Br. J. clin. Pharmac. (1988), **25**, 9–15

Disposition of fosinopril sodium in healthy subjects

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1 Fosinopril sodium is the first phosphorus-containing angiotensin-converting enzyme (ACE) inhibitor to be studied clinically as an antihypertensive agent. It is an ester prodrug that is hydrolysed *in vivo* to the active diacid ACE inhibitor, SQ 27, 519.

2 In a three-way crossover study, nine healthy male subjects (age range 20–34 years) each received an intravenous 7.5 mg dose of SQ 27, 519-[^{14}C] and two oral 10 mg doses of [^{14}C]-fosinopril sodium, administered as a capsule and in solution.

3 After the intravenous dose of SQ 27, 519, the 0 to 96 h recovery of radioactivity averaged 44 and 46% of the dose in urine and faeces, respectively, indicating substantial biliary secretion. Only intact SQ 27, 519 was detected in the plasma, urine, and faeces following the intravenous dose of SQ 27, 519.

4 After oral doses of fosinopril sodium, about 75% of the radioactivity in plasma and urine was present as SQ 27, 519; the remainder corresponded mainly to a β-glucuronide conjugate of SQ 27, 519 (15–20%), and a monohydroxylated analogue of SQ 27, 519 (about 5%). Negligible amounts of fosinopril sodium were present, indicating complete hydrolysis of the prodrug.

5 For the solution and capsule doses, respectively, the oral absorption of fosinopril sodium averaged 32% and 36% and the oral bioavailability of SQ 27, 519 averaged 25% and 29%.

6 The average values for clearance (39 ml min^{-1}), renal clearance (17 ml min^{-1}), V_{ss} (10 l), and plasma protein binding (~ 95%), indicated that SQ 27, 519 was slowly cleared from the body and not distributed extensively into extravascular sites.

Keywords pharmacokinetics metabolism absorption angiotensin-converting enzyme inhibitor fosinopril sodium SQ 27, 519

Introduction

Fosinopril sodium (SQ 28, 555), an ester prodrug sodium salt of the diacid SQ 27, 519, is an orally-effective inhibitor of the angiotensin I-induced pressor response and serum ACE activity in healthy subjects (Duchin *et al.*, 1984, 1985). It has also been shown to be effective in lowering arterial blood pressure in patients with essential hypertension (Duchin, 1986). This drug is designated chemically as [1(±), 4*S*]-4-cyclohexyl-1-[[[2-methyl-1-(oxopropoxy) propoxy] (4-phenyl butyl) phosphinyl]-acetyl]-L-proline, monosodium salt (Figure 1), and is hydrolysed in the body to the active ACE inhibitor, SQ 27, 519 (Figure 1).

The primary objectives of this study in healthy human volunteers were to determine the oral

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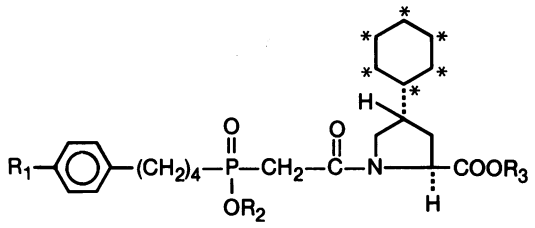


*denotes sites labeled with ^{14}C

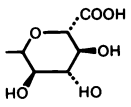
Compound	R_1	R_2	R_3
Fosinopril sodium	H	$(H_3C)_2-CH-\dot{C}H-O-\overset{O}{\overset{\|}{C}}-C_2H_5$	Na
SQ 27,519	H	H	H
Acyl β-glucuronide conjugate of SQ 27,519	H	H	
Hydroxy analog of SQ 27,519	OH	H	H

Figure 1 Structures of fosinopril sodium, SQ 27, 519, and metabolites.

absorption of fosinopril sodium and bioavailability of SQ 27, 519 after oral administration of fosinopril sodium and to evaluate the pharmacokinetics of SQ 27, 519 after its intravenous administration.

Methods

Clinical protocol

Nine healthy male Caucasian subjects ranging in age from 20 to 34 years (mean = 27 years) were studied. They weighed between 64.3 and 93.2 kg (mean = 77.0 kg), and ranged in height from 168 to 184 cm (mean 178 cm). The subjects received no medication for at least 2 weeks before the study. The study protocol was reviewed and approved by the Institutional Review Committee of the Princeton Medical Center and all subjects gave written informed consent to participate in the study.

In this open three-way crossover study, each of the nine subjects received two formulations of 10 mg of [^{14}C]-fosinopril sodium (radiochemical and chemical purity more than 99%) administered orally, as a solution and a dry-filled capsule, and 7.5 mg of SQ 27, 519-[^{14}C] (radiochemical and chemical purity more than 98%) administered intravenously over a 3 min period (dose equimolar to 10 mg of fosinopril sodium). Each dose was separated by a 1 week washout period. Each subject received about 50 μCi of ^{14}C radioactivity with each dose.

Samples of venous blood (12 ml) were drawn just before drug administration and at frequent times over the 48 h interval after dosing. Each blood sample was centrifuged under refrigeration for 15 min and plasma was collected. Urine and faeces were collected for 4 days after drug administration.

Sample extraction and quantitation

The radioactivity in plasma samples (2 ml) was quantitatively extracted into methanol (6 ml of methanol three times), and the combined methanol extract was concentrated to dryness *in vacuo* at 25°C. The dried residue was then reconstituted in 0.6 ml methanol containing non-radiolabelled SQ 27, 519 (100 μg ml^{-1}) and the radioactive components were measured using the thin-layer radiochromatography (t.l.r.c.) procedure described later. Aliquots (2 g) of pooled aqueous homogenates of faeces (0–96 h) of all subjects for each formulation were extracted with 10 ml of methanol (30 min on shaker), centrifuged, and the supernatant was decanted and saved. The remaining pellet was suspended with 4 ml of pH 7 phosphate buffer (30 min on shaker), adjusted to pH 3 with HCl (1M), and then extracted three times with 20 ml of ethyl acetate. The initial methanol extract and the

three ethyl acetate extracts were combined and evaporated to dryness *in vacuo* at 25°C. The dried residue was then reconstituted with 2 ml of methanol/water (1:1 v/v) and analysed by both t.l.r.c. and h.p.l.c. procedures as described later.

Aliquots (50 μl) of urine samples, and aliquots (100 μl) of the reconstituted extracts of plasma and faeces were analysed by t.l.r.c. on silica gel GF plates in a *n*-butyl acetate/glacial acetic acid/water (3:2:1 v/v) solvent system. Non-radioactive compounds (fosinopril sodium and SQ 27, 519) were used as reference standards and were visualized with short-wave ultraviolet light; the R_F values were 0.75 for fosinopril and 0.50 for SQ 27, 519.

For determination of metabolites in urine and faeces by h.p.l.c., a Whatman M9-ODS-3 reverse phase column was used. The mobile phase initially consisted of 30% methanol/67% 0.01 M potassium phosphate (pH 6.0)/3% tetrahydrofuran. The methanol content of the mobile phase was increased in a linear gradient to a final composition of 60% methanol/37% 0.01 M potassium phosphate (pH 6.0)/3% tetrahydrofuran. This final composition of the mobile phase was maintained for 20 min. The retention times of SQ 27, 519 and its two metabolites (SQ 27, 519 acyl β-glucuronide and hydroxylated SQ 27, 519) were approximately 27, 24, and 17 min, respectively. For quantitation of unchanged prodrug (fosinopril sodium), a modified mobile phase was used. Initially the composition was 50% methanol/50% 0.01 M potassium phosphate (pH 6.0); this was changed in a linear manner to a final composition of 80% methanol/20% aqueous potassium phosphate (pH 6.0). The retention times of SQ 27, 519 and fosinopril were 15 and 39 min, respectively.

The mobile phase flow rate was 4 ml min^{-1} for both systems and the effluent was monitored by an ultraviolet detector (210 nm). The fractions collected at 0.5 or 1 min intervals were mixed with liquid scintillation cocktail and counted for radioactivity. Urine samples were injected directly (after centrifugation) and faecal samples were extracted (as described previously) before injection.

Liquid scintillation counting

The scintillation cocktail of Anderson & McClure (1973) was used to count all samples. Soluene 350® (Packard Instrument Co.) was used to solubilize blood, plasma, and urine samples. Faeces were homogenized with water, and samples were counted after digestion with Soluene 350. All samples were counted in either a Packard Tri-Carb® or an Intertechnique Model SL-4200 liquid scintillation spectrometer. Counting efficiencies were determined using automatic external standardization.

Binding to plasma proteins and cellular elements of whole blood

Additional aliquots of blood withdrawn from each subject at 1 and 3 h were used for analysis of radioactivity in blood, plasma, and protein-free filtrate (PFF). For preparation of PFF, about 4 ml of plasma were transferred into Centriflow® ultrafiltration membrance cones CF-50A (Amicon Corp.) and centrifuged at 2200 rev min^{-1} for 5 min (Singhvi *et al.*, 1977).

Calculations and analysis of data

Concentrations of total radioactivity and individual radioactive components in plasma were expressed as equivalents of SQ 27, 519. For SQ 27, 519, the area under the plasma concentration *vs* time curve from time zero to time t AUC (0, t) was determined by the integration method of Lagrange (Yeh & Kwan, 1978). AUC from time zero to infinity (∞) was determined by the sum of AUC (0, t) and C_t/λ_2, where C_t = concentration of SQ 27, 519 at time t (16 h) after drug administration and λ_2 = slope of the terminal portion of the log concentration *vs* time curve.

Model-independent pharmacokinetic parameters of SQ 27, 519 were calculated using standard procedures (Gibaldi & Perrier, 1982).

Pharmacokinetic parameters of SQ 27, 519 following i.v. administration were also determined by a model-dependent, curve-fitting method. In all subjects, plasma concentrations of SQ 27, 519 declined in an apparent biexponential fashion, and the concentrations of SQ 27, 519 in plasma at any time (C) were fitted to the following equation: $C = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}$.

Initial estimates of pharmacokinetic parameters for SQ 27, 519 were obtained by graphical methods and data were analysed using the non-linear least squares regression program NONLIN (Metzler *et al.*, 1974). In this case, the best fits were obtained when all plasma concentrations were weighted according to $1/C^2$.

The percentage of radioactivity in the blood associated with cellular elements was calculated using the equation:

$$\% \text{ association} = \frac{\text{Blood conc.} - \text{Plasma conc.}\ (1-H)}{\text{Blood conc.}} \times 100$$

where individual haematocrit (H) values obtained just before drug administration were used to determine the percent association.

Results

Excretion in urine and faeces

During 96 h after i.v. administration of SQ 27, 519-[^{14}C], an average of 90% of the dose was recovered in the excreta; excretion averaged 44% in urine and 46% in faeces. After p.o. dosing of [^{14}C]-fosinopril sodium, recovery of the radioactivity in the excreta averaged 86% (solution) and 94% (capsule) of the dose; urinary excretion averaged 13% (solution) and 16% (capsule) and faecal excretion averaged 73% (solution) and 78% (capsule).

Biotransformation products in urine

Thin-layer radiochromatography analysis of individual samples (0–24 h) and h.p.l.c. analysis of pooled samples (0–24 h) indicated that after i.v. administration only intact SQ 27, 519 was present in urine. After p.o. administration of the prodrug, only a trace amount (less than 1%) of the radioactivity was present in urine as intact prodrug (fosinopril sodium), and an average of 78% (solution) and 77% (capsule) of the radioactivity was represented by SQ 27, 519. Thus, the cumulative urinary excretion of SQ 27, 519 in the 0–24 h urine averaged 9.1% (solution) and 10.9% (capsule) of the dose. Autoradiograms of pooled urine samples indicated the presence of two distinct radioactive components in addition to SQ 27, 519. High-performance liquid chromatography analysis of pooled urine samples also indicated the presence of two metabolites of SQ 27, 519 and intact SQ 27, 519.

Incubation of the pooled urine samples with crude β-glucuronidase enzyme indicated that the predominant urinary metabolite was a glucuronide conjugate of SQ 27, 519. In both t.l.r.c. and h.p.l.c. analyses, this metabolite was apparently stable when incubated in either buffer alone (pH 5.2) or when 1, 4-saccharolactone, a specific β-glucuronidase inhibitor, was added to the crude enzyme mixture. In a separate experiment, using h.p.l.c., quantitative conversion of this metabolite to SQ 27, 519 was observed when the urine was made alkaline (pH 12) and allowed to stand at ambient temperature for 2 h. Instability in mildly alkaline conditions is characteristic of acyl glucuronides (Faed, 1984); this suggested that the glucuronide conjugate of SQ 27, 519 may be an acyl-linked β-glucuronide conjugate (Figure 1). The other metabolite had the same h.p.l.c. and t.l.r.c. retention time as authentic *p*-hydroxylated SQ 27, 519 (Figure 1).

Biotransformation products in faeces

Extracts of the pooled faecal samples prepared from the 0 to 96 h faecal collections were analysed by both t.l.r.c. (autoradiographic visualization) and h.p.l.c. Both methods indicated that the radioactivity in faeces following i.v. administration was essentially all (about 95%) associated with SQ 27, 519. Based on h.p.l.c. and t.l.r.c. analyses, about 85% of the radioactivity in the faeces of subjects who received oral fosinopril sodium was associated with SQ 27, 519. High-performance liquid chromatography results indicated that the remaining radioactive component corresponded chromatographically to the *p*-hydroxy analogue of SQ 27, 519. Although the glucuronide conjugate of SQ 27, 519 was not detected in faeces, it is possible that the conjugate was excreted in the bile and hydrolysed back to SQ 27, 519 in the intestines before excretion in faeces.

Concentrations and biotransformation profiles in plasma

After i.v. administration of SQ 27, 519-[^{14}C], virtually all of the radioactivity in plasma was present as the unchanged diacid. After p.o. administration, fosinopril sodium was rapidly and extensively hydrolysed *in vivo*; at 20 and 40 min after the solution dose, an average of only 11 and 3%, respectively, of the radioactivity in plasma was present as unchanged prodrug. From 1–24 h, less than 1% of the radioactivity was present as unchanged prodrug after either formulation. At all time points, an average of 70 to 80% of the radioactivity in plasma was present as SQ 27, 519; the remainder of the radioactivity corresponded to the glucuronide conjugate of SQ 27, 519.

The mean concentrations of SQ 27, 519 in plasma after the p.o. doses of [^{14}C]-fosinopril sodium and the i.v. dose of SQ 27, 519-[^{14}C] are shown in Figure 2. The concentrations of SQ 27, 519 and total radioactivity were slightly higher for the capsule formulation than for the oral solution from 1.5–24 h after dosing. The bioavailability parameters for SQ 27, 519 and kinetic parameters for total radioactivity are shown in Table 1. Maximum concentrations of SQ 27, 519 and total radioactivity in plasma were attained at about 3 h after oral administration of the prodrug.

Binding to plasma proteins and cellular elements of whole blood

After either oral dose of [^{14}C]-fosinopril sodium the binding of radioactivity to plasma proteins

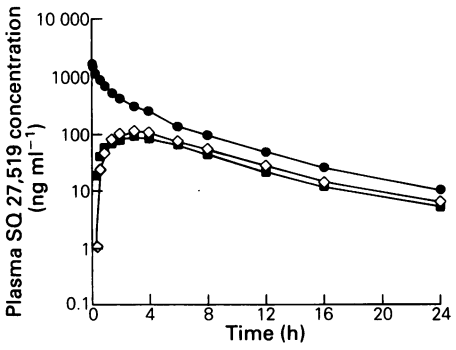


Figure 2 Average concentrations of SQ 27, 519 in plasma after intravenous administration of 7.5 mg of SQ 27, 519-[^{14}C] and oral administration of 10 mg of [^{14}C]-fosinopril sodium to nine healthy subjects. ● intravenous, ◇ dry-filled capsule, ■ oral solution.

averaged 89% at 1 h and 92% at 3 h. After the intravenous dose of SQ 27, 519-[^{14}C], the protein binding averaged 95% at both time points. Thus, SQ 27, 519 is extensively bound to plasma proteins. On the other hand, association of radioactivity with cellular elements of whole blood was negligible (an average of less than 6%) at 1 and 3 h after administration of either compound.

Absorption of total radioactivity and bioavailability of SQ 27, 519

Based on a comparison of plasma AUC values for total radioactivity (Table 1) after p.o. doses of [^{14}C]-fosinopril sodium and i.v. dose of SQ 27, 519, the absorption of the radioactive dose averaged 32% (solution dose) and 36% (capsule dose); a similar comparison of urinary excretion values indicated an average oral absorption of 29% (solution) and 36% (capsule). The absolute bioavailability of SQ 27, 519 after oral doses of [^{14}C]-fosinopril sodium averaged 25% (solution) and 29% (capsule) based on plasma AUC values for SQ 27, 519 (Table 1), and 21% (solution) and 25% (capsule) based on urinary excretion values for SQ 27, 519. The oral absorption and bioavailability values for the capsule dose were slightly, but not statistically significantly ($P > 0.05$, Student's t-test for paired data), higher than those for the solution dose.

Pharmacokinetic evaluation

The total renal, and non-renal clearances of SQ 27, 519 averaged 39, 17, and 22 ml min^{-1}, respectively (Table 2). The average elimination half-life of SQ 27, 519 from plasma, calculated between the time interval of 4 and 16 h, ($t_{½,z}$) was 3.7, 4.2, and 4.3 h for the i.v., oral solution and capsule formulations, respectively. Mean residence time (MRT) was 4.2 h following i.v. administration and 7.4 and 7.7 after the solution and capsule doses. Thus, the mean absorption time (MAT) for the two oral formulations averaged about 3.2–3.5 h. The average steady-state volume of distribution of SQ 27, 519 was 9.8 l.

Clinical results

Fosinopril sodium and SQ 27, 519 were well tolerated by all subjects. There were no adverse effects and no significant changes attributable to

Table 1 Kinetic parameters for total radioactivity and SQ 27, 519 after oral administration of [^{14}C]-fosinopril sodium and intravenous administration of SQ 27, 519-[^{14}C]

Parameter	*Component(s) analysed*	*Oral dose*[a] *Solution*	*Capsule*	*Intravenous dose*[a]
C_{max} (ng ml^{-1})	Total radioactivity[b]	126 ± 13	140 ± 10	—
	SQ 27, 519	99 ± 11	112 ± 8.0	1556 ± 73[c]
t_{max} (h)	Total radioactivity[b]	2.7 ± 0.3	3.0 ± 0.2	—
	SQ 27, 519	2.8 ± 0.3	3.1 ± 0.2	—
AUC (0, 24) (ng ml^{-1} h)	Total radioactivity[b]	1035 ± 93	1132 ± 68	3166 ± 194
AUC (0–∞) (ng ml^{-1} h)	SQ 27, 519	788 ± 79	920 ± 57	3302 ± 205

[a] Mean ± s.e. mean for nine subjects.
[b] Expressed as ng-equivalents of SQ 27, 519 ml^{-1}.
[c] Corresponds to the concentration at 5 min after dosing, the initial sampling time following administration of SQ 27, 519-[^{14}C].

Table 2 Mean (± s.e. mean) pharmacokinetic parameters for SQ 27, 519 after intravenous administration of SQ 27, 519-[^{14}C] and oral administration of [^{14}C]-fosinopril sodium

Parameter	*Intravenous dose (SQ 27, 519)*	*Oral dose (Fosinopril sodium)* Solution	Capsule
1. *Model independent*			
Total clearance (ml min^{-1})	39.1 ± 2.6	—	—
Renal clearance (ml min^{-1})	17.0 ± 1.1	—	—
Non-renal clearance (ml min^{-1})	22.1 ± 2.4	—	—
V_{ss} (l)	9.8 ± 0.7	—	—
$t_{½,z}$ (h)[a]	3.7 ± 0.2	4.2 ± 0.2	4.3 ± 0.3
Mean residence time (h)	4.2 ± 0.2	7.4 ± 0.2	7.7 ± 0.5
Mean absorption time (h)	—	3.2 ± 0.2	3.5 ± 0.5
2. *Model dependent*			
Total clearance (ml min^{-1})	38.5 ± 2.5	—	—
$t_{½}$ (λ_1) (h)	0.55 ± 0.04	—	—
$t_{½}$(λ_2) (h)[a]	3.7 ± 0.02	—	—
C_1 (ng ml^{-1})	1144 ± 59	—	—
C_2 (ng ml^{-1})	449 ± 28	—	—

[a] Determined between 4 and 16 h; these values are underestimates of the true elimination half-life values (see text for details)

the drug were noted in the physical, electrocardiographic, or clinical laboratory examinations, except for a slight increase in serum glutamic-pyruvate transaminase (SGPT) levels [57 (pre) to 85 iu l^{-1}; normal range (10–55 iu l^{-1})] in one subject 4 days after intravenous administration of SQ 27, 519. Three days later, SGPT levels in that subject returned to the normal range (47 iu l^{-1}).

Discussion

Fosinopril sodium was absorbed to the extent of 32% after the solution dose and 36% after the capsule dose and was almost completely hydrolysed *in vivo* to SQ 27, 519. Plasma, urine, and faecal samples indicated that more than 70% of the radioactivity in all samples consisted of SQ 27, 519; thus, the active diacid (SQ 27, 519) was the most prominent metabolite of fosinopril sodium. The acyl β-glucuronide conjugate of SQ 27, 519 and a hydroxy analogue of SQ 27, 519 were the only other metabolites present in significant amounts in these samples. The absolute bioavailability of SQ 27, 519 averaged about 25% (solution) and 29% (capsule) after the oral doses of fosinopril sodium.

After intravenous administration of SQ 27, 519, essentially all of the radioactivity in plasma, urine, and faeces was present as unchanged SQ 27, 519. Recovery of 46% of the dose in faeces indicated substantial biliary excretion of SQ 27, 519. This would provide an alternate route for elimination of SQ 27, 519 and its metabolites after an oral dose of fosinopril sodium in hypertensive patients with compromised kidney function. More than 50% of the total clearance (39 ml min^{-1}) of SQ 27, 519 was attributed to non-renal clearance (22 ml min^{-1}). This is in contrast to the properties of currently available ACE inhibitors (captopril and enalapril) which are eliminated primarily by the kidneys (Singhvi *et al.*, 1982; Ulm, 1982).

The mean residence time for SQ 27, 519 was much higher (an average of about 7–8 h) after p.o. doses of fosinopril sodium compared with about 4 h after the intravenous dose of SQ 27, 519. Therefore, slow absorption of the prodrug contributes to prolongation of the plasma levels of the active ACE inhibitor after oral administration. The lower total clearance of SQ 27, 519 (about 40 ml min^{-1}) relative to normal creatinine clearance (about 120 ml min^{-1}) indicates that SQ 27, 519 is cleared slowly from the body.

Although the elimination half-life of SQ 27, 519 was estimated to be about 4 h, the actual terminal elimination half-life could be much longer than 4 h. The concentrations of SQ 27, 519 in plasma at 24 h after dosing, in most cases, showed a deviation from the log-linear terminal phase used for determination of λ_2 values (see Figure 2), suggesting the existence of a longer terminal phase for decline of plasma concentration versus time beyond 16 h after dosing. Since, in this study, the concentrations of SQ 27, 519 in

plasma could not be reliably estimated beyond 24 h after dosing, this longer terminal phase could not be characterized resulting in an overestimation of λ_2 values, and, consequently, an underestimation of the actual terminal half-life of SQ 27,519 in plasma. In future studies, an attempt will be made to characterize this longer terminal elimination phase by improving the detection limits (about 1 ng ml^{-1}) of the assay used in this study. In early clinical studies, fosinopril sodium given once daily has been shown to be effective in lowering arterial blood pressure for 24 h in patients with essential hypertension (Duchin, 1986).

We wish to thank Ms Jane Blue, Ms Susan Lubin and the nursing staff at the Clinical Pharmacology Unit of the Medical Center at Princeton for thier outstanding cooperation and Drs Doris McKinstry and Bruce Migdalof for editorial assistance. James Shaw, Callixtus Ita, Carmen Mondi and Robert Ratoff provided technical assistance. The synthesis of radiolabeled fosinopril sodium and SQ 27, 519 was carried out by Peter Egli.

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(Received 16 July 1987, accepted 4 August 1987)