (up to 30 mg/kg, i.v.) had no effect on gross behavior nor had any effect on pentylenetetrazole induced convulsions.

2. **Effect on Acetic Acid Induced Writhing Syndrome in Mice**: In mice, E3810 (up to 30 mg/kg, i.v.) had no significant effect on acetic-induced writhing syndrome. Hence, E3810 has no analgesic effect.

3. **Effects of E3810 on Respiration in Anesthetized Rats**: In anesthetized rats, E3810 (up to 10 mg/kg, i.v.) had no significant effect on respiratory rate or tidal volume.

4. **Effects of E3810 on Hemodynamics and ECG in Anesthetized Open-Chest Beagle Dogs**: In anesthetized dogs, E3810 (up to 3 mg/kg, i.v.) had no significant effect on LvdP/dt, heart rate, aortic pressure, aortic flow, systemic vascular resistance or ECG.

5. **Effects of E3810 on Water and Electrolyte Metabolism in Rats**: In rats, E3810 (up to 10 mg/kg, i.v.) had no significant effect on urinary volume nor had any effect on $Na^+$, $K^+$ and Cl excretion during 5 hours following drug administration.

6. **Effects of E3810 on Gastric Emptying and Intestinal Transit in Mice**: E3810 (up to 30 mg/kg, i.v.) had no significant effect on gastric emptying and intestinal transit in mice.

7. **Effects of E3810 on Biliary and Pancreatic Secretion in Anesthetized Rats**: In anesthetized rats, E3810 (up to 30 mg/kg, i.v.) had no significant effect on biliary or pancreatic secretion.

8. **Effects of E3810 on Contractile Responses Induced by Acetylcholine, Histamine and Barium Chloride in Isolated Ileum of Rat and Guinea Pig**: E3810 (up to 100 $\mu$M) had no significant effect on contractile responses induced by acetylcholine, histamine and barium chloride in isolated ileum of rat and guinea pig.

9. **Effects of E3810 on Blood Coagulation and Fibrinolysis of Human Plasma In Vitro**: In vitro, E3810 (up to 100 $\mu$M for 2 hr at 37°C) had no significant effect on human plasma activated partial thromboplastin time (APTT: intrinsic coagulation), hepaplastin-test (HPT: extrinsic coagulation) and plasminogen (fibrinolytic activity) level.

10. Effect of E3810 on $Na^+/K^+$-ATPase Activity: E3810, omeprazole and ouabain concentration dependently inhibited $Na^+/K^+$-ATPase activity in vitro (assay was done at pH 7.5). At 100 $\mu$M, ouabain, E3810 and omeprazole inhibited $Na^+/K^+$ ATPase activity by 95%, 67% and 35% respectively.

Effects on the contractile tension of isolated rat aoric strips: It (at concentrations of ($10^{-7}$ - $10^{-4}$M) was without effect on aortic strips contracted with norepinephrine and only slightly relaxed aortic strips contracted with KCl at the highest concentration.

Effects on human platelet aggregation in vitro: At concentration of $5x10^{-4}$M, it inhibited Platelet activating factor-induced platelet aggregation by 65%. No effect on collagen-induced aggregation was seen at concentration up to $5x10^{-4}$M.

Cardiohemodynamics and renal function of anesthetized dogs: Neither compounds influenced the heart rate, blood pressure, left ventricular pressure (LV dp/dt), renal blood flow or respiratory rate at a dose of 1 mg/kg (i.v.). However, at 10 mg/kg, blood pressure, LV dp/dt and renal blood flow were decreased transiently for 1-2 minutes. One of three dogs tested had increased in heart rate (60%), blood pressure (7%) and respiratory rate (10%) following the 10 mg/kg dose of E3810. No effects on EKG and renal plasma flow, GFR and urine volume, urine excretion or plasma concentrations of electrolytes or hematocrit were observed.

Gastrointestinal system: In conscious rats, no effects on gastric or duodenal motility were observed after E3810 was administered at doses of 50 mg/kg intraduodenally or 20 mg/kg intravenously. After 100 or 200 mg/kg (i.d.) of E3810, inhibition of gastric and dudenal motilities was seen.

Effects on urine volume and urinary electrolytes excretion in conscious rats: At intravenous doses up to 10 mg/kg, it did not alter urine volume or the urinary excretion of electrolytes two hours following dosing.

CNS: At intravenous dose up to 30 mg/kg, E3810 did not affect the times of recovery of righting reflex after pentobarbital, locomotor activity, tonic hindlimb extension in response to electroshock, pentylenetetrazole-induced mortality and rectal temperature.

Effects on blood glucose levels and glucose tolerance in rats: No effects were observed at i.v. doses up to 30 mg/kg.

Effects on the corneal reflex in guinea pigs: At concentrations up to 3%, it had no inhibitory effect on the corneal reflex of guiena pigs elicited by stylet stimulation. Thus, it did not possess local anesthetic property.

In summary, rabeprazole sodium (E3810), a potent proton pump inhibitor, inhibits gastric acid secretion by inhibiting the $H^+/K^+$-ATPase, the final step of gastric acid secretion, with $IC_{50}$ of ~2.0-2.6 x $10^{-7}$ M in porcine gastric parietal cells. It was demonstrated that E3810 interacts with SH-groups on the inner part and surface of the enzyme which undergoes conformational change and then leads to the inhibition of the enzyme activity. Its desmethyl metabolite (M3) also inhibits the activity of $H^+/K^+$-ATPase with $IC_{50}$ of 2.9 x $10^{-7}$ M. It was also demonstrated that (±)-E3810 (racemic mixture), R-(+)-E3810 [R-(+) enantiomer], and S-(-)-E3810 [S-(-) enantiomer] are equipotent in inhibiting $H^+/K^+$-ATPase in porcine gastric parietal cells. E3810 was demonstrated to inhibit gastric acid secretion in rats, rabbits and dogs. E3810 inhibits cAMP-induced acid secretion in the isolated rabbit gastric glands with $IC_{50}$ of 1.6 x $10^{-7}$ M. In rats, E3810 inhibited histamine-induced gastric acid secretion with $ID_{50}$ of 1 mg/kg. The $ID_{50}$ of E3810 for inhibition of histamine-induced gastric acid secretion in dogs was 0.06 mg/kg. Both thioether (M1, 2 mg/kg) and desmethyl (M3, 0.5 mg/kg) metabolites inhibit histamine-induced gastric acid secretion in dogs by 41% and 47% at 1 hour after dosing. These results suggest that E3810 is therapeutically useful in the diseased conditions such as GERD, gastric and duodenal ulcers and pathological hypersecretory conditions.

**ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):**

MICE:

Plasma Level of E3810 After a Single or 14-Day Repeated Oral Dose of E3810 in Mice
(S96614)

Methods: To determine the systemic exposure of E3810 and its metabolites, E3810 was given orally to CD-1 mice at 100 mg/kg. The pH of the drug solution was not provided. The plasma levels of E3810 and its metabolites, thioether-E3810 (M1), sulfone-E3810 (M2) and desmethyl-E3810 (M3) were determined using HPLC-UV.

Results: The results were summarized in a table on page 9 in volume 1.74 and this table is attached below.

| Dose (mg/kg) | Study day | | Cmax (μg/ml) | | Tmax (hr) | | AUC (0-3hr) (μg·hr/ml) | |
|---|---|---|---|---|---|---|---|---|
| | | | Male | Female | Male | Female | Male | Female |
| 100 | 1 | E3810 | 9.364 | 4.753 | 0.083* | 0.083* | 1.432 | 1.375 |
| | | M-1 | 0.919 | 1.106 | 0.250 | 0.250 | 0.322 | 0.353 |
| | | M-2 | - | - | - | - | - | - |
| | | M-3 | 0.628 | - | 0.083* | - | - | - |
| | 14 | E3810 | 15.022 | 5.231 | 0.083* | 0.083* | 2.669 | 1.369 |
| | | M-1 | 1.034 | 1.115 | 0.250 | 0.250 | 0.516 | 0.423 |
| | | M-2 | - | - | - | - | - | - |
| | | M-3 | 0.906 | - | 0.083* | - | - | - |

*: first time point monitored.
-: not calculated; too few quantifiable levels to determine parameter(s).

Metabolite, M2, was not identified. AUC levels of E3810 and M1 were similar between males and females on days 1 and 14, suggesting that there was no apparent drug accumulation over time and no difference of the drug levels between the sexes. The AUC values (~1.4 μg.hr/ml) of E3810 on day 1 are approximately 1.6 folds of the AUC values (0.88 μg.hr/ml) following oral dose of 20 mg/day in healthy volunteers.

## Plasma Level of E3810 After a Single or 4-Day Repeated I.P. Dose of E3810 in Mice (S96615)

Methods: To assess the systemic exposure of E3810 and its metabolites in mice, E3810 was given intraperitoneally to CD-1 mice at 100 and 200 mg/kg. Route of administration (i.p.) was chosen based on the route of administration in the mouse i.p. micronucleus assay. The plasma levels of E3810 and its metabolites, thioether-E3810 (M1), sulfone-E3810 (M2) and desmethyl-E3810 (M3), were determined using [redacted]

Results: The results on the first day after dosing were summarized in a table on page 80 in volume 1.74 and this table is attached below.

| E3810 Dose (mg/kg/day) | Cmax (μg/ml) | | Tmax (hr) | | AUC (0-2 hr) (μg·hr/ml) | | t1/2 (hr) | |
|---|---|---|---|---|---|---|---|---|
| | 100 | 200 | 100 | 200 | 100 | 200 | 100 | 200 |
| E3810 | 83.095 | 165.670 | 0.083* | 0.083* | 17.085 | 59.115 | 0.119 | 0.179 |
| M-1 | 2.849 | 6.524 | 0.083* | 0.083* | 1.518 | 5.798 | 0.315 | 0.638 |
| M-2 | 1.704 | 7.495 | 0.083* | 0.250 | - | 4.931 | - | 0.314 |
| M-3 | 3.176 | 8.134 | 0.083* | 0.250 | 0.919 | 4.240 | 0.139 | 0.254 |

*: first time point monitored
-: not calculated; Too many M-2 levels were below the quantification limit to calculate AUC and t1/2.

On day 4, $C_{max}$ was 82.9, 2.5, 2.9 and 6.3 µg/ml for E3810, M1, M2 and M3 and AUC was 16.7 and 1.5 µg.hr/ml for E3810 and M1, respectively following i.p. dose of 100 mg/kg. The half life was ~7 minutes for E3810 and 22 minutes for M1. The plasma levels of E3810 and its metabolites were similar on days 1 and 4, suggesting that there was no apparent drug accumulation over time.

A Pharmacokinetic Study of E3810 Following Oral Administration to the Mouse
(CHE726/17)

Methods: To study the pharmacokinetics of E3810, $^{14}$C-E3810 was given to mice by oral gavage at 100 mg/kg (specific radioactivity = 4.44 MBq/mg). The pH of the drug solution was not provided. The radioactivity levels in the plasma, urine and feces were determined using liquid scintillation counter. Metabolites in the urine were characterized using HPLC and tandem mass spectroscopy.

Results: The maximum plasma level of radioactivity was detected at 30 minutes after dosing (7.4 and 4.0 µg eq./g for males and females). The radioactivity was recovered mainly in the feces (42-50%) and urine (20-23%). M6 (carboxylic acid) was the major metabolite in the urine. Human metabolites, Unk2 (4-hydroxy-pyridine metabolite) and Unk3 (unknown structure), were also identified in the urine.

RATS:

A Study of the Absorption, Metabolism and Excretion Following Oral Administration to the Rat
(CHE726/16)

Methods: To study the pharmacokinetics of E3810, $^{14}$C-E3810 was given to rats (fasted males and females, and non-fasted females) by oral gavage at 25 mg/kg (specific radioactivity = 4.44 MBq/mg). The pH of drug solution was not provided. The radioactivity in the plasma, urine and feces were determined using liquid scintillation counter and expressed as µg equivalents of test article per g of sample. The metabolites were characterized using HPLC and tandem mass spectroscopy.

Results: The maximum plasma level of radioactivity was detected at 30 minutes after dosing (7.2, 3.85 and 3.01 µg eq./g for fasted males and females and non-fasted females). In the fasted rats, the radioactivity was recovered mainly in the feces (46-53%) and urine (39-44%). In the non-fasted females, the majority of radioactivity was recovered in feces (63%) and urine (28%). The human metabolite (4-hydroxypyridine, UnK2) was identified in urine.

Evaluation of Hepatic First Pass Effect of E3810 in Rats
(SHKA950221)

Methods: To study the first pass effect in rats, E3810 was given to rats by intravenous and intraportal administrations at 2, 6, 20 and 60 mg/kg. The plasma level of E3810 was then determined using HPLC-UV. The hepatic extraction ratios were calculated using the following equation: 1 - ratio of $AUC_{portal}/AUC_{i.v.}$.

Results: The results were summarized in Table A-7-1 on page 44 in volume 1.12 and this table is attached below.

**Table A-7-1: Pharmacokinetic parameters after intravenous and intraportal administration of E3810 to rats.**

| Route of Admin. | E3810 Dose (mg/kg) | $t_{1/2}$ (hr) | | $AUC_{(0-\infty)}$ (μg.hr/mL) | | Cl (L/hr/kg) | |
|---|---|---|---|---|---|---|---|
| | | male | female | male | female | male | female |
| i.v.[a] | 2 | 0.23 | 0.29 | 0.740 | 0.618 | 2.71 | 3.24 |
| | 6 | 0.36 | 0.48 | 2.385 | 3.214 | 2.53 | 1.89 |
| | 20 | 0.33 | 0.43 | 7.499 | 9.671 | 2.69 | 2.07 |
| | 60 | 0.39 | 0.48 | 28.589 | 34.969 | 2.11 | 1.76 |
| i.p.v.[b] | 2 | 0.11 | 0.18 | 0.121 | 0.325 | 17.24 | 6.53 |
| | 6 | 0.30 | 0.53 | 0.884 | 0.850 | 7.81 | 7.09 |
| | 20 | 0.33 | 0.35 | 3.896 | 3.089 | 5.44 | 6.70 |
| | 60 | 0.31 | 0.41 | 17.492 | 24.091 | 3.48 | 2.49 |

*Each value is the mean of data from 3 animals*
*a: $Cl_{iv}$*
*b: $Cl_{ipv}$*

The hepatic extraction ratios were 0.84, 0.63, 0.48 and 0.39 for 2, 6, 20 and 60 mg/kg dosed males and 0.47, 0.74, 0.68 and 0.31 for 2, 6, 20 and 60 mg/kg dosed females, respectively. These results suggest that the hepatic first pass effect of E3810 was at least partially saturated at higher doses.

Tissue Metabolic Profiles After a Single Intraduodenal Administration of $^{14}$C-E3810 in Rats
(SHKA950201)

Methods: To study the metabolism and distribution in rats, $^{14}$C-E3810 was given to rats intraduodenally at 20 mg/kg (specific activity = 3.96 MBq/mg). The samples of blood, tissue, urine and feces were collected for determination of the radioactivity using liquid scintillation counter and thin layer chromatography.

Results: At 15 minutes after dosing, the highest radioactivity was detected in the stomach (target organ) and duodenum (site of administration) followed by the liver, kidney and plasma. Mercapturic acid conjugate (M5) and carboxylic acid derivative (M6) were the major metabolites identified in all tissues except the stomach where thioether-E3810 (M1) was the major metabolite. M5 and M6 were also the major metabolites in the urine which accounted for 13.4% and 18.5% of the dose given, respectively. In feces, M6 was the major metabolite which accounted for 15.2% of the dose given.

Metabolites in Bile Related to the Enterohepatic Circulation After Intraduodenal Administration of $^{14}$C-E3810 in Male Rats
(Report #19961676)

Methods: To study the enterohepatic circulation in rats, $^{14}$C-E3810 was given to rats intraduodenally at 20 mg/kg (specific activity = 3.94 MBq/mg). The radioactive bile was collected via a catheter and then infused into the ligated duodenal sacks of other rats cannulated in the splenogastric vein. The portal blood that drained from this cannula was then collected. The metabolites were analyzed using [redacted]

Results: Mercapturic acid conjugate (M5), carboxylic acid derivative (M6) and corresponding cysteine and glutathione conjugates were identified in the bile which accounted for 2.9, 45.1, 7.2 and 12.8% of the total radioactivity recovered in the bile. After administration of radioactive bile into the splenogastric cannulated rats, M5, M6 and the cysteine-conjugate were the major radioactive compounds recovered in the portal plasma with only trace amounts of the glutathione conjugate.

Effects of E3810 on Hepatic Drug Metabolizing Enzymes in the Rat Liver Microsomes
(T93046)

Methods: The effects of E3810 on the hepatic enzymes were studied using rat liver microsomes in vitro. The activity of the hepatic drug metabolizing enzymes (aminopyrine N-demethylation, benzphetamine N-demethylation, aniline hydroxylation and p-nitrosole O-demethylation) was assayed in the presence and absence of E3810 or omeprazole at 60, 300 and 1500 µg/g liver.

Results: E3810 inhibited aminopyrine N-demethylation by 18.3% and 80.4% at 300 and 1500 µg/g liver. It inhibited benzphetamine N-demethylation, aniline hydroxylation and p-nitrosole O-demethylation only at the high concentration of 1500 µg/g liver

by 44.1%, 30.7% and 23.6%, respectively. Omeprazole produced similar degrees of inhibition at comparable concentrations. These results suggest that E3810 may interfere in the metabolism of the drugs which are metabolized by these hepatic enzymes.

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

**Methods**: Sprague-Dawley rats were given a single i.v. (20 mg/kg), I.d. (5, 20 or 80 mg/kg) or oral (gavage: 2, 6 or 20 mg/kg) dose of E3810 (dissolved in normal saline). Blood samples were collected from abdominal aorta at 5, 15, 30 and 45 min, 1, 2 and 3 hr after drug administration to measure plasma drug levels (3 rats/time point were used). Concentrations of E3810 and its metabolites in plasma were measured by HPLC methods. Various pharmacokinetic parameters were calculated.

**Results**: In rats, irrespective of route of administration, $t_{1/2}$ was about 6-8 min. Increases in AUC values with respect to dose were not linear. Oral or i.d. bioavailability was highly variable, probably due to hepatic first pass effect and/or instability of E3810 in gastric juice.

**Table 1 Pharmacokinetic parameters of E3810 in plasma following administration of E3810 in rats**

| Sex | 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. (%) |
| Male | i.v. | 20 | - | - | 2.46 | 0.10 | 1.02 | 8.1 | - |
| | i.d. | 5 | 0.80 | 0.083 | 0.12 | - | 1.85 | 41.7 | 19.5 |
| | | 20 | 4.56 | 0.083 | 0.90 | 0.11 | 1.17 | 22.2 | 36.6 |
| | | 80 | 45.90 | 0.083 | 11.03 | 0.13 | 0.74 | 7.3 | 112.1 |
| | p.o. | 2 | 0.12 | 0.083 | 0.03 | 0.10 | - | 69.0 | 12.2 |
| | | 6 | 0.11 | 0.083 | 0.03 | 0.07 | - | 222.2 | 4.1 |
| | | 20 | 1.10 | 0.25 | 0.52 | 0.10 | - | 38.4 | 21.1 |
| Female | i.v. | 20 | - | - | 3.21 | 0.09 | 0.67 | 6.2 | - |
| | i.d. | 20 | 16.58 | 0.083 | 2.91 | 0.07 | 0.37 | 6.9 | 90.7 |
| | p.o. | 2 | 0.05 | 0.083 | 0.02 | - | - | 87.0 | 6.2 |
| | | 6 | 1.03 | 0.25 | 0.11 | - | - | 53.1 | 11.4 |
| | | 20 | 0.89 | 0.083 | 0.34 | 0.17 | - | 59.7 | 10.6 |

All values are shown as mean (N=4).
B.A. (%) = AUC (i.d. or p.o.)/AUC (i.v.) × Dose (i.v.)/Dose (i.d. or p.o.) × 100

Addendum: The report number is W-19961539 / W-19971272.

## Pharmacokinetics of E3810 in Rats Following Multiple Oral Dose (10 mg/kg) (Report # F-4)

**Methods**: Male Sprague-Dawley rats were given $^{14}C$ labeled E3810 (10 mg/kg/day) in normal saline for 14 consecutive days. Animals were starved for about 19 hr each day before drug administration. Blood samples were collected from tail vein at 30 min and 24 hr after drug administration. Urine and feces were collected at 14 hr intervals. Three rats were sacrificed at 24 hr after drug administration on days 1, 3 10 and 14 of the study for tissue distribution study. In all samples radioactivity was measured by liquid scintillation counter. Metabolites of E3810 in plasma, urine and feces were measured by TLC methods.

**Results**: Blood concentrations of radioactivity at 24 hr after each dose increased with duration of treatment and reached to steady state on day 12 of the study. Radioactivity was distributed throughout the body, and concentrations in thyroid, liver, bone marrow, pituitary gland and gastric mucosa were higher than that seen in blood cells. Significant amount of radioactivity was still present in thyroid at 11 weeks after the last dose. About 47.6% and 55.1% of the administered radioactivity were eliminated in urine and feces respectively.

Unchanged drug was not seen in urine or feces sample. Carboxylic acid derivative was the main metabolite in urine (51-54% of urinary radioactivity) and feces (26-33% of the fecal radioactivity). Other minor metabolites were mercapturic acid conjugate (8-112%) and desmethyl thioether derivative (3.6-3.8%) in urine, and thioether derivative (12-14%) and desmethyl-thioether derivative in feces (3.2=4.7%). Sponsor did not report any other pharmacokinetic parameters.

**Table 2** Tissue concentrations of radioactivity at 24 hr after 1st, 3rd, 10th and 14th dose of repeated oral administration of $^{14}C$-sodium pariprazole 14 times (10 mg/kg/day) to rats

| Tissues | 1st | 3rd | 10th | 14th |
|---|---|---|---|---|
| Brain | 0.056 ± 0.008 | 0.020 ± 0.002 | 0.048 ± 0.013 | 0.050 ± 0.007 |
| Pituitary gland | 0.400 ± 0.102 | 0.378 a) | 0.126 ± 0.030 | 0.117 ± 0.014 |
| Eyeball | 0.014 ± 0.003 | 0.020 ± 0.006 | 0.034 ± 0.007 | 0.029 ± 0.002 |
| Harderian gland | 0.112 ± 0.028 | 0.098 ± 0.025 | 0.088 ± 0.015 | 0.133 ± 0.025 |
| Submaxillary gland | 0.038 ± 0.012 | 0.053 ± 0.017 | 0.059 ± 0.008 | 0.055 ± 0.010 |
| Lymph node | 0.060 ± 0.017 | 0.045 ± 0.010 | 0.081 ± 0.015 | 0.049 ± 0.004 |
| Thyroid | 2.251 ± 0.077 | 2.501 ± 0.359 | 3.715 ± 0.745 | 4.039 ± 1.131 |
| Thymus | 0.043 ± 0.014 | 0.315 ± 0.278 | 0.038 ± 0.005 | 0.045 ± 0.010 |
| Lung | 0.211 ± 0.073 | 0.089 ± 0.029 | 0.149 ± 0.015 | 0.236 ± 0.007 |
| Heart | 0.136 ± 0.090 | 0.060 ± 0.008 | 0.093 ± 0.006 | 0.110 ± 0.005 |
| Skin | 0.073 ± 0.011 | 0.079 ± 0.003 | 0.112 ± 0.025 | 0.139 ± 0.038 |
| Skeletal muscle (femoral part) | 0.035 ± 0.011 | 0.048 ± 0.010 | 0.048 ± 0.009 | 0.045 ± 0.005 |
| Sciatic nerve | 0.033 ± 0.004 | 0.061 ± 0.023 | 0.063 ± 0.005 | 0.075 ± 0.027 |
| Pancreas | 0.039 ± 0.009 | 0.075 ± 0.025 | 0.071 ± 0.008 | 0.081 ± 0.004 |
| Spleen | 0.195 ± 0.070 | 0.142 ± 0.064 | 0.163 ± 0.026 | 0.219 ± 0.054 |
| Bone marrow | 0.429 ± 0.173 | 0.152 ± 0.031 | 0.334 ± 0.132 | 0.233 ± 0.057 |
| Liver | 0.558 ± 0.025 | 1.160 ± 0.033 | 2.218 ± 0.498 | 1.584 ± 0.359 |
| Kidney | 0.248 ± 0.008 | 0.418 ± 0.021 | 0.375 ± 0.111 | 0.617 ± 0.291 |
| Adrenal | 0.178 ± 0.022 | 0.317 ± 0.045 | 0.479 ± 0.050 | 0.760 ± 0.269 |
| Prostate | 0.047 ± 0.005 | 0.033 ± 0.006 | 0.034 ± 0.004 | 0.051 ± 0.009 |
| Urinary bladder | 0.050 ± 0.008 | 0.160 ± 0.035 | 0.435 ± 0.302 | 0.129 ± 0.026 |
| Forestomach | 0.143 ± 0.011 | 0.317 ± 0.090 | 0.422 ± 0.044 | 0.372 ± 0.132 |
| Glandular stomach | 0.191 ± 0.064 | 0.362 ± 0.030 | 0.248 ± 0.013 | 0.144 ± 0.018 |
| Gastric mucosa | 0.317 ± 0.071 | 0.600 ± 0.367 | 0.310 ± 0.042 | 0.206 ± 0.057 |
| Small intestine | 0.182 ± 0.050 | 0.211 ± 0.019 | 0.228 ± 0.051 | 0.188 ± 0.023 |
| Testis | 0.022 ± 0.001 | 0.024 ± 0.005 | 0.029 ± 0.006 | 0.041 ± 0.008 |
| Fat (renal side) | 0.031 ± 0.015 | 0.046 ± 0.005 | 0.037 ± 0.002 | 0.043 ± 0.002 |
| Blood | 0.143 ± 0.014 | 0.289 ± 0.021 | 0.864 ± 0.148 | 0.896 ± 0.108 |
| Plasma | 0.082 ± 0.001 | 0.074 ± 0.007 | 0.098 ± 0.006 | 0.066 ± 0.003 |
| Blood cells | 0.222 ± 0.040 | 0.481 ± 0.025 | 1.743 ± 0.320 | 1.598 ± 0.072 |

Data are expressed as mean ± standard error of 3 animals, in μg sodium pariprazole eq./g or ml.
a) Data denote the mean of 2 animals.

Addendum: The report number is W19961211 / W19961532.

## Transfer of Radioactivity Into Fetus and Milk Following Single Oral Administration of $^{14}$C-E3810 to Rats (Report # F-8)

**Methods**: Rats were given a single oral dose of $^{14}$C-E3810 (dissolved in purified water and 2% NaHCO3) on day 12 or 19 of gestation or on 14th day after delivery. In this experiment animals were starved for about 20 hr prior to drug administration. At 15 min, 2 hr and 24 hr after drug administration animals were sacrificed, blood and various tissues were collected to measure levels of radioactivity. Levels of radioactivity were also measured in the milk of lactating females at 15, 30 min, 1, 2, 4, 6, 8, 24 and 48 hr after drug administration. In all samples radioactivity was measured by LSC methods.

**Results**: Very little radioactivity (about 0.01% of the dose) was seen in the fetus when drug was administered on day 12 of gestation. However, the amount of total radioactivity detected in the whole fetus at 15 min, 2 hr and 24 hr after drug administration on day 19 of gestation were 1.16%, 0.44% and 0.13% of the dose. Hence, radioactivity of E3810 crosses placental barrier. Radioactivity was also seen in the milk of lactating rats. The $T_{max}$ in the milk was 6 hr, at 6 hr the level of radioactivity in the milk was about 6.5 fold higher than that seen in blood. Thus, radioactivity of E3810 was excreted in the milk.

Addendum: (1) the report number is W-19961158 / W19980279 and (2) the oral dose of $^{14}$C-E3810 was 20 mg/kg with specific activity of 109.4 μCi/mg.

## Excretion of Thioether Derivative Into Gastric Juice Following I.V. Administration of E3810 to Rats (Report # F-13)

**Methods**: Pyloric ligated rats were given a single i.v. dose of 1, 3, 10 or 30 mg/kg of E3810. Gastric juice samples were collected at 0.5, 1, 2, 4 and 8 hr after drug administration and blood samples were collected at 0.5, 1 and 2 hr after drug administration (3 rats/time point were used). In gastric juice and plasma samples levels of thioether derivative of E3810 were measured by HPLC methods.

**Results**: In gastric juice at 2 hr after i.v. dose, the levels of thioether derivative of E3810 increased with increasing dosages. Total amount of E3810-thioether excretion also increased with increasing dosages and correspondingly acidity of gastric juice were decreased.

| | Thioether Derivative in Gastric Juice | | Acidity of Gastric Juice |
|---|---|---|---|
| Dose (mg/kg) | (μg/ml) | Total Amount (μg) | μeq/2 hr/Rat |
| 0 | --- | --- | 357.0 ± 67.1 |
| 1 | 0.50 ± 0.13 | 1.73 ± 0.20 | 253.4 ± 35.3 |
| 3 | 1.08 ± 0.20 | 3.31 ± 0.53 | 117.8 ± 22.8 |
| 10 | 1.72 ± 0.33 | 5.57 ± 1.08 | 35.5 ± 8.4 |
| 30 | 2.84 ± 0.42 | 8.75 ± 1.03 | 0.8 ± 0.8 |

At 0.5 hr after the drug administration, the levels of E3810 and its thioether derivative in plasma were 0.46 ± 0.04 and 0.13 ± 0.01 μg/ml respectively. At 2 hr after the drug administration (i.e. $T_{max}$ for E3810-thioether in the gastric juice) drug and its thioether derivative were not detected in plasma.

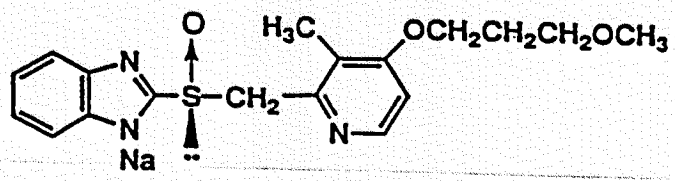
Addendum: The report number is W-19940654 / W-19961424.

## Pharmacokinetics of E3810 and Its Optical Isomers in Rats (Report # F-15)

Methods: Rats (n=4/group) were given a single i.v. dose of E3810 (racemic mixture, 40 mg/kg), R(+) E3810 (20 mg/kg) or S(-) E3810 (20 mg/kg). The volume of administration was fixed at 0.1 ml/100 g and animals were fasted for 16 hr prior to drug administration. Blood samples were collected from jugular vein at 5, 15, 30, 45 min, 1, 1.5 and 2.0 hr after drug administration. The levels of optical isomers of E3810 and their metabolites in rat 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, and $t_{½}$ values were comparable (about 10 min). However, $C_p$ (plasma clearance) and $V_d$ (volume of distribution) were about 1.5 fold greater in S(-) E3810 treated rats than that obtained in R(+)-E3810 treated rats. Similar results were seen when drug was administered in racemic mixture. There were no significant chiral conversion in vivo (about 2%). The thioether nd demethylated derivatives of E3810 were also seen in plasma as metabolite. Overall, pharmacokinetic parameters of racemic mixture and each individual isomers were similar.

R(+)E3810



S(-)E3810

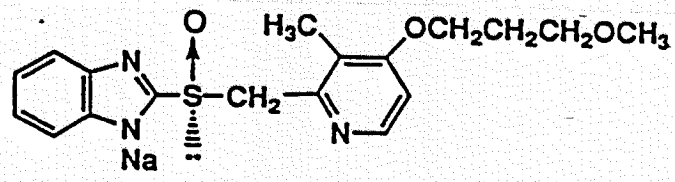


**Fig. 1 Chemical structures of E3810 enantiomers**

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

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) |
|---|---|---|---|---|---|---|
| Rat | R(+)E3810 | 20 | 9.8 | 905 | 64.4 | 331 |
| | S(-)E3810 | 20 | 10.6 | 1490 | 95.1 | 249 |
| | R/S ratio | | 0.92 | 0.61 | 0.68 | 1.33 |

Data shows mean of 4 rats.

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

| Species | Dose (mg/kg) | | t1/2 (min) | Vd (ml/kg) | Cl tot (ml/min/kg) | AUC (μg•min/ml) |
|---|---|---|---|---|---|---|
| Rat | 40 | R(+)E3810 | 9.5 | 692 | 51.4 | 420 |
| | | S(-)E3810 | 10.4 | 1142 | 79.7 | 276 |
| | | R/S ratio | 0.91 | 0.61 | 0.64 | 1.52 |

Data shows mean of 4 rats.

Addendum: The report number was W-19940597 / W19961240.

## Oral Bioavailability of E 3810 in Pregnant Rats (Study # 920516)

**Testing Laboratories**: Department of Drug Safety Research, Eisai Co., Ltd., Takehaya, Japan.

**Study Started**: December 2, 1992

**Study Completed**: December 9, 1992

**GLP Requirements**: Non-GLP study

**Animals**: Pregnant female Sprague Dawley rats.

**Drug Batch No.**: 11042522

**Methods**: Groups of pregnant rats (12/group) were given a single oral (via gavage: 25 mg/kg, 5 ml/kg) or i.v. (6 mg/kg, 1 ml/kg) dose of E 3810 on day 14 of gestation. Pregnant rats receiving oral dose of E 3810 were on restricted food regimen i.e. from gestation days 0-13 dams were given food for only 5 hr/day (9:00 AM to 2:00 PM) and were fasted from 2:00 PM to 9:00 AM. To these rats water were available ad libitum. Pregnant rats receiving i.v. dose had free access to food and water. Blood samples were collected at 5, 15, 30, 60 and 120 min after drug administration. Levels of E 3810 in samples were measured by HPLC methods and various pharmacokinetic parameters were calculated.

**Results**: Irrespective of route of administration the plasma terminal half-life was 7.6-7.7 min. The $T_{max}$ after oral administration was 5 min (first sampling point) and $C_{max}$ was 1.8608 ± 0.6796 mcg/ml. AUC value after 25 mg/kg oral dose was 42.7669 mcg x min/ml and after 6 mg/kg of i.v. dose was 88.4323 mcg x min./ml. Based on AUC values the absolute bioavailability (normalized for dose) was 11.6%.

## Effect of E 3810 on Hepatic Drug Metabolizing Enzymes Activities and Thyroid Function in Rats (Study # T92038)

**Testing Laboratories**: Department of Drug Safety Research, Eisai Co., Ltd., Takehaya, Japan.

**Study Started**: November 10, 1992

**Study Completed**: October 29, 1993

**GLP Requirements**: Non-GLP study

**Animals**: 7 weeks old SD male rats.

**Drug Batch No.**: 11042522