Stability			
	RBP	Sulfone	Thioether
RT for 30 minutes	100%	100%	101%
-20°C for 10 months	101%	101%	105%
Freeze/thaw cycles x4	>95%	>92%	>93%

RBP Pre-study Validation - Urine				
			Quality Control	
	UM-2 (with β-glucuronidase)	UM-2 (without β-glucuronidase)	UM-2 (with β-glucuronidase)	UM-2 (without β-glucuronidase)
Linearity	>0.998	>0.998	-	-
LOQ	102.4 ng/ml	102.4 ng/ml	-	-
Interday Precision	54% CV at LOQ <7% CV all others	45% CV at LOQ 5% CV all others	ND	<6% CV
Interday Accuracy	20-161% at LOQ 86-112% all others	49-161% at LOQ 92-112% all others	ND	92-116%
Intraday Precision	ND	<6.5% CV	ND	8% CV
Intraday Accuracy	ND	ND	ND	ND
Specificity: No blanks from individual subjects submitted.				
Recovery: 84% at 1000 ng/ml and 83% at 2000 ng/ml for UM-2 and IS, respectively.				
Stability: 97% residual after 3 months at -20°C, 97% residual following 4 freeze/thaw cycles				

RESULTS:

Demographics: There were no dropouts in this study. All subjects were Japanese males ranging in age from 24-50 years. Mean weights and heights were 63.5 kg and 171 cm, respectively.

Pharmacokinetics: Due to insufficient reliable plasma concentration data, no data analysis was performed for RBP and its metabolites after either the 1 mg or 3 mg dose. For all other doses, N=6 unless indicated otherwise.

Table 1. Mean±SD PK parameters for RBP.

	10 mg	20 mg	40 mg[2]	80 mg
Cmax (ng/ml)	247±59	406±156	1,351±453	2,499±618
tmax (hr)	3.8±1.3	3.1±0.4	2.9±0.5	3.3±0.9
AUC_{0-t}(ng*hr/ml)	423±56	788±438	2,128±607	4,988±1,934
$AUC_{0-\infty}$(ng*hr/ml)	440±59	809±456	2,153±628	5,212±2,158
Half-life (hr)[1]	0.85±0.09	1.02±0.39	1.06±0.09	1.21±0.32
Cl/F (ml/min/kg)	6.46±1.07	8.4±5.95	5.71±2.74	4.37±1.79

[1]Determined from the terminal 5 points.

[2]n=4 (2 subjects excluded from data analysis due to low plasma levels)

Two subjects exhibited extremely low RBP plasma concentrations after receiving the 40 mg dose and were, therefore, excluded from the data analysis. The sponsor attributed these low levels to a dosing error. Although there was a trend for greater than proportional increases in $AUC_{0-\infty}$ at higher doses, the values did not reach statistical significance. Likewise, half-life values tended to increase while Cl/F values tended to decrease following higher doses. Again, these did not reach

statistical significance. Furthermore, when oral clearance was plotted and regressed as a function of RBP dose, the slope was not statistically significantly different from zero, indicating dose-independence for this parameter. Results of dose-normalized values (based on a 10 mg dose) for RBP AUC and Cmax values are provided in Table 2.

Table 2. Mean dose-normalized PK parameters for RBP.

	10 mg	20 mg	40 mg[1]	80 mg
Cmax (ng/ml)	247	203	338	312
$AUC_{0-\infty}$(ng*hr/ml)	440	405	538	652

[1]n=4

DM and DMTE could not be detected in the plasma of any subject. The sulfone metabolite appeared in limited quantities and was only detected in the plasma of every individual after the 80 mg dose. The half-life of the S was similar to that of RBP, indicating formation rate-limited elimination. The mean half-life value provided for the 40 mg dose was invalid, as it was determined from only 2 plasma concentration time points for one of the subjects. Recalculation of the data indicates that a more accurate estimate of half-life is 0.93±0.29 hours (n=3). Figure 1 (attached to the study report) displays the S plasma concentration profile paralleling that of RBP for the 80 mg dose in each individual subject.

Table 3. Mean±SD Sulfone PK pararmeters.

	10 mg[1]	20 mg	40 mg	80 mg
Cmax (ng/ml)	ND	22±20[2]	71±28[2]	157±62
tmax (hr)	ND	3.4±0.6[2]	2.9±0.5[2]	3.3±0.9
AUC_{0-t}(ng*hr/ml)	ND	28±47[2]	89±50[2]	341±223
$AUC_{0-\infty}$(ng*hr/ml)	ND	109±134[2]	162±64[2]	387±238
Half-life (hr)	ND	ND[3]	2.11±2.38[2,4]	1.17±0.49[4]

[1]Could not be calculated as all data were <LOQ.
[2]n=4 (some subjects did not provide enough data for analysis)
[3]Not determined as <50% data obtained.
[4]Determined from terminal 3 points.

The TE metabolite was the primary metabolite of RBP detected in plasma. Its half-life was longer than that of RBP as can be observed in Figure 1. Furthermore, the mean values provided for TE half-life in the above table may be underestimated, as plasma sampling did not include much of the terminal elimination phase (AUC_{0-t} contributed only 54-74% of the $AUC_{0-\infty}$ after dosing for all treatments). Plasma concentrations of TE exceeded that of the parent compound by about 6 hours post-dose.

Table 4. Mean±SD Thioether PK parameters.

	10 mg	20 mg	40 mg[1]	80 mg
Cmax (ng/ml)	51±26	73±40	193±40	419±162
tmax (hr)	5.0±1.1	4.3±0.8	4.3±0.7	4.9±0.8
AUC_{0-t}(ng*hr/ml)	164±139	256±191	789±155	1,763±759
$AUC_{0-\infty}$(ng*hr/ml)	302±177	378±221	1,069±240	2,998±1,923
Half-life (hr)[2]	3.15±1.10	2.77±0.75	2.95±1.18	3.53±1.60

[1]n=4 (some subjects did not provide enough data for analysis)
[2]Determined from terminal 4 points.

Because no RBP, S, nor DM could be detected in the urine of the first 2 subjects in both studies, and because the sum of the cumulative amounts of TE and DMTE excreted into the urine were

not more than 0.10% of the dose in either individual, these compounds were not quantified in the other subjects. UM-2 was the primary metabolite found in urine; cumulative excreted amounts of unconjugated UM-2, UM-2 glucuronide, and total UM-2 into the urine after RBP dosing are provided in Table 5. The difference between the urine concentration of UM-2 without and with β-glucuronidase incubation was calculated to be the amount of UM-2 glucuronide present.

Table 5. Cumulative Excretion of UM-2 in the Urine.

	Cumulative Excretion over 72 hours (% of administered RBP dose)		
	Unconjugated UM-2	UM-2 glucuronide	Total UM-2
10 mg dose	22.8±5.1%	10.2±4.5%	33.0±2.3%
20 mg dose	19.1±4.1%	10.8±2.3%	29.9±4.4%
40 mg dose	18.6±1.9%	11.1±0.4%	29.7±1.5%
80 mg dose	21.4±5.7%	8.4±1.7%	29.8±6.7%

The mean cumulative urinary excretion of total UM-2 after administration of all four RBP doses is depicted in Figure 2 (attached to the study report). Excretion of these metabolites was not dose-dependent.

Because analytical methods for the determination of RBP and its metabolites in feces had not been established by the time the samples had been stored for 2 years, no analysis was undertaken.

Serum Gastrin:

The concentrations of gastrin exhibited a diurnal variation and increased after meals in both active and placebo groups. (Meals were taken just after blood sampling for the determinations of serum gastrin at 5 and 8 hours after RBP administration.) Although it was difficult to draw a clear conclusion because of the large interday and intersubject variability observed in both active and placebo groups, the data suggest that RBP administration did not result in dose-dependent increases in serum gastrin. Mean change of serum gastrin values for the 6 subjects in the active subgroup for each dose were compared with the mean change of serum gastrin of the same 6 subjects after placebo and results are listed in the table below.

Table 6. Mean±SD changes in serum gastrin[1] concentrations (pg/ml): RBP-placebo.

	1 mg RBP	3 mg RBP	10 mg RBP	20 mg RBP	40 mg RBP	80 mg RBP
Predose	0	0	0	0	0	0
Hour 2	-4.0±20	-6±46	-4±16	-2±8	-7±18	-7±17
Hour 4	2±19	-16±35	-6±23	14±10	26±19*	8±13
Hour 5	-17±20	17±35	11±9**	19±26	54±32**	29±23
Hour 6	72±71	106±92**	81±87	75±92	99±77	168±154
Hour 8	63±64	97±91*	132±119*	78±85	91±61	143±138
Hour 24	-11±21	16±7*	37±36**	27±33	10±48	51±53

*p<0.05, **p<0.01(paired t-test vs placebo for each group)
[1]Normal range (fasting): 37-172 pg/ml

Safety: Abnormal symptoms, which were considered to be possibly related to the study drug, were reported in 2 subjects; these were rated as mild in severity and included upset nausea and heavy-headedness. No clinically significant changes were observed in ECG recordings, vital signs, or clinical laboratory tests. Several abnormal clinical laboratory results were observed, but none were considered likely to be related to RBP, although a relationship could not be excluded.

CONCLUSIONS:

RBP was generally well tolerated by all subjects. The PKs of RBP were roughly linear following single oral doses of 10 to 80 mg, however, did show a trend for greater than proportional kinetics at higher doses. The S and TE metabolites were observed in plasma at most doses. Based on half-life values and corresponding plasma concentration vs time profiles, the elimination of the S metabolite appears to be formation rate-limited while that of the TE appears to be elimination rate-limited. Only the UM-2 and UM-2 glucuronide could be detected in urine in appreciable quantities; their excretion was not dose-dependent.

REVIEWER'S COMMENTS:

1. The method of statistical analysis used to determine differences between the PK parameters for the different dosing regimens was not included in the study protocol.
2. The to-be-marketed formulation or strength of RBP tablets were not used in this study. Furthermore, there were no linking BE studies with any of the formulations used in any of the PK or clinical studies.
3. Analytical validation for RBP and its metabolites in both plasma and urine was inadequate and/or unacceptable. Furthermore, the sponsor admits that "final procedures" for assay validation criteria were not established at the time this study was performed, therefore, no in-study validation data are available.
4. Overall, the validity of the results obtained in the current study are tentative due to the uncertainty regarding the BA of the formulation used and the inability of the analytical assay to reliably and accurately quantitate RBP and its metabolites at lower concentrations in plasma and urine.

1 Page(s) Redacted

RAW DATA

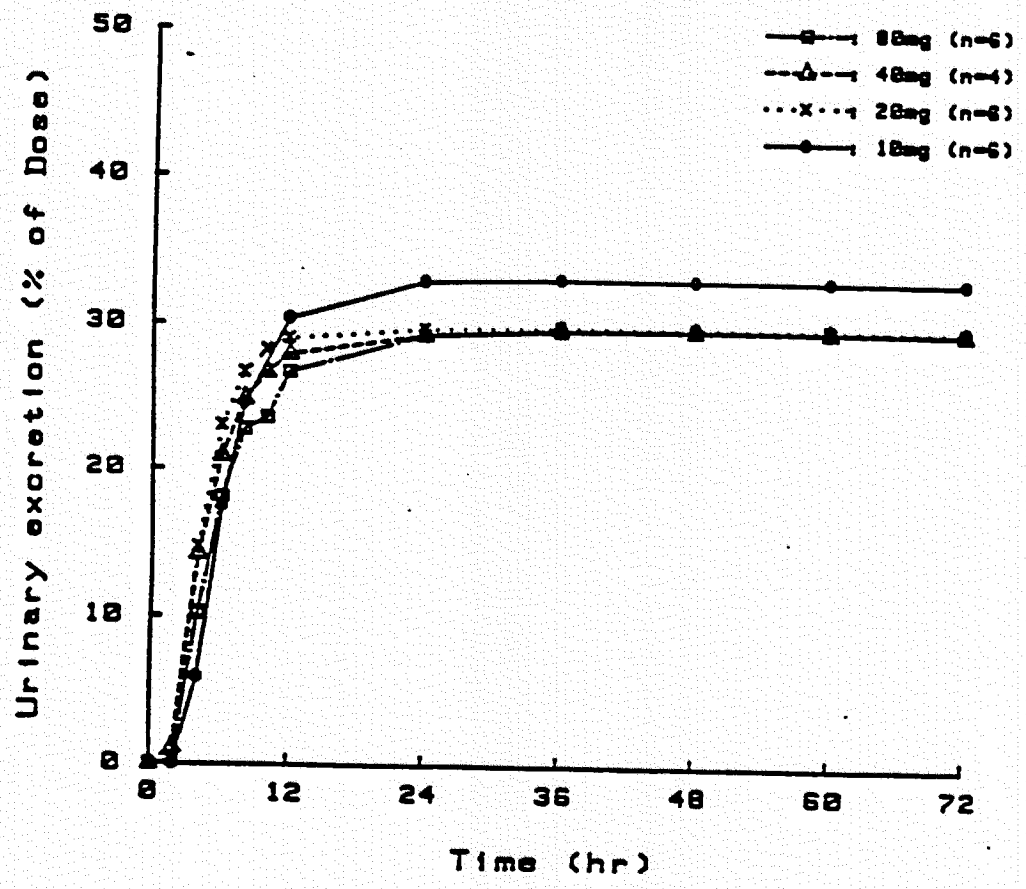

Figure 2.
Plots of Mean Cumulative Urinary Excretion of Metabolites of E3810 (Sum of UM-2 and UM-2 glucuronide).

Each point represents the mean ± S.D. of 4 (40 mg dose study) or 6 subjects.
Results of Subjects 7 and 8 in 40 mg-dose study were excluded from averaging since these two subjects had much lower plasma levels.

TITLE: An Ascending, Multiple-Dose Safety and Tolerance Study of an Oral Formulation of E3810 in Healthy Male Volunteers

Protocol Number: E3810-A001-002

Study Dates: August-October, 1992

OBJECTIVES:
Primary Objective:
To assess the safety and tolerance of ascending dose strengths of RBP in healthy male volunteers following administration of the drug for 14 consecutive days as single, daily, oral doses.
Secondary Objectives:
To measure the plasma concentrations of RBP which resulted from these doses and to obtain information regarding the influence of the drug on plasma gastrin and intragastric pH.

METHODS:
Study Design: double-blind, randomized, placebo-controlled, sequential-group

Study Population: 25 normal, healthy, males aged 18-45 years

Treatment and Administration:
A total of 25 subjects were enrolled. RBP was administered as follows:
Period I: 6 subjects received 10 mg RBP and 2 received placebo
Period II: 7 subjects received 20 mg RBP and 2 received placebo
Period III: 6 subjects received 40 mg RBP and 2 received placebo
All Periods: Test medication was administered orally with 250 ml water, daily for 14 days during each Period. Meals were closely monitored and provided at 0.5, 5, 10, and 14 hours after drug administration.

Study Drug Supplies:
10 mg RBP tablets; #K16001BZZ. ***This was not the to-be-marketed formulation or strength.***
Placebo tablets; #K9X0700

Biological Sampling:
Plasma: Blood was collected immediately prior to and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours after drug administration on Days 1, 5, and 14 of the protocol for determination of plasma RBP levels. Blood was collected immediately prior to and at 1.5, 5, 6, 10, and 24 hours after RBP administration on Days 1, 5, 14, and 15 for the determination of plasma gastrin levels.
Intragastric pH : monitored for 24 continuous hours after RBP administration on Day 1 and 5, and for 48 hours after the final dose on Day 14. Intragastric pH was measured using the [redacted] glass electrodes inserted into the antral portion of the stomach.

Pharmacokinetic Analysis:
PK parameters were determined from the plasma concentration data using standard non-compartmental techniques. Parameters computed were as follows: AUC_{0-t}, $AUC_{0-\infty}$, Cmax, tmax, $t_{½}$, and Cl_T/F for Days 1, 5, 14 and 15. Oral clearance was plotted and regressed as a function of RBP dose to assess PK linearity.

Pharmacodynamic Analysis:
Intragastric pH was measured at 32-second intervals for 24 hr periods on Days 1, 5, 14, and 15. Plasma gastrin was measured on Days 1, 5, 14, and 15.

Statistical Methods:
PK data - rigorous statistical anaysis was not performed. As appropriate comparisons were made among and between the placebo- and RBP-treated subjects at each dose level.
PD data - for plasma gastrin and intragastric pH data, ANOVA was used to test for dose group differences. If the overall p-value was ≤ 0.05, pairwise comparisons were made using Fisher's least significant difference procedure at the 0.05 level of significance.

Safety and Tolerability:
Assessed via clinical laboratory evaluations, vital signs, physical examinations, and ECGs. All adverse events were recorded with notation of duration, severity, (i.e., mild, moderate, or severe) and outcome.

Analytical Methods:
Plasma RBP concentrations were measured at [redacted] detection. Assay pre-validation parameters are provided below. Plasma gastrin was quantified using a validated [redacted]

[redacted]

Linearity - 5.5 - 444 ng/ml, $r^2 > 0.999$
Sensitivity – LOQ=6 ng/ml
Interday Precision - ≤5% CV
Interday Accuracy - 98 to 110%
Intraday Precision - <12% CV
Intraday Accuracy - 90 to 105%

Quality Control Samples:
Interday Precision - <5% CV
Interday Accuracy - 100 to 106%
Intraday Precision - <6% CV
Intraday Accuracy - 87 to 106%
Recovery - RBP: 86.4% at 5.5 ng/ml, 92.4% at 444 ng/ml
IS: 104%
Stability - recovery after storage at -20°C for 11 weeks; 92% at 16 ng/ml, 87% at 88 ng/ml, and 80% at 333 ng/ml
- 83-86% recovered after standing at room temperature for 30 hr.
- 88-94% recovered after standing at room temperature for 22 hr.
- <5% loss after 3 freeze/thaw cycles

Specificity - [redacted]

Study plasma samples were analyzed October/November of 1992. Assay validation parameters were acceptable and comparable to the results obtained above, however, the LOQ was set at 5.5 ng/ml. [redacted] were provided for the subjects in the 10 mg dosing group only. RBP peaks for some of the 5.5 ng/ml samples were poorly resolved.

RESULTS:

Demographics:

One subject (20 mg dose) withdrew due to inability to tolerate the intragastric electrode and was replaced. The study population was all male, predominately white, and ranged in age from 22 to 45 years. Subjects' heights ranged from 170 to 188 cm and weights from 63.4 to 86.2 kg. Baseline characteristics were similar between the treatment groups.

Safety:

There were no deaths or serious adverse events. All reported AEs were mild to moderate in nature. There was no increase in frequency or severity for any clinical symptom with increasing dose. One 40-mg subject had a reversible increase in circulating eosinophils. Another 40-mg subject had elevated creatine kinase on Days 5 and 16 accompanied by elevated ALT on Day 16; at follow-up, the ALT returned to normal, but the creatine kinase remained elevated. A third 40-mg subject had elevated ALT on Days 5 and 16 accompanied by slight elevations in AST; the ALT returned to slightly above normal at follow-up.

One 10-mg subject showed transient occasional PVCs on the ECG on Day 5, but not on Day 14 or follow-up. In addition, one subject developed first-degree AV block approximately 4 hrs after 40 mg RBP on Day 1, however, the ECG had returned to normal 4 days later. There were no clinically significant changes in vital signs.

Pharmacokinetics:

On at least one sampling day, the plasma concentrations were above the limit of detection at only two or three time points for each of the subjects in the 10-mg group, three of the six subjects in the 20-mg group, and one subject in the 40-mg group. Thus, there was greater intersubject variability at the lower doses which decreased with higher doses.

The mean PK parameters for each dose of RBP on Days 1, 5, and 14 are given in Table 1 below. Cl_T/F appeared to decrease with higher doses of RBP, however, these values were based on calculations which included AUC, therefore, contain a large degree of variability. Likewise, $t_{½}$ appeared to increase with higher doses, but due to the large intersubject variability in RBP plasma concentrations (some of the plasma concentration vs time curves tended to be erratic at the 10 mg and 20 mg doses), it was difficult to accurately characterize the terminal elimination phase. In addition, more than one "peak" concentration was seen for some of the profiles, with a secondary peak noted in the two hour post-prandial period corresponding to lunch. The reason for these additional peaks is unknown. The mean RBP plasma concentration vs time profiles for each dose are attached to the study report as Figures 1-3.

Table 1. Mean±SD PK parameters for RBP.

	Dose								
	10 mg (N=6)			20 mg (N=7)			40 mg (N=6)		
PK parameter	*Day 1*	*Day 5*	*Day 14*	*Day 1*	*Day 5*	*Day 14*	*Day 1*	*Day 5*	*Day 14*
AUC_{0-t} (ng*hr/ml)	247± 198	326± 154	283± 180	407± 162	435± 260	684± 489	1302± 458	1388± 590	1505± 523
$AUC_{0-\infty}$ (ng*hr/ml)	247± 198	328± 158	283± 180	408± 164	435± 260	684± 489	1310± 458	1389± 591	1508± 527
Cmax (ng/ml)	153± 110	215± 72	202± 126	238± 116	353± 184	402± 319	775± 296	851± 507	960± 462
Tmax (hr)	5.83± 3.37	3.42± 3.37	3.25± 1.33	2.33± 0.98	2.67± 1.72	5.00± 3.79	3.42± 2.06	2.08± 0.74	2.58± 1.24
half-life (hr)	0.68± 0.21	0.64± 0.17	0.69± 0.23	0.95± 0.51	1.15± 0.39	0.77± 0.17	1.12± 0.43	1.14± 0.33	0.97± 0.30
Cl_T/F (L/hr/kg)	0.73± 0.49	0.52± 0.25	0.67± 0.40	0.74± 0.27	0.92± 0.67	0.45± 0.33	0.45± 0.18	0.46± 0.21	0.40± 0.15
Vd (L/kg)	0.76± 0.73	0.47± 0.26	0.70± 0.60	0.94± 0.47	1.62± 1.34	0.49± 0.40	0.65± 0.17	0.74± 0.38	0.53± 0.19

Dose-adjusted values for $AUC_{0-\infty}$ and Cmax are provided in Table 2. Although there appeared to be a trend for $AUC_{0-\infty}$ to increase with higher doses, the differences can most likely be attributed to the high intersubject variability at lower doses and the sensitivity limits of the analytical assay at the plasma concentrations observed after the 10 mg dose. In addition, it should be remembered that different subjects were studied at each treatment regimen, thus introducing further variability into the data. When oral clearance was plotted and regressed as a function of RBP dose for Days 1, 5, and 14, the slope was not statistically significantly different from zero, indicating dose-independence for this parameter.

There was also a trend for a time-dependent increase in PK parameters within the 20 and 40 mg dosage groups, however, these differences did not achieve statistical significance according to the sponsor (statistical data not provided).

Table 2. Mean±SD dose-adjusted PK parameters (adjusted to 10 mg dose).

	$AUC_{0-\infty}$ (ng*hr/ml)			Cmax (ng/ml)		
	10 mg	*20 mg*	*40 mg*	*10 mg*	*20 mg*	*40 mg*
Day 1	247	204	328	153	119	194
Day 5	328	217	347	215	177	213
Day 14	283	342	377	202	201	240

Pharmacodynamics:

There were dose-related increases in the pharmacological response to RBP as measured by mean 24-hr intragastric pH, mean nocturnal pH, and percentages of 24-hr or nocturnal time pH≥3. The increases achieved statistical significance for all three RBP treatment regimens when compared to placebo.

The 24-hr values for mean pH and % of time that pH ≥ 3 were similar for Days 5 and 14, indicating that overall, the pharmacological response had reached a steady state by Day 5. Upon termination of RBP treatment (Day 15), mean values for all dose groups for mean pH had decreased to approximately 67% of Day 14 values, and those for % of time pH≥3 to 55% of Day 14 values.

Values for nocturnal mean pH and % of time pH≥3 continued to increase between Days 5 and 14 for the 10 and 20 mg groups. However, for the 40 mg dose these values were within 88% of the Day 14 value after only a single dose.

Plasma gastrin concentrations and total gastrin AUC also increased in a dose- and time-related manner in response to RBP treatment, with the increases in gastrin AUC reaching statistical significance compared to placebo by Day 5 of treatment. While there was a trend toward larger gastrin AUCs with higher RBP doses, the differences noted between dosage groups were not significantly different. The increases produced during RBP treatment were substantially reversed within 24 hr after the last dose (Day 15).

PK/PD Correlation:
Correlation plots were constructed for the following PK/PD relationships from data obtained on Day 14: gastrin $AUC_{0-\infty}$ vs RBP $AUC_{0-\infty}$, gastrin $AUC_{0-\infty}$ vs RBP Cmax, mean intragastric pH vs RBP $AUC_{0-\infty}$, mean intragastric pH vs RBP Cmax, % time pH>3 vs RBP $AUC_{0-\infty}$, and % time pH>3 vs RBP Cmax. There were no apparent relationships between any of the variables observed in any of the plots.

CONCLUSIONS:
- RBP was well-tolerated when administered for 14 consecutive days as single, oral doses of 10, 20, or 40 mg.
- The PK data suggest that the disposition of RBP was rapid and dose-proportional (based on oral clearance data) over a 10 to 40 mg dosing range. Some accumulation of drug appeared to occur over time, however, the extent was not statistically significant.
- Statistically significant dose-related increases in intragastric pH and plasma gastrin were observed. The effects on intragastric pH were significant for all doses studied.
- There were no apparent PK/PD relationships.
- At steady-state, the relative effectiveness of the four treatment regimens at raising intragastric pH was: Placebo<10 mg RBP/day<20 mg RBP/day≡40 mg RBP/day.

REVIEWER'S COMMENTS:
1. Formal statistical analysis of differences in PK parameters between treatment groups was not performed.
2. Different subjects were used in each treatment group, increasing the intersubject variability.
3. There were no trough levels (Cmin) of drug reported; it is unclear how the sponsor arrived at the conclusion that there was no accumulation of RBP with repeated dosing.
4. A number of the [redacted] which were poorly resolved at the assay LOQ.

TITLE: A placebo-controlled ascending multiple oral dose study to evaluate the safety, tolerance, and pharmacokinetics of E3810 in healthy male volunteers.

Protocol Number: E3810-J081-004

Study Dates: December, 1988 – January, 1989

OBJECTIVE: to evaluate the safety, tolerance, and the pharmacokinetic profile of RBP and its metabolites in plasma and urine

METHODS:
Study Design: randomized, placebo-controlled, double-blind, ascending multiple oral dose

Study Population: 18 healthy male Japanese volunteers, aged 20-26 years

Treatment and Drug Administration:
Subjects were randomly divided into two groups of 9 subjects each:
Group A - received 20 mg oral RBP.
Group B – received 40 mg oral RBP.
In each group, 6 subjects were given RBP while 3 received placebo. Drug was administered with 120 ml water, 30 minutes after breakfast, at 9:00 AM on Days 1-7. Meals were served at 8:30, 14:00, 18:30, and 21:00 (breakfast, lunch, dinner, and snack, respectively) on Days 1-7.

Study Drug Supplies:
10 mg enteric-coated RBP tablets; #K891503. ***This was not the to-be-marketed formulation nor strength.***
10 mg placebo enteric film-coated tablets; #K891501.

Biological Sampling:
RBP and metabolites - blood was collected prior to each dosing from Day 1 - Day 7 and at 2, 3, 4, 4.5, 5, 5.5, 6, 7, 8, 10, and 12 hours after RBP administration on Day 1 and Day 7, and 24 hours after the final dosing (Day 8). Urine was collected for 12 hours prior to and for hours 0-2, 2-4, 4-6, 6-8, 8-10, 10-12, and 12-24 hours after the first dose. Twenty-four hour urine samples were collected after each dose administration from Day 2-Day 6. Urine collection after the final dose of RBP was the same as after the first dose except a period of 24-48 hours was added.
Serum protein binding - blood was collected for the determination of serum protein binding at 5 and 7 hours after the first and the final dose of RBP.
Serum gastrin - blood was collected prior to breakfast and just prior to each dose of RBP from Days 1-7, just prior to breakfast on Day 8, and in the morning of Day 14 (20 mg dose) or Day 21 (40 mg dose).

Pharmacokinetic Analysis:
Non-compartmental PK parameters were calculated using standard methods. Values were reported for $AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$, Cmax, tmax, half-life, and Cl/F for RBP and its metabolites. Urinary excretion of the metabolites was expressed as % of RBP dose.

Safety:
Assessed by physical exams and monitoring of vital signs, ECGs, subjective symptoms, and clinical laboratory tests.

Statistical Methods:
No information was provided regarding statistical analysis of PK parameters.

Analytical Methods:
Serum gastrin - [redacted] **method using a commercially available kit. The LOQ was 25 pg/ml.**

Serum protein binding of RBP - [redacted] method followed by [redacted] LOQ was 14-25 ng/ml. RBP was stable for up to 70 minutes after [redacted] and after storage for 6 days at -20°C. No other validation data was provided.

RBP and metabolites in plasma and urine - [redacted] was performed by Eisai Co., Ltd., Tokyo. Pre-study validation: May-Sept, 1988. Analysis of study samples: Plasma - 1/89, Urine - 12/88-3/89.

Pre-study Validation - Plasma						
[redacted]				Quality Control (determined retrospectively from in-study data)		
	RBP	Sulfone	Thioether	RBP (206.7 ng/ml)	Sulfone (205.2 ng/ml)	Thioether (222.8 ng/ml)
Linearity	>0.999	>0.997	>0.999	-	-	-
LOQ	5 ng/ml	20 ng/ml	20 ng/ml	-	-	-
Interday Precision	<15% CV	<5%CV	22% CV at LOQ	<2% CV	3% CV	2% CV
Interday Accuracy	95-130% LOQ 95-126% all others	102-109% LOQ 96-102% all others	85-113% LOQ 94-104% all others	102-112%	92-108%	92-107%
Intraday Precision	<7% CV	<8% CV	<10% CV	<3% CV	<7% CV	<5% CV
Intraday Accuracy	42-122% LOQ 78-113% all others	ND at LOQ 93-112% all others	ND at LOQ 96-109% all others	ND	ND	ND
Specificity: No blank [redacted] nor [redacted] rom individual subjects submitted.						
Recovery: RBP – ranged from 112% at 5 ng/ml to 86% at 400 ng/ml. Sulfone - ≥79% at all concentrations. Thioether - ≥91% at 50-400 ng/ml; no data at assay LOQ, but only 44% recovery at 10 ng/ml.						

Stability			
	RBP	Sulfone	Thioether
RT for 30 minutes	100%	100%	101%
-20°C for 10 months	101%	101%	105%
Freeze/thaw cycles x4	>95%	>92%	>93%

APPEARS THIS WAY
ON ORIGINAL

Pre-study Validation - Urine				
			Quality Control	
	UM-2 (with β-glucuronidase)	UM-2 (without β-glucuronidase)	UM-2 (with β-glucuronidase)	UM-2 (without β-glucuronidase)
Linearity	>0.998	>0.998	-	-
LOQ	102.4 ng/ml	102.4 ng/ml	-	-
Interday Precision	54% CV at LOQ <7% CV all others	45% CV at LOQ 5% CV all others	ND	ND
Interday Accuracy	20-161% at LOQ 86-112% all others	49-161% at LOQ 92-112% all others	ND	ND
Intraday Precision	ND	<6.5% CV	ND	ND
Intraday Accuracy	ND	ND	ND	ND
Specificity: No blanks from individual subjects submitted.				
Recovery: 84% at 1000 ng/ml and 83% at 2000 ng/ml for UM-2 and IS, respectively.				
Stability: 97% residual after 3 months at -20°C, 97% residual following 4 freeze/thaw cycles				

RESULTS:

Demographics:

There were no dropouts in this study. All subjects were Japanese males ranging in age from 20-26 years. Mean weights and heights were 66.4 kg and 174.5 cm, respectively. No information was provided with respect to baseline characteristics of the two treatment groups.

Pharmacokinetics:

Plasma concentrations of RBP were all below the assay LOQ (5 ng/ml) prior to each daily dose, indicating lack of accumulation with multiple dosing at both 20 and 40 mg. This conclusion is also confirmed by the Cmax and AUC values for each dose after the first and last doses. The tmax after the last dose of 40 mg RBP was statistically significantly shorter than that observed after the first dose. The sponsor suggested that this could have been caused by the neutralization of gastric juice after initial RBP doses (due to the pharmacologic effect of the drug), with partial dissolution of the enteric tablet in the stomach as it is designed to release at higher pHs. Mean values for half-life, tmax, and Cl/F were not significantly different between the 20 and 40 mg doses. Mean values for Cmax and AUC did not increase proportionally with dose, with the values after the 40 mg dose less than predicted based on values observed after the 20 mg dose. The results of the PK calculations for RBP are provided in Table 1.

Table 1. Mean±SD PK parameters for RBP (N=6 for each group).

	20 mg		40 mg	
	First[1]	Last[2]	First	Last
$AUC_{0-\infty}$ (ng*hr/ml)[3]	863±492	897±336	1,296±552	1,036±308
Cmax (ng/ml)	478±124	407±136	595±199*	418±177
tmax (hr)	4.6±1.1	4.0±1.4	5.1±0.6**	3.8±0.7
Half-life (hr)[4]	1.03±0.56	1.34±0.70	0.90±0.19	1.49±0.78
Cl/F (ml/min/kg)	7.49±3.39	6.49±1.95	8.91±4.00	10.08±2.53

[1,2]Results are provided for RBP, S, and TE after the first and last doses (Days 1 and 7, respectively).

[3]AUCs were calculated as $AUC_{0-\infty}$ after the first dose and as AUC_{0-24hr} after the last dose.

[4]Calculated from the terminal 4 plasma concentration points

*$p<0.05$ for First vs Last, **$0.05<p<0.10$ for First vs Last

Although DM and DMTE could not be detected in the plasma of any subject during repeated qd dosing of 20 mg and 40 mg RBP, the TE and S metabolites were observed. There were very few plasma samples that contained detectable levels of S, whereas the TE metabolite appeared in greater quantities. Even though not statistically significant, there did appear to be some accumulation of TE by the last dose in both the 20 and 40 mg dosing regimens based on individual and mean Cmax and AUC values. The results of the PK calculations for both the S and TE metabolites are provided in Tables 2 and 3.

Table 2. Mean±SD PK parameters for S.

	20 mg		40 mg	
	First[1]	Last[2]	First	Last
$AUC_{0-\infty}$ (ng*hr/ml)	ND[3]	ND	ND	ND
Cmax (ng/ml)	27±14	13±14	24±19	10±16
tmax (hr)	4.4±1.1 (n=5)[4]	3.0±1.0 (n=3)	5.1±1.3 (n=4)	4.3 (n=2)
Half-life (hr)	ND	ND	ND	ND
Cl/F (ml/min/kg)	ND	ND	ND	ND

[1,2]Results are provided for RBP, S, TE after the first and last doses (Days 1 and 7, respectively).
[3]ND - not determined
[4]Means are provided for 6 subjects unless indicated otherwise.

Table 3. Mean±SD PK parameters for TE (N=6 for each group).

	20 mg		40 mg	
	First[1]	Last[2]	First	Last
$AUC_{0-\infty}$ (ng*hr/ml)[3]	401±242	614±452	911±1,123	1,366±1,735
Cmax (ng/ml)	89±43	123±85	139±123	232±235
Tmax (hr)	5.3±0.8	4.8±0.8	5.4±0.5	5.3±0.6
Half-life (hr)[4]	2.66±0.65	2.29±0.27	2.53±0.68	2.44±1.02
Cl/F (ml/min/kg)	ND	ND	ND	ND

[1,2]Results are provided for RBP, S, and TE after the first and last doses (Days 1 and 7, respectively).
[3]AUCs were calculated as $AUC_{0-\infty}$ after the first dose and as AUC_{0-24hr} after the last dose.
[4]Calculated from the terminal 3 plasma concentration points.

Because no RBP, S, nor DM could be detected in the urine of the first 2 subjects in both studies, and because the sum of the cumulative amounts of TE and DMTE excreted into the urine were not more than 0.10% of the dose in either individual, these compounds were not quantified in the other subjects.

UM-2 and its glucuronide conjugate were the primary metabolites detected in urine. Stable quantities were excreted from the second day of dosing forward. Excretion of these metabolites did not appear to be dose-dependent.

Serum protein-binding was measured using serum samples taken 5 and 7 hours after the last RBP dose in both the 20 and 40 mg dosing regimens. However, RPB concentrations of most of the samples were less than the detection limit (14-25 ng/ml) of the assay and only 5/48 samples could be quantified. Mean protein-binding was 96.3±1.0%, however, in view of the small sample size and lack of information regarding the analytical assay sensitivity, this value is of limited use.

Serum Gastrin:
There were statistically significant increases noted after both the 20 and 40 mg RBP doses when compared to predose values. Although it was difficult to draw a clear conclusion because of the