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INTESTINAL ABSORPTION OF CAPTOPRIL AND TWO THIOESTER ANALOGS IN RATS AND DOGS

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ABSTRACT

The objectives of this study were (i) to determine whether the reduced absorption of captopril from the colon of humans also occurs in rats and (ii), after confirmation of the relevance of a new rat model, to evaluate the intestinal absorption of captopril and several of its analogs. A model was developed and validated in which specific sites within the GI tract of rats were surgically implanted with a cannula such that animals could be dosed while conscious and unrestrained.

The absorption of captopril after administration into the lower GI tract of rats was significantly reduced relative to the upper GI tract, which was consistent with results reported previously in humans. In rats, the absorption of the S-benzoyl thioester prodrug of captopril (SQ-25868) from the lower GI tract was substantially greater than that of captopril. However, the absorption of the S-benzoyl thioester prodrug of 4-phenyl thio-captopril (SQ-26991) from the lower GI tract was only marginally better than that of captopril. In additional studies in dogs, a 12 h controlled-release formulation of SQ-25868 provided sustained blood levels of captopril while maintaining acceptable bioavailability ($>80\%$).

Two approaches were tried, without success, to stabilize captopril *in vivo*: (i) complexation with zinc (SQ-26284) and (ii) use of ascorbic-acid-buffered (pH 3·5) vehicle. The zinc complex might have failed because it has very low solubility, whereas the pH-3·5-buffered vehicle was quickly neutralized within the colonic lumen in rats, and did not stabilize captopril against oxidation. Rapid neutralization might explain why the colonic bioavailability of captopril was not substantially increased when this pH-3·5-buffered vehicle was tried in humans.

KEY WORDS: captopril; absorption; GI sites; S-benzoyl prodrug; rats; dogs

INTRODUCTION

Because captopril has a relatively short $t_{1/2}$ in blood (~ 2 h), a controlled-release formulation of captopril that would provide anti-hypertensive efficacy for 24 h was considered to be desirable. As such, a variety of pre-clinical studies

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were performed in order to evaluate the site(s) of the intestinal absorption of captopril and several of its analogs, some of which have modified thiol groups. In many cases, these exploratory studies were conducted in parallel to (i) understand the *in vivo* pharmacokinetic behavior of controlled-release captopril formulations or (ii) evaluate modification of the thiol group and/or its stability as it pertains to the absorption of captopril. These studies were done in several laboratories over a 15 year period while bioanalytical methods and animal surgery techniques evolved. Nonetheless, because each study was independently controlled, valuable information on the intestinal absorption of captopril and several thiol analogs was obtained and is summarized in the present report.

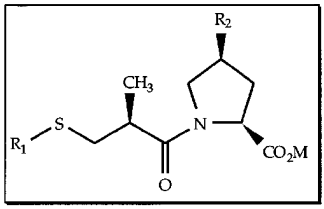
Brennan *et al.*[1] reported that the bioavailability of captopril after administration into the intubated colon of human volunteers was substantially reduced to about 14–21% of that obtained after oral administration of a conventional immediate-release tablet. In addition captopril bioavailability was low when delivered into the distal ileum or colon of humans with a pulsed-release formulation.[2] The initial objective of the present study was to determine whether the reduced extent of colonic absorption of captopril observed in humans also occurs in rats, thus providing a convenient model to conduct exploratory studies. A rat model consisting of cannulated GI tract sites was developed and validated for the present study. After confirmation of the relevance of the rat model, [^{14}C]-captopril was then used to evaluate (i) the absorption of captopril after administration into various sites of the GI tract of rats and (ii) the disposition of captopril in rats when administered in the same pH-3·5-buffered vehicle that was administered into the colon of humans.[1]

Additional exploratory studies were conducted in rats and dogs to explore the effect of modification of the thiol group of captopril and a captopril analog on intestinal absorption. The absorption of the following compounds was evaluated after administration into various sites along the upper and lower GI tract of rats: the S-benzoyl thioester of captopril (SQ-25868), the S-benzoyl thioester of 4-phenyl thiocaptopril (SQ-26991), and a zinc complex of captopril (SQ-26284) (Figure 1). These studies in rats suggested that SQ-25868 would perform best in a controlled-release (CR) formulation. Three formulations of SQ-25868 were prepared (12, 18 and 24 h *in vitro* release rates) and evaluated after oral administration to dogs.

MATERIALS AND METHODS

Materials

[^{14}C]-captopril (specific activity, 9·96 μCi mg^{-1}), [^{14}C]-SQ26991 (zofenopril; specific activity 4·1 μCi mg^{-1}), [^{14}C]-SQ-25868 (S-benzoyl captopril; specific



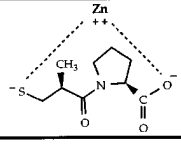
Compound	Number	R_1	R_2	M
Captopril	SQ-14225	H	H	H
S-Benzoyl Captopril	SQ-25868		H	H
4-Phenylthio Captopril	SQ-26333	H		H
S-Benzoyl-4-Phenylthio Captopril	SQ-26991			1/2 Ca^{++}
Captopril-Zinc Complex (1:1)	SQ-26284			

Figure 1. Structures of captopril and several analogs

activity 3·35 μCi mg^{-1}), and non-radiolabeled SQ-26284 (zinc–captopril complex) were synthesized at Bristol–Myers Squibb. [^{14}C]-inulin (specific activity 2·5 μCi mg^{-1}) was purchased from Dupont-NEN (Wilmington, DE). Radiochemical purity was greater than 97% for all ^{14}C compounds.

N-ethylmaleimide (NEM) was purchased from Sigma Co. (St Louis, MO). Soluene-350® was obtained from Packard Instrument Co. (Downers Grove, IL) and scintillation cocktail (TX-114) was purchased from National Diagnostics Co. (Somerville, NJ). Silica gel plates (GF; 0·2 mm thick) were purchased from Analtech, Inc. (Newark, DE). All other reagents were reagent grade or better.

Surgical preparation of rats

Prior to surgery, anesthesia was induced with ether and then maintained with an intramuscular injection of a 0·5% aqueous solution of urethane. Each male Sprague–Dawley rat (250–350 g) was surgically prepared with an indwelling cannula in one of the following sections of the intestine: duodenum, jejunum, ileum, or proximal colon. The ligament of Treitz (near the duodenum–jejunum juncture) and the ileo-cecal junction were used to identify the proper sites along the GI tract for cannulation (Figure 2). The

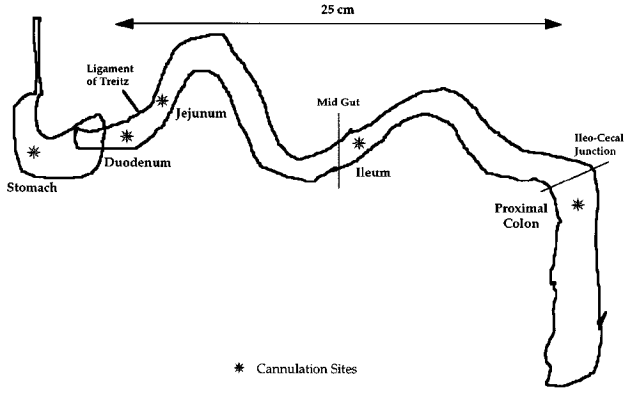


Figure 2. A schematic representation of the location of the cannulated sites along the GI tract of rats

cannula consisted of PE-50 tubing with a 1 cm piece of soft silastic tubing at the tip to prevent puncturing the intestine. After the appropriate intestinal section had been located (duodenum, jejunum, ileum, and proximal colon), the cannula was inserted through a 2 mm incision and was secured by application of cyanoacrylate glue. The end of the cannula was approximately 1 cm distal to the insertion site. On the day of surgery and the day of dosing, each rat was hydrated with five separate 5 mL subcutaneous injections of 5% dextrose in saline administered at various sites on the back. This hydration regimen substantially reduced the recovery time from surgery and the incidence of lethargy ($\sim$5%), which was about 20% for non-hydrated rats. At least 24 h elapsed between surgery and drug administration. The placement and patency (dye test) of each cannula were confirmed at necropsy.

Validation of the cannulated intestine model in rats

Barrier properties. In order to confirm that the barrier properties of the intestine were not adversely affected by cannulation and surgery, the absorption of [^{14}C]-inulin was determined in rats surgically implanted with an indwelling cannula in the colon, as described in detail above. Each rat received a single 3 mg kg^{-1} dose of [^{14}C]-inulin into the colon *via* a cannula ($N=2$), and intact control rats each received the same dose of [^{14}C]-inulin by iv and oral (gavage) routes of administration ($N=3$ each). The intestinal absorption of [^{14}C]-inulin was determined by the recovery of the radioactive dose in the 0–24 h urine relative to the recovery for the iv route.

Movement of dose within lumen. For evaluation of the movement of the dosing vehicle within the intestine after dosing, separate rats were given a dye into the

intestinal lumen. Single 1 mg doses of phenol red were administered as an aqueous solution into the jejunum of (i) conscious rats *via* an exteriorized cannula (the model described above) and (ii) anesthetized rats *via* syringe and needle by direct injection into the lumen. The animals were sacrificed 2 h after dosing, and the intestinal mucosa near the site of administration was exposed; phenol red was located visually by addition of 0·1 N NaOH.

Drug administration

Each rat and dog was fasted for at least 24 h prior to dosing. A fasting time of 72 h was used to clear the lower intestine when the administration site was the ileum or colon. Rats were excluded from the study at the time of surgery if food-related material was observed near the cannulation (dosing) site.

Captopril in rats. [^{14}C]-captopril was administered at a dose of 5 mg kg^{-1} (23 μmol kg^{-1}) as an aqueous solution. In addition, two cannulated rats also received a colonic dose of [^{14}C]-captopril in a vehicle, consisting of EDTA (13 mM), ascorbic acid (244 mM), sodium ascorbate (16 mM), and [^{14}C]-captopril (7·0 mM), buffered at pH 3·5. This vehicle was designed to reduce oxidative degradation of captopril in the intestine after dosing, and was the same vehicle that was used when captopril was introduced into the human colon.[1]

A limited exploratory *in situ* study was conducted in a separate group of two rats to evaluate the time course of the pH of the fluid within the lumen of the colon after administration of (i) the pH 3·5 ascorbate buffer used as a vehicle described above and (ii) captopril dissolved in water (pH 3·1; control). The pH of the lumenal fluid was measured for up to 2 h with a microelectrode (model M1-410, Microelectrodes Inc., Londonderry, NH) while the rats were anesthetized with urethane. It was technically too difficult to perform this experiment in conscious rats, and as such the effect of anesthesia on these pH measurements could not be determined.

Analogs of captopril in rats. [^{14}C]-SQ-25868 was administered at a dose of 45 mg kg^{-1} (138 μmol kg^{-1}) as an aqueous solution containing an equimolar amount of $NaHCO_3$. SQ-25868 was the first compound of the series tested (prior to development of the cannulated model described above) and it was directly injected by syringe into the appropriate GI site of an anesthetized rat, which was opened by midline incision. The rat was then surgically closed and allowed to recover from anesthesia. [^{14}C]-SQ-26991 and SQ-26284 were administered as suspension in 0·15% agar at a 23 μmol kg^{-1} dose (equimolar to captopril) to conscious rats with cannulated intestinal sites as described above.

SQ-25868 in dogs. Four female beagle dogs received single 148 mg doses of non-radiolabeled SQ-25868 formulated as a controlled-release hydrogel matrix tablet with *in vitro* release rates (t_{90}) of 12, 18, and 24 h.[3]

Sample collection and analysis

Rat studies. Urine was collected for 24 h after dosing, at which time the rats were sacrificed and their GI tracts were removed. N-Ethylmaleimide (NEM), which stabilizes sulfhydryl-containing compounds in biologic fluids,[4] was present in the urine collection bottles to provide a final concentration of at least 1 mg NEM/mL urine. For determination of total radioactivity in urine, aliquots (0·2 mL) were mixed with 1 mL Soluene-350®, neutralized and mixed with 15 mL of scintillation cocktail. Each carcass was repetitively processed with a meat grinder without addition of water. Aliquots (0·2 g) of each processed carcass were analyzed for total radioactivity as described above for urine samples. After administration of SQ-26284, urine was analyzed with a previously reported GC/ECD assay.[5]

In the rat study involving colonic administration of [^{14}C]-captopril dissolved in the pH 3·5 ascorbate-buffered vehicle, the GI tracts were removed 24 h after dosing and immediately homogenized in a saturated solution of NEM in water. The homogenate was extracted with acetonitrile twice and the supernatant was evaporated to dryness, reconstituted in methanol, and developed with the thin-layer chromatographic (TLC) procedure described in detail below.

Dog studies. After administration of [^{14}C]-SQ-25868, blood samples were collected for up to 12 h and urine samples were collected for 24 h. All samples were analyzed for total radioactivity. The biotransformation profile in pooled urine samples was determined with the TLC method described below. The blood samples collected after administration of controlled-release formulations of SQ-25868 were analyzed by the GC/ECD method. All biologic samples were stabilized with NEM immediately after collection.

Thin-layer chromatography. For studies involving radiolabeled compound, biotransformation profiles were determined in urine samples and extracts of the GI tract–contents (captopril colonic dose only) with previously developed TLC methods.[4] The GI tract–contents were immediately homogenized in three volumes of an aqueous NEM solution (2 mg mL^{-1}); the homogenate was extracted three times with methanol and the supernatants were combined and evaporated to dryness with N_2. An aliquot (0·05 mL) of each urine sample or reconstituted extract was spotted directly on silica gel plates and developed in one of the following solvent systems along with non-radiolabeled reference standards. For captopril

and SQ-25868 studies, the solvent system consisted of chloroform : ethyl acetate : glacial acetic acid (4:5:3, by volume). For SQ-26991 studies, the solvent system consisted of chloroform : glacial acetic acid: 37% formaldehyde (60:7:1, by volume).

Data analysis

For the studies involving GI sites of absorption of [^{14}C]-labeled drug, absorption estimates were based on summation of the radioactivity in 0–24 h urine and the carcass. After [^{14}C]-SQ-25868 administration, the absorption value does not include the carcass; this does not adversely affect the interpretation of the study since SQ-25868 is rapidly converted to captopril and the half-life of captopril in rats is less than 2 h; thus, there is typically less than 5% of an administered dose associated with the carcass at 24 h. For studies with SQ-26284, absorption estimates were based on urinary excretion (0–24 h) of 'total' captopril, which includes captopril and its reducible disulfides; bioavailability estimates were based on urinary excretion (0–24 h) of intact captopril.

After administration of controlled-release formulations of SQ-25868 to dogs, relative absorption was estimated based on the recovery of 'total' captopril in 0–24 h urine, and relative bioavailability was based on $AUC_{0-12\,h}$ of intact captopril in blood. The absorption and bioavailability values are expressed relative to results obtained when gavage doses of SQ-25868 were given as an aqueous solution.

RESULTS AND DISCUSSION

Validation of the absorption-site model in rats

Other investigators have used various *in vitro* and *in situ* approaches to evaluate absorption of drugs from different sites of the GI tract.[6] Single-pass intestinal perfusion *in situ* has been used extensively; however, it does not appear to be suitable to meet the present objectives because oxidation of captopril in the lumen is high when perfusates are prepared at relevant captopril concentrations.[7] It would be very difficult to optimize the *in situ* or *in vitro* experimental conditions such that the extent of intestinal permeability/uptake and thiol oxidation matched the *in vivo* conditions. Therefore, an *in vivo* model is the most appropriate when the competing processes of permeability through the intestinal wall (absorption) and pre-systemic degradation occur simultaneously, as is the case for captopril.

Rats were selected because they are similar to humans with respect to oral absorption ($\sim$70%), bioavailability ($\sim$60%), and metabolism of captopril.[8] In addition, for general utility of this model, rats are ideal because they

require smaller doses when there is a limited supply of investigational compound. The model developed for the present series of studies is novel in that the compound can be administered at a specific intestinal site while the rat is conscious and unrestrained. Validation experiments were conducted with two 'non-absorbable markers': (i) [^{14}C]-inulin, for evaluation of the integrity of the absorption barrier after surgery, and (ii) phenol red, for evaluation of the movement of the dosing vehicle within the intestinal lumen after dosing.

The recovery of the radioactive dose of [^{14}C]-inulin in the 0–24 h urine averaged 95, 4, and 2%, after administration to rats into the systemic circulation (iv), stomach (by gavage), and colon (*via* a cannula), respectively, indicating that absorption was less than 5% in either case. Inulin, a polysaccharide (molecular weight about 5500 daltons) is not absorbed well from the healthy GI tract. Because the absorption of [^{14}C]-inulin from the cannulated colon was similar to the absorption after dosing by gavage, the surgical procedure did not apparently compromise the integrity of the intestinal absorption barrier.

Obviously, direct injection of a drug into the intestinal lumen of an anesthetized rat is a simpler method than the cannulated model that was developed and utilized in the present study. However, exploratory experiments with phenol red showed that retrograde movement of the dose occurs after direct injection into the small intestine, probably because the rats are supine and under anesthesia. As expected, there was minimal retrograde movement between the duodenum and stomach or between the colon and ileum because of the physiologic properties of the pyloric sphincter and ileo-cecal junction, respectively. The model that was used in the present study (except for SQ-25868—see below) involved conscious and ambulatory rats, for which phenol red was found only at the site or distal to the site of administration.

Absorption of captopril

As shown in Figure 3, the absorption of [^{14}C]-captopril averaged 62% after dosing into the stomach and progressively decreased to about 28% after dosing into the colon. Based on the recovery of intact captopril in 0–24 h urine, the bioavailability of captopril averaged about 26% (duodenum), 37% (jejunum), 18% (ileum), and 15% (proximal colon). These data are consistent with the data in humans[1,2] and suggest that the rat is an appropriate animal model.

The extent of absorption of captopril from a particular site within the GI tract would be reduced if oxidation to the disulfide dimer or formation of mixed disulfides with endogenous compounds occurred within the lumen, because these compounds exhibit low intestinal permeability (e.g., absorption of captopril disulfide dimer is less than 5% in rats).[9] Colonic administration of captopril in a pH 3·5-buffered vehicle containing ascorbic acid and EDTA did not substantially increase the colonic bioavailability of captopril in humans.[1]

The biotransformation profiles on the *unabsorbed radioactive dose* remaining in the GI tract (and contents) at 24 h after colonic administration of [^{14}C]-captopril to rats are shown in Figure 4. The profiles were similar for the water and pH-buffered vehicles. Thus, these data suggest that the pH-3·5-buffered vehicle unexpectedly did not provide additional stabilization of captopril

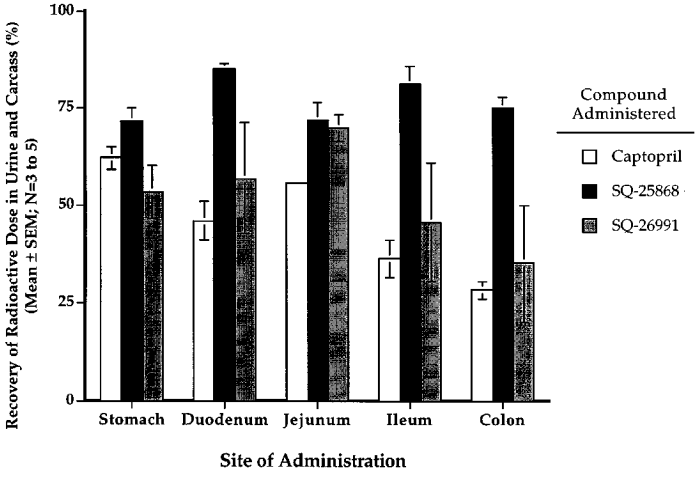


Figure 3. Estimates of mean ± SEM oral absorption after administration of [^{14}C]-captopril, [^{14}C]-SQ-26991, or [^{14}C]-SQ-25868 into various sites of the GI tract of rats (no error bar is shown for captopril dosed into the jejunum, since one animal died and there were only two values)

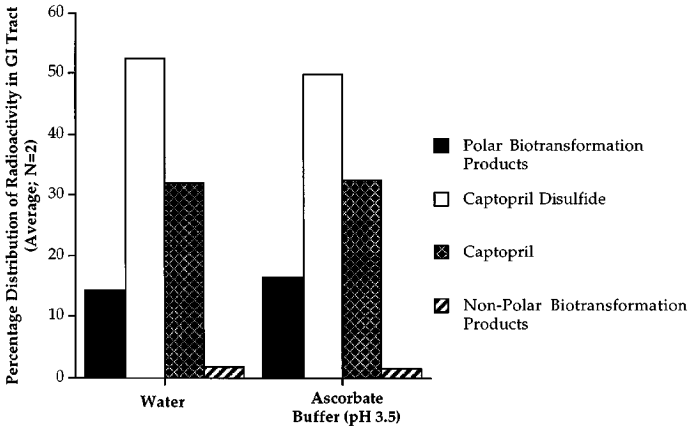


Figure 4. Thin-layer radiochromatography of the unabsorbed dose remaining in the GI tract at 24 h after colonic administration of [^{14}C]-captopril to rats

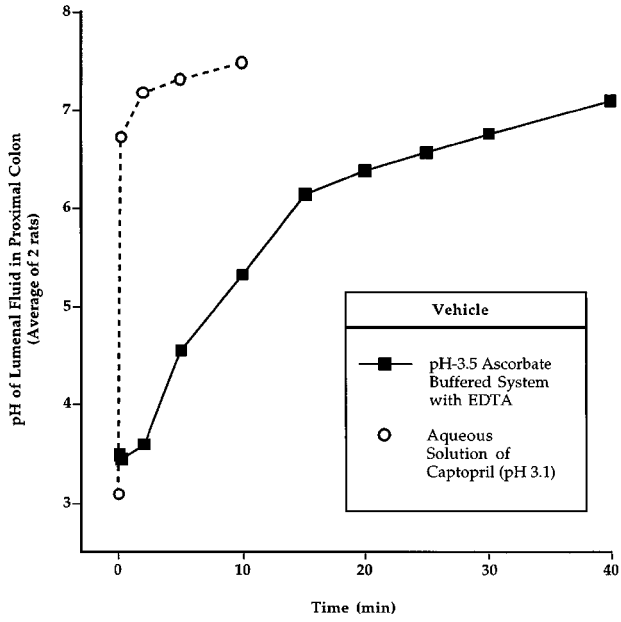


Figure 5. The time course of lumenal pH in the colon of rats after administration of captopril dissolved in water or in a pH 3·5 ascorbate-buffered solution

within the colon. Limited *in situ* studies with a microelectrode inserted into the colonic lumen of rats indicated that after administration of the pH 3·5-buffered vehicle the pH of the lumenal fluid quickly increased from pH 3·5 to more than 7 within 40 min after dosing (Figure 5). Based on these data in rats, it seems likely that the so-called 'pH-stabilized vehicle' that was used in humans was quickly neutralized within the colon, such that it could only provide stabilization for a very short period of time. Nonetheless, the observation that a substantial fraction of the unabsorbed colonic dose ($\sim$30%) corresponded to intact captopril, even after residing in the colon for 24 h, indicates that the colonic intestinal wall was adequately exposed to intact captopril. Thus, these data suggest that low intestinal permeability in addition to degradation contributes substantially to explaining the low colonic bioavailability of captopril. This hypothesis is consistent with the low *in situ* colonic permeability of captopril in rats reported by Hu and Amidon.[7]

Absorption of captopril analogs

SQ-26991. [^{14}C]-SQ-26991 was well absorbed (>50%) after dosing into the upper GI tract (stomach, duodenum, and ileum), but there was a trend towards

Table 1. Biotransformation profiles in 0–24 h urine after administration of [^{14}C]-captopril, [^{14}C]-SQ-26991, or [^{14}C]-SQ-25868 into various sites of the GI tract of rats

	Percentage distribution of radioactivity in 0–24 h urine (mean ± SEM; $N = 3$)					
	[^{14}C]-captopril		[^{14}C]-SQ-26991		[^{14}C]-SQ-25868[a]	
Administration site	Captopril	Captopril disulfide	SQ-26991	SQ-26333	Captopril	Captopril disulfide
Stomach	58·0 ± 1·9	12·2 ± 1·3	0·7 ± 0·4	22·3 ± 6·6	73·5	9·1
Duodenum	56·3 ± 5·4	12·3 ± 2·6	0·5 ± 0·0	19·3 ± 1·9	69·7	7·3
Jejunum	66·0[b]	9·2[b]	0·4 ± 0·1	24·2 ± 2·7	65·8	9·4
Ileum	50·9 ± 2·7	12·9 ± 1·1	ND[c]	ND[c]	69·6	6·2
Colon	54·5 ± 3·4	6·6 ± 0·8	1·1 ± 0·5	19·6 ± 7·9	59·7	3·7

[a]Values represent analysis of pooled urine obtained from three rats. Excretion of intact prodrug (SQ-25868) was negligible (< 1%).
[b]Average of two values; one rat that received the dose into the jejunum was sacrificed in poor condition.
[c]Not determined because the stabilizer (NEM) was accidentally omitted.

reduced absorption of SQ-26991 in the ileum and colon (Figure 3). For all dosing sites, less than 1% of the dose was excreted in 0–24 h urine as the intact thioester prodrug, SQ-26991 (Table 1). The active ACE inhibitor, SQ-26333, accounted for about 19–24% of the radioactivity in urine for all administration sites.

SQ-26284. Interaction of the sulfhydryl group of captopril with Zn^{2+} in the active site of ACE (a metalloprotease) is a critical component of the enzyme inhibition mechanism. A zinc–captopril complex (1:1) was prepared (Figure 1) to determine whether the thiol would be stabilized and absorption would be increased in the lower intestine. The results for SQ-26284, along with new control data for captopril [this study was separately controlled because it used a different assay (GC/ECD)], are shown in Figure 6. The absorption of captopril administered into the jejunum as the zinc complex was significantly lower ($p < 0{\cdot}05$) than when captopril itself was administered. This difference was not observed for the stomach or ileum. At the low pH conditions in the stomach, protonation of the complex probably occurs leading to rapid dissociation (and solubilization) and conversion of captopril to its free thiol, free acid form. As such, it is not surprising that the absorption values are similar for captopril and its zinc complex when administered into the stomach. Complexation with zinc would theoretically deactivate the sulfhydryl group and protect captopril from oxidation in the small intestine; however, the aqueous solubility of the complex at the pH of the jejunum and ileum (pH 6–8) is less than 0·001 mg mL^{-1}, which might have offset any protective effect.

SQ-25868. In rats, the S-benzoyl thioester prodrug of captopril (SQ-25868) was well absorbed (>70%) from all GI sites, including the colon (Figure 3) and the bioavailability of captopril was similar for each site based on the observation that between 60 and 73% of the urinary radioactivity corresponded to intact captopril (Table 1). As mentioned in the methods

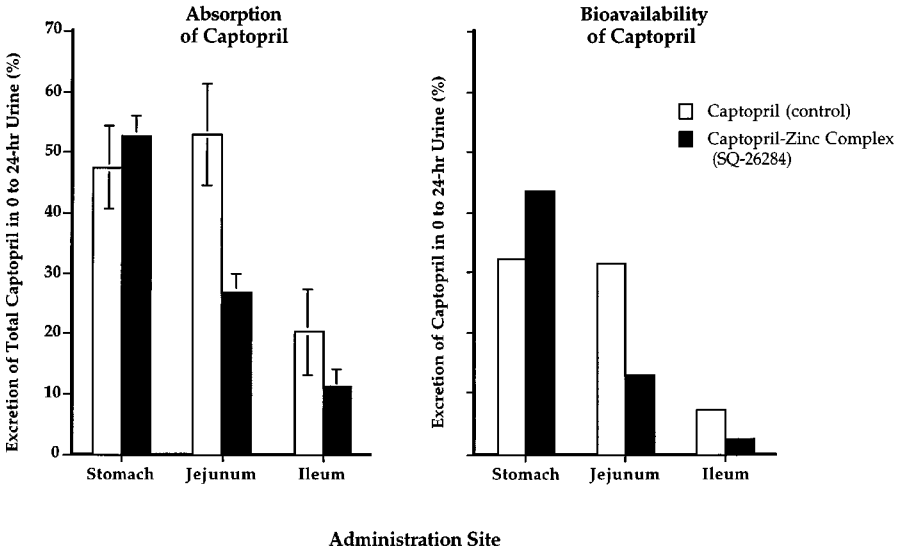


Figure 6. Mean ± SEM oral absorption and bioavailability of captopril after administration of captopril as a 1:1 zinc complex (SQ-26284) to rats

Table 2. Pharmacokinetic parameters (mean ± SEM) of captopril in female dogs after administration of SQ-25868 in a non-eroding matrix forumlation

		In vitro release rate			
Parameter	Units	0 h	12 h	18 h	24 h
N		5	4	4	4
$AUC_{0-12\,h}$	$\mu g\,h\,mL^{-1}$	8·01 ± 0·22	9·28 ± 1·3	6·83 ± 1·3	3·76 ± 1·4
C_{max}	$\mu g\,mL^{-1}$	5·45 ± 0·50	2·05 ± 0·39	1·14 ± 0·17	1·06 ± 0·40
$C_{12\,h}$	$\mu g\,mL^{-1}$	BQL[a]	0·26 ± 0·080	0·27 ± 0·091	0·27 ± 0·098
T_{max}	h	0·9 ± 0·1	4·0 ± 1·4	4·5 ± 2·4	4·5 ± 1·3
Recovery of total captopril in urine[b]	% of dose	73·4 ± 2·1	59·2 ± 4·9	53·5 ± 5·1	29·7 ± 10·1

[a]Below quantifiable limit of $10\,ng\,mL^{-1}$.
[b]Includes captopril and all of its reducible metabolites.

section, [^{14}C]-SQ-25868 was administered to anesthetized rats by direct injection into the appropriate GI site because this compound was studied before development of the cannulated intestine model used subsequently for other compounds. Exploratory studies with phenol red indicated that there is substantial movement of the dosing vehicle within the *small intestine* after direct injection; however, when injected directly into the colon, the vehicle apparently does not move up into the small intestine because of the natural barrier properties of the ileo-cecal junction (Figure 2). Therefore, absorption values for SQ-25868 within the *small intestine* might be influenced by some retrograde movement of the vehicle in these anesthetized rats to a more proximal site prior to drug absorption; however, the values for the absorption of SQ-25858 from the rat colon are reliable, with respect to the site of administration.

Chromatographic analyses of urine samples indicated that $< 1\%$ of the dose of [^{14}C]-SQ-25868 was excreted as the intact thioester prodrug (Table 1). Limited *in vitro* incubation studies with porcine liver esterase (Sigma Co.) indicated that SQ-25868 is considerably more stable to thiol ester hydrolysis than SQ-26991. If SQ-25868 is relatively resistant to hydrolysis in the gut lumen then this might explain why it was well absorbed throughout the GI tract.

Evaluation of SQ-25868 in dogs

Controlled release formulations of SQ-25868 were prepared to evaluate whether release rates of 12–24 h (*in vitro* t_{90}) would provide sustained blood levels of captopril, without substantially reducing bioavailability. Compared to administration as an immediate-release aqueous solution, blood levels of captopril were indeed sustained when SQ-25868 was given in a controlled-release form (Figure 7 and Table 2). The peak concentrations of captopril were blunted and blood levels were sustained when SQ-25868 was given in a controlled-release form to dogs; however, release rates greater than 12 h caused the relative bioavailability to fall below 80% (Figure 8). Based on these results in dogs and rats, limited clinical trials were conducted which indicated that SQ-25868 was orally absorbed to a significantly greater extent than equimolar doses of captopril and that it provided sustained blood levels of captopril.[10]

CONCLUSIONS

Obtaining a once-a-day controlled-release formulation of captopril is particularly challenging because it degrades in the lower GI tract to poorly absorbed disulfides and it has low intrinsic permeability through the colon intestinal wall. Esterification of the thiol of captopril (SQ-25868) substantially increased the extent of colonic absorption in rats, presumably because the thiol was protected and/or the prodrug has greater intestinal

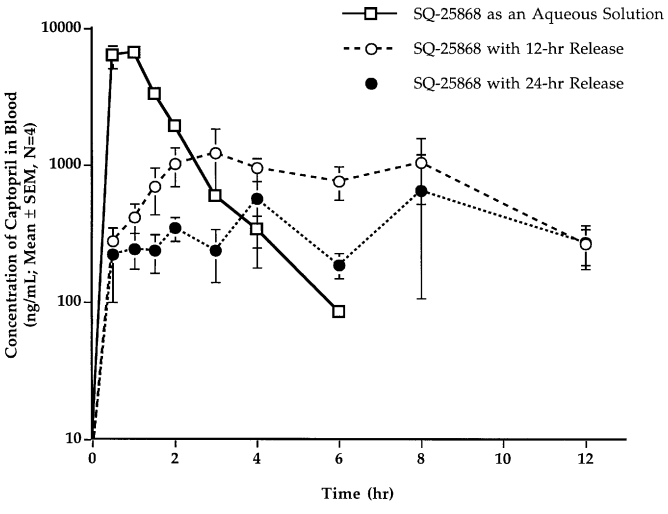


Figure 7. Mean ± SEM concentration–time profiles of captopril in blood after administration of controlled-release formulations of SQ-25868 to female dogs

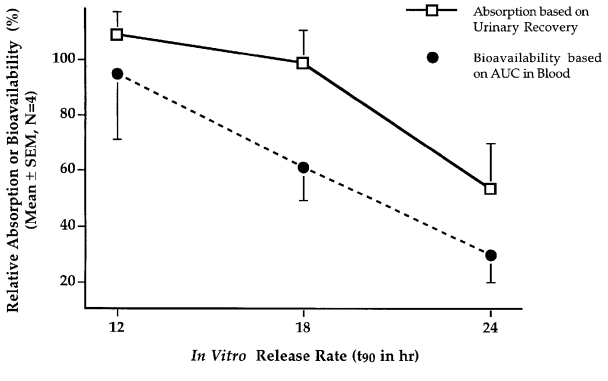


Figure 8. Mean ± SEM oral absorption and bioavailability of captopril after administration of controlled-release formulations of SQ-25868 to female dogs

permeability. After oral administration of SQ-25868, formulated as a controlled-release tablet (12 h release) to dogs, concentrations of captopril in blood were sustained and relative bioavailability was acceptable (>80%). These encouraging data in rats and dogs provided justification for limited clinical trials with SQ-25868.

Two approaches were tried, without success, to stabilize captopril in the intestinal lumen *in vivo*: (i) complexation with zinc and (ii) use of ascorbic-acid-buffered (pH 3·5) vehicle. The zinc complex might have failed because it has very low aqueous solubility, whereas the pH-3·5-buffered vehicle (the same vehicle used in a clinical study) was quickly neutralized within the colonic lumen in rats, and did not provide long-term stabilization of captopril within the colonic lumen. Rapid neutralization of this vehicle might explain why the colonic bioavailability of captopril was not substantially increased when this pH-3·5-buffered vehicle was tried in humans.

The experimental model in rats that was developed and validated allows for drug administration to a particular intestinal site while the animal is conscious and unrestrained. This simple model in rats can be used to perform exploratory studies and thus it can be a useful addition to other studies (e.g., oral administration to dogs) that are typically conducted during pre-clinical optimization of a controlled-release dosage form.

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