The Pharmacokinetics of Topotecan and Its Carboxylate Form Following Separate Intravenous Administration to the Dog

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Purpose. To determine the relationship between topotecan and its ring opened hydrolysis product (SK&F 105992) following intravenous administration of the two agents separately, and to determine the bioavailability of topotecan in female beagle dogs.

Methods. The pharmacokinetics of topotecan and SK&F 105992 were determined following separate administration as 30 minute intravenous infusions in a cross-over design. Topotecan was also administered orally to the same dogs.

Results. When administered intravenously to dogs, SK&F 105992 underwent interconversion to topotecan. Plasma concentrations of both topotecan and SK&F 105992 appeared to decline multi-exponentially following IV infusion of either compound. A 2-compartment model was found to adequately characterize the data.

Conclusions. The clearance of topotecan by other routes proceeded at a faster rate than its interconversion to SK&F 105992, whereas the clearance of SK&F 105992 by other routes was slower than the rate of its interconversion to topotecan. Any SK&F 105992 formed in the GI tract did not appear to be well absorbed following oral administration of topotecan to dogs. The steady-state volume of distribution for topotecan was approximately 8- to 9-fold greater than that for SK&F 105992 in the dog. After intravenous administration of topotecan, the amount of topotecan in the dog was much greater than that of the carboxylate, even though their respective plasma concentrations were similar. The bioavailability of topotecan, calculated from oral topotecan data or from SK&F 105992 data, was approximately 50%.

KEY WORDS: topotecan; pharmacokinetics; topoisomerase I inhibitor; reversible metabolism.

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ABBREVIATIONS: CL_{10}, elimination clearance of topotecan; CL_{12}, interconversion clearance—topotecan to SK&F 105992; CL_{Dp}, distribution clearance of topotecan; CL_{20}, elimination clearance of SK&F 105992; CL_{21}, interconversion clearance—SK&F 105992 to topotecan; CL_{Dm}, distribution clearance of SK&F 105992; V_{ss}, volume of distribution at steady-state; V_c, volume of the central space; F_{p1}, bioavailability of topotecan, calculated to include any contribution of the reversible metabolite; F_{p2}, bioavailability of topotecan, calculated by the conventional method; F_{p3}, bioavailability of topotecan, calculated from SK&F 105992 data obtained following oral and IV dosing with topotecan; f^p_m, the fraction of the oral dose of topotecan which is absorbed into the circulation as intact metabolite.

INTRODUCTION

Topotecan (SK&F 104864) is a semisynthetic analogue of the alkaloid camptothecin which has been shown to be a potent inhibitor of topoisomerase I, an enzyme essential for replication of DNA (1). Topoisomerase I is present in both proliferating and non-proliferating cells, and therefore, inhibition of this enzyme provides a unique therapeutic approach to the treatment of slow growing tumor types. At the pH at which topotecan is administered by infusion (pH 3), greater than 95% of the administered drug is present as topotecan (lactone form). However, at physiological pH, approximately 80% of the drug is present as SK&F 105992, the carboxylate form of topotecan, which has been shown to be inactive *in vitro* (2).

Attempts have been made to understand the pharmacokinetic relationship between topotecan and SK&F 105992, but many issues remain unresolved (3). In addition, considerable interest has been expressed in understanding the pharmacological relevence of the carboxylate form as a possible "source" of topotecan. Hence, in order to improve our understanding of the pharmacokinetic relationship between topotecan and SK&F 105992, both compounds were administered to dogs on separate occasions at doses of 4.0 mg/m^2 topotecan and approximately 25 mg/m^2 SK&F 105992. Both topotecan and SK&F 105992 plasma concentrations were determined. SK&F 105992 was administered at a higher dose than topotecan to ensure that any topotecan formed from SK&F 105992 would be measurable in the plasma samples obtained.

Although the majority of clinical studies to date have been conducted using an intravenous formulation of topotecan, animal studies conducted in tumor-bearing mice have shown efficacy of the drug following oral administration (4). Therefore, topotecan was also administered orally in this study, and bioavailability determined by conventional methodology compared with that using equations derived for a reversible system.

MATERIALS AND METHODS

Animals

Four adult female Beagle dogs (Marshall Farms USA, North Rose, NY) were used for this study, with a recovery period of at least six days between dosing days. Dogs were selected for this study based on detailed clinical examinations, body weight, clinical chemistry and hematology results. Animals were fasted overnight prior to dosing with either topotecan or SK&F 105992. Food was provided approximately 2 hours after dosing. All experimental procedures were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication #85-23).

Preparation of Drug Solutions

Topotecan (SK&F 104864-A, SmithKline Beecham, King of Prussia, PA) and the ring-opened carboxylate (SK&F 105992, SmithKline Beecham, King of Prussia, PA) were used throughout the study. For both oral and intravenous (IV) administration, topotecan was dissolved in 0.9% saline (pH 3). For the intravenous administration of SK&F 105992, the dose solution was

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adjusted to pH 9 to ensure that the compound remained in the ring-opened form. For intravenous administration of both topotecan and SK&F 105992, solutions were prepared so that the correct dose (4.0 or 25 mg/m^2, respectively) would be achieved with a 30 minute infusion, which was administered at a rate of 1 ml/min into the cephalic vein using a constant rate infusion pump. The oral dose of topotecan (4.0 mg/m^2) was administered (in solution) in size 12 gelatin capsules (Torpac, Ltd.) using a dose volume of 0.5 ml/kg.

Experimental Procedure

Each dog received topotecan by both oral and intravenous routes of administration. Each dog also received an IV dose of SK&F 105992. Doses of topotecan and SK&F 105992 were administered one week apart. A series of blood samples (approximately 1.5–2.0 ml) was drawn for pharmacokinetics evaluation from a jugular vein of each dog. After oral dosing, blood samples were drawn prior to dosing and at 15 and 30 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours following dosing. Following IV dosing, blood samples were collected prior to dosing and at 15, 30, 35, 40 and 45 minutes, and at 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours following the start of the intravenous infusion of topotecan or SK&F 105992. Blood samples were collected into tubes containing heparin, and plasma immediately separated by centrifugation (10,000 g for 60 sec.). A plasma aliquot (400 ul) was immediately transferred to polypropylene tubes containing 800 ul ice cold methanol, vortex mixed (20 sec.) and centrifuged (10,000 g for 60 sec.). The methanol extract was then transferred to clean polypropylene tubes, and stored at approximately −70°C until analyzed.

Analysis of Plasma Samples

Plasma concentrations of topotecan and SK&F 105992 were determined using an automated reverse phase HPLC system and fluorescence detection, based on a method previously described by Beijnen *et al.* (5). The lower limit of quantitation (LLQ) was 0.20 ng/ml for both topotecan and SK&F 105992.

Pharmacokinetics

Pharmacokinetic analysis of intravenous and oral topotecan and SK&F 105992 plasma concentration-time data was performed non-parametrically with the computer program #PROTOCOL (SmithKline Beecham, Version 1.2) and parametrically with the program MODFIT, version 5.0 (6). All calculations were based on actual sampling times. Clearances and volumes of distribution were calculated from the area under the plasma concentration-time curve (AUC) values for topotecan and SK&F 105992 using a set of equations described by Cheng and Jusko (7,8,9). These equations are based on the general model pictured in Figure 1, which was developed to describe the pharmacokinetics of drugs which undergo reversible metabolism.

The inter-conversion and elimination clearances were calculated using the following equations. Superscripts refer to the dosed compound, subscripts to the measured compound (p = parent, m = metabolite):

$$CL_{10} = \frac{Dose^p AUC_m^m - Dose^m AUC_m^p}{AUC_p^p AUC_m^m - AUC_m^p AUC_p^m}$$

$$CL_{20} = \frac{Dose^m AUC_p^p - Dose^p AUC_p^m}{AUC_p^p AUC_m^m - AUC_m^p AUC_p^m}$$

$$CL_{12} = \frac{Dose^m AUC_m^p}{AUC_p^p AUC_m^m - AUC_m^p AUC_p^m}$$

$$CL_{21} = \frac{Dose^p AUC_p^m}{AUC_p^p AUC_m^m - AUC_m^p AUC_p^m}$$

The following equations describe the calculation of the distribution clearances for drug and metabolite following intravenous dosing:

$$CL_{D_p} = -[Dose^p C_p^{p'}(0)/C_p^p(0)^2] - CL_{10} - CL_{12}$$

$$CL_{D_m} = -[Dose^m C_m^{m'}(0)/C_m^m(0)^2] - CL_{20} - CL_{21}$$

where $C_p^p(0)$ and $C_m^m(0)$ are the respective plasma concentrations at time zero, and $C_p^{p'}(0)$ and $C_m^{m'}(0)$ are their first derivatives at time zero.

Assuming that all clearance terms remained constant, the following equation was used to estimate the bioavailability of topotecan:

$$F_{p1} = \frac{(CL_{10}AUC_p^{p,po} + CL_{20}AUC_m^{p,po})Dose^p}{(CL_{10}AUC_p^p + CL_{20}AUC_m^p)Dose^{p,po}}$$

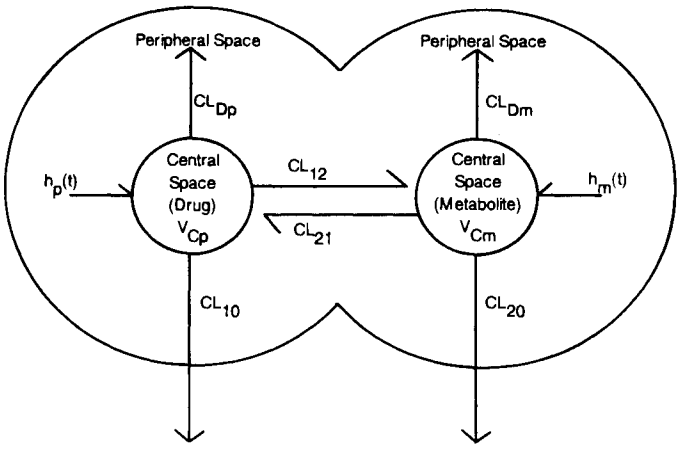

Fig. 1. General model of reversible metabolism proposed by Cheng and Jusko (7,8,9).

Bioavailability was also calculated for topotecan and SK&F 105992 using the conventional equation:

$$F_{p2} = \frac{AUC_p^{p,po}}{AUC_p^{p,iv}} \times \frac{Dose^{p,iv}}{Dose^{p,po}}$$

$$F_{p3} = \frac{AUC_m^{p,po}}{AUC_m^{p,iv}} \times \frac{Dose^{p,iv}}{Dose^{p,po}}$$

The following equation evaluated the fraction of the absorbed oral dose of topotecan entering the circulation as intact metabolite:

$$f_m^p = \frac{(AUC_m^{p,po}AUC_p^p - AUC_p^{p,po}AUC_m^p)D^m}{(AUC_p^pAUC_m^m - AUC_m^pAUC_p^m)D^{p,po}}$$

The equations below describe the central and steady-state volumes of distribution for topotecan and SK&F 105992 after separate intravenous administration of both compounds:

$$V_{C_p} = Dose^p/C_p^p(0)$$

$$V_{C_m} = Dose^m/C_m^m(0)$$

$$V_{ss}^p = \frac{Dose^p[(AUC_m^m)^2AUMC_p^p - AUC_m^pAUC_p^mAUMC_m^m]}{(AUC_p^pAUC_m^m)^2 - (AUC_m^pAUC_p^m)^2}$$

$$V_{ss}^m = \frac{Dose^m[(AUC_p^p)^2AUMC_m^m - AUC_p^mAUC_m^pAUMC_p^p]}{(AUC_p^pAUC_m^m)^2 - (AUC_m^pAUC_p^m)^2}$$

The above mentioned equations apply to intravenous bolus administration only, and therefore, the coefficients and exponents obtained by fitting an infusion model to the data using non-linear regression analysis (MODFIT) were used to calculate C(0) and AUMC(0-inf). Goodness-of-fit was assessed by comparison of the fitted line to the observed data, the size of the errors on the fitted parameters, examination of the correlation matrices and residuals, and by statistical comparison using Akaike Information Criteria.

RESULTS

Plasma concentrations of both topotecan and SK&F 105992 visually appeared to decline multi-exponentially following IV infusion of either compound (Figures 2–4). Mean (SD) maximum observed concentration (Cmax) and AUC for topotecan and SK&F 105992 following administration of either topotecan or SK&F 105992 are summarized in Table I. A 2-compartment IV infusion model was found to adequately characterize the plasma concentration-time data, as determined by visual fits of the model to the data, as well as the magnitudes of the errors associated with each of the parameters estimated. Following IV infusion of topotecan, the ratio of topotecan plasma concentration to the SK&F 105992 plasma concentration was initially greater than one, then decreased below unity at approximately 1 hour after dosing, and eventually became constant at a value of approximately 0.5 (Figure 5). Following the IV infusion of SK&F 105992, the ratio of the topotecan plasma concentration to the SK&F 105992 plasma concentration slowly increased until it also became constant at approximately 0.5.

The fastest clearance process for topotecan was elimination by the renal and biliary routes (CL_{10}), and the slowest clearance was conversion to the carboxylate (Table II). For the carboxylate, the fastest clearance was the conversion to topotecan, with

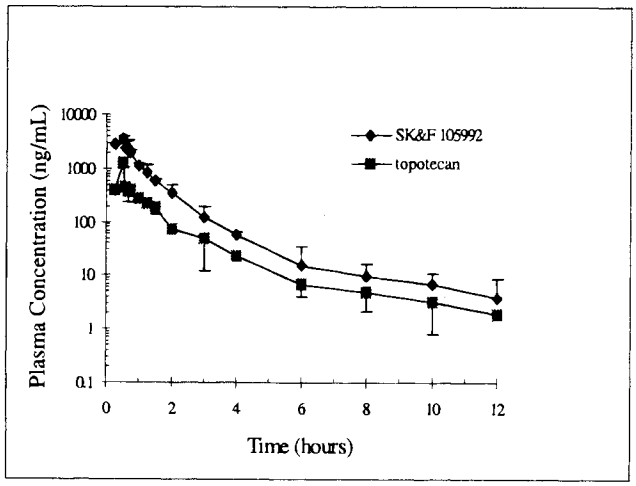

Fig. 3. Mean topotecan and SK&F 105992 plasma concentrations (ng/mL) following intravenous administration of SK&F 105992.

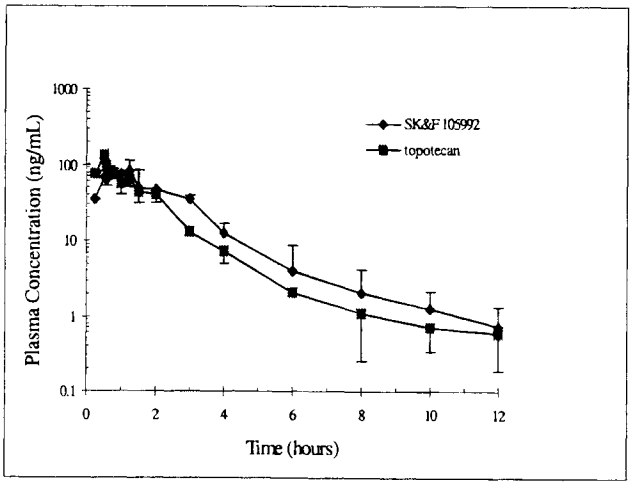

Fig. 2. Mean topotecan and SK&F 105992 plasma concentrations (ng/mL) following intravenous administration of topotecan.

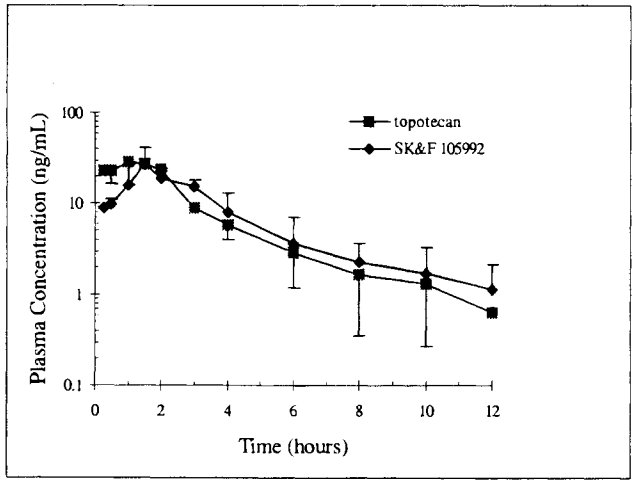

Fig. 4. Mean topotecan and SK&F 105992 plasma concentrations (ng/mL) following oral administration of topotecan.

Table I. Mean (SD) Values of Maximum Observed Plasma Concentrations and AUC Values Following Intravenous and Oral Administration of Topotecan and Intravenous Administration of SK&F 105992

Compound administered (dose)	Compound measured	Cmax (ng/ml)	AUC(0-inf) (ng.h/ml)
Topotecan (IV) (4.0 mg/m^2)	SK&F 104864	137 (33)	173 (38)
	SK&F 105992	95.7 (25.4)	192 (41)
Topotecan (Oral) (4.0 mg/m^2)	SK&F 104864	30.3 (0.2)	74.1 (28.2)
	SK&F 105992	27.9 (3.8)	86.5 (36.1)
SK&F 105992 (IV) (25 mg/m^2)	SK&F 104864	611 (72)	653 (101)
	SK&F 105992	3727 (587)	3314 (326)

little drug distributing from the central space into the peripheral space.

The bioavailability calculated using the conventional method was 47%, whereas using equations incorporating reversibility gave a value of 50%. Calculation of the bioavailability from the SK&F 105992 data gave a figure of 54%.

DISCUSSION

The volume of distribution (V_{SS}) for topotecan was larger than the V_{SS} for SKF 105992, which suggests that topotecan was more widely distributed into the peripheral space than SKF 105992 (Table II). The mean distribution clearance (CL_D) for topotecan was also relatively large, which further suggests that the lactone form was rapidly distributed into tissues. Elimination from the body (CL_{10}) occurred at a faster rate than the conversion of topotecan to SK&F 105992, as $CL_{10} > CL_{12}$.

The CL_D values calculated for SK&F 105992 were all less than zero. Since the steady-state volume of distribution (V_{SS}) and the volume of the central space (V_C) for SK&F 105992 were very similar, it would appear that there was little or no distribution of this form of the drug to the peripheral space.

Table II. Mean (±SD) Values of Pharmacokinetic Parameters of Topotecan After Oral and Intravenous Administration and SK&F 105992 After Intravenous Administration

Topotecan		SK&F 105992	
CL_{10} (ml/min)	176 ± 40.6	CL_{20} (ml/min)	40.2 ± 9.00
CL_{12} (ml/min)	118 ± 48.0	CL_{21} (ml/min)	60.2 ± 23.2
CL_{Dp} (ml/min)	190 ± 70.1	CL_{Dm} (ml/min)	[a]
F_{p1}	0.499 ± 0.028[b]	F_{p3}	0.535 ± 0.041[b]
F_{p2}	0.469 ± 0.051[b]	f_m^p	0.025 + 0.025[b]
V_{ss} (l)	17.4 ± 5.07	V_{ss} (l)	2.27 ± 0.920
V_c (l)	13.5 ± 2.93	V_c (l)	2.13 ± 0.320

[a] Mean ± SD not presented as all values calculated were less than zero.
[b] Indicates that n = 3.

The differences in the V_{SS} for topotecan and SK&F 105992 suggest that although higher AUC values were achieved for SK&F 105992 following oral and IV administration of topotecan, there was less SK&F 105992 in the body. The apparent lack of distribution of SK&F 105992 out of the central space, suggests that it's pharmacokinetics could be characterised by a one compartment model. However, the apparent tri-exponential decline in plasma concentrations can be explained, as the rate of change of SK&F 105992 concentrations will mirror those of topotecan, due to the interconversion.

The bioavailability of topotecan was found to be approximately 50% when calculated using the equation which includes contributions of the reversible hydrolysis product to the bioavailability estimate. The bioavailability of topotecan calculated using the conventional equation was 47%, which indicates that interconversion has little or no effect on bioavailability estimates for this drug. It is evident from the foregoing analysis that topotecan itself is the driving force in its clearance rather than conversion to and elimination of SK&F 105992. The two estimates of bioavailability may be similar because clearance of the interconversion product contributes little to the overall pharmacokinetic model.

When SK&F 105992 data obtained following oral and IV administration of topotecan was used to calculate bioavailability, the value obtained, approximately 50%, was nearly identical to the value obtained from the topotecan data. The data on SK&F 105992 would support the view of there being little effect on the calculation of bioavailability in the case of this reversible chemical reaction. Other authors have suggested that bioavailability and bioequivalence could be calculated by using metabolite data rather than parent drug data (10,11). The data generated in this study would appear to support that view.

The fraction of the absorbed oral dose of topotecan that enters the central circulation intact as the carboxylate form has been calculated in this study to be approximately 2.5%, which suggests that SK&F 105992 was not well absorbed. A similar finding for a related drug has been reported by Scott, *et al.* (12) who demonstrated that less than 1% of a dose of the carboxylate form of camptothecin was absorbed when administered directly into the duodenum of rats.

This general model of reversible metabolism proposed by Cheng and Jusko should be considered an approximation for topotecan, as the equations derived assume that interconversion of drug only occurs in the central space. It is likely that the interconversion of topotecan occurs in all tissues, as it is a chemical change driven by pH. Therefore, all parameters calculated based on this model should be considered estimates. If interconversion does occur in the peripheral space as well as the central space, CL_{21} and CL_{12} become the global interconversion clearances accounting for reversibility in the whole body, assuming that the rate of reversibility is the same in all tissues (8,9).

By 12 hours following the intravenous dose of either topotecan or SK&F 105992, the ratios of the plasma concentrations of the two analytes (topotecan:SK&F 105992) were equal, approximately 0.5, reflecting the equilibrium between the two compounds during the final exponential phase of the profile. It is interesting that the ratio of CL_{21} to CL_{12} is also approximately 0.5. *In vitro*, when topotecan is incubated in plasma (pH 7.4) at 37°C, interconversion occurs, and at equilibrium, the ratio of topotecan to SK&F 105992 is 0.11 (3). This difference

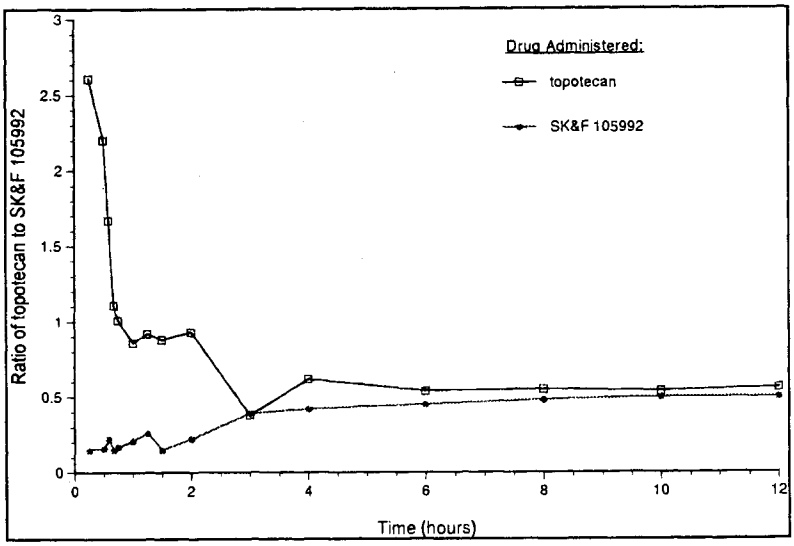

Fig. 5. Mean Ratio of Topotecan to SK&F 105992 Plasma Concentrations as a Function of Time Following Intravenous Dosing With Either Topotecan or SK&F 105992.

in the *in vitro* and *in vivo* ratios of topotecan:SK&F 105992 demonstrates that *in vitro* equilibrium data does not reflect the dynamics of the *in vivo* system.

The relative values for individual clearance and volume terms calculated in this study are in a similar ratio to those from a study in which the pharmacokinetics of camptothecin were determined after IV administration of camptothecin or its carboxylate form to rats (13). The clearance and volume terms for the lactone form of camptothecin were larger than those for the carboxylate form, suggesting the same extensive distribution of the lactone form to tissues, and limited distribution of the reversible metabolite for camptothecin.

Since reversible interconversion occurs in the dog, it is likely that this interconversion will also occur in man, as it does with camptothecin (14). However, SK&F 105992 is inactive *in vitro*, and is only active *in vivo* as a result of its conversion to topotecan. Based on the fact that the interconversion is pH dependent and reversible, it is likely that pharmacokinetics similar to that seen in the dog will be observed in man allowing for allometric adjustment for species differences.

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