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ORIGINAL ARTICLE

Preclinical pharmacokinetic, toxicological and biomarker evaluation of SR16157, a novel dual-acting steroid sulfatase inhibitor and selective estrogen receptor modulator

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Abstract
Purpose SR16157 is a novel dual-acting inhibitor of estrogen action that irreversibly inhibits the estrogen biosynthetic enzyme steroid sulfatase (STS) and releases the selective estrogen receptor modulator SR16137, which blocks the estrogen receptor. SR16157 is a promising agent for the endocrine therapy of breast cancer. We conducted preclinical in vivo toxicity evaluations to determine the maximum-tolerated dose (MTD), target organ(s) of toxicity, reversibility, dose-limiting toxicity, no observable adverse effect level (NOAEL), and toxicokinetics (TK) and to investigate a potential biomarker for use in SR16157 clinical trials.
Methods SR16157 was administered to female Fischer 344 rats or beagle dogs by oral gavage (po) or capsule. Intravenous (iv) groups were included for the determination of bioavailability. Endpoints evaluated included clinical observations, body weights, hematology, serum chemistry, pharmacokinetics, TK, pathology of tissues, and STS activity in liver, or peripheral blood mononuclear cells (PBMCs).
Results For rats, the MTD (i.e., the highest dose that did not cause lethality but produced toxicity) was 33 mg/kg/day (198 mg/m^2/day), and the NOAEL was <10 mg/kg/day (60 mg/m^2/day). For dogs, the MTD was estimated to exceed 10 mg/kg/day (200 mg/m^2/day), and the NOAEL was estimated to be at or above 2.5 mg/kg/day (50 mg/m^2/day).
Conclusions Our studies demonstrate that SR16157 has excellent pharmacokinetic properties and an acceptable toxicological profile. Modulation of STS activity in PBMCs appeared to be a possible biomarker for use in future clinical trials of SR16157.

Keywords Estrone sulfatase · Steroid sulfatase · Biomarker · SERM · Breast cancer

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Introduction

Estrogens have been shown to stimulate the growth of hormone-dependent breast cancers, and the goal of endocrine (antihormonal) therapy in breast cancer is to reduce the effect of estrogenic stimuli on breast tumor growth. Two general strategies have been clinically validated for endocrine therapy of breast cancer: (1) blockade of the action of estrogens at the estrogen receptor (ER) with antiestrogens and (2) inhibition of estrogen biosynthesis. Antiestrogens such as tamoxifen have been used for decades in breast cancer treatment, whereas only inhibitors of the aromatase pathway of estrogen biosynthesis are clinically established thus far for breast cancer. Despite the success of aromatase inhibitors for breast cancer therapy, their overall response rates (40–50%) [1] suggest that total estrogen suppression is not achieved with this strategy and that alternative sources of estrogenic stimuli may still be present. Indeed, conclusive evidence indicates that the hydrolysis of estrone sulfate to estrone in breast tissues by the enzyme estrone sulfatase (also known as steroid sulfatase, STS) is a major source of estrogen found in breast tumors [2]. The steroid sulfatase enzyme is also responsible for the hydrolysis of dehydroepiandrosterone sulfate (DHEA-S) to DHEA (Fig. 1), which is an immediate precursor of androstenediol

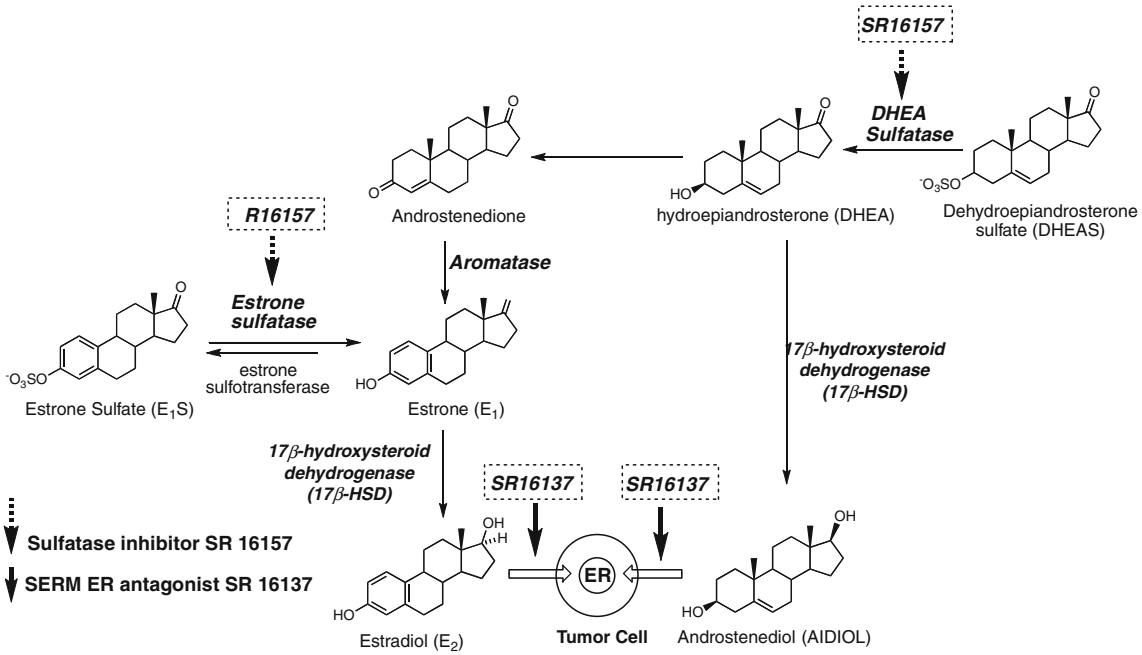

Fig. 1 Hydrolysis of dehydroepiandrosterone sulfate (DHEA-S) to DHEA

(ADIOL, Fig. 1), a potent estrogenic steroid, whose formation is not inhibited by aromatase inhibitors [3–5]. Therefore, inhibition of STS is a potentially new strategy for achieving a more complete suppression of estrogenic stimuli for breast cancer treatment. The development of sulfatase inhibitors has been hampered by the lack of potency and presence of unwanted estrogenic effects of inhibitors reported thus far [6, 7]. Nevertheless, several potent STS inhibitors have now been reported, and a novel coumarin-based STS inhibitor 667COUMATE (STX64) has been evaluated in a phase I clinical trial in post-menopausal women with advanced breast cancer, with promising results [8].

In a program to develop novel STS inhibitors, we identified a potent sulfamate-based STS inhibitor, SR16157, with a unique 'dual-target' profile, synthesized on an 'antiestrogen SERM' platform. SR16157 is a potent inhibitor of steroid sulfatase in intact MCF-7 cells and inhibits the growth of estrone sulfate-stimulated MCF-7 cells in culture. In vivo, SR16157 shows potent oral suppression of estrone sulfate-stimulated tumor growth of MCF-7 xenografts in nude mice [9]. SR16157 is particularly interesting because it has a dual mode of action: the sulfamate moiety irreversibly inhibits the STS enzyme, releasing the phenolic metabolite, SR16137 (Fig. 2), which is a tissue-selective antiestrogen and a *S*elective *ER M*odulator (SERM), with beneficial effects in the bone and cardiovascular system [10]. SR16157 thus has a novel therapeutic profile for the endocrine therapy of breast cancer, with the ability to not only block the biosynthesis of estrogen and other estrogenic substances (such as ADIOL, see Fig. 1) but also block the action of estrogens (both estradiol and ADIOL) [5] at the estrogen receptor, thus providing a dual mode of antiestrogenic action. SR16157 has a promising profile for further development as a viable new modality for breast cancer therapy. There have been limited toxicology assessments previously conducted with SR16157. A battery of genotoxic tests have also been carried out and demonstrated weak clastogenicity in the Chinese hamster ovary (CHO) chromosomal aberration assay, but this finding was not confirmed in a mouse micronucleus assay. Furthermore, SR16157 was judged to be nonmutagenic in the bacterial mutagenicity test (Ames assay) [11]. SR16157 therefore is considered to have minimal genotoxic activity that would not be of significant concern in the future clinical development of this promising anticancer compound.

We have conducted preclinical pharmacokinetic and safety studies of SR16157 to assess the oral bioavailability, pharmacokinetics, toxicity, and toxicokinetics. The toxicology was carried out in two species (rat and dog). In addition, we report here a method for the noninvasive determination of STS inhibition in peripheral blood mononucleocytes as a potential biomarker for the evaluation of pharmacologic activity that could be easily adapted to human clinical trials.

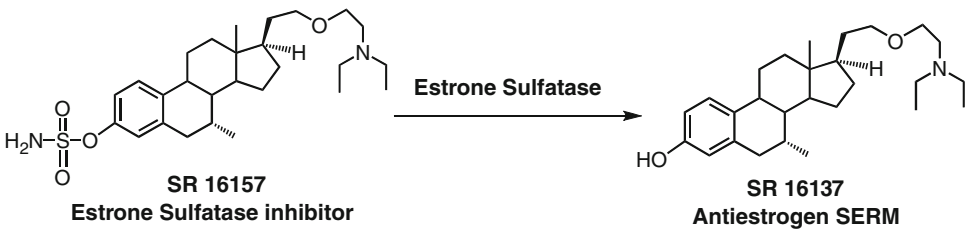

Fig. 2 Chemical structure of SR16157 (**a**) and chemical structure of SR16137, metabolite of SR16157 (**b**)

Materials and methods

Study designs

Table 1 summarizes the designs for all animal studies. Single-dose range-finding studies (rat and dog), bioavailability studies (rat and dog), repeat-dose range-finding studies (dog: 7 days, rat: 14 days), and biomarker toxicity studies (28 days for both species) were performed. Clinical observations, body weight measurements, clinical pathology, and histopathology were performed for all toxicity studies. In addition, ophthalmology and cardiology parameters were performed on the 28-day study in dogs. Preliminary mouse and rat range-finding studies (not presented here) were used to estimate doses for subsequent studies. Body surface area dose conversions, or human-equivalent dose (HED) [12], are also presented in Table 1 to facilitate comparison. All animal protocols were approved by SRI's Institutional Animal Care and Use Committee (IACUC).

Animals

Rats

Sexually mature female Fischer 344 rats (9–11 weeks old) were supplied by Charles River Laboratories (Raleigh, NC). Rats were housed up to 3 per cage in polycarbonate cages with hardwood bedding and were acclimated for 3–7 days before study initiation. Rooms were maintained under environmentally controlled conditions (18–26°C and 30–70% humidity) with a minimum of 10 room air volumes exchanges per hour and a 12-h light/dark cycle. Rats had ad libitum access to water (purified by reverse osmosis) and certified rodent chow.

Dogs

Female beagle dogs were obtained from Covance Research Products, Inc. (Cumberland, VA) and were 5–10 months of age at study initiation. All dogs were acclimated before study start for at least 14 days. During that time, they received a complete physical examination and were determined to be healthy at the time of release from quarantine. Dogs were housed 1–2 per enclosure (4 × 8 ft) with a minimum of 10 room air volumes exchanges per hour, at approximately 18–26°C and 30–70% humidity, and a 12-h light/dark cycle. All dogs had ad libitum access to municipal tap water and access to certified canine chow 4 h postdose for approximately 2 h daily.

Test article and formulations

SR16157 (NSC-732011) was obtained from the National Cancer Institute's (NCI) repository (McKesson Bioservices, Rockville, MD). Purity was 96.8–99% as determined by high-performance liquid chromatography (HPLC). For rat toxicology studies, SR16157 was administered by oral gavage [prepared in a 0.5% hydroxypropyl methylcellulose (HPMC) suspension], except for the bioavailability study in which it was also administered iv via the lateral tail vein. For all iv administrations, SR16157 was dissolved in 5% dimethyl sulfoxide and 95% polyethylene glycol 300. For the dog toxicology studies, SR16157 was administered neat (in gelatin capsules). For the dog bioavailability study, dogs received SR16157 neat by gelatin capsule, po, or iv by cephalic vein administration.

Clinical and microscopic evaluations

Blood was obtained by retro-orbital sinus collection under CO_2 anesthesia for rats and via the cephalic vein for dogs at scheduled sampling times (Table 1). Blood samples for hematology were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Hematology evaluations, which were performed using a fully automated ADVIA 120 hematology analyzer (Bayer Healthcare, Wayne, NJ), included hematocrit (HCT), hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCC), platelet count (PLC), and reticulocyte count (RET). Absolute neutrophil (ANS), absolute lymphocyte (ALY), absolute monocyte (AMO), absolute eosinophil (AEO), and absolute basophil (ABA) were obtained by blood smears stained with Wright–Giemsa (ThermoFisher Scientific, Kalamazoo, MI) prepared for WBC differential parameters. Coagulation was evaluated in dogs only. Parameters included clot formation [prothrombin time (PT)], activated partial thromboplastin time (APT), and fibrinogen (FIB), assessed with an ACL 100 automated

Table 1 SR16157 study designs

Species	No./Gender	Regimen	Dose level (HED, Mg/m^2)	Clinical pathology days evaluated	Pharmacokinetics days and time points evaluated	Biomarker or metabolite days evaluated	Histopathology days evaluated
Rat	9M/9M/12F	Single dose (iv or po)	Male at 5 mg/kg (30), male and female at 50 mg/kg (300)	None	Pre-dose, 10, 20, 30 min, 1, 2, 4, 6, 8, 12, 24 h	None	None
	12F	Single-dose escalation (po)	6.25 mg/kg (37.5), 12.5 mg/kg (75), 25 mg/kg (150), 50 mg/kg (300), 100 mg/kg (600), 200 mg/kg mg/kg (1,200), 400 mg/kg (2,400), 600 mg/kg (3,600), 800 mg/kg (4,800), 1,000 mg/kg (6,000)	3 and 7	None	None	None
	5F[a]/3F[b]	14-day repeat (po)	0, 50, 200, 1,000 mg/kg/day (300, 1,200, 6,000 mg/m^2)	5, 12, 19, 26, and 29	None	5, 12, 15	29[b]
	5F[a]/5F[b]/9F[c]	28-day repeat (po)	0, 10, 33, 100 mg/kg/day (6, 198, 600 mg/m^2)	Main: 7, 21 Recovery: 7, 14, 21, 29, 42	Day 1, 28 (0.5, 1, 2, 4, 8, 24 and 48 h)	29, 42	29, 42
Dog	2F	Single dose (iv, po, or oral capsule)	0, 2 (iv), 20(iv, po), 200 (po) mg/kg (40, 400, 4,000 mg/m^2)	Pre-dose, 6, 48 h	Pre-dose, 5, 15, 30 min, 1, 2, 4, 6 h or 12, 24, 48 h	6, 48 h	6, 48 h
	1F	7-day repeat (oral capsule)	0, 1.25, 2.5, 5, 10, 25, 50 mg/kg/day (25, 50, 100, 200, 500, 1,000 mg/m^2)	3, 8	None	None	8
	3F[a]/2F[b]	28-day repeat (oral capsule)	0, 0.5, 2.5, 10 mg/kg/day (10, 50, 200 mg/m^2)	Pretest, 29, 42	Day 1, 28 (pre-dose, 20, 60 min, 2, 4, 24 h)[a]	6, 29, 42[d] 2, 23[e] 15, 29, 42[f]	29, 42

[a] Main

[b] Recovery

[c] TK satellite

[d] PBMCs

[e] Necropsy

[f] Ophthalmology and cardiovascular evaluation also performed

analyzer (Instrumentation Laboratory, Bedford, MA) using nephelometry.

Both rat and dog serum chemistry parameters, which were evaluated on a Hitachi 911 automated chemistry analyzer (Roche, Nutley, NJ) using colorimetric wet chemistry methodologies, included alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin [total (TBI), direct (DBI), and indirect (IBI)], blood urea nitrogen (BUN), calcium (CAL), chloride (CHL), cholesterol (CHO), creatinine (CRE), creatinine phosphokinase (CPK), gamma glutamyl transferase (GGT), globulin (GLO), glucose (GLU), phosphorus (PHO), potassium (POT), total protein (TPR), sodium (SOD), triglycerides (TRI), and albumin/globulin ratio (AGR).

Necropsy, with complete gross and microscopic examination, was conducted on all organ systems for rats and dogs in the 28-day studies. Animals euthanized in moribund condition for preliminary studies received a gross necropsy evaluation. Sections (5 μm) of paraffin-embedded tissues were prepared and stained with hematoxylin and eosin for microscopic evaluation.

Pharmacokinetics

Blood was obtained by retro-orbital sinus collection under CO_2 anesthesia for rats and via the cephalic vein for dogs at scheduled sampling times (Table 1). Blood was collected into Vacutainer® (Becton Dickinson, Franklin Lakes, NJ) collection tubes containing disodium EDTA, processed to plasma by centrifuging for 8 min at 6,000 rpm, and stored at −70°C (±10°C) until processing.

SR16157 and SR16137 plasma levels in rat and dog plasma were determined using a single liquid chromatography–tandem mass spectrometry (LC-MS/MS) method validated in both species. This method used a stable-isotope internal standard of SR16157 (SR16157-D_2, $^{13}C_2$) provided by NCI. The sample extraction procedure and analytical method were as follows: methyl tert-butyl ether (MTBE, 1.0 ml) containing 10 ng/ml of deuterated SR16157 internal standard (IS) was added to 100 μl of plasma [blanks, standards, quality controls (QCs), and study samples] in a 2.0-ml microcentrifuge tube. The tubes were vortexed at maximum speed on a plate vortexer for 20 min and centrifuged for 5 min at 14,000×*g*; then, 0.8 ml of the MTBE supernatant was removed to a 1.5-ml microcentrifuge tube. The extracts were then evaporated to dry overnight using a centrifugal evaporator. To the dried evaporate was added 0.15 ml of 20.0:79.9:0.1 acetonitrile/MilliQWater/formic acid (v/v/v), and the tube was vortexed for 20 min at maximum speed and centrifuged for 2 min at 14,000×*g*; 120 μl of the supernatant was transferred to an HPLC vial containing a 150-μl glass insert for subsequent LC-MS/MS analysis.

Plasma samples were analyzed using a Waters 2795 HPLC and a Luna C18(2) 5 μm, 50 × 2 mm column (Phenomenex, Torrance, CA). The mobile phase comprised water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B); the gradient conditions were as follows—at a flow rate of 0.35 ml/min: 20% B: 0–1.5 min, 55% B: 2.5–4.2 min, 20% B: 4.5–7.5 min. The autosampler was maintained at 15°C, and the column was at ambient temperature. The injection volume was 20 μL, and LC detection was obtained using a Micromass Quattro Micro triple quadrupole MS (Waters Corporation, Milford, MA) with electrospray ionization operated in positive ion mode. The following mass spectrometric settings were used: capillary voltage 1.0 kV, cone voltage 40 V, source temperature 120°C, desolvation temperature 350°C, collision energy 35 eV with Ar at 2.3×10^{-3} mbar. The analytes were detected by multiple reaction monitoring (MRM) at the following transitions: SR16157, 493 → 100; SR16137, 414 → 100; IS, 497 → 100.

Plasma calibration standard curves in each analytical run were generated using a nonweighted linear regression based on analyte-to-internal-standard peak area ratios. The lower limit of quantitation of the method was 2.00 ng/ml for both SR16157 and SR16137, and the upper limit of quantitation was 2,000 ng/ml for SR16157 and 200 ng/ml for SR16137. QC standards were analyzed in each analytical run (5.00, 900, and 1,800 ng/ml for SR16157; and 5.00, 80.0, and 180 ng/ml for SR16137); in all runs, at least 67% of the QCs yielded accuracy within ±15% of the nominal concentration. Stability of the analytes was determined during validation, and it was found that SR16157 and SR16137 were stable (<15% change) in rat and dog plasma upon benchtop exposure for 1 h, after 3 freeze/thaw cycles, or after storage at −70°C for 5 months.

The plasma drug level data were analyzed with WinNonlin® Professional (Pharsight, Mountain View, CA) using noncompartmental modeling. Because the dose administered was input to the program as mg/kg, no additional corrections for individual body weights of the animals were necessary. The following parameters and constants were determined: terminal elimination half-life ($t_{1/2}$), maximal plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), area under the plasma concentration–time curve determined to the last time point (AUC_{last}) and extrapolated to infinity (AUC_{inf}), apparent volume of distribution (V), clearance (Cl), and bioavailability in the pharmacokinetic studies. Toxicokinetic parameters were determined using WinNonlin® noncompartmental modeling with the sparse sampling feature.

STS quantitation activity in liver samples and in PBMCs

The reagents used were ^{3}H-estrone sulfate (57.3 Ci/mM; Perkin Elmer, Waltham, MA); ^{14}C-estrone (^{14}C-E, 50.3 mCi/mM; NEN Life Sciences Products, Inc., Boston, MA); bicinchoninic acid assay kit (BCA; Pierce Chemical Co., Rockford, IL); BD Vacutainer® cell preparation tubes (CPTs; Becton Dickinson, Franklin Lakes, NJ) with sodium citrate.

At necropsy, liver (rat and dog) specimens were immediately frozen in liquid N and stored at ≤70°C. For microsome preparations, the liver pieces were homogenized briefly in approximately 3 volumes of 100 mM phosphate buffer, pH 7.4 (w/v). Homogenates were centrifuged at 9,000×*g* for 30 min at 4°C to remove the tissue debris. The supernatant was removed and centrifuged at 105,000×*g* for 90 min at 4°C. The microsomes in the pellet were suspended in 100 mM phosphate buffer, pH 7.4, and stored at approximately −135°C.

PBMCs separated from whole blood (~8 ml) were collected in CPTs following the procedure provided with the tubes. Samples were centrifuged at 1,600×*g* for 25–30 min at room temperature within 2 h of collection. After centrifugation, the CPTs were inverted gently (5–10 times) to resuspend the separated PBMCs into the plasma and then stored at room temperature overnight. The PBMCs were washed twice with phosphate buffered saline (PBS) and were centrifuged at 300×*g* for 15 min at room temperature. The resulting supernatant was discarded without disturbing the cell pellet. The cell pellet was suspended in PBS containing 0.2% Triton X-100 (Sigma–Aldrich, St. Louis, MO) to form a lysate that was clarified by centrifuging at 10,000×*g* for 5 min. The PBMC lysates were stored at ≤−70°C. 20 μM ^{3}H-estrone sulfate [~300,000 disintegrations per min (dpm) per tube] at 37°C in 1-ml incubations with 100 mM phosphate buffer, pH 7.4 for 5 min with rat liver mcs and 20 min with dog liver mcs. The PBMCs (0.05 mg) were incubated with 3 nM ^{3}H-estrone sulfate (~300,000 dpm per tube) at 37°C in 1-ml incubations with 100 mM phosphate buffer, pH 7.4, for 20 h. These conditions were found to yield the optimum rate of enzyme activity. Control incubations included heat-inactivated microsomes (Hk-mcs) or Hk-PBMCs added to the 0-min incubation after 4 ml of toluene, with no protein samples incubated for the full time. The incubations were terminated by the addition of 4 ml of toluene along with an internal standard of approximately 10,000 dpm of ^{14}C-E. The samples were vortexed for 1 min and then centrifuged to separate the organic and aqueous layers. An aliquot of the toluene layer was added to scintillation vials with 10 ml of Ultima Gold (Perkin Elmer, Norwalk, CT). Radioactivity (^{3}H) in dpm was determined in each sample using a Tri-Carb 2900TR liquid scintillation counter (Beckman Coulter, Fullerton, CA). The extraction efficiencies ranged from 92.8 to 100.3%. ES activity was reported as nM/mg protein/min for rat or dog liver microsomes and fM/mg protein/h for dog PBMCs.

Statistical evaluations

Effects of dose on body weights, body weight gains, and clinical pathology data in rodents and dogs were evaluated by one-way analysis of variance followed by Dunnett's test comparing each treated group with the respective control group (null hypothesis rejection, $P < 0.5$).

Results

Rats

Single IV or po dose and pharmacokinetics

All male rats administered 5 mg/kg SR16157 iv had discolored red urine, which is considered to be associated with the adverse effect of dosing iv since the formulations were not red. A single male rat administered 50 mg/kg SR16157 po was observed to be slightly hypoactive. After iv administration of 5 mg/kg, the elimination $t_{1/2}$ was 8.6 h (Table 2). Cl was 1,035 ml/h/kg. The apparent volume of distribution (V) was 12.8 l/kg suggesting distribution to tissues. After a po dose of 50 mg/kg (300 mg/m^2), the C_{max} was achieved at about 4 h and was nearly 1.5 times higher in female rats (4.18 μM) than in males (2.51 μM) as summarized in Table 2. This difference was also observed in the AUC_{inf} values of 36.10 h μM (males) versus 60.08 h μM (females). The oral bioavailability of SR16157 in male rats was estimated to be 36% (Table 2). AUC by iv route was not determined in females so oral bioavailability was not calculated, but use of the AUC_{iv} from male rats suggests that bioavailability is high in female rats, ~60%.

Single oral gavage dose escalation range-finding with 14-day recovery

Based on bioavailability and no adverse oral dose effects during the single-dose study, experiments were continued using oral routes of administration with increased po dose levels. Female rats were administered a single po administration of 200, 800, or 1,000 mg/kg SR16157 (1 rat per dose level) with adverse clinical signs seen on days 3 through 7, including hypoactivity, convulsions, and death on day 3 for the rat treated with 1,000 mg/kg SR16157, and slight hypoactivity, ruffled fur, and hunched posture on days 7 through 9 for rats treated with 200, 600, or 800 mg/kg SR16157. Serum chemistry parameters that showed a slight

Table 2 Pharmacokinetic Parameters of SR16157 in rats

Parameter	Male[a]		Female[a]
	IV	po	po
	(5 mg/kg; 30 mg/mm^2)	(50 mg/kg; 300 mg/mm^2)	(50 mg/kg; 300 mg/mm^2)
$t_{1/2}$ (h)	8.60	5.43	7.94
T_{max} (h)[b]	0.17	4	4
C_{max} (μM)[b]	1.74	2.51	4.18
AUQ_{nf} (h μM)	9.80	36.10	60.08
Cl (ml/h/kg)	1,035	NC[c]	NC
V (l/kg)	12.8	NC	NC
Bioavailability		37%	NC[d]

[a] $n = 3$ rats per time point

[b] T_{max} and C_{max} for the iv groups represent time of first collection

[c] *NC* not calculated

[d] Pharmacokinetic parameters were not determined by the iv route in female rats; thus, bioavailability could not be calculated

dose-related decrease on day 3 included WBC and ALY, in rats administered 600, 800, and 1,000 mg/kg SR16157 (6.7–3.7 × 10^3/mm^3 and 72–83%, respectively) compared with controls (10.5 × 10^3/mm^3 and 83%, respectively). These values were comparable with control levels by day 7. Serum AST and ALT were elevated on day 3 (128 and 105 I/UL, respectively) in the animal administered 1,000 mg/kg SR16157 compared with the control rat (56 and 38 I/UL, respectively).

14-Day oral gavage repeat dose toxicity with 14-day recovery

Mortality was observed for rats treated with 200 (days 11 and 12) and 1,000 (days 4 and 5) mg/kg/day. Clinical observations for all treatment groups included chromodacryorrhea, red nasal discharge alopecia, hunched posture, and ruffled fur. All of these observations are associated with stress when seen in rats. Hematology increases for rats treated with 200 and 1,000 mg/kg/day SR16157 were seen in RBC (9.84 and 11.46 × 10^6/mm^3), HGB (17.4 and 20.4 g/dl), and HCT (153.6 and 62.2%) when compared with controls (8.51, 15.2, and 47.7, respectively). All SR16157-treated groups showed clinical chemistry increases in BUN (25 and 44 mg/dl), AST (88 and 282 IU/l), and ALT (135 and 233 IU/l) compared with controls (25, 81, and 39, respectively). Histopathology dose-related decreases were observed in circulating lymphocytes (57 and 100%) and lymphoid depletion in spleen (255 and 50% of rats), thymus (38% at 200 mg/kg/day), and regional lymph nodes (75 and 100%). Only slight changes noted above were seen for the 50 mg/kg/day group, which persisted to recovery. On the basis of these results, a high dose of 100 mg/kg was selected for the 28-day rat toxicity study.

28-Day oral gavage repeat dose toxicity and toxicokinetics with 14-day recovery

Mortality was observed in the 100 mg/kg/day group by day 12; the group was euthanized early on day 15. Food consumption for rats treated with 100 mg/kg/day exhibited a 38% decrease by day 2. Clinical observations for rats treated with SR16157 at 100 mg/kg/day included red nasal discharge, hunched posture, and ruffled fur starting in week 2; emaciation (these rats lost 22 g (∼20% of body weight) during the first week of dosing and an additional 10 g of body weight the second week of dosing for a total body weight loss of approximately 27% until their early scheduled necropsy on day 15), squinting, chromodacryorrhea, and vocalization, with 4 rats found dead before the day 15 necropsy. Clinical observations for rats treated with 33 and 10 mg/kg/day were similar, in addition to body weight loss for high-dose-treated rats, with recovery by day 38. Hematology increases were seen in ALY (12,558 and 11,236/mm^3), ANS (1,438 and 1534/mm^3), WBC (14.3 and 13.1 × 10^3/mm^3), and RET (7.6 and 6.9%) in all treated groups and persisted through the recovery period for the 10 and 33 mg/kg/day groups compared with controls (ALY: 8,451/mm^3; WBC: 9.5 × 10^3/mm^3; and RET: 5.4%). Clinical chemistry changes included increased ALT (67 and 76 IU/l) and decreased CHO (30 and 33 mg/dl) or TRI (41 and 35 mg/dl) for all treated groups compared with controls (ALT: 50 IU/l, CHO: 86 mg/dl, and TRI: 84 mg/dl); these changes persisted through the recovery period for the 33 and 10 mg/kg/day groups.

Plasma levels of SR16157 increased with dose level (Fig. 3), although the increases in C_{max} and AUC were not proportional to dose (Table 3). The $t_{1/2}$, ranging from 7.4 h (33 mg/kg/day, day 14) to 14 h (100 mg/kg/day, day 14), is long enough to suggest a potential for drug accumulation when administered daily. AUC values were higher on day 28 compared to day 14, confirming that the drug was incompletely cleared within 24 h. SR16137, the phenolic metabolite of SR16157, was readily detected in plasma samples from all dose groups and time points, with C_{max} values as high as 0.124 μM, approximately 4% of the C_{max} of the parent drug under the same conditions.

Histopathology findings included changes in liver (fatty increases in all treated groups and hyperplasia at 100 mg/kg/day), kidney nephrosis, atrophy in the thymus and all reproductive organs, and lymphoid depletion; CHO and TRI changes were most likely due to marked changes in body weight and food consumption of treated animals.

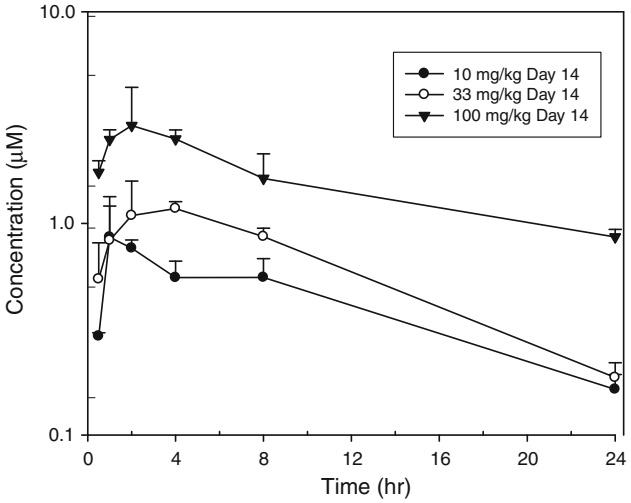

Fig. 3 Plasma concentrations of SR16157 in female rats after po administration of 10, 33, or 100 mg/kg/day for 14 days

Table 3 Toxicokinetic parameters of SR16157 and SR16137 in rats

Study day	Dose (mg/kg)	$t_{1/2}$ (h)	C_{max} (μM)		AUC_{last} (h μM)	
			Mean	SE	Mean	SE
SR16157						
14	10	10.6	0.86	0.27	10.46	0.78
	33	7.4	1.17	0.05	16.19	0.71
	100[a]	14.0	2.89	0.86	37.76	3.16
28	10	7.6	0.97	0.12	13.96	1.12
	33	NC[b]	2.67	0.04	41.41	2.22
SR16137						
14	10	40.5	0.013	0.003	0.197	0.004
	33	31.1	0.020	0.007	0.332	0.027
	100[a]	50.7	0.124	0.031	1.823	0.171
28	10	10.8	0.020	0.002	0.335	0.014
	33	11.0	0.055	0.012	0.909	0.049

[a] All rats in the 100 mg/kg group were euthanized on day 15 due to toxicity

[b] *NC* not calculated because there were insufficient data in the terminal phase

Dogs

Single-dose IV toxicity and pharmacokinetics

For the iv study, all animals survived until scheduled killing, except for one dog that was initially administered 20 mg/kg iv; that animal evidenced adverse clinical signs (retching, twitching, cyanosis) and died within minutes post-dose. The dose level was decreased to 2 mg/kg in an additional dog with no adverse affects observed. Following iv administration of 2 mg/kg (40 mg/m^2), the $t_{1/2}$ for SR16157 was 5.91 h. The apparent volume of distribution (V) was 32.5 l/kg, suggesting tissue binding of the drug, and the Cl was 3,214 ml/h/kg (Table 4).

Single-dose oral toxicity and pharmacokinetics

Clinical signs observed appeared to be dose-related with emesis, diarrhea, dehydration, or tremors occurring in the 200 mg/kg po and capsule groups. ANS (2.4-fold over controls) and WBC (2.0-fold over controls) were elevated on day 1 in the 200 mg/kg groups, especially in the 200 mg/kg capsule group (5,720/mm^3 and 8.8×10^3/mm^3, respectively); however, the pre-study values were also elevated in those animals. WBC and ANS values returned to control levels by day 3. AST (193 IU/l) and ALT(215 IU/l) showed marked elevations in the 200 mg/kg gavage group (6.2- and 6.7-fold) on day 1 relative to control values (31 and 32 IU/l, respectively). Similar increases were not observed in the 200 mg/kg capsule group. Levels returned to control and pre-study values by day 3.

Oral administration of 20 or 200 mg/kg (400 or 4,000 mg/m^2) as neat drug in capsules resulted in markedly higher SR16157 plasma levels compared with po administration as a suspension in 0.5% HPMC. The C_{max} and AUC were both higher (three- to fivefold), and capsule administration gave essentially 100% oral bioavailability compared with about 30–40% bioavailability with the gavage dose. There was a trend toward T_{max} at a later time when administered by capsules (4 h) compared with gavage (2 h), suggesting that absorption of SR16157 by dogs after capsule administration is more prolonged than after gavage, allowing the AUC to reach higher values and improving oral bioavailability (Table 4).

7-Day capsule repeat dose toxicity

SR16157 was administered orally via gelatin capsule daily for 7 days with a single dog per dose group. The dose level for Groups 2 and 3 were escalated to 25 and 50 mg/kg/day, respectively, for an additional 7 days, after a washout period of 13 days. The Group 1 control was dosed for 7 additional days with an empty capsule to provide a control for the dose-escalated Groups 2 and 3.

All animals survived until scheduled sacrifice. Clinical signs observed included soft stool, emesis, and diarrhea but were intermittent and did not appear to be dose-related. Reticulocyte counts were higher on day 3 (2.6% RBC) and day 8 (2.1% RBC) for the dog administered 50 mg/kg/day SR16157 compared with the control (0.6 and 0.4% RBC, respectively). All clinical chemistry parameters were comparable with the control dog. Body weight gain was very slightly depressed in SR16157-treated dogs, but this effect was very small and not clearly dose-related. No gross necropsy or histopathology findings were associated with the

Table 4 Pharmacokinetic parameters of SR16157 in beagle dogs

Parameter	iv (2 mg/kg; 40 mg/mm^2)	Gavage (20 mg/kg; 400 mg/mm^2)	Capsule (20 mg/kg; 400 mg/mm^2)	Gavage (200 mg/kg; 4,000 mg/mm^2)	Capsule (200 mg/kg; 4,000 mg/mm^2)
$t_{1/2}$ (h)	5.91	7.97	5.66	11.13	6.79
T_{max} (h)[a]	0.17	2	4	2	4
C_{max} (μM)[a]	0.26	0.50	1.21	1.93	14.96
AUQ_{nf} (h μM)	1.06	2.89	14.10	40.84	122.66
Cl (ml/h/kg)	3,836	NC[b]	NC	NC	NC
V (l/kg)	32.7	NC	NC	NC	NC
Bioavailability		27%	133%	39%	116%

$n = 1$ dog

[a] T_{max} and C_{max} for the IV groups represent the first collected time point

[b] *NC* not calculated

test article. Ovary weights were much lower (~50%) in the dog treated with 10 (0.76 g) or 50 mg/kg/day (0.72 g) than in the control (1.38 g), but not for the dog treated with 25 mg/kg/day (1.71 g).

28-Day oral gavage repeat dose toxicity and TK with 14-day recovery

Because of the significant toxicity observed in the rat 28-day toxicity study, doses for the study in dogs were lowered to 0.5, 2.5, and 10 mg/kg/day (10, 50, and 200 mg/m^2, respectively). Various significant but reversible changes relating to WBC were observed in SR16157-treated dogs at day 29, including reduced WBC (8.80×10^3/mm^3), ALY (3.03×1000/UL), PEO (2.6%), AEO (0.23×1000/UL), and AMO (0.42×1000/UL) compared with controls (WBC: 12.73×10^3/mm^3, ALY: 3.99×1000/UL, PEO: 3.8%, AEO: 0.46, and AMO: 0.69×1000/UL). Clinical observations, clinical chemistry, ophthalmology, cardiology, and histopathology revealed no treatment-related findings. Body weights were noted to be slightly lower for high-dose-treated dogs (7.6 kg) compared with controls (7.8 kg), which persisted through the recovery period. Food consumption at day 29, although not significantly different among groups, was slightly lower in the high-dose group (2.74 g) compared with controls (296 g), which persisted through the recovery period of day 42 (289 and 247 g, respectively). Reversible hematology changes, including those in WBC (12.01×10^3/mm^3), ALY (3.58×1000/UL), and AEO (0.48×1000/UL), were observed in high-dose-treated dogs. Mean thymus weights for high-dose dogs decreased 30% (8.9 g) compared with controls (12.8) at day 29 and remained 20% lower at day 42 recovery (13.6 and 18.1 g, respectively). Microscopic examination of all tissues revealed no drug-related findings.

SR16157 plasma concentrations were higher than the lower limit of quantitation (LLOQ; 2 ng/ml) in only one dog in the low-dose group (dog 7 on day 28); however, all dogs on both sampling days (day 1 and 28) had quantifiable plasma drug concentrations in the 2.5 and 10 mg/kg/day dose groups. Exposure to SR16157 tended to increase with dose level, as indicated by C_{max} and AUC (Table 5). SR16137 was detected in plasma samples that contained higher concentrations of the parent drug; levels of the phenolic metabolite were as high as 20 nM in the high-dose dogs (data not shown).

Table 5 Toxicokinetics of SR16157 in Dogs

Study day	Dose (mg/kg)	C_{max} (nM)		AUC_{last} (h nM)	
		Mean	SD	Mean	SD
1	0.05[a]	<LLOQ		<LLOQ	
	2.5	41.2	17.6	359.9	212.9
	10	363.1	296.6	3,286.7	2,389.9
28	0.05[a]	13.9		121.1	
	2.5	61.3	39.0	529.4	584.9
	10	94.4	98.4	1,102.9	1,109.1

[a] SR16157 was <LLOQ in all dogs on day 1 and in 2 of 3 on day 28

Biomarker results

Hepatic STS levels in the initial rat and dog studies showed significant inhibition for all treated groups.

In the 14-day toxicity study in rats, STS activity was significantly inhibited in the livers of all SR16157 treatment groups. The activity in control animals was 2.08 ± 1.12 nmol estrone/mg protein/min. In SR16157-treated rats, STS activity was below the level of detection in all groups. In the 28-day toxicity study in rats, hepatic STS activity was also completely inhibited after 28 days of administration of 10 or 33 mg/kg/day SR16157 and after 14 days of administration of 100 mg/kg/day SR16157. After cessation of SR16157 treatment for 14 days, STS activity had recovered to only 23, 7, and 4% of control for rats in the 10, 33, and 100 mg/kg/day dose groups, respectively.

In the single-dose toxicity study in dogs, hepatic STS activity was not detectable by 6 h post-dose for all treated

groups. In the 28-day toxicity study in dogs, hepatic STS activity was completely inhibited by day 29 at the 0.5, 2.5, and 10 mg/kg/day levels. As seen in the rat, enzyme activity was partially regained only in an inversely proportional manner to dose for all treated groups by day 42 (Fig. 4a). PBMC STS was completely inhibited by day 6 in the 28-day study in dogs, with very little recovery of activity on day 29, 1 day after SR16157 administration was discontinued. STS activity increased in the recovery group by day 42, in an inversely proportional manner to dose (Fig. 4b).

Discussion

SR16157 (NSC 732011) is a novel dual-acting STS inhibitor and SERM. SR16157's advantage is that it is an irreversible sulfatase inhibitor designed on a tissue-selective antiestrogen SERM platform and is metabolized to release the SERM, thus providing a dual-targeted inhibition of estrogen action. SR16157 has demonstrated in vivo antitumor activity in MCF-7 breast cancer xenografts [9]. The tissue-selective effects of the precursor SERM SR16137 in the breast, uterus, bone, and cardiovascular system have also been demonstrated in animal models [10].

SR16157 exhibits pharmacokinetic parameters consistent with the profile of an oral drug that can be administered once per day. Oral bioavailability was high in both rats and dogs, without the need for developing a specialized formulation. Oral bioavailability in male rats (50 mg/kg; 300 mg/m^2) was 37% (gavage) and in female dogs (20 mg/kg; 400 mg/m^2) 27% after gavage or ~100% after capsule administration. SR16137, the SERM metabolite that is released when SR16157 interacts with estrone sulfatase, was readily detected in plasma samples. Plasma concentrations were observed that exceeded 100 nM in rats and 20 nM in dogs; the local concentration in target tissues is likely to be higher than in plasma and is expected to be sufficient to exert the dual action that is hypothesized for this drug.

Table 6 summarizes the maximum-tolerated dose (MTD), no observable adverse effect level (NOAEL), and target organs of toxicity for rats and dogs. From the 28-day repeat dose studies, the MTD for rats and dogs was determined to be approximately 200 mg/m^2. Significant target organ toxicity was observed in rats, including liver hyperplasia, kidney nephrosis, atrophy in the thymus (Fig. 5a) and all reproductive organs, and lymphoid depletion. No significant target organ effects were seen in dogs. The high dose used in the 28-day study in rats (100 mg/kg/day), in retrospect, was too high, and this group was euthanized on day 14. The effects seen at that dose level therefore represent toxicity at a dose greater than the MTD, which has little relevance to clinical exposure. For dogs treated with

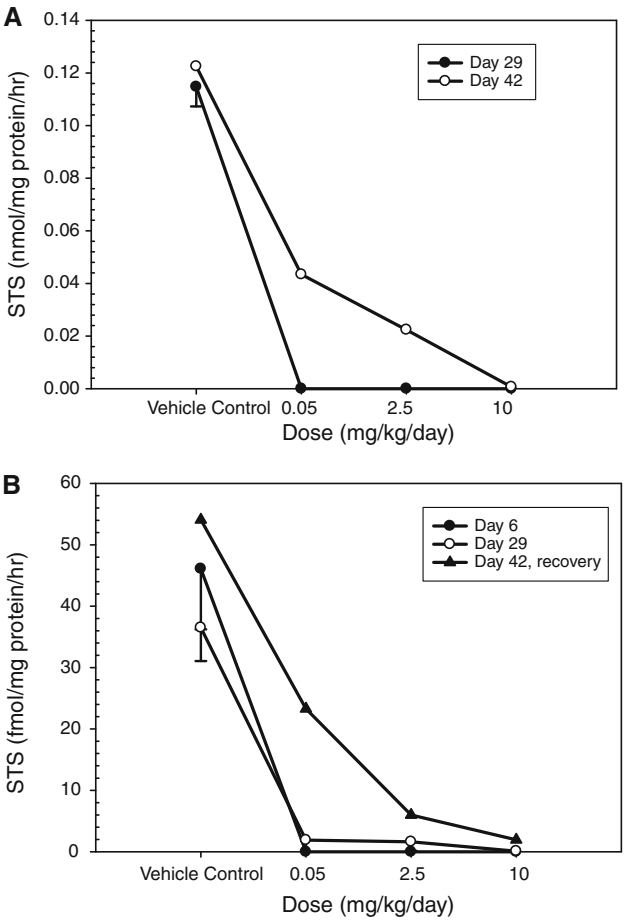

Fig. 4 STS activity in beagle dog liver (**a**) and in beagle dog PBMC (**b**) on study day 6 (PBMC) or 29 (liver and PBMC) of capsule administration followed by a 14-day recovery period on day 42. Values represent the mean ± SD of $n = 3$ dogs for days 6 and 28. The recovery group data (day 42) are the mean of $n = 2$ dogs

SR16157 daily for 28 days, minimal adverse effects were seen. Those effects included reversible changes relating to WBC, with reduced WBC, ALY, PEO, AEO, and AMO. Mean thymus weights for high-dose dogs decreased 30% compared with controls at necropsy on day 29 and remained 20% lower at necropsy on day 42 (Fig. 5b). Similar effects on the thymus were also observed in earlier dose range–finding studies in both rats and dogs. Although no histopathologic changes were seen in the dog thymus, decreased mean thymus weights, combined with clinical pathology changes, indicate possible steroidal effects of SR16157. Given the expected pharmacology of this drug on its intended patients, this effect on the thymus is not considered to be dose-limiting for human clinical trials.

SR16157 was highly effective at inhibiting STS activity in rats and dogs in all studies in which it was evaluated. This inhibitory effect clearly takes place very rapidly, given that dog hepatic STS was completely inhibited in SR16157-treated groups 6 h after the first dose administration. In 28-day

Table 6 Summary of major findings

Species	Dosing frequency	MTD mg/kg (mg/m^2)	NOAEL	Primary target organs
Rat	14-day repeat (po)	>50 mg/kg/day and <200 mg/kg/day (300 mg/m^2 and 1,200 mg/m^2)	Not achieved	Mandibular and mesenteric lymph nodes, spleen, thymus
	28-day repeat (po)	33 mg/kg/day (198 mg/m^2)	<10 mg/kg/day (60 mg/m^2)	Liver, kidney, thymus, adrenal glands
Dog	Single dose (iv, po, or oral capsule)	200 mg/kg (1,200 mg/m^2)	20 mg/kg (400 mg/m^2)	Gross necropsy only; no gross findings
	7-day repeat (oral capsule)	≥50 mg/kg/day (300 mg/m^2)	5 mg/kg/day (100 mg/m^2)	None
	28-day repeat (oral capsule)	≥10 mg/kg/day (200 mg/m^2)	≥2.5 mg/kg/day (50 mg/m^2)	Thymus

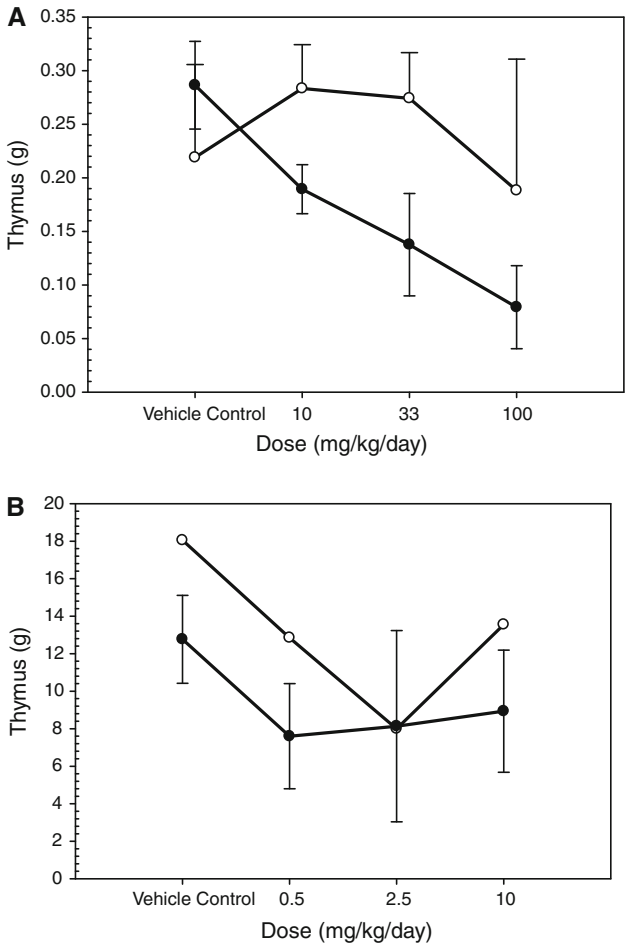

Fig. 5 Mean (±SD) rat thymus weights after 28 days of po administration at 10 or 33 mg/kg/day and after 14 days at 100 mg/kg/day (**a**) and for dogs administered 0.5, 2.5, or 10 mg/kg/day by capsule (**b**)

toxicity studies, all dose groups showed essentially complete inhibition of STS activity throughout the duration of the treatment period. STS activity was only partially restored 14 days after cessation of treatment, confirming that SR16157 causes irreversible inhibition of STS.

Although initial studies of STS activity were conducted using liver specimens, collection of liver samples is not feasible for clinical studies. We therefore evaluated the use of PBMC as a surrogate biomarker for STS activity. Results with PBMC were similar to those for the liver, with significant inhibition of STS activity. PBMC should therefore serve as an excellent biomarker of pharmacologic activity of SR16157 in human clinical trials.

In conclusion, our studies demonstrate that SR16157 has excellent pharmacokinetic properties and an acceptable toxicological profile. The activity of the drug may be monitored in human clinical trials by assessing the surrogate biomarker PBMC for STS activity. Based on the results of these studies, we recommend an initial phase I clinical starting dose of 50 mg/m^2 (~1.3 mg/kg/day) administered as a single dose in humans. Based on the NOAEL determined in dogs, and the relative lack of toxicity in rats in single-dose studies, this appears to be a reasonable starting dose when combined with the estrone sulfatase biomarker for the assessment of human effects.

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