(10) R. Wachowiak, *Arzneim-Forsch.*, **29**, 725 (1979).
(11) K. G. Mooney, M. Rodriguez-Gaxiola, M. Mintun, K. J. Himmelstein, and V. J. Stella, *J. Pharm Sci.*, **70**, 1358 (1981).
(12) J. W. Turczan and B. A. Goldwitz, *J. Pharm. Sci.*, **62**, 1705 (1973).
(13) J. W. Turczan and B. A. Goldwitz, *J. Pharm. Sci.*, **61**, 1309 (1972).
(14) E. B. Sheinin, W. R. Benson, and M. M. Smith, Jr., *J. Assoc. Off. Anal. Chem.*, **56**, 124 (1973).

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Influence of Food on the Bioavailability of Enalapril

B. N. SWANSON *, P. H. VLASSES *, R. K. FERGUSON *, P. A. BERGQUIST *, A. E. TILL ‡, J. D. IRVIN ‡, and K. HARRIS ‡

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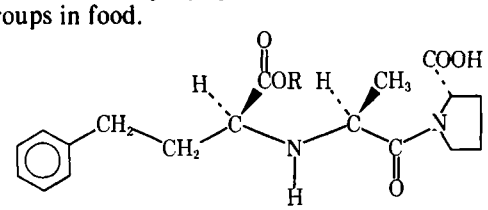
Abstract □ In a randomized, two-period crossover study in 12 normal volunteers, serum and urine concentrations of the angiotensin-converting enzyme inhibitor enalapril and its active metabolite enalaprilat were determined following administration of a single 40-mg tablet of enalapril maleate administered both in the fasting state and with a standard breakfast. A 7-d interval separated the two treatment periods. Area under the serum concentration-time curves for enalaprilat and urinary recoveries for enalaprilat and total drug did not differ significantly between the fed and fasted conditions. The mean observed maximum serum concentration of enalaprilat was slightly higher for the fasting treatment, but the time to peak concentration was almost identical for the two treatments. Enalapril maleate is unlike the prototype angiotensin-converting enzyme inhibitor captopril in that a standard meal does not appear to influence absorption of this new drug.

Keyphrases □ Enalapril maleate—bioavailability, influence of food □ Enalaprilat—metabolite of enalapril, bioavailability, influence of food □ Angiotensin-converting enzyme—enalapril maleate, bioavailability, influence of food

Food is known to alter the bioavailability of many drugs (1). The presence of food in the GI tract has been shown to reduce, by 30–40%, the absorption of the recently marketed oral angiotensin-converting enzyme (ACE) inhibitor captopril (2). Compromised absorption of captopril could affect the magnitude or duration of the antihypertensive effect of this drug (3). Enalapril maleate is a new ACE inhibitor which, like captopril, has been shown to be effective in the treatment of hypertension and congestive heart failure (4). Enalapril ((*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline; I) is a prodrug which is deesterified to an active diacid form, enalaprilat ((*S*)-1-[*N*-(1-carboxy-3-phenylpropyl)-L-alanyl]-L-proline; II) (4). Since enalapril lacks the sulfhydryl moiety of the chemical structure of captopril, it may have a more favorable benefit-to-risk ratio. Oral absorption of enalapril may also be more complete than captopril since the sulfhydryl group of the latter binds to other thiol groups in food.



I, R = CH_2CH_3
II, R = H

The purpose of this study was to investigate whether consumption of food alters the rate or extent of absorption of enalapril when a single dose is administered to healthy male volunteers.

EXPERIMENTAL SECTION

Subjects—Twelve healthy, normotensive male subjects (age, 23–37 years) volunteered to participate in this study. They were judged to be in good health on the basis of history, physical examination, routine laboratory data, standard electrocardiogram, and diastolic blood pressure (≤90 mm Hg). All subjects weighed within ±10% of the ideal body weights for their ages and heights. No medications other than enalapril maleate were taken by the subjects from 1 week prior to the study until its completion. The protocol was approved by the Committee on Research of Thomas Jefferson University.

Study Design—This was an open, randomized, two-way crossover study in which enalapril maleate (40 mg) was administered as a single dose in its market image. Subjects fasted from midnight of the previous night. On the first day of each treatment period, subjects received doses of enalapril maleate either in a fasting state or immediately after a standard prescribed breakfast by a randomized design. The breakfast consisted of one egg, two pieces of toast or bread, two strips of bacon or two sausages, 150 mL of low fat milk or 100 mL of orange juice, tea, or coffee. All volunteers resumed their normal diets each day at lunch (4 h after dosing). Treatments were separated by 7 d.

Blood was drawn at 0, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, and 72 h postdrug administration. Urine was collected at −1–0, 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, and 96–120 h after each treatment. Blood pressure and pulse rate were measured in the supine and sitting positions at regular intervals for 24 h postadministration. Adverse effects were monitored throughout the study.

Biochemical Analysis and Statistical Methods—The assay value for the enalapril maleate tablet was 42.02 mg, equivalent to 29.88 mg of enalaprilat per tablet. A radioimmunoassay procedure (5) was used to analyze the serum and urine samples for enalaprilat and for total drug (enalaprilat after enzymatic hydrolysis of enalapril). After mixing and incubation overnight at room temperature, the hydrolysis of enalapril to enalaprilat was virtually complete. The assay was considered to be accurate and reproducible at enalaprilat concentrations >1 ng/mL in serum and 0.5 μg/mL in urine; the interassay coefficient of variation was 8%. Concentrations below these values were not considered detectable and, hence, zero for the purpose of data analysis.

All parameters were analyzed by using an analysis of variance for a two-period crossover design (6). These results were corroborated by a nonparametric method based on ranks of the data (7). The posterior probabilities were calculated by the method of Rodda and Davis (8). The power analysis was based on the *t* test.

RESULTS

The mean serum profiles for enalaprilat after administration of enalapril maleate in the fasting and fed states are depicted in Fig. 1. Profiles for the two treatments are virtually superimposable. Mean urinary excretion rate plots

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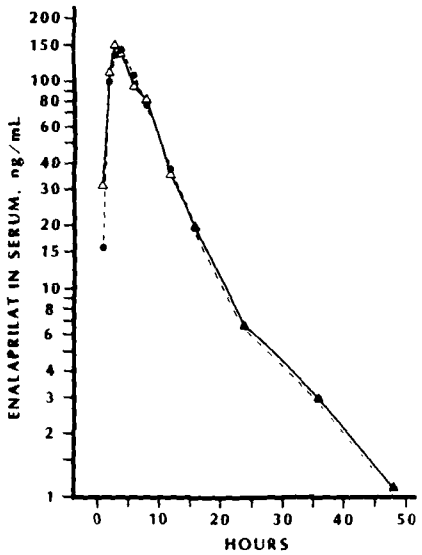

Figure 1—*Mean enalaprilat serum concentrations following administration of an enalapril maleate 40-mg tablet to fasting (●) and fed (△) healthy subjects (n = 12).*

for enalaprilat and total drug (Figs. 2 and 3, respectively) were similar for the fasting and fed states as well. Serum parameters for enalaprilat and urine parameters for enalaprilat and total drug are presented in Table I, as are power analyses and posterior probability results. Mean peak serum concentrations of enalaprilat were 154 and 147 ng/mL for the fasting and nonfasting treatments and were observed at 3.3 and 3.4 h, respectively, after administration of enalapril maleate. Mean serum $AUC_{0\text{-}24}$ values for enalaprilat obtained from the fasting and nonfasting treatments were essentially the same and accounted, on the average, for 93% of the total AUC (from time zero to the time of the lowest detectable serum concentration) for both treatments. For most subjects, the maximum serum concentration for enalapril (obtained as the difference between enalaprilat equivalents before and after sample hydrolysis) was observed at 1 h, the first sampling time, and tended to be slightly higher during the fasting state. In two subjects, the maximum was at 2 h (data not shown).

Mean urinary recoveries (0–48 h) of enalaprilat were 31 and 32% of the dose for the fasting and nonfasting treatments, respectively; recoveries of total drug after hydrolysis of urinary enalapril were 53 and 58% of the dose, respectively. The mean urinary recovery ratio of enalaprilat–total drug was 0.58 for the fasting treatment and 0.54 for the nonfasting treatment. No statistically significant differences were found between treatments for any of the serum or urine parameters considered.

Table I—Mean Serum Parameters for Enalaprilat and Urine Parameters for Enalaprilat and Total Drug [a]

Parameter[b]	Fasting (Mean ± *SD*)	Fed (Mean ± *SD*)	Comparison[c]
C_{max} (enalaprilat), ng/mL	154 ± 39	147 ± 36	NS
t_{max} (enalaprilat), h	3.3 ± 0.5	3.4 ± 0.5[d]	NS
$AUC_{0\text{-}24}$ (enalaprilat), ng·h/mL	1209 ± 203	1173 ± 212	NS
$AUC_{0\text{-}LSC}$ (enalaprilat), ng·h/mL	1304 ± 240	1262 ± 220	NS
Urinary recovery, 0–48 h (percent of administered enalaprilat equivalents)[d]			
Enalaprilat	30.5 ± 7.5	31.6 ± 8.8	NS
Total drug	53.1 ± 10.1	57.6 ± 12.4	NS
Urinary recovery ratio of enalaprilat/total drug, 0–48 h[d,e]	0.58	0.54	NS

[a] Following oral administration of enalapril maleate (40 mg) in healthy fasting and fed volunteers; $n = 12$; total drug was measured after hydrolysis, representing enalaprilat which was present in the urine as enalapril itself and that which was present as enalaprilat. [b] C_{max}, observed maximum serum concentration; t_{max}, time of observed maximum serum concentration; $AUC_{0\text{-}24}$ and $AUC_{0\text{-}LSC}$, area under the serum concentration–time curve from 0–24 h and 0–last observed serum concentration, respectively. [c] No significant differences (NS) were found for any comparison. The power of detecting a 20% difference in urinary recovery of enalaprilat and total drug, with $\alpha = 0.05$, is 0.82 and 0.81, respectively; posterior probability that the true difference is 20% or less is 0.97 for enalaprilat and 0.94 for total drug. [d] $n = 11$. [e] Geometric mean of individual ratios.

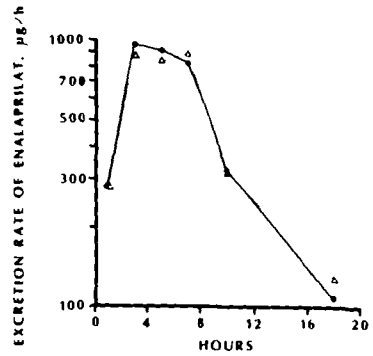

Figure 2—*Mean enalaprilat urinary excretion plots following oral administration of enalapril maleate to fasting (●) and fed (△) healthy subjects (n = 9 and 10, respectively).*

Mean diastolic blood pressure significantly decreased from predose values by 2 h after each dose, and these decreases were maintained for 4–8 h. No adverse effects were reported.

DISCUSSION

Enalapril maleate is a new nonsulfhydryl ACE inhibitor which is converted *in vivo* to an active diacid metabolite (enalaprilat). Although both enalapril and its metabolite are ACE inhibitors, it is the metabolite that exhibits the greatest potency and is the presumed active compound *in vivo*. In the present study, the effect of food on the serum concentration–time profile of enalaprilat and urinary recovery of enalaprilat and total drug (enalapril plus enalaprilat) after administration of a single dose of enalapril maleate were evaluated. This is of interest because food has been reported to decrease by 30% the extent of absorption of captopril, the currently available oral ACE inhibitor (2). In addition, maximum inhibition of ACE, as reflected by enzyme activity in serum, appears to be required for the full therapeutic effect of this class of drugs (4). Since serum ACE activity is directly related to circulating drug concentrations, this maximal degree of inhibition may not occur if drug absorption is impaired. Interestingly, food has recently been reported to decrease, by ~50%, the oral absorption of *d*-penicillamine, another sulfhydryl-containing compound (9).

This study in normal volunteers showed that a standard breakfast did not alter the serum concentration–time profile and urinary recovery of enalaprilat following a single oral dose of enalapril maleate (40 mg). We have demonstrated previously that an enalaprilat concentration of ~14 ng/mL is required to inhibit serum ACE levels by 90% (10, 11). In the current study, this serum concentration was maintained for at least 16 h after each 40-mg dose in most subjects, regardless of diet.

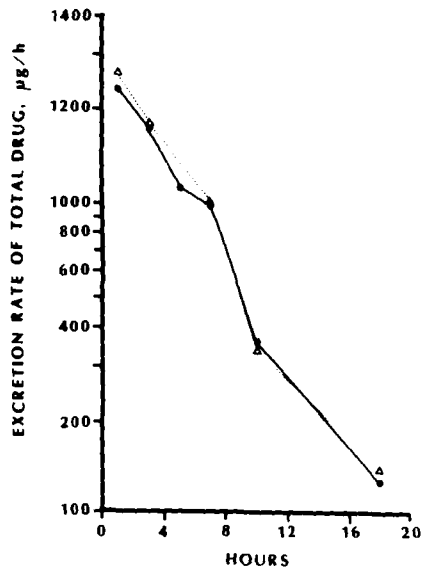

Figure 3—*Mean urinary excretion rate plots for total drug (enalaprilat measured after hydrolysis) following oral administration of enalapril maleate to fasting (●) and fed (△) healthy subjects (n = 9 and 10, respectively).*

In conclusion, the serum parameters for enalaprilat and urine parameters for enalaprilat and total drug (enalapril-enalaprilat) were similar following administration of a single enalapril maleate 40-mg tablet to healthy volunteers under fasting and nonfasting conditions. Thus, food did not appreciably alter the absorption of enalapril or the bioavailability of enalaprilat in this study.

REFERENCES

(1) R. D. Toothaker and P. G. Welling, *Annu. Rev. Pharmacol. Toxicol.*, **20**, 173 (1980).

(2) S. M. Singhvi, D. N. McKinstry, J. M. Shaw, D. A. Willard, and B. H. Migdalof, *J. Clin. Pharmacol.*, **22**, 135 (1982).

(3) R. K. Ferguson, G. A. Turini, H. R. Brunner, H. Gavras, and D. N. McKinstry, *Lancet*, **i**, 775 (1977).

(4) R. K. Ferguson, P. H. Vlasses, B. N. Swanson, P. Mojaverian, M. Hichens, and J. D. Irvin, *Clin. Pharmacol. Ther.*, **32**, 48 (1982).

(5) M. Hichens, E. L. Hand, and W. S. Mulcahy, *Ligand Quart.*, **4**, 43 (1981).

(6) J. E. Grizzle, *Biometrics*, **21**, 467 (1965).

(7) G. G. Koch, *Biometrics*, **28**, 577 (1972).

(8) B. E. Rodda and R. L. Davis, *Clin. Pharmacol. Ther.*, **28**, 247 (1980).

(9) M. A. Osman, R. B. Patel, A. Schuma, W. R. Sundstrom, and P. G. Welling, *Clin. Pharmacol. Ther.*, **33**, 465 (1983).

(10) R. K. Ferguson, P. H. Vlasses, J. D. Irvin, B. N. Swanson, and R. B. Lee, *J. Clin. Pharmacol.*, **22**, 281 (1982).

(11) B. N. Swanson, M. Hichens, P. Mojaverian, R. K. Ferguson, P. H. Vlasses, and M. Dudash, *Res. Commun. Chem. Path. Pharmacol.*, **33**, 525 (1981).

Metabolism of Salsalate in Normal Subjects

SYDNEY H. DROMGOOLE ˣ, DANIEL E. FURST, and HAROLD E. PAULUS

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Abstract □ The metabolism of salsalate (I) was characterized in two normal volunteers. The drug was almost completely absorbed and was excreted primarily in the urine; only ~1% of the total dose was found in the stools. Although I is a salicylate derivative, which on hydrolysis yields two molecules of salicylic acid (II), ~7-10% of the dose was not hydrolyzed to salicylic acid and appeared in the urine either as unchanged drug or glucuronide conjugates. Thus, the incomplete availability of salicylate from salsalate that has been previously reported may not be due to incomplete absorption of the drug but to incomplete hydrolysis to salicylic acid.

Keyphrases □ Salsalate—metabolism, normal subjects □ Absorption—salsalate, normal subjects

Salsalate (I) is a salicylate derivative which on hydrolysis yields two molecules of salicylic acid (II). The results of several studies have indicated that the availability of salicylate from salsalate is lower than from either choline magnesium trisalicylate (1) or aspirin (2, 3). It has been suggested that the lower availability of salicylate from salsalate may be due to incomplete absorption (1). However, the recovery of total salicylate in the urine samples of patients receiving equivalent doses of salsalate and aspirin indicated that the absorption of the two salicylate formulations was essentially complete. The hydrolysis of salsalate to salicylate was incomplete, with <1% of the dose being excreted as unchanged salsalate and ≤13% of the dose being excreted in the urine as conjugates of salsalate (3). The only limitation of this latter study was the fact that the biological samples had been stored for several months prior to analysis, during which time salsalate (both nonconjugated and conjugated) may have partially hydrolyzed to salicylic acid.

The purpose of this study was to characterize the metabolism of salsalate in two normal volunteers following the administration of 2000 mg of salsalate containing ^{14}C-labeled salsalate.

EXPERIMENTAL SECTION

Two normal healthy male volunteers (age 29 and 35) entered into the 4-d study after initial screening procedures. The volunteers fasted for 12 h prior to and 4 h after drug administration. They ingested 2000 mg (four 500-mg capsules) of salsalate containing ^{14}C-labeled salsalate[1] (64 μCi) with water. Compound I was radiolabeled at both carboxyl positions.

Blood samples were taken just before drug ingestion, at hourly intervals up to 12 h, and then at 16, 20, 24, 30, 36, 48, 60, and 72 h following drug administration. Immediately after the blood samples were drawn into heparinized tubes, they were centrifuged, and the plasma was frozen until analysis. Urine samples were collected during the following intervals: 0-1, 1-2, 2-4, 4-6, 6-8, 8-12, 12-16, and 16-24 h. For the next 3 d, urinary output was collected as consecutive 12-h aliquots. The volume of urine and pH were recorded for each period, and aliquots were frozen in plastic containers until analysis. All stools were collected, weighed, and frozen until analysis. Due to the possibility of hydrolysis of salsalate and its conjugates to salicylic acid, all assays were completed within 2 weeks of collection.

Aliquots of plasma (0.1 mL with 0.9 mL of water) or urine (1.0 mL) were transferred into scintillation vials containing scintillation fluid[2] and counted in a scintillation counter[3]. Standards containing 500 μg of the radiolabeled salsalate[1] in propanol were also counted. All counts were corrected for quenching.

The plasma and urine samples were assayed for unchanged salsalate and salicylic acid by a previously reported HPLC technique (4). Salsalate[1], salicylic acid[4], and α-phenylcinnamic acid[4], the internal standard, were extracted from acidified plasma and urine samples. Methylene chloride[5] was used to extract plasma, whereas urine was extracted into hexane[6]. The organic phases were evaporated to dryness, redissolved in methanol (0.5 mL)[7], and analyzed by HPLC with an automatic sample injector accessory[8]. The mobile phase of methanol-1% acetic acid (60:40, v/v) was pumped at a rate of 2.0 mL/min through a 4.6 × 150-mm column[9]. Peaks were detected with a UV detector[10] (300 nm) coupled to a recorder[11] and peak integrator[12]. Plasma samples were assayed for total salicylate by HPLC after heating with HCl (18 M) overnight at 100°C. Extraction and chromatographic conditions were the same as described above for unchanged I.

Total urinary salicylate was determined by a modification of the colorimetric method described by Chiou and Onyemelukwe (5). Aliquots of urine

[1] Riker Laboratories, 3M Center, St. Paul, Minn.
[2] Ready-Sol EP; Beckman Instruments, Inc., Fullerton, Calif.
[3] Isocap 300; Scarle Analytical Inc., Des Plaines, Ill.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Spectroquality; Matheson, Coleman and Bell, Norwood, Ohio.
[6] Spectroquality; Mallinckrodt, St. Louis, Mo.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Model 1500; Altex Scientific, Berkeley, Calif.
[9] Ultrasphere ODS; Altex Scientific.
[10] Model 100-10 spectrophotometer; Hitachi Scientific Instruments, Mountain-View, Calif.
[11] Model 250-2, two channel; Curken Scientific, Danbury, Conn.
[12] Model 485; Varian Instrument Division, Palo Alto, Calif.

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