Interconversion Pharmacokinetics of Eplerenone, a Selective Aldosterone Blocker, and Its Lactone-Ring Open Form

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ABSTRACT: The interconversion pharmacokinetics of eplerenone and its lactone-ring open form, SC-70303, were examined in dogs using a stable isotope method. [$^{13}CD_3$]EP and SC-70303 were coadministered orally (10 mg/kg) and intravenously (5 mg/kg) as aqueous solutions under fasted conditions. After I.V. administration of [$^{13}CD_3$]EP, the mean AUC of [$^{13}CD_3$]EP was 16.0 h·μg/mL, while the C_{max}, T_{max}, and AUC for [$^{13}CD_3$]SC-70303 acid were 0.744 μg/mL, 0.5 h, and 3.49 h·μg/mL, respectively. After I.V. administration of SC-70303, the AUC for SC-70303 acid was 6.36 h·μg/mL, while the C_{max}, T_{max}, and AUC for EP were 2.26 μg/mL, 0.5 h, and 9.48 h·μg/mL, respectively. After oral administration of [$^{13}CD_3$]EP, the C_{max}, T_{max}, and AUC for [$^{13}CD_3$]EP were 6.01 μg/mL, 0.5 h, and 27.7 h·μg/mL, respectively, and the corresponding values for [$^{13}CD_3$]SC-70303 acid were 0.972 μg/mL, 0.75 h, and 5.52 h·μg/mL, respectively. After oral administration of SC-70303, the C_{max}, T_{max}, and AUC for EP were 1.38 μg/mL, 0.83 h, and 9.29 h·μg/mL, respectively, and the corresponding values for SC-70303 acid were 0.330 μg/mL, 0.67 h, and 2.19 h·μg/mL, respectively. The systemic availability was 90% for [$^{13}CD_3$]EP and 17.5% for SC-70303 acid. EP and SC-70303 acid were rapidly interconvertible in the dog. The percentage of dose converted to [$^{13}CD_3$]SC-70303 acid following I.V. administration of [$^{13}CD_3$]EP was 55.0%, while the percentage of dose converted to EP following I.V. administration of SC-70303 was 60.2%. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:1383–1389, 2002

Keywords: eplerenone; dog; interconversion pharmacokinetics; lactone ring

INTRODUCTION

Clinical and preclinical studies have linked elevated levels of the mineralocorticoid hormone aldosterone to a variety of negative outcomes, including hypertension, cardiac hypertrophy, cardiac and vascular fibrosis, renal injury, magnesium loss, baroreceptor sensitivity, ventricular arrhythmias, and increased mortality in patients with heart failure.[1–3] Consequently, blockade of aldosterone is beneficial in the treatment of cardiovascular and renovascular diseases. Because receptor-mediated activation of aldosterone production is triggered by angiotensin II, inhibitors of angiotensin II production (ACE inhibitors) or angiotensin II activity (angiotensin II receptor blockers) have, until recently, been assumed to also inhibit aldosterone synthesis. However, several studies have shown that aldosterone levels rise to baseline or even higher levels after chronic treatment with ACE inhibitors or angiotensin II receptor blockers, a phenomenon known as aldosterone escape.[1,4,5] Aldosterone escape likely results from the activation of aldosterone synthesis by other mechanisms independent of angiotensin II receptor binding.

The existence of aldosterone escape, coupled with the fact that aldosterone and Angiotensin II have independent roles in the development of cardiovascular and end-organ disease, suggest that both hormones need antagonism to ensure

optimum therapeutic intervention. Strong evidence supporting this hypothesis was recently provided by the Randomized Aldactone Evaluation Study (RALES).[1] In RALES, patients with severe heart failure who were already being treated with ACE inhibitors were randomly assigned to receive either placebo or spironolactone, a nonselective aldosterone inhibitor. The group receiving spironolactone displayed a 30% decrease in mortality compared to the placebo group. However, because spironolactone also blocks androgen and progesterone receptors, gynocomastia was observed in 10% of men in the spironolactone arm.

To avoid these side effects, a new selective aldosterone blocker known as EP is currently under development. EP has been shown to effectively block aldosterone at receptor sites in tissues, but because of the presence of a stable 9,11-epoxide group (Figure 1), EP has dramatically reduced progestational and antiandrogenic side effects compared with spironolactone.[6,7] As a result, EP is expected to provide important clinical benefits not previously available with spironolactone.

EP is a steroid nucleus-based antimineralocorticoid that is chemically and enzymatically interconvertible with an open lactone-ring form. SC-70303 is the potassium salt of this pharmacologically inactive open form. Under basic conditions, EP is converted to SC-70303 acid, whereas under acidic conditions, SC-70303 acid is converted to EP. The primary objective of this study was to determine the interconversion pharmacokinetics of EP and SC-70303 acid after I.V. and oral administration in the dog.

MATERIALS AND METHODS

Test Compounds

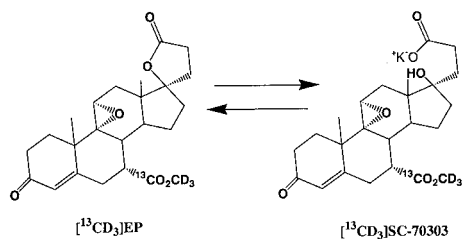[$^{13}CD_3$]EP (Lot No. GDS8181-49A) and SC-70303 (Lot No. YW3089) were supplied from Pharmacia

Figure 1. Chemical structures of [$^{13}CD_3$]EP and [$^{13}CD_3$]SC-70303.

Corporation (Skokie, IL). The compounds were stored at room temperature on desiccant and protected from light. The purity of the test compounds was greater than 97%.

Animals

The study adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23. Revised 1985). During the study, the animals were housed at controlled room temperature (18 to 29°C) in individual stainless steel cages modified for the separation and collection of urine and feces. Dogs were fasted prior to dosing and fed *ad libitum* 4 h after dose administration. All dogs were provided free access to water.

Study Design

Three female beagle dogs (8.3 to 9.1 kg) were coadministered either an oral dose of 10 mg/kg [$^{13}CD_3$]EP and SC-70303 or an intravenous (I.V.) dose of 5 mg/kg [$^{13}CD_3$]EP and SC-70303 as an aqueous solution under fasted conditions.

Blood samples were collected in heparinized Vacutainer at specified time points from the jugular vein after I.V. or oral administration. Plasma samples were analyzed for [$^{13}CD_3$]EP, [$^{13}CD_3$]SC-70303, EP, and SC-70303 using a liquid chromatographic/tandem mass spectrometric (LC-MS/MS) procedure described below.

Sample Analysis

The concentrations of [$^{13}CD_3$]EP, [$^{13}CD_3$]SC-70303, EP, and SC-70303 in unacidified plasma were analyzed at CEDRA Corp. (Austin, TX) using a validated LC-MS/MS assay as follows: internal standard containing SC-70440 was added to 500 µL of heparinized dog plasma containing SC-66110, [$^{13}CD_3$]EP, SC-70303, and [$^{13}CD_3$]SC-70303. The plasma sample was extracted with a C-18 Bond Elut solid-phase extraction cartridge (SPEC), which was previously conditioned with acetonitrile and water. After the cartridge was washed with water, the sample was eluted from the SPEC with 500 µL of acetonitrile and evaporated to dryness. After reconstituting with 125 µL of mobile phase (ammonium acetate in acetonitrile/water), the sample was injected onto a short C-18 HPLC column. Peak areas of the m/z 415.3*163.1 product ion of SC-66110, the m/z 419.3*163.1 product ion of [$^{13}CD_3$]SC-66110, the m/z 431.3*337.4 product ion of SC-70303, and the m/z 435.3*337.4 product ion of [$^{13}CD_3$]SC-70303

were measured against the m/z 399.3*322.3 product ion of the internal standard using multiple reaction monitoring mode. The assay sensitivity of the dog plasma assay using the standard curve was 0.0100 μg EP/mL of plasma, with a precision (expressed as coefficient of variation) of 4.16% and an accuracy (expressed as analytical recovery) of 112% for the between-run calculations. For the within-run calculation, the precision was 4.28% and the accuracy was 104%. For SC-70303, the sensitivity limit was also 0.0100 μg/mL with a precision of 23.0% and an accuracy of 94.8% for the between-run calculations. For the within-run calculations, the precision was 6.69% and the accuracy was 113%. Acceptable precision and accuracy were obtained for concentrations over the balance of the standard curve range of 0.025 to 2.50 μg/mL for both EP and SC-70303 acid. Stability of EP and SC-70303 acid in dog plasma was confirmed after storage of the samples for 9 months. Plasma concentrations of SC-70303 were reported as the free acid concentrations.

Calculations and Pharmacokinetic Analysis

The observed peak plasma concentrations (C_{max}) of [$^{13}CD_3$]EP, [$^{13}CD_3$]SC-70303 acid, EP, and SC-70303 acid, and time to reach peak plasma concentrations (T_{max}) were obtained for each dog. The areas under the plasma concentration–time curve (AUC) were calculated using the linear trapezoidal rule.

Plasma concentration–time curves of [$^{13}CD_3$]EP, [$^{13}CD_3$]SC-70303 acid, EP, and SC-70303 acid after coadministration of I.V. and oral doses of [$^{13}CD_3$]EP and SC-70303 were simultaneously fit according to the pharmacokinetic model (Figure 2) using the SAAM II (SAAM Inst.,

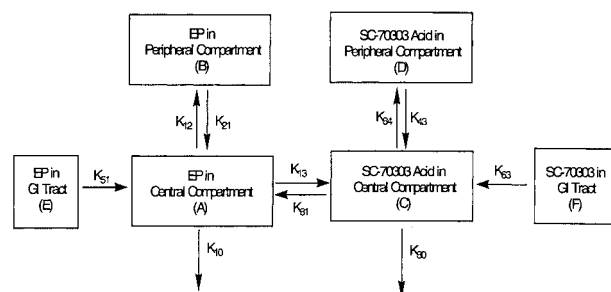Seattle, WA) computer program.[8] The differential equations for the proposed pharmacokinetic model for the I.V. dose are given in eqs 1 through 4, and the differential equations for the oral doses are given in eqs 5–10:

$$dA/dt = -(k_{10} + k_{12} + k_{13})A + k_{21}B + k_{31}C \quad (1)$$

$$dB/dt = -k_{21}B + k_{12}A \quad (2)$$

$$dC/dt = -(k_{30} + k_{31} + k_{34})C + k_{13}A + k_{43}D \quad (3)$$

$$dD/dt = -k_{43}D + k_{34}C \quad (4)$$

$$dA/dt = -(k_{10} + k_{12} + k_{13})A \\ + k_{21}B + k_{31}C + k_{51}E \quad (5)$$

$$dB/dt = -k_{21}B + k_{12}A \quad (6)$$

$$dC/dt = -(k_{30} + k_{31} + k_{34})C \\ + k_{13}A + k_{43}D + k_{63}F \quad (7)$$

$$dD/dt = -k_{43}D + k_{34}C \quad (8)$$

$$dE/dt = -k_{51}E \quad (9)$$

$$dF/dt = -k_{63}F \quad (10)$$

Plasma concentrations of [$^{13}CD_3$]EP (C_{ep}) and [$^{13}CD_3$]SC-70303 acid (C_{sc}) after I.V. administration of [$^{13}CD_3$]EP can be described by the tetra-exponential equations shown in Appendix. The values for α_1, α_2, α_3 and α_4 were calculated using eqs. 17–20 in the Appendix.

The percentage of the dose converted from EP to SC-70303 acid, or from SC-70303 acid to EP, after I.V. administration was calculated using the following equation after corrections were made for the difference in doses.

$$\frac{\begin{array}{c}\text{AUC of SC-70303 acid or EP formed} \\ \text{after IV does of EP or SC-70303}\end{array}}{\begin{array}{c}\text{AUC of SC-703303 acid or EP formed} \\ \text{after IV does of SC-70303 or EP}\end{array}} \times 100$$

(11)

The percentage of the dose systemically available after oral administration was calculated by dividing the AUC values of EP or SC-70303 acid for the oral doses by the respective I.V. data after corrections were made for the difference in doses.

RESULTS

The plasma concentrations of [$^{13}CD_3$]EP, [$^{13}CD_3$]SC-70303 acid, EP, and SC-70303 acid

Figure 2. Pharmacokinetic model for [$^{13}CD_3$]EP, EP, [$^{13}CD_3$]SC-70303 acid, and SC-70303 after coadministration of [$^{13}CD_3$]EP and SC-70303 intravenously and orally.

after I.V. or oral doses of [^{13}CD$_3$]EP and SC-70303 are shown in Figures 3 and 4, respectively. Model-independent pharmacokinetic parameters derived from these data are given in Table 1.

After I.V. administration of [^{13}CD$_3$]EP, the C_{max} of [^{13}CD$_3$]SC-70303 acid was achieved within 0.5 h, indicating [^{13}CD$_3$]EP was rapidly converted to [^{13}CD$_3$]SC-70303 acid. Plasma concentrations of [^{13}CD$_3$]SC-70303 acid were lower than those of [^{13}CD$_3$]EP, and the mean AUC value for [^{13}CD$_3$]SC-70303 acid was approximately 24% of the [^{13}CD$_3$]EP AUC value. After I.V. administration of SC-70303, plasma concentrations of SC-70303 acid was initially more rapidly declined than EP. The C_{max} of EP after I.V. administration of SC-70303 was also achieved within 0.5 h. Thus, SC-70303 acid also was rapidly converted to EP. The mean AUC value for SC-70303 acid was approximately 73% of the EP AUC value.

After oral administration of [^{13}CD$_3$]EP, [^{13}CD$_3$]EP was rapidly absorbed and C_{max} of [^{13}CD$_3$]EP and [^{13}CD$_3$]SC-70303 acid were achieved within 0.5 and 0.75 h, respectively. The plasma concentrations of [^{13}CD$_3$]EP were much
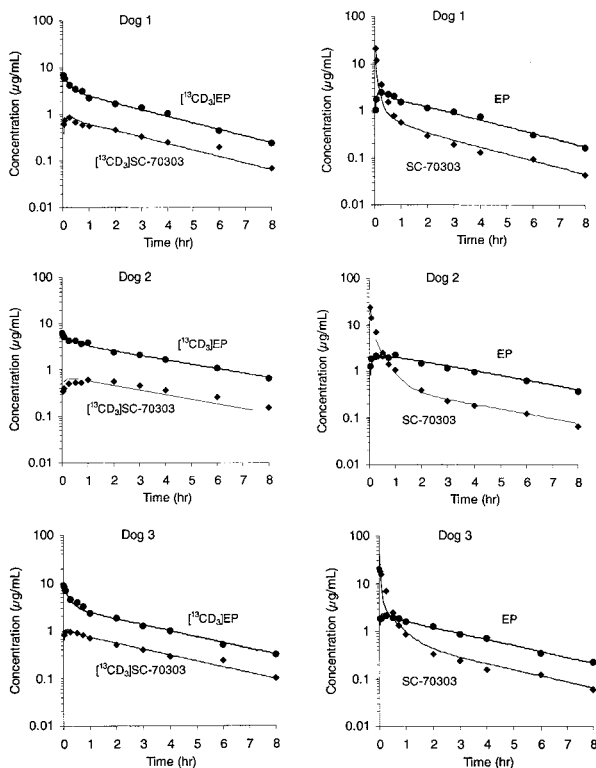

Figure 3. Plasma concentrations and model predicted curves of [^{13}CD$_3$]EP, [^{13}CD$_3$]SC-70303 acid, EP, and SC-70303 acid after coadministration of [^{13}CD$_3$]EP and SC-70303 intravenously.
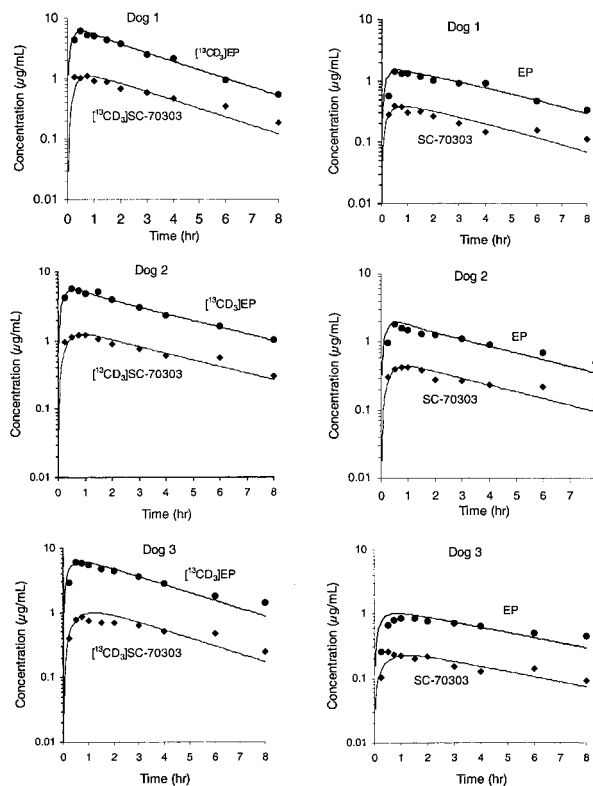

Figure 4. Plasma concentrations and model predicted curves of [^{13}CD$_3$]EP, [^{13}CD$_3$]SC-70303 acid, EP, and SC-70303 acid after coadministration of [^{13}CD$_3$]EP and SC-70303 orally.

higher than those of [^{13}CD$_3$]SC-70303 acid. The mean AUC values for [^{13}CD$_3$]SC-70303 acid after oral administration was approximately 22% of that of [^{13}CD$_3$]EP. The systemic availability of [^{13}CD$_3$]EP was $90.0 \pm 0.15\%$ after oral administration of [^{13}CD$_3$]EP. After oral administration of SC-70303, plasma concentrations of SC-70303 acid also were much lower than those of EP. The mean AUC value for SC-70303 acid was approximately 26% of that of EP. The systemic availability of SC-70303 acid was $17.5 \pm 0.03\%$ after oral administration of SC-70303.

An attempt was made to simultaneously analyze plasma concentration−time curves for [^{13}CD$_3$]EP, EP, [^{13}CD$_3$]SC-70303 acid, and SC-70303 acid after I.V. and oral administration of [^{13}CD$_3$]EP and SC-70303 according to the pharmacokinetic model depicted in Figure 2. However, plasma concentration−time curves of EP and SC-70303 acid after oral administration of SC-70303 did not fit reasonably to the model (data not shown). Therefore, all plasma concentration−time curves for [^{13}CD$_3$]EP, EP, [^{13}CD$_3$]SC-70303 acid, and SC-70303 acid after I.V. administration

Table 1. Model Independent Pharmacokinetic Parameters After I.V. and Oral Coadministration of $[^{13}CD_3]EP$ and SC-70303

Dose Route	Compound Administered	Analytes	Pharmacokinetic Parameters		
			C_{max} (μg/mL)	T_{max} (h)	AUC (h$\cdot\mu$g/mL)
I.V.	$[^{13}CD_3]EP$	$[^{13}CD_3]EP$	NA[a]	NA[a]	16.0 ± 2.6
		$[^{13}CD_3]$SC-70303 acid	0.74 ± 0.09	0.50 ± 0.25	3.49 ± 0.31
	SC-70303	EP	2.26 ± 0.11	0.50 ± 0.25	9.48 ± 1.12
		SC-70303 acid	NA[a]	NA[a]	6.36 ± 0.64
Oral	$[^{13}CD_3]EP$	$[^{13}CD_3]EP$	6.01 ± 0.13	0.50 ± 0	27.7 ± 3.0
		$[^{13}CD_3]$SC-70303 acid	0.97 ± 0.10	0.75 ± 0	5.52 ± 0.62
	SC-70303	EP	1.38 ± 0.29	0.83 ± 0.33	9.29 ± 0.82
		SC-70303 acid	0.33 ± 0.05	0.67 ± 0.17	2.19 ± 0.33

[a]Not applicable.
AUC = area under the plasma concentration–time curve; C_{max} = maximum concentration; EP = Eplerenone; I.V. = intravenous; T_{max} = time to maximum concentration.

of $[^{13}CD_3]EP$ and SC-70303, and after oral administration of $[^{13}CD_3]EP$ were simultaneously fitted to the model. The rate constants obtained from these fits were subsequently fixed, and the absorption rate constant (k_{63}) of SC-70303 was estimated assuming that SC-70303 is partially converted to EP in the stomach and that either SC-70303 plus converted EP or only converted EP were absorbed. Because the goodness of fit appeared to be approximately the same for the prior assumptions, all plasma concentration–time curves after I.V. and oral administration were finally fitted assuming that both EP and SC-70303 were absorbed after oral administration of SC-70303.

The pharmacokinetic parameters obtained from the preceding analysis are summarized in Table 2. The experimental values and fitted curves for each dog are shown in Figure 3 for the I.V. dose and Figure 4 for the oral dose. The mean rate constant (k_{31}) for the conversion of SC-70303 acid to EP was approximately 11 times greater than the rate constant (k_{13}) for the conversion of EP to SC-70303 acid. The elimination rate constant for EP (k_{10}) was approximately 11 times lower than that of SC-70303 acid (k_{30}). The percentage of the dose converted to $[^{13}CD_3]$ SC-70303 acid following I.V. administration of the $[^{13}CD_3]EP$ was $55.0 \pm 0.01\%$. The percentage of the dose converted to EP following I.V. administration of SC-70303 was $60.2 \pm 0.03\%$. Because EP and SC-70303 are in equilibrium, the rate constants α_1, α_2, α_3 and α_4 are the same for these compounds (eqs. 12 and 13) and the half-life for terminal phase (α_4 phase) were 2.58 ± 0.31 h.

DISCUSSION

The present study demonstrates that EP and SC-70303 acid were rapidly interconvertible in the dog. After I.V. administration of $[^{13}CD_3]EP$, 55% of the dose was rapidly converted to its lactone-ring open form, SC-70303 acid, reaching a C_{max} within 0.5 h. Conversely, after I.V. administration of SC-70303, approximately 60% of the dose was converted to EP. Pharmacokinetic analysis of EP and SC-70303 according to the proposed model showed that the conversion rate

Table 2. Pharmacokinetic (PK) Parameters for EP and SC-70303 Acid After I.V. Administration

Parameters(h^{-1})	Dog 1	Dog 2	Dog 3	Mean	SE
α_1	11.2	27.5	21.5	20.1	4.8
α_2	3.91	2.56	4.87	3.78	0.67
α_3	1.50	2.11	1.93	1.85	0.18
α_4	0.33	0.22	0.28	0.28	0.03
k_{10}	0.30	0.18	0.46	0.31	0.08
k_{12}	1.48	0.55	2.32	1.45	0.51
k_{13}	0.98	0.30	0.70	0.66	0.20
k_{21}	2.12	1.72	1.92	1.92	0.12
k_{30}	2.96	7.30	4.33	4.87	1.28
k_{31}	5.08	9.20	6.75	7.01	1.20
k_{34}	2.14	9.40	8.40	6.65	2.27
k_{43}	1.91	3.72	3.70	3.11	0.60
k_{51}	2.75	3.70	1.66	2.70	0.59
k_{63}	0.35	0.00	0.18	0.18	0.10
V_1	0.67	0.85	0.55	0.69	0.09
V_2	0.37	0.10	0.13	0.20	0.09

α, k = rate constant; SE = standard error of the mean; I.V. = intravenous; V_1 = volume of distributions for EP; V_2 = volume of distributions for SC-70303 acid.

constant of SC-70303 acid to EP (k_{31}) was approximately 11 times greater than the conversion rate constant of EP to SC-70303 acid (k_{13}). However, the elimination rate constant of SC-70303 acid (k_{30}) was 11 times greater than that of EP (k_{10}), which counteracted the conversion of SC-70303 acid to EP. As a result, the total amount of EP converted to SC-70303 acid were similar to the amount of SC-70303 converted to EP. Although EP can be converted to its open form under basic conditions, conversion after I.V. or oral administration in this study likely occurred by enzymatic mechanisms in the liver. This conclusion is based on the findings that EP was stable in dog plasma for more than 2 h and not converted to SC-70303 acid. Following oral dose administration, the lactone ring in statins (mevastatin, lovastatin, and simvastatin) and spironolactone are also opened in the liver and plasma by enzymatic hydrolysis with esterases to form the hydroxy acid.[9–11] For statins, the open forms are active, whereas for spironolactone, as well as canrenone and EP, only closed forms are active. Although the open forms of the latter three compounds are pharmacologically inactive, they are different from other inactive metabolites in that they can be readily converted to the pharmacologically active forms and, in fact, the open and closed forms exist in equilibrium. The conversion of SC-70303 acid to EP also appeared to be mediated enzymatically. This conclusion is based following two results. First, blood pH is 7.4, and therefore, the open form is not expected to be rapidly converted to the closed form at this pH. Second, SC-70303 was stable in dog plasma and not converted to EP.

EP was rapidly and well absorbed after oral administration to the dog, despite the fact that it is highly lipophilic, with an aqueous solubility of less than 0.5 mg/mL. A site absorption study in the dog demonstrated that the systemic availability of EP following intraduodenal, intrajejunal, and rectal administration were similar to that following oral administration. Although absorption of EP was most rapid in the jejunum and slowest in the colon, the systemic availability after colonic administration was approximately 75% that of jejunal administration.[12] Clinical study results show that EP is also well absorbed in humans (absorption greater that 70%), and increases in AUC values are approximately proportional to dose over the clinically relevant dose range (50 to 300 mg).[12] Thus, the dog is a good animal model for a study of absorption and pharmacokinetics of EP.

In contrast to EP, SC-70303 was poorly absorbed when administered orally. Interestingly, the AUC ratios of SC-70303 acid to EP after oral administration of SC-70303 were similar to those after I.V. and oral administration of EP rather than to the ratios after I.V. administration of SC-70303. Furthermore, plasma concentration data obtained after oral administration of SC-70303 could not be fitted simultaneously with the data obtained after I.V. and oral administration of EP, and after I.V. administration of SC-70303 unless partial conversion of SC-70303 to EP and absorption of resulting EP were assumed. These results suggest that some SC-70303 may have been converted to its closed form EP in the gastro-intestinal tract, and that the converted EP was absorbed at least in part. Because the stomach pH is acidic (pH 1 to 3), it is conceivable that SC-70303 was cyclized in the stomach and upper small intestine. However, the following alternative possibilities cannot be excluded: first, that SC-70303 could be first-pass metabolized to EP in the liver after oral administration, producing more EP compared with the I.V. dose of SC-70303; and second, that the conversion rate could be concentration dependent and, at low concentrations of SC-70303 acid, the proportion of EP produced could be greater than that at the high concentrations of SC-70303 acid, resulting in a higher proportion of EP after oral administration of SC-70303 than after I.V. administration.

In conclusion, after I.V. administration of either EP or SC-70303, more than half the dose was interconvertible between the lactone-ring closed and open forms. Because EP is highly lipophilic, with a water solubility of less than 0.5 mg/mL, whereas, SC-70303 is highly water soluble, SC-70303 could be used as a prodrug for I.V. uses. Although the rate constants for conversion of SC-70303 acid to EP were greater than the conversion rate constants of EP to SC-70303 acid, the rapid elimination rate constant of SC-70303 acid counteracted the conversion of SC-70303 acid to EP and the total amounts of SC-70303 acid converted to EP were approximately the same as the amounts of EP converted to SC-70303 acid.

ACKNOWLEDGMENTS

APPENDIX

Plasma concentrations of EP (C_{ep}) and SC-70303 acid (C_{sc}) after I.V. administration of EP

$$C_{ep} = \frac{X_0(\beta_1 - \alpha_1)(\beta_2 - \alpha_1)(\beta_1 - \alpha_1)}{V_1(\alpha_2 - \alpha_1)(\alpha_3 - \alpha_1)(\alpha_4 - \alpha_1)}e^{-\alpha_1 t}$$
$$+ \frac{X_0(\beta_1 - \alpha_2)(\beta_2 - \alpha_2)(\beta_3 - \alpha_2)}{V_1(\alpha_1 - \alpha_2)(\alpha_3 - \alpha_2)(\alpha_4 - \alpha_2)}e^{-\alpha_2 t}$$
$$+ \frac{X_0(\beta_1 - \alpha_3)(\beta_2 - \alpha_3)(\beta_3 - \alpha_3)}{V_1(\alpha_1 - \alpha_3)(\alpha_2 - \alpha_3)(\alpha_4 - \alpha_3)}e^{-\alpha_3 t}$$
$$+ \frac{X_0(\beta_1 - \alpha_4)(\beta_2 - \alpha_4)(\beta_3 - \alpha_4)}{V_1(\alpha_1 - \alpha_4)(\alpha_2 - \alpha_4)(\alpha_3 - \alpha_4)}e^{-\alpha_4 t} \quad (12)$$

$$C_{sc} = \frac{X_0(k_{21} - \alpha_1)(k_{43} - \alpha_1)}{V_2(\alpha_2 - \alpha_1)(\alpha_3 - \alpha_1)(\alpha_4 - \alpha_1)}e^{-\alpha_1 t}$$
$$+ \frac{X_0(k_{21} - \alpha_2)(k_{43} - \alpha_2)}{V_2(\alpha_1 - \alpha_2)(\alpha_3 - \alpha_2)(\alpha_4 - \alpha_2)}e^{-\alpha_2 t}$$
$$+ \frac{X_0(k_{21} - \alpha_3)(k_{43} - \alpha_3)}{V_2(\alpha_1 - \alpha_3)(\alpha_2 - \alpha_3)(\alpha_4 - \alpha_3)}e^{-\alpha_3 t}$$
$$+ \frac{X_0(k_{21} - \alpha_4)(k_{43} - \alpha_4)}{V_2(\alpha_1 - \alpha_4)(\alpha_2 - \alpha_4)(\alpha_3 - \alpha_4)}e^{-\alpha_4 t} \quad (13)$$

where X_0 is the I.V. dose, V_1 and V_2 are volume of distribution of EP and SC-70303 acid, respectively.

$$\beta_1 + \beta_2 + \beta_3 = k_{21} + k_{30} + k_{31} + k_{34} + k_{43} + k_{21} \quad (14)$$

$$\beta_1\beta_2 + \beta_1\beta_3 + \beta_2\beta_3 = k_{21}k_{30} + k_{21}k_{31}$$
$$+ k_{21}k_{34} + k_{21}k_{43}$$
$$+ k_{21}k_{43} + k_{30}k_{43}k_{31}k_{43} \quad (15)$$

$$\beta_1\beta_2\beta_3 = k_{21}k_{30}k_{43} + k_{21}k_{31}k_{43} \quad (16)$$

$$\alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 = k_{10} + k_{12}$$
$$+ k_{13} + k_{21} + k_{30}$$
$$+ k_{31} + k_{34} + k_{43} \quad (17)$$

$$\alpha_1\alpha_2 + \alpha_1\alpha_3 + \alpha_1\alpha_4 + \alpha_2\alpha_3 + \alpha_2\alpha_4 + \alpha_3\alpha_4$$
$$= k_{10}k_{30} + k_{10}k_{31} + k_{10}k_{34} + k_{10}k_{43} + k_{10}k_{21}$$
$$+ k_{12}k_{30} + k_{12}k_{31} + k_{12}k_{34} + k_{12}k_{43} + k_{13}k_{21}$$
$$+ k_{13}k_{30} + k_{13}k_{34} + k_{13}k_{43} + k_{21}k_{30} + k_{21}k_{31}$$
$$+ k_{21}k_{34} + k_{21}k_{43} + k_{30}k_{43} + k_{31}k_{43} \quad (18)$$

$$\alpha_1\alpha_2\alpha_3 + \alpha_1\alpha_2\alpha_4 + \alpha_1\alpha_3\alpha_4 + \alpha_2\alpha_3\alpha_4$$
$$= k_{10}k_{21}k_{30} + k_{10}k_{21}k_{31} + k_{10}k_{21}k_{43}$$
$$+ k_{10}k_{30}k_{43} + k_{10}k_{31}k_{43} + k_{12}k_{30}k_{43} + k_{12}k_{31}k_{43}$$
$$+ k_{13}k_{21}k_{30} + k_{13}k_{21}k_{34} + k_{13}k_{21}k_{43}$$
$$+ k_{13}k_{30}k_{43} + k_{21}k_{30}k_{43} + k_{21}k_{31}k_{43} \quad (19)$$

$$\alpha_1\alpha_2\alpha_3\alpha_4 = k_{10}k_{21}k_{30}k_{43}$$
$$+ k_{10}k_{21}k_{31}k_{43}$$
$$+ k_{13}k_{21}k_{30}k_{43} \quad (20)$$

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