Pharmacokinetics of a Novel Antiarrhythmic Drug, Actisomide

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The pharmacokinetics of a novel antiarrhythmic drug, actisomide, were examined in the rat, dog, monkey, and human. The terminal half-life of actisomide was similar (1.15–1.89 hr) across species, regardless of dose. The total plasma clearance was higher in the monkey (13.5–16.4 mL/min/kg) than in the dog (9.01–9.32 mL/min/kg), rat (8.6–9.8 mL/min/kg), or human (6.79 ± 1.07 mL/min/kg). Excretion of the parent drug was higher in urine than in feces in the dog and rat, whereas in the monkey and human, urinary and fecal excretions of actisomide were similar. In humans, atypical plasma concentration–time curves with double peak concentrations were observed following oral doses. Systemic availability of actisomide was higher in the dog than in the rat, monkey, and human. Further, the systemic availability appeared to increase with dose in the rat and monkey. The species-dependent systemic availability appeared to be due primarily to species-dependent absorption of actisomide, and not to species-dependent first-pass metabolism, biliary excretion, and/or renal elimination. The absorption of actisomide in the rat and its *in vitro* uptake in CaCo-2 cells were pH dependent. The higher systemic availability of actisomide observed in the dog may be due partly to the higher pH in the gastrointestinal (GI) tract of the dog. However, the pH differences in the GI tract of the different species alone did not appear to be enough to explain the difference in systemic availability of actisomide.

KEY WORDS: actisomide; species-dependent absorption; pH effect.

INTRODUCTION

Actisomide (SC-36602) is a novel antiarrhythmic agent (Fig. 1) that blocks sodium channels in a manner similar to that of lidocaine and mexiletine (1,2). In preclinical studies, actisomide was well tolerated and had negligible negative inotropic activity in comparison to other class 1 antiarrhythmic agents. The present study was undertaken to evaluate the pharmacokinetics of actisomide in laboratory animals and humans after administration of the drug intravenously and orally.

EXPERIMENTAL PROCEDURE

Materials

[^{14}C]Actisomide, unlabeled actisomide, and N-dealkylated metabolite were supplied by G. D. Searle & Co. (Skokie, IL). All chemical reagents were commercially available.

In Vivo/in Situ Study Protocol

Rats. Male rats (Charles River) weighing 250 to 300 g were fasted overnight prior to drug administration. [^{14}C]Actisomide was administered as aqueous solutions intravenously (i.v.) at doses of 8 and 32 mg/kg and orally at doses of 16 and 160 mg/kg to each group of eight rats for the radioactivity recovery and plasma concentration–time course study. Blood, urine, and fecal samples were collected from the appropriate groups at specified time intervals.

For the biliary excretion study, rats were anesthetized with ethyl ether and anesthesia was maintained by sodium pentobarbital (intraperitoneal dose of 55 mg/kg). The common bile duct was catheterized with polyethylene tubing (PE 10, Clay Adams, Parsippany, NJ). After the animals had recovered from anesthesia, [^{14}C]actisomide was given i.v. at an 8 mg/kg dose and orally at doses of 8, 64, and 160 mg/kg. Bile, urine, and feces were collected periodically for 72 hr.

For the absorption study over a range of pH, an 8 mg/kg dose of [^{14}C]actisomide was administered in 0.1 *M* phosphate buffer solutions of pH 5, 7.4, and 8.5 to three groups of three rats each. The rats which received a pH 8.5 dose solution were pretreated with Maalox (5 mL/kg) 1 hr prior to dosing.

An *in situ* absorption study was conducted using a single-path perfusion method reported by Sinko and Amidon (3) with minor modifications. The dose solution (0.5 mg/mL), prepared using Ringer's solution (pH 7.5), was perfused at a flow rate of 0.15 mL/min through approximately 10 cm of the upper jejunum, ileum, and colon of three rats each. Jejunum perfusion was conducted with and without bile duct ligation. Changes in water volume in the *in situ* gastrointestinal (GI) tract were corrected for by using [^{3}H]inulin.

Dog. Eight male beagle dogs weighing 7.0–12 kg were fasted overnight prior to dosing. Four dogs received i.v. doses of [^{14}C]actisomide at 8 and 16 mg/kg in a randomized crossover manner. The remaining four dogs received unlabeled actisomide as an oral solution at a dose of 20 mg/kg. Blood, urine, and fecal samples were collected at specified time intervals.

Monkey. Four female rhesus monkeys weighing 4.7–6.7 kg were fasted overnight prior to drug administration. Each animal received [^{14}C]actisomide i.v. doses of 8 and 16 mg/kg body weight and oral doses of 16 and 64 mg/kg in a solution in a randomized crossover manner with a washout period of at least 1 week. During the first 10 hr of the study, animals were placed in a primate chair and saline (20 mL/hr) was infused through a catheter. The animals were then transferred to stainless-steel metabolism cages. Blood, urine, and feces were collected at appropriate time intervals.

Man. Twelve healthy male subjects between 21 and 28 years of age participated in the study. A complete medical history and results of physical and laboratory examinations were obtained for each subject. These included complete blood count, urinalysis, creatinine, serum bilirubin (total), total protein, albumin, calcium, inorganic phosphorus, cho-

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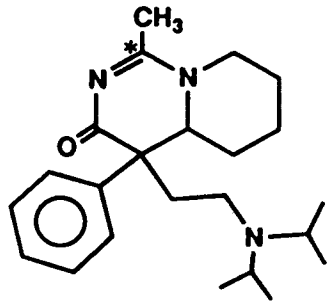

Fig. 1. Chemical structure of actisomide. Asterisk indicates labeled carbon atom.

lesterol, uric acid, LDH, alkaline phosphatase, BUN, glucose, and SGOT. These values in all six subjects were within the normal range. No other medications besides actisomide were taken by the subjects during the study. The six subjects received an i.v. dose of 50 mg [^{14}C]actisomide/person (approx. 0.8 mg/kg). For the oral study, three subjects received a dose of 400 mg unlabeled actisomide/person (approx. 5.3 mg/kg) and three subjects received 500 mg unlabeled drug (approx. 7.3 mg/kg). The i.v. dose solution was prepared by dissolving 50 mg of [^{14}C]actisomide (approx. 100 μCi) in 10 mL of isotonic citrate buffer. The dose solution (10 mL) of the drug was administered as an i.v. injection over a 5-min period. The oral dosage form was a gelatin capsule containing 100 mg neat actisomide. [The bioavailability of the drug was the same as a solution or neat chemical in gelatin capsules in laboratory animals (data not shown).] The subjects were fasted approximately 10 hr prior to and 4 hr after dosing. On the day of the oral study, each subject drank 250 mL of water 1 hr before receiving the drug. An additional 50 mL of water was taken with the drug. After i.v. administration, blood samples were taken at 0 (immediately after cessation of infusion), 1, 3, 5, 7, 10, 15, 30, and 45 min and 1, 2, 4, 6, 8, 10, 12, 16, 24, 48, and 72 hr. Urine was collected at 0, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hr. Feces was collected every 24 hr up to 168 hr. After oral administration, blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.75, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 24, 28, 32, 48, 52, 56, and 72 hr.

In Vitro CaCo-2 Cell Uptake

An *in vitro* uptake study of [^{14}C]actisomide was conducted with CaCo-2 cells (American Type Culture Collection, Rockville, MD) at pH's of 6.5, 7, and 8 and at drug concentrations of 0.1, 0.5, 1, and 5 μg/mL using a procedure reported by Ruben *et al.* (4).

Sample Analysis

Plasma. Total radioactivity in animal and human plasma was determined by liquid scintillation counting (LSC) after the addition of a 50–500 μL aliquot to a mixture of 2 mL of water and 5 mL of PCS scintillant (Amersham Co., Arlington Heights, IL). To quantitate the plasma concentrations of the parent drug in all species, plasma samples were extracted as follows: an aliquot of plasma (0.5–1 mL) containing an internal standard (SC-13957) was made basic by the addition of 0.5 *N* NaOH and applied to a CN Bond Elut column (size, 3 cm^3; AnalytiChem International Inc., Harbor City, CA) which was preactivated with methanol followed by a water rinse. Actisomide was eluted with a solvent mixture of methanol:water:1 *M* dibutylamine phosphate (91:8:1, vol). The eluent was evaporated to dryness. The residue was reconstituted with a mixture of CH_3CN and 0.01 *M* dibutyl amine phosphate (90:10, vol) and analyzed using a high-performance liquid chromatographic (HPLC) procedure.

Urine. Total radioactivity in animal and human urine was determined by counting triplicate 0.5- to 1.0-mL aliquots directly in a mixture of 1 mL of distilled water and 5 mL of PCS scintillant. To measure actisomide concentrations, aliquots of the pooled or individual urine samples (1 mL) were made basic by the addition of 0.5 *N* NaOH and placed in the activated Bond Elut column. Radioactive compounds were eluted from the Bond Elut column and the eluants were analyzed using a high-performance liquid radiochromatographic (HPLRC) procedure.

Feces. Each animal and human fecal sample was mixed with an equal weight of distilled water and the mixture was homogenized in a Stomacher (Lab-Blender 400, A. J. Seward, London). Triplicate aliquots of each homogenized sample were dried at room temperature for at least 15 hr and oxidized with a Packard Tri-Carb sample oxidizer (Model 306, Packard Instruments Co., Downers Grove, IL). The combustion products were mixed with 9 mL of Carbosorb and 12 mL of Permafluor V (Packard Instruments Co., Downers Grove, IL) and the total radioactivity was determined by LSC. To determine the distribution of radioactivity in feces, an aliquot (0.6–1.0%) was pooled from each of the selected fecal samples proportionally to its weight. The pooled fecal samples were extracted by refluxing with methanol for approximately 16 hr in Soxhlet extraction tubes. The methanol extract was applied to a Bond Elut column and eluted according to the same procedure as that described for the urine samples.

Liquid Scintillation Counting (LSC). All radioactivity determinations were carried out using a liquid scintillation spectrometer (Mark II or III, Tracor Analytic Inc., Elk Grove, IL). Chemical quenching was corrected by the automatic external standard channel ratio method.

HPLC and HPLRC. The HPLC system consisted of pumps (Waters M-6000), a system controller (Waters Associates), a WISP automatic injector (Waters Model 710B, Waters Associates), a UV detector (Kratos Spectrolow 783 absorbance detector, Kratos Analytical Instruments, Ramsey, NJ), and a reverse-phase column (Radial-Pak CN column, Waters Associates). The mobile phase, which consisted of water, acetonitrile, and 1 *M* dibutylamine phosphate (88:11:1, vol), was used at a flow rate of 1.5 mL/min. Actisomide was detected at a fixed wavelength of 254 nm. HPLRC was performed using a C-18 radial compression cartridge (8-mm ID, 10-μm particle size). The mobile phase consisted of a linear gradient system from 5% acetonitrile in 0.01 *M* dibutylamine phosphate to 90% acetonitrile in 0.01 *M* dibutylamine phosphate over a 60-min period. The flow rate was 1 mL/min. Eluant fractions from the column were collected every minute in liquid scintillation vials using a Foxy fraction collector (Foxy ISCO, Lincoln, NE). Five milliliters of PCS was added to each vial and total radioactivity was determined by LSC.

Data Analysis

The plasma concentration–time curves of actisomide in all species following i.v. doses were analyzed according to a biexponential equation [Eq. (1)] using the NONLIN computer package (5).

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_t is plasma concentration at time t, A and B are coefficients, and α and β are first-order rate constants for the distribution and elimination phases, respectively.

The volume of distribution (V_d) was calculated from Eq. (2):

$$V_d = X_0/\beta \cdot AUC_0^\infty \quad (2)$$

where X_0 is the i.v. dose and AUC_0^∞ is the area under the plasma concentration–time curve from time 0 to infinity. The steady-state volume of distribution (V_{dss}) was obtained from the noncompartmental Eq. (3):

$$V_{dss} = \frac{X_0\left[\int_0^\infty t \cdot Ctdt\right]}{\left[\int_0^\infty Ctdt\right]^2} \quad (3)$$

The total-body clearance (CL) were estimated from Eq. (4).

$$CL = X_0/AUC_0^\infty \quad (4)$$

The mean residence time (MRT) was calculated from Eq. (5):

$$MRT = \frac{\int_0^\infty t \cdot Ctdt}{\int_0^\infty Ctdt} \quad (5)$$

RESULTS

Plasma Concentrations and Kinetics

Animals. Figures 2 and 3 show the mean plasma concentration–time curves of actisomide after i.v. administration of [^{14}C]actisomide to the rat, dog, monkey and oral administration to the dog and monkey. Following an i.v. dose (8 mg/kg), plasma concentrations of actisomide declined biexponentially, with terminal elimination half-lives of 1.15–1.51 hr in all animal species. All pharmacokinetic parameters in all animal species were independent of dose (Table I). The total clearance of actisomide was higher in the monkey than in the rat and dog.

After oral administration to the rat, the systemic availability of actisomide was low at doses of both 16 and 160 mg/kg (Table II). At the 16 mg/kg dose plasma concentrations were below the assay sensitivity (0.2 μg/mL) at all time points examined. In the monkey systemic availability of the drug was also low (Table II). In contrast to the monkey and rat, actisomide was rapidly and well absorbed in the dog, as evidenced by C_{max} values being achieved within 1 hr and by the high systemic availability (67.5%).

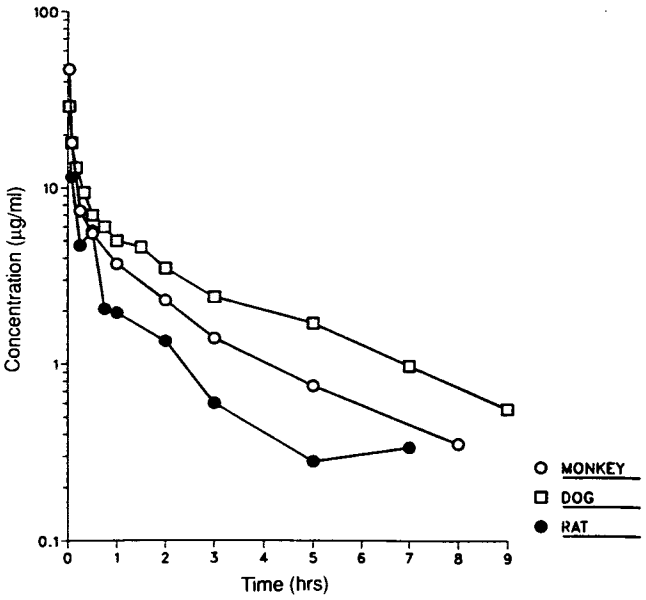

Fig. 2. Mean plasma concentration–time curves of actisomide after i.v. administration to the rat, dog, and cynomolgus monkey at a dose of 8 mg/kg.

Man. The mean plasma concentration–time curves of actisomide after i.v. and oral administration of [^{14}C]actisomide to humans are shown in Fig. 4. Pharmacokinetic parameters obtained following i.v. and oral doses are given in Tables III and IV, respectively. Following an i.v. dose (50 mg), plasma concentration–time curves of the drug were adequately described by a biexponential equation. The V_d and terminal half-lives of actisomide in man were similar to those in the laboratory animals examined. After oral administration, the first peak plasma concentration (C_{1max}) was ob-

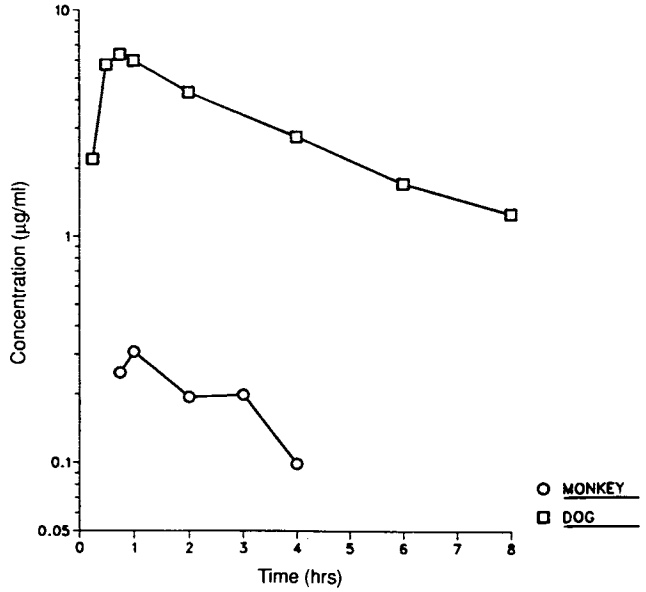

Fig. 3. Mean plasma concentration–time curves of actisomide after oral administration to the dog and cynomolgus monkey at a dose of 16 mg/kg. All concentrations in rat plasma at a dose of 16 mg/kg were below assay sensitivity (0.2 μg/mL).

Table I. Pharmacokinetic Data Following i.v. Administration of [^{14}C]Actisomide to Rats, Dogs, and Cynomolgus Monkeys (All Values are Mean ± SE Where Appropriate)

	Rat		Dog		Monkey	
	8 mg/kg[a] (N = 3)	32 mg/kg (N = 3)	8 mg/kg (N = 4)	16 mg/kg (N = 4)	8 mg/kg (N = 4)	16 mg/kg (N = 4)
A (μg/mL)	5.98[b]	27.1[b]	24 ± 4	32 ± 7	27 ± 3	76 ± 23
α $t_{1/2}$ (hr)	0.086[b]	0.095[b]	0.0344 ± 0.0084	0.0587 ± 0.0142	0.0312 ± 0.0034	0.0351 ± 0.0090
B (μg/mL)	1.05[b]	6.29[b]	5.1 ± 0.5	32 ± 7	3.3 ± 0.2	7.5 ± 1.3
β $t_{1/2}$ (hr)	1.35[b]	1.29[b]	1.33 ± 0.12	1.51 ± 0.22	1.15 ± 0.13	1.19 ± 0.08
CL_T (mL/min/kg)	9.8[b]	8.6[b]	9.01 ± 0.45	9.32 ± 0.46	16.4 ± 1.9	13.5 ± 0.7
V_d (L/kg)	1.14[b]	0.96[b]	1.05 ± 0.11	1.23 ± 0.21	1.59 ± 0.03	1.40 ± 0.17
Urinary excretion (%)						
Total ^{14}C (0–168 hr)	44.0 ± 4.3[c]	47.3 ± 3.1[c]	39.3 ± 2.8	43.7 ± 1.7	26.0 ± 0.9	24.2 ± 2.1
Actisomide (0–48 hr)	35.1[d]	32.9[d]	30.2 ± 2.8	36.9 ± 3.1	15.1 ± 0.9	13.5 ± 1.5
Fecal excretion (%)						
Total ^{14}C (0–168 hr)	44.5 ± 3.2[c]	46.2 ± 2.9[c]	50.6 ± 3.3	48.3 ± 3.2	57.5 ± 2.0	58.9 ± 1.4
Actisomide (0–24 hr)	24.8[d]	25.3[d]	20.0[d]	18.0[d]	10.0[d]	15.5[d]

[a] Dose of [^{14}C]actisomide.
[b] Values determined using pooled plasma concentrations and SE value not calculated.
[c] Value obtained from 0- to 120-hr sample.
[d] Value obtained from 0- to 24-hr pooled sample.

served within an hour, followed by a second peak plasma concentration (C_{2max}) between 4 and 6 hr, regardless of the dose. The systemic availability, calculated by comparison of the mean AUC values after the i.v. and oral dose administration, was approximately 30 and 43% at doses of 400 and 500 mg, respectively, after corrections were made for the difference in doses.

Excretion of ^{14}C, Actisomide, and Actisomide Metabolites

After i.v. administration, the mean excretions of total carbon-14 in the rat urine and feces were similar regardless of i.v. dose (Table I). The majority of the carbon-14 in urine (about 80%) and feces (about 60%) was present as the parent drug. After oral administration of actisomide to the rat, the majority of the radioactive dose was excreted in the feces (Table II). The majority of urinary carbon-14 was also parent drug following oral doses. Greater than 90% of fecal radioactivity was the parent drug regardless of oral dose. In the bile duct-cannulated rats, there was a trend toward higher percentages of the radioactive dose excreted in urine and lower percentages excreted in feces as doses increased from 8 to 64 to 160 mg/kg (Table V). The mean percentages of the radioactive dose excreted in bile increased slightly at

Table II. Pharmacokinetic Data Following Oral Administration of [^{14}C]Actisomide to Rats, Dogs, and Cynomolgus Monkeys (All Values are Mean ± SE Where Appropriate)

	Rat		Dog	Monkey	
	16 mg/kg[a] (N = 3)	160 mg/kg (N = 3)	16 mg/kg[b] (N = 4)	16 mg/kg (N = 4)	64 mg/kg (N = 4)
C_{max} (μg/mL)	<0.2[c]	2.04[c]	7.05 ± 0.67	0.357 ± 0.079	2.71 ± 0.34
T_{max} (hr)	—[d]	3.0[c]	0.81 ± 0.12	1.58 ± 0.71	3.00 ± 0.58
Urinary excretion (%)					
Total ^{14}C (0–168 hr)	4.31 ± 0.35[e]	8.85 ± 1.71[e]	—[d]	5.11 ± 0.74	12.5 ± 0.3
Actisomide (0–72 hr)	2.89[f]	7.95[f]	—[d]	2.13 ± 0.21	7.46 ± 0.67
Fecal excretion (%)					
Total ^{14}C (0–168 hr)	81.1 ± 5.0[e]	81.5 ± 2.7[e]	—[d]	90.0 ± 2.2	83.9 ± 1.9
Actisomide (0–72 hr)	79.6[f]	78.2[f]	—[d]	67.2 ± 1.7	36.0 ± 3.6
Bioavailability (%)	—[g]	14.2[h]	64.6 ± 2.1	—[g]	43.4[h]

[a] Dose of [^{14}C]actisomide.
[b] The actual dose was 20 mg/kg but pharmacokinetic parameters were normalized to 16 mg/kg for species comparison.
[c] Value obtained from pooled plasma and SE not determined.
[d] Value not determined.
[e] Value obtained from 0- to 120-hr urine and fecal samples.
[f] Value obtained from 0- to 24-hr pooled urine and fecal samples.
[g] Accurate value could not be determined because of low plasma concentrations.
[h] Oral and i.v. doses were given to separate group of animals and SE could not be calculated.

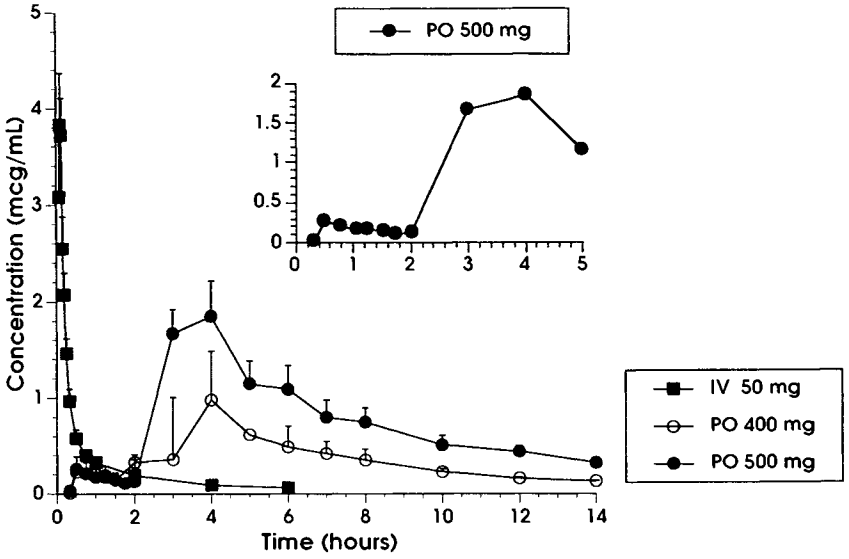

Fig. 4. Mean plasma concentrations of actisomide after i.v. (50 mg) and oral (400 and 500 mg) administration to humans.

the 64 and 160 mg/kg doses compared to that at the 8 mg/kg dose.

The effects of the dose solution pH on actisomide absorption were examined after oral administration of [^{14}C]actisomide to the rat at a dose of 8 mg/kg. As the pH of the dose solution increased from 5.0 to 7.4 and 8.5, the percentages of the radioactive dose excreted in 0- to 48-hr urine increased from 3.3 ± 0.44 to 6.08 ± 0.97 and 8.71 ± 1.95%, respectively.

In the monkey, the fecal-to-urinary radioactivity ratio was about 2 following i.v. doses (Table I). The mean percentages of the dose excreted in urine as the parent drug were approximately half the values for total radioactivity regardless of i.v. doses. Following oral administration, the urinary excretion of radioactivity was much lower than fecal excretion (Table II). In addition, urinary excretion of total radioactivity and actisomide increased approximately 2.4- and 3.5-fold, respectively, when the dose increased from 16 to 64 mg/kg. In monkey feces, excretion of the parent drug was lower at the high dose than at the low dose, whereas excretion of metabolites was higher at the high dose than at the low dose. The percentages of the dose recovered as the

Table III. Pharmacokinetic Data Following i.v. Administration of [^{14}C]Actisomide to Humans at a Dose of 50 mg/Person (Approx. 0.796 mg/kg)

Pharmacokinetic parameter	Subject ID						Mean ± SE
	SUB1	SUB2	SUB3	SUB4	SUB5	SUB6	
Body wt (kg)	61.9	65.6	63.6	57.2	68.5	60.1	62.8 ± 1.6
A (μg/mL)	11.91	3.74	10.74	3.72	6.00	4.50	6.77 ± 1.49
α (hr^{-1})	8.51	4.99	7.87	6.00	6.47	5.17	6.50 ± 0.58
B (μg/mL)	0.69	0.40	0.60	0.40	0.31	0.30	0.45 ± 0.07
β (hr^{-1})	0.27	0.50	0.30	0.50	0.40	0.35	0.39 ± 0.04
α $t_{1/2}$ (hr)	0.08	0.14	0.09	0.12	0.11	0.13	0.11 ± 0.01
β $t_{1/2}$ (hr)	2.57	1.39	2.28	1.39	1.72	1.98	1.89 ± 0.20
MRT (hr)	2.44	1.13	1.99	1.20	1.20	1.51	1.58 ± 0.22
V_d (L/kg)	0.77	0.96	0.78	1.24	1.08	1.34	1.03 ± 0.10
V_{ss} (L/kg)	0.51	0.54	0.47	0.74	0.52	0.71	0.58 ± 0.05
CL							
mL/min/kg	3.48	7.98	3.93	10.32	7.25	7.80	6.79 ± 1.07
mL/min	215	523	250	590	497	469	424 ± 62.9
AUC (μg · hr/mL)	3.96	1.55	3.32	1.42	1.69	1.73	2.28 ± 0.441
Urinary excretion (%)							
Total ^{14}C (0–168 hr)	34.6	32.9	36.4	32.3	33.0	33.8	33.8 ± 0.8
Actisomide (0–72 hr)	27.7	24.5	29.6	25.3	24.2	—[a]	26.3 ± 1.0
Fecal excretion							
Total ^{14}C (0–168 hr)	62.9	71.4	54.3	62.6	31.3	10.1	56.5 ± 6.9
Actisomide (0–72 hr)	24.6	36.4	32.0	34.0	20.1	—[a]	29.4 ± 3.1

[a] Value not determined.

Table IV. Pharmacokinetic Data Following Oral Administration of Actisomide to Humans

Dose (mg)	Pharmacokinetic parameter	Subject ID SUB7	SUB8	SUB9	Mean ± SE
400	Body wt (kg)	74.8	74.6	76.6	75.3 ± 0.6
	C_{max1} (μg/mL)	0.88	0.12	0.47	0.49 ± 0.22
	T_{max1} (hr)	0.25	1.0	0.25	0.50 ± 0.25
	C_{max2} (μg/mL)	1.58	1.04	0.31	0.98 ± 0.37
	T_{max2} (hr)	4.0	4.0	4.0	4.0 ± 0
	AUC (μg · hr/mL)	8.37	3.81	1.46	4.55 ± 2.03
		SUB10	SUB11	SUB12	
500	Body wt (kg)	83.2	66.8	59.6	69.9 ± 7.0
	C_{max1} (μg/mL)	0.35	0.17	NA[a]	0.26 NA
	T_{max1} (hr)	0.5	0.5	NA	0.50 NA
	C_{max2} (μg/mL)	1.34	2.36	1.53	1.74 ± 0.31
	T_{max2} (hr)	4.0	4.0	6.0	4.67 ± 0.67
	AUC (μg · hr/mL)	7.59	10.24	9.30	9.04 ± 0.78

[a] Value not available.

parent drug in urine were greater at the high dose than at the low dose.

In the dog, the fecal-to-urinary radioactivity ratio following an i.v. dose of [^{14}C]actisomide was approximately 1 at both doses of 8 and 16 mg/kg. The percentages of the dose excreted at the parent drug in urine were approximately the same as these doses (Table I).

In humans, excretion of radioactivity was greater in feces than in urine following an i.v. dose, where the fecal to urinary ratio was about 1.7 (Table III). The majority of urinary radioactivity was the parent drug. The percentage of the dose recovered as the parent drug in feces was similar to that in urine, although the percentage of total radioactivity was higher in feces than in urine.

In Situ Absorption and *in Vitro* CaCo-2 Cell Uptake

When absorption of [^{14}C]actisomide in Ringer's solution (pH 7.4) was examined using the rat *in situ* model, absorption from the upper jejunum was negligible. Absorption in the upper ileum and colon was 0.24 ± 0.05 and 0.85 ± 0.03 mg, respectively, during a 30-min perfusion period. Concentrations of actisomide in the jejunum perfusate were not changed substantially over a 3-hr period with and without bile ligation. Thus, lack of disappearance of the drug from jejunum perfusate was not due to its extensive biliary excretion, but due to poor absorption.

When *in vitro* uptake of [^{14}C]actisomide was examined using the CaCo-2 cells, the cell uptake of the drug was pH dependent, with the percentages of drug accumulated in the cells being higher at the higher pH regardless of the drug concentrations (Table VI). At a given pH, the cell uptake of the drug was approximately the same regardless of the concentration, although at pH 6.5 uptake of the drug decreased as the drug concentration increased.

DISCUSSION

The present study demonstrates that the systemic availability of actisomide after oral administration is species dependent, although the pharmacokinetics following i.v. doses are similar. Some possible reasons for species-dependent systemic availability of actisomide include (i) differences in first-pass metabolism, (ii) differences in biliary excretion, and (iii) differences in apparent absorption.

Actisomide metabolism was less extensive in the dog than in the monkey but similar to that in the rat and human following i.v. administration. When a drug is not extensively metabolized, the metabolism rate is limited by the metabolic capacity, and not by blood flow, after either i.v. or oral administration. Therefore, the i.v. data can be used to predict oral first-pass liver metabolism. Thus, the greater systemic availability of actisomide in the dog does not appear to be due to lower first-pass metabolism.

After i.v. administration of [^{14}C]actisomide, fecal excretion of actisomide in the dog was not substantially different

Table V. Mean (±SE) Percentages of the Radioactive Dose Excreted in 0- to 72-hr Urine, Feces, and Bile After i.v. and Oral Administration of [^{14}C]Actisomide to the Rat

Dose route	Dose (mg/kg)	Urine	Bile	Feces
i.v.	8	48.6 ± 6.5	35.1 ± 4.3	9.9 ± 4.3
Oral	8	6.5 ± 1.6	10.2 ± 2.9	70.7 ± 5.0
Oral	64	9.0 ± 2.2	16.5 ± 0.3	65.9 ± 4.3
Oral	160	13.1 ± 4.2	13.5 ± 4.7	63.8 ± 8.3

Table VI. Mean (±SE) Percentages of [^{14}C]Actisomide Accumulated in CaCo-2 Cells After Incubation at 37°C for 6 hr at Various Drug Concentrations and pH's

Concentration (μg/mL)	pH 6.5	7.0	8.0
0.5	1.50 ± 0.05	1.88 ± 0.07	2.50 ± 0.07
1.0	1.26 ± 0.03	2.06 ± 0.01	2.67 ± 0.08
5.0	0.99 ± 0.13	2.04 ± 0.06	2.52 ± 0.06

from that in the rat and human. In addition, actisomide absorption in the bile-ligated rat was not enhanced compared to the control rats. Based on these results, the species difference in the systemic availability of actisomide does not appear to be due to differences in biliary excretion of the drug. Therefore, the species-dependent systemic availability of actisomide is most likely due to species difference in apparent absorption of the drug.

Species difference in acid secretion into the GI tract has been reported. In the dog (6), the pH ranges in the GI tract are generally higher than those in the rat (7), monkey (8), and human (9,10). Transport of ionized diamines through cellular membranes is reported to be more difficult than that of monoamines because of their dicationic nature and, consequently, greatly diminished probability of doubling deprotonating to neutral molecules (11). These, together with the present findings that systemic availability of actisomide in the rat and its *in vitro* uptake in CaCo-2 cells were pH dependent, suggest that pH differences in the GI tract among the species may have contributed in part to the species difference in absorption of dicationic actisomide (pK_a values of 4.66 and 9.81). A monoamine drug, bidisomide (pK_a of 9.3), also exhibits species-specific systemic availability (approximately 20, 50, and 66% in the rat, monkey, and dog, respectively), although the extent of the difference is less remarkable compared with that of actisomide (12). However, other monocationic drugs, such as disopyramide (pK_a of 10.2) do not show substantial differences in systemic availability among the dog, rhesus monkey, and human. In the *in situ* rat study, actisomide was not substantially absorbed in the upper jejunum, but absorption of the drug increased in the lower GI tract such as the ileum and colon, although the pH of the perfusate was fixed (pH 7.5). Thus, in addition to pH differences in the GI tract, some other factors (e.g., species differences in membrane permeability, morphology of the intestine, binding to bile salts or other materials in the GI tract) appeared to be involved in this species difference in systemic availability and site-dependent absorption of the drug.

In humans, atypical plasma concentration–time curves with two C_{max} values were observed after oral administration. Similar phenomena have been reported for many drugs such as pafenolol (13), penicilamine (14), cimetidine (15), ranitidine (16), and veralipride (17). For the majority of these drugs, two absorption sites were postulated to explain these double-peak phenomena. For actisomide, C_{2max} values observed between 4 and 6 hr were much higher than C_{1max} values, suggesting that the majority of the drug was absorbed at the second absorption site. This is consistent with delayed absorption of actisomide in the rat and monkey, where the pH of the GI tract is lower than in the dog. However, unlike the rat and monkey, actisomide was initially rapidly absorbed in humans, with C_{1max} achieved within 1 hr. Thus, the pH effect alone could not explain the atypical absorption phenomena of actisomide as discussed above for the rat.

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