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ORIGINAL ARTICLE

Pharmacokinetics and enhanced bioavailability of candidate cancer preventative agent, SR13668 in dogs and monkeys

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Abstract
Purpose SR13668 (2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo-(2,3-b)carbazole), is a new candidate cancer chemopreventive agent under development. It was designed using computational modeling based on a naturally occurring indole-3-carbinol and its in vivo condensation products. It showed promising anti-cancer activity and its preclinical toxicology profile (genotoxicity battery and subchronic rat and dog studies) was unremarkable. However, it exhibited a very poor oral bioavailability (<1%) in both rats and dogs. Therefore, a study was initiated to develop and evaluate in dogs and non-human primates formulations with a more favorable oral bioavailability.
Methods Two formulations utilizing surfactant/emulsifiers, PEG400:Labrasol® and Solutol®, were tested in dogs and monkeys. Levels of SR13668 were measured in plasma and blood using a high-performance liquid chromatograph–tandem mass spectrometer system. Non-compartmental analysis was used to derive pharmacokinetic parameters including the bioavailability.
Results The Solutol® formulation yielded better bioavailability reaching a maximum of about 14.6 and 7.3% in dogs and monkeys, respectively, following nominal oral dose of ca. 90 mg SR13668/m^2. Blood levels of SR13668 were consistently about threefold higher than those in plasma in both species. SR13668 did not cause untoward hematology, clinical chemistry, or coagulation effects in dogs or monkeys with the exception of a modest, reversible increase in liver function enzymes in monkeys.
Conclusions The lipid-based surfactant/emulsifiers, especially Solutol®, markedly enhanced the oral bioavailability of SR13668 over that previously seen in preclinical studies. These formulations are being evaluated in a Phase 0 clinical study prior to further clinical development of this drug.

Keywords Formulation · Pharmacokinetics · Absorption enhancer · Bioavailability · Chemoprevention · Liver function enzymes

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Introduction

Indole-3-carbinol (I3C) is a naturally occurring compound found in cruciferous vegetables (broccoli, cabbage, cauliflower) with a reported broad-spectrum cancer chemopreventive activity [15]. Its activity has been attributed to a variety of mechanisms and antiproliferative and/or apoptic activity at a number of molecular targets [9], including the Akt pathway. However, I3C is a prodrug and is highly unstable in acidic media. In gastric acid environment, at least 20 condensation products are formed in an unpredictable and variable manner. Only four of these formed

condensation products (15% of total) have anti-cancer activity. One of its dimer condensation products, 3,3′-diindolylmethane (DIM), was proposed as a better alternative because of its more stable form, similar activity, and a more predictable and safer response [2]. Using four active I3C oligomers as lead compounds, Chao et al. [3], applied computational modeling to design a synthetic compound, SR13668 (Fig. 1), with I3C-like anti-cancer mechanisms but improved potency and activity. This agent was selected for development by the RAPID Program [12] in the Division of Cancer Prevention of the National Cancer Institute. It was shown negative in the genotoxicity battery [7] and essentially free of significant toxicity in the 28-day rat and 14-day dog subchronic preclinical toxicology studies. It was shown to have anti-cancer activity in number of in vitro and in vivo models, including breast, lung, prostate, and ovary [10, 11]. Inhibition of PI3K/Akt pathway was proposed as its mechanism of action [10, 11]. This pathway is up-regulated in preneoplastic lesions across a broad range of target tissues and its down-regulation is considered to be a viable strategy for cancer prevention [4]. While hyperinsulinemia and glucose intolerance commonly result from the inhibition of PI3K pathway, SR13668 did not cause these effects even at doses ten times higher than needed for anti-cancer activity in mice [10]. In the prevention as opposed to therapy, only non-invasive routes of administration (e.g. oral, topical, and inhalation) are acceptable. While SR13668 is orally available and effective, initial studies in rats and dogs showed a very poor bioavailability, less than 1%. Based on its limited aqueous solubility and expected high permeability, SR13668 is projected to be a Biopharmaceutics Drug Classification II drug [1]. Class II drugs exhibit dissolution rate limited absorption in vivo, except at very high dose levels. The present study was designed to develop and evaluate alternate formulations with improved bioavailability of SR13668. Specifically, the goals were to determine the absolute and relative bioavailability of oral SR13668 formulations in dogs and non-human primates, compare pharmacokinetic parameters in both species after a single dose and seven consecutive days of dosing, and evaluate effects of food and dose on pharmacokinetics.

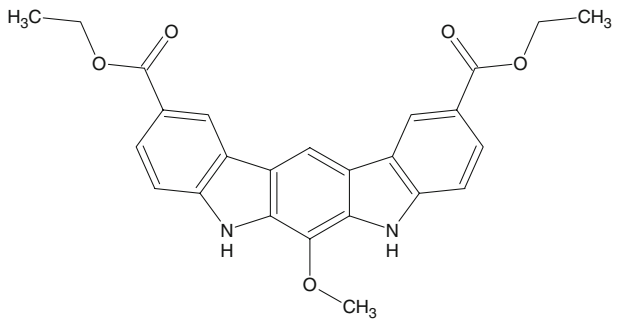

Fig. 1 Chemical structure of SR13668

Experimental methods

Test article and formulation vehicles

SR13668 (lot number 70810AR001) was provided to the Division of Cancer Prevention, National Cancer Institute by ScinoPharm, Taiwan, with a Certificate of Analysis confirming identity by NMR, IR and MS and reported purity of 99% by HPLC. The formulation vehicles PEG 300, PEG 400, and DMSO (dimethyl sulfoxide) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO); Solutol® HS 15 (lot number 52919668E0) was obtained from BASF (Florham Park, NJ); and Labrasol® was purchased from Gattefosse USA (Paramus, NJ).

Animals

Four non-naïve male beagle dogs (approximately 3 years of age; Ridglan Farms Inc., Mt. Horeb, WI) and four non-naïve male cynomolgus monkeys [cynos (macaca fasicularis); approximately seven to eight years of age; Charles River Laboratories, Inc., Houston, TX] were used in this study. Prior to experimental initiation for the present study, the attending veterinarian certified that the animals were healthy and free from disease and parasites.

Monkeys were housed individually in stainless steel cages and dogs were housed individually in pens. The animals were housed in accordance with standards set forth in the Guide for Care and Use of Laboratory Animals (National Research Council, 1996) and by the US Department of Agriculture through the Animal Welfare Act (7 USC 2131, 1985) and Animal Welfare Standards incorporated in Title 9, Part 3 of the Code of Federal Regulations, 1991.

Animal rooms were held within a temperature range of approximately 18–29°C and a humidity range of approximately 30–70%. Fluorescent lighting in the animal room was provided for 12 h followed by 12 h of darkness.

Certified commercial dog or monkey chow was provided once (dogs) or twice (monkeys) daily. Primate diets were supplemented with fresh fruit and/or other primate dietary supplements. City of Chicago municipal water was available ad libitum by automatic watering systems in all cages and pens.

Study design and dosage

For both species, each animal was part of each experimental group, with a 7-day interval between treatments to

allow for washout of the dosing formulation and its effects. Dosing formulations of SR13668 were administered by oral gavage (intragastric) or intravenous (i.v.) injection as a single dose or once daily for seven consecutive days (oral gavage). Oral doses were administered at a dosing volume of 5 mL/kg of body weight, while intravenous doses were administered at a dosing volume of 0.5 mL/kg of body weight. Dogs were dosed with 93.6 mg/m^2 SR13668 intravenously in DMSO:PEG300 15:85 (*v*/*v*) or orally in Solutol® vehicle (two groups, fed and after an overnight fast). It was necessary to use a chemical restraint for dosing and blood sampling in monkeys only. For these procedures, they were anesthetized with 0.35–0.4 mL Ketaset (ketamine hydrochloride) using a sterile, 1-mL plastic syringe with a 26-gauge, 3/8 in. needle. Monkeys were dosed with 84.2 mg/m^2 SR13668 intravenously in DMSO:PEG300 15:85 (*v*/*v*) or orally (336.7 mg/m^2 SR13668 for the oral high-dose group) in PEG400:Labrasol® 1:1 (*v*/*v*) or Solutol® vehicles. Vehicle control groups were used to assess the tolerability of the vehicle and for clinical pathology evaluations.

Animals were observed at 0.5 (dogs)/1 (monkeys) and 4 h post-dose on dosing days, as well as daily during washout periods, for any unusual behavioral activity, observable changes in appearance, and/or adverse clinical signs. At the end of each dosing period, the animals were examined for detailed clinical signs and symptoms; i.e., alterations of teeth, nose, eyes, perineum, pelage and body orifices; changes in appearance or behavior; and/or presence of any tissue masses. Body weights were recorded prior to treatment and daily during each testing interval. Quantitative (g/day) food consumption data were recorded for dogs on a daily basis during each testing interval. Qualitative food consumption observations were recorded for monkeys on a daily basis during each testing interval. Blood samples for clinical pathology (clinical chemistry, hematology, and coagulation) parameter evaluation were collected from dogs and monkeys prior to dosing and on Days 2 and 8 post-dose, as well as from monkeys on Days 15 and 30 post-dose.

Blood collection for analysis of SR13668

Blood samples were collected from each animal at ten time points (0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose) during the first and last 24-h segment of each dosing regimen. For single intravenous dose experiments, samples were collected during the 24-h post-dose period only. Samples were transferred to Vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA; Fisher Scientific, Pittsburgh, PA). The tubes were inverted several times to mix and then placed on ice until storage or centrifugation for plasma preparation. After centrifugation, the plasma was transferred into storage tubes (0.5 mL), which were placed on dry ice and then stored frozen (approximately −70°C).

Analytical method

Levels of SR13668 in plasma and blood were measured using a tandem mass spectrometer (API 3000; Applied Biosystems/MDS Sciex, Foster City, CA) equipped with a high-performance liquid chromatograph (Agilent 1200; Agilent Technologies, Wilmington, DE). For SR13668 determination, a 100 μL blood or plasma aliquot was mixed with 1 mL of acetonitrile (ACN; Sigma–Aldrich, St. Louis, MO). After vortex-mixing for 1 min, the sample was centrifuged at 4°C and 7,000 RPM for 10 min to remove precipitated proteins, and the supernatant was transferred to a clean tube and dried under nitrogen flow at room temperature (approximately 25°C). After the evaporation was completed, the residue was reconstituted in 500 μL of ACN/water (*v*/*v* 70:30), and vortex-mixed and centrifuged again. An aliquot of the resulting supernatant was transferred to an autosampler tube for instrumental analysis.

A freshly prepared SR13668 standard curve was analyzed along with samples on each day of analysis. Instrument calibrators and quality control (QC) samples were prepared by adding 10 μL of a stock SR13668 solution in ACN/water mixture (*v*/*v* 70:30) to 100 μL of blank monkey or dog blood or plasma (Bioreclamation Inc., Westbury, NY). Calibrator concentrations were approximately 1, 5, 25, 50, 100, 500, 1,000, 1,500, and 2,500 ng/mL. QC samples were prepared at approximately 2.4, 1,000, and 2,000 ng/mL. Calibrators and quality control samples were processed for analysis following the procedure described above.

The chromatographic column was a Luna 3μ C18(2) 110 Å 30 × 2.0 mm (Phenomenex, Torrance, CA). The column temperature was maintained at 25°C, and a flow rate of 0.30 mL/min was used. The mobile phase consisted of MPA: formic acid in water (0.05%, *v*/*v*) and MPB: formic acid in ACN (0.05%, *v*/*v*). The mobile phase gradient was as follows: after injection, initial conditions with MPA at 40% were held for 0.01 min, decreased to 5% and held constant for 3 min, returning to initial conditions for another 3 min of re-equilibration time. Retention time of SR13668 was approximately 2.4 min. Total run time was 6 min. A turbo ion spray interface was used as the ion source operating in negative ion mode. Acquisition was performed in multiple reaction monitoring mode using ions 429.15 (Q1) and 414.12 (Q3) Dalton. Ion spray voltage was −4,200 V, ion source temperature was 340°C, and collision energy was −30 V.

Method's inter-day average accuracy was 92% or better, with precision (CV%) of less than 10% for all blood and

plasma matrices. The limit of quantitation (LOQ) was 0.5 ng/mL for both plasma and blood for dogs and monkeys.

Pharmacokinetic and statistical analysis

Pharmacokinetic (PK) analysis was performed on plasma and whole blood SR13668 concentration data on an individual animal basis using WinNonlin Professional Edition version 4.1 (Pharsight Inc., Mountain View, CA). The non-compartmental model for extravascular input was used for all PK analyses for oral (intragastric gavage) administration groups. The non-compartmental model for i.v.–bolus input was used for all PK analyses for i.v. administration groups. Area under the plasma concentration–time curve (AUC) from time zero to the last measured concentration was estimated by the linear trapezoidal rule up to C_{max} (maximum observed plasma concentration), followed by the log trapezoidal rule for the remainder of the curve. Area under the plasma concentration–time curve extrapolated to infinity is defined as $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$, where λ_z is the disposition rate constant estimated using log-linear regression during the terminal elimination phase and C_t is the last measureable plasma concentration. For oral gavage groups on Day 7, the interdose AUC_{0-t} ($t = 24$ h) instead of $AUC_{0-\infty}$ values were calculated and used to derive other pharmacokinetic parameters. Statistical analyses were performed for AUC and F (systemic availability of the administered dose) using log-transformed PK parameter data. For AUC, the data were normalized to the body surface area dose (i.e., mg SR13668/m^2) prior to log-transformation. Systat software (Systat Software Inc., Chicago, IL; version 10.2) was used to analyze pharmacokinetic parameter data via repeated measure design and using general linear model computations to test changes across the repeated measures (within subjects) as well as differences between groups of subjects (between subjects). For each pharmacokinetic parameter, the tests were performed either by paired t tests or repeated measure analysis followed, as necessary, by the post-hoc Tukey's test ($p \leq 0.05$).

Results

Animals were observed at least at 0.5 and 4 h post-dosing and daily for any unusual behavioral activity, observable changes in appearance, and clinical signs. There were no mortalities or morbidity in the study. The only adverse treatment-related clinical observations included vomiting in fasted dogs and soft stools and diarrhea in orally but not i.v. dosed monkeys. Vomiting was present even in vehicle control animals which suggests that it was at least in part due to the vehicle. The animals seemed to have acclimatized to dosing with daily administration. No significant treatment-related effects on body weights, body weight gains or food consumption were seen in either the dogs or monkeys during this study.

Plasma SR13668 concentration–time profiles following i.v. and oral gavage administration of SR13668 are presented for dogs and monkeys in Figs. 2 and 3, respectively. Summaries of pharmacokinetic parameters in plasma and blood for both dogs and monkeys are presented in Tables 1 and 3, respectively. The data are presented following the first day of i.v. dosing and the seventh oral dose of SR13668. Vomiting was problematic in some animals on initial dosing and therefore only data for the last (seventh) day of oral dosing are presented. However, there were no discernible differences between the first and seventh day of oral dosing (Table 2) in groups where adequate Day 1 data were available. The corresponding dog data for whole blood are presented in Table 3. Whole blood concentrations of SR13668 were consistently nearly threefold greater than those in plasma throughout the study in both species. The clearance was similar in dogs and monkeys following the i.v. dosing. Oral bioavailability tended to be slightly higher in whole blood as compared to plasma. Plasma and blood oral bioavailability ranged from 0.6 to 13.3% and 0.97 to 15.6%, respectively. Greater bioavailability following a comparable dose in the Solutol® vehicle based on a body surface area was observed in

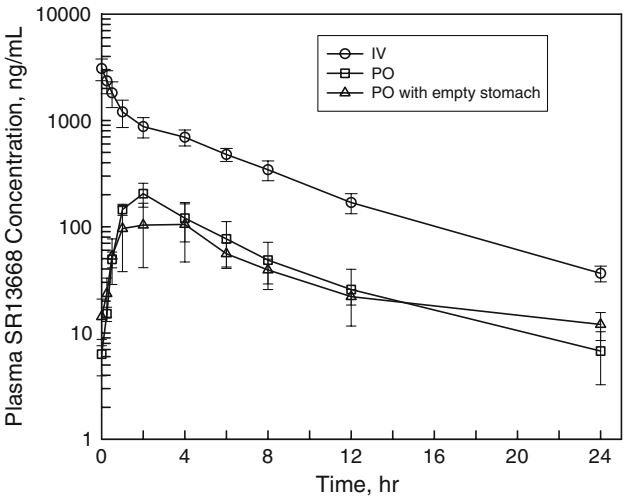

Fig. 2 Pharmacokinetic profile of SR13668 following i.v. dosing in fed and oral gavage dosing in fed and fasted dogs. Data are presented for a single dose i.v. and seventh daily oral dose at 93.6 mg/m^2 (4.7 mg/kg) in DMSO:PEG300 (15:85, *v/v*) and Solutol®, respectively. Data were graphed using SigmaPlot® version 9.0 (Systat Software, Inc., Chicago, IL)

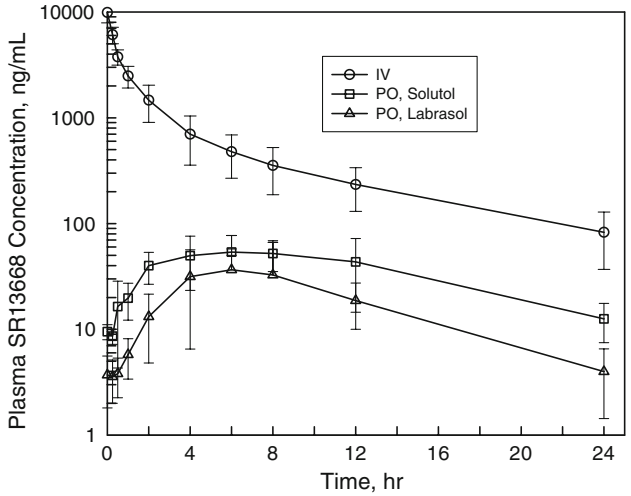

Fig. 3 Pharmacokinetic profile of SR13668 following i.v. dosing in fed and oral dosing in monkeys. Data are presented for a single dose i.v. and seventh daily oral gavage dose at 84.2 mg/m^2 (7.0 mg/kg) in DMSO:PEG300 (15:85, *v/v*) and Solutol® or PEG400:Labrasol® (1:1, *v/v*), respectively. Data were graphed using SigmaPlot® version 9.0 (Systat Software, Inc., Chicago, IL)

dogs than in monkeys, 13.6 versus 6.1% in plasma (Table 1) and 15.6 vs. 7.32% in blood (Table 3). Solutol® yielded greater bioavailability in monkeys than PEG400:Labrasol® vehicle. However, due to data variability, this was only statistically significant in blood. Increasing the dose four-fold in PEG400:Labrasol® in monkeys resulted in a lower oral bioavailability. There was no significant difference in oral bioavailability between fed and fasted dogs. In order to distinguish low absorption from high first-pass presystemic clearance as the contributing factor for the low bioavailability, the fraction absorbed, F_{abs}, was estimated as $F/(1-CL/Q)$ where Q represents hepatic blood flow in the respective species [5] and CL is the calculated blood clearance for SR13668 (Table 4). Values for bioavailability (%F) were slightly lower but very close to corresponding the fraction absorbed (%F_{abs}) values.

During the study, clinical pathology (clinical chemistry, hematology, and coagulation) parameters were also monitored. No treatment-related effects on any clinical pathology variables were observed for dogs. The same was true for the monkeys except for modest increases in liver enzymes, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Table 5). These increases appeared to be due to SR13668, since there were no increases in any of these enzymes in the two corresponding vehicle groups. These changes were reversible on discontinuation of dosing (Table 6).

Table 1 Summary of pharmacokinetic properties of SR13668 in plasma of dogs and monkeys

	Dogs			Monkeys			
	93.6 mg/m^2 i.v. DMSO:PEG300 15:85 (*v/v*) Fed	93.6 mg/m^2 Oral gavage Solutol® Fed	93.6 mg/m^2 Oral gavage Solutol® Fasted	84.2 mg/m^2 i.v. DMSO:PEG300 15:85 (*v/v*) Fed	84.2 mg/m^2 Oral gavage PEG400:Labrasol® 1:1 (*v/v*) Fed	336.7 mg/m^2 Oral gavage PEG400:Labrasol® 1:1 (*v/v*) Fed	84.2 mg/m^2 Oral gavage Solutol® Fed
$t_{½}$ (h)	5.0 ± 0.2	5.6 ± 1.4	9.7 ± 2.4	7.6 ± 0.8	6.0 ± 1.3	5.0 ± 1.2	7.4 ± 0.7
T_{max} (h)	NA	1.8 ± 0.5	2.8 ± 1.5	0.25	5.0 ± 1.2	3.5 ± 1.9	8.0 ± 3.3
C_{max} (ng/mL)	NA	207 ± 48	113 ± 58	9,888 ± 1,991	40.5 ± 38	43.7 ± 9.2	71.4 ± 16
AUC (h×ng/mL)	8,779 ± 1,242	1,180 ± 386	925 ± 334	14,570 ± 5,166	405 ± 297[c]	347 ± 138[c]	825 ± 226
V_z/F (L/kg)	3.87 ± 0.51	34.0 ± 10	79.5 ± 38	5.78 ± 1.9	224 ± 147	635 ± 256	105 ± 13
CL/F (L/h/kg)	0.541 ± 0.076	4.37 ± 1.6	5.57 ± 1.8	0.527 ± 0.17	24.4 ± 14	90.3 ± 32	8.94 ± 2.1
MRT (h)	6.1 ± 0.6	5.98 ± 0.79	7.63 ± 1.1	6.3 ± 1.1	9.7 ± 0.73	7.6 ± 2.3	10.0 ± 1.3
F (%)	100	13.3 ± 3.6[d]	10.6 ± 3.4	100	2.73 ± 1.7[c]	0.62 ± 0.23[c]	6.12 ± 2.0[d]

Values are presented as mean ± SD

Oral gavage groups—AUC, V_z, CL, MRT and F values are based on "0–24 h" (i.e., steady-state) calculations rather than "0–∞"

Statistically significant differences resulting from the following comparisons for AUC and F are indicated in the table (comparisons "a" and "b" yielded no statistically significant differences)

NA not applicable

[a] Oral gavage, fed dogs and oral gavage, fasted dogs for given parameter

[b] Oral gavage, low-dose (84.2 mg/m^2) monkeys (Solutol®) and oral gavage, low-dose (84.2 mg/m^2) monkeys (Labrasol®) for given parameter

[c] Oral gavage, high-dose (336.7 mg/m^2) monkeys (Labrasol®) and oral gavage, low-dose (84.2 mg/m^2) monkeys (Labrasol®) for given parameter

[d] Oral gavage, low-dose (84.2 mg/m^2) monkeys (Solutol®) and oral gavage, fed dogs (Solutol®) (F only)

Table 2 Summary of AUC properties of SR13668 in plasma of dogs and monkeys

	Dogs		Monkeys
	93.6 mg/m^2	93.6 mg/m^2	336.7 mg/m^2
	Oral gavage	Oral gavage	Oral gavage
	Solutol®	Solutol®	PEG400:Labrasol® 1:1 (*v*/*v*)
	Fed	Fasted	Fed
$AUC_{0-\infty}$ (h×ng/mL) (Day 1)	1,415 ± 385	703 ± 226	258 ± 153
$AUC_{0-24\ h}$ (h×ng/mL) (Day 7)	1,180 ± 386	925 ± 334	347 ± 138

Values are presented as mean ± SD

No statistically significant differences were observed between Days 1 and 7

Table 3 Summary of pharmacokinetic properties of SR13668 in blood of dogs and monkeys

	Dogs			Monkeys			
	93.6 mg/m^2	93.6 mg/m^2	93.6 mg/m^2	84.2 mg/m^2	84.2 mg/m^2	336.7 mg/m^2	84.2 mg/m^2
	i.v.	Oral gavage	Oral gavage	i.v.	Oral gavage	Oral gavage	Oral gavage
	DMSO:PEG300 15:85 (*v*/*v*)	Solutol®	Solutol®	DMSO:PEG300 15:85 (*v*/*v*)	PEG400:Labrasol® 1:1 (*v*/*v*)	PEG400:Labrasol® 1:1 (*v*/*v*)	Solutol®
	Fed	Fed	Fasted	Fed	Fed	Fed	Fed
$t_{½}$ (h)	5.0 ± 0.1	5.9 ± 1.5	7.0 ± 1.8	8.6 ± 1.7	5.8 ± 0.9	6.8 ± 1.2	7.7 ± 0.6
T_{max} (h)	NA	1.8 ± 0.5	2.8 ± 1.5	0.25	7.0 ± 0.35	3.5 ± 1.9	6.5 ± 3.8
C_{max} (ng/mL)	NA	575 ± 152	277 ± 87	16,463 ± 2,853	129 ± 140	168 ± 35	227 ± 56
AUC (h×ng/mL)	20,441 ± 3134[e]	3,253 ± 1,234[e]	2,370 ± 600[e]	37,487 ± 12410[e]	1,235 ± 1,102[b,e]	1,372 ± 390[e]	2,629 ± 1,037[b,e]
V_z/F (L/kg)	1.68 ± 0.21	13.5 ± 5.2	21.9 ± 11	2.46 ± 0.60	67.3 ± 47	216 ± 82	37.0 ± 5.3
CL/*F* (L/h/kg)	0.233 ± 0.031	1.62 ± 0.63	2.08 ± 0.53	0.204 ± 0.067	8.80 ± 5.1	21.9 ± 7.1	2.93 ± 0.89
MRT (h)	6.4 ± 0.7	6.0 ± 0.59	7.6 ± 0.90	8.5 ± 2.2	9.8 ± 0.69	8.5 ± 1.6	10.1 ± 1.2
F (%)	100	15.6 ± 3.9[d,e]	11.9 ± 4.0	100	3.14 ± 2.3[b]	0.97 ± 0.38[e]	7.32 ± 2.4[b,d,e]

Values are presented as mean ± SD

Oral gavage groups—AUC, V_z, CL, MRT and *F* values are based on "0–24 h" (i.e., steady-state) calculations rather than "0–∞"

Statistically significant differences resulting from the following comparisons for AUC and *F* are indicated in the table (comparisons "a" and "c" yielded no statistically significant differences)

NA not applicable

[a] Oral gavage, fed dogs and oral gavage, fasted dogs for given parameter

[b] Oral gavage, low-dose (84.2 mg/m^2) monkeys (Solutol®) and oral gavage, low-dose (84.2 mg/m^2) monkeys (Labrasol®) for given parameter

[c] Oral gavage, high-dose (336.7 mg/m^2) monkeys (Labrasol®) and oral gavage, low-dose (84.2 mg/m^2) monkeys (Labrasol®) for given parameter

[d] Oral gavage, low-dose (84.2 mg/m^2) monkeys (Solutol®) and oral gavage, fed dogs (Solutol®) (*F* only)

[e] Blood and plasma for given group and parameter

Discussion

SR13668 is poorly water soluble (<2 μg/ml) and very lipophilic candidate cancer chemopreventive drug with XLogP3 value of 5.3.[1] This high-log *P* value constitutes a violation of commonly employed "Rule of 5" for drug-like properties [8] and predicts poor oral bioavailability. Consistent with this prediction, a very poor bioavailability, less than 1%, was observed in both rats and dogs. Due to promising pharmacodynamic properties of this candidate drug, an effort was made to develop formulation that would improve its bioavailability. Lipid-based delivery systems have been proposed as effective means for enhancing oral bioavailability of lipophilic drugs [13]. Several lipid based formulations were evaluated in rats and bioavailability as high as 40% was achievable in rats using PEG400:Labrasol® (1:1, *v*/*v*). In anticipation of clinical studies, bioavailability studies were extended to non-rodent species, beagle dogs, and non-human primates, cynomolgus monkeys. The optimum vehicle in rats, PEG400:Labrasol®, and another lipid-based delivery vehicle, Solutol®, were

[1] http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=9845566&loc=ec_rcs#Properties.

Table 4 Summary of fraction absorbed (F_{abs}) estimates in dogs and monkeys

	Dogs			Monkeys			
	93.6 mg/m^2	93.6 mg/m^2	93.6 mg/m^2	84.2 mg/m^2	84.2 mg/m^2	336.7 mg/m^2	84.2 mg/m^2
	i.v.	Oral gavage	Oral gavage	i.v.	Oral gavage	Oral gavage	Oral gavage
	DMSO:PEG300 15:85 (*v*/*v*)	Solutol®	Solutol®	DMSO:PEG300 15:85 (*v*/*v*)	PEG400:Labrasol® 1:1 (*v*/*v*)	PEG400:Labrasol® 1:1 (*v*/*v*)	Solutol®
	Fed	Fed	Fasted	Fed	Fed	Fed	Fed
CL (mL/min/kg)	3.88	27.0	34.7	3.40	147	365	48.8
Q Blood flow (hepatic) [5] (mL/min/kg)	30.9	30.9	30.9	43.6	43.6	43.6	43.6
ER $CL_{i.v.}/Q$	0.13			0.078			
F (%)	100	15.6	11.9	100	3.1	1.0	7.3
F_{abs} (%) $F/(1-ER)$		17.8	13.6		3.4	1.1	7.9

Table 5 Summary of liver enzymes in dogs and monkeys following SR13668

					ALT (IU/L)	AST (IU/L)	LDH (IU/L)
Dogs	0 mg/m^2	Oral	Solutol®	Fed	42.3 ± 4.8	34.5 ± 6.8	84.8 ± 35
	93.6 mg/m^2	i.v.	DMSO:PEG300 15:85 (*v*/*v*)	Fed	41.8 ± 5.4	30.8 ± 5.2	54.8 ± 23[c]
	93.6 mg/m^2	Oral gavage	Solutol®	Fed	39.5 ± 4.7	29.3 ± 3.6	30.3 ± 6.2[c]
	93.6 mg/m^2	Oral gavage	Solutol®	Fasted	44.8 ± 10	30.8 ± 7.2	56.3 ± 9.4
Monkeys	0 mg/m^2	Oral	PEG400:Labrasol® 1:1 (*v*/*v*)	Fed	48.8 ± 13	46.0 ± 4.8	304 ± 66
	84.2 mg/m^2	i.v.	DMSO:PEG300 15:85, *v*/*v*	Fed	88.5 ± 37	134 ± 41[b]	730 ± 210[c]
	84.2 mg/m^2	Oral gavage	PEG400:Labrasol® 1:1 (*v*/*v*)	Fed	86.0 ± 24	138 ± 75	795 ± 455
	336.7 mg/m^2	Oral gavage	PEG400:Labrasol® 1:1 (*v*/*v*)	Fed	108 ± 28[a]	188 ± 34[b]	753 ± 242[c]
	84.2 mg/m^2	Oral gavage	Solutol®	Fed	95.5 ± 33	150 ± 40[b]	779 ± 403

Values are presented as mean ± SD for $n = 4$ on Day 2 following a single dose of SR13668

Statistically significant difference between treated and control (0 mg/m^2) group, with each group per species compared separately to its control group

[a] *ALT* alanine aminotransferase

[b] *AST* aspartate aminotransferase

[c] *LDH* lactate dehydrogenase

selected for evaluation in dogs and monkeys. Under the conditions tested, the highest bioavailability achieved was about 15.6% using Solutol® as a vehicle in dogs. Similarity in $\%F$ and $\%F_{abs}$ values suggests that the low bioavailability of SR13668 is mainly due to its low absorption. Presystemic clearance does not appear to play a major role. Dogs exhibited a twofold higher bioavailability than monkeys with comparable doses in Solutol®. SR13668 tended to have higher bioavailability in Solutol® than in PEG400:Labrasol®. Fed or fasted condition did not have an effect on bioavailability. SR13668 tended to concentrate in blood cells with a whole blood:plasma concentration range of almost three in all cases. Bioavailability estimates were similar between whole blood and plasma. Increasing the dose fourfold in PEG400:Labrasol® resulted in about a fourfold decrease in the bioavailability of SR13668 in monkeys. Reasons for this decrease are not clear, but it is conceivable that SR13668 may have come out of suspension upon administration and precipitated in the gastrointestinal tract. This would not be surprising for very lipophilic, dissolution limited drugs. It is also possible that the decrease in bioavailability may have been due to a decreased gastro-intestinal transit time resulting from high load of PEG400:Labrasol®. Dose dependent bioavailability may be important in selection and use of the optimum human dose. Human bioavailability of SR13668 is currently being investigated in a Phase 0 study.

SR13668 exhibited relatively high volume of distribution. This is suggestive of extensive tissue distribution. It is also consistent with its high blood/plasma concentration ratio. In addition and also consistent with this, SR13668 was shown to preferentially concentrate in lungs of rats with more than fivefold concentration ratio relative to blood (unpublished results).

Clinical chemistry and pathology parameters were monitored during the study. The only abnormalities noted were

Table 6 Reversibility of liver enzymes changes in monkeys following SR13668

Monkeys	Day	ALT (IU/L)	AST (IU/L)	LDH (IU/L)
84.2 mg/m^2 oral gavage PEG400:Labrasol® 1:1 (*v*/*v*) Fed	1 (predose)	41.0 ± 10	25.5 ± 4.1	221 ± 75
	2	112 ± 52	234 ± 115	1,022 ± 401
	8	100 ± 38[a]	178 ± 86[b]	1,035 ± 596
	15	41.3 ± 10	24.0 ± 2.9	184 ± 39
	30	33.3 ± 2.2	22.5 ± 4.3	168 ± 39
Range of normal historical values in the testing laboratory		16–80	39–80	313–1,034
Range of normal values [6]		20–60	25–60	300–600

Values are presented as mean ± SD for $n = 4$ except for $n = 2$ on Day 2

Single daily dose of SR13668 was administered to monkeys on Days 1–7. Liver enzymes were monitored 24 h after a single dose on Days 2 and 8 and after discontinuation of dosing, Days 15 and 30

Statistically significant difference between given day and Day 1

[a] *ALT* alanine aminotransferase

[b] *AST* aspartate aminotransferase

[c] *LDH* lactate dehydrogenase

modest increases in liver enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase), and LDH (lactate dehydrogenase) in monkeys. These increases were modest, reversible, and statistically significant only in few instances. They were less than threefold of the upper limit of normal and without concomitant increases in total bilirubin and prothrombin time and thus not predictive of hepatocyte injury according to a recent review [14]. However, SR13668 is being developed as a cancer preventatitive agent, which implies chronic or prolonged administration. Therefore, more chronic studies in relevant species should be considered to further address the observation made in monkeys.

Although a considerable improvement in bioavailability of SR13668 has been achieved preclinically, its bioavailability using lipid-based delivery systems is being studied in human subjects in a Phase 0 clinical study prior to further clinical development of this drug.

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