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Plasma pharmacokinetics of the indenoisoquinoline topoisomerase I inhibitor, NSC 743400, in rats and dogs

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Abstract

Purpose—NSC 743400 is a novel synthetic indenoisoquinoline analog under development as an anticancer agent. It is a potent topoisomerase I inhibitor with potential therapeutic advantages over FDA-approved camptothecin derivatives. In preparation for clinical development of NSC 743400, we determined the pharmacokinetics after administration to rats and dogs.

Methods—NSC 743400 was administered intravenously at a dose of 12 or 24 mg/m² to rats (single bolus) or 10, 50, 100, 215, 430, or 646 mg/m² (intravenous infusion) or 860 or 1720 mg/m² (orally) to dogs.

Results—Intravenously administered NSC 743400 was eliminated from both species with an estimated $t_{1/2}$ of 2–5 h in rat and 6–14 h in dog. Elimination $t_{1/2}$ increased with dose in dog. Area

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Ethical Standards: “all human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments

under the plasma concentration-versus-time curve (AUC) was comparable in both species, at about 300-400 h•ng/mL for the approximately 10 mg/m² dose groups. Overall, AUC values increased proportionally with dose for both species but had evidence of more than proportional exposure at the highest doses. Oral dosing resulted in variable drug absorption.

Conclusions—The pharmacokinetic data were used to plan first-in-human clinical trials.

Keywords

NSC 743400; indenoisoquinolines; topoisomerase I; pharmacokinetics; anticancer; rat; dog

Introduction

Topoisomerase I (Topo I) is a recognized target for ovarian, lung, and colorectal cancer therapy [1]. The FDA-approved camptothecin (CPT) Topo I inhibitors, topotecan and irinotecan, suffer from lactone instability and rapid reversibility of the Topo I cleavage complexes that they induce [2]. To circumvent these limitations, indenoisoquinoline derivatives have been developed as an alternative class of agents and tested for their ability to inhibit Topo I and tumor growth [2]. NSC 743400 (Fig 1), which is one of these novel indenoisoquinoline Topo I inhibitors, has been advanced to clinical evaluation.

In vitro, NSC 724998 (the free base of NSC 743400) produces Topo I cleavage at unique genomic positions compared with those resulting from CPT treatment. NSC 743400 causes cell cycle arrest in both S and G(2)-M. As with other known Topo I inhibitors, resistance to NSC 724998 was shown in cells deficient for Topo I [2].

The protein-linked DNA breaks characteristic for Topo I poisons were detected in cells treated with nanomolar concentrations of NSC 743400. These Topo I cleavage complexes persisted longer after removal of drug (1 μM) when induced by NSC 743400 compared with those induced by CPT or SN-38 (the active metabolite of irinotecan) [2]. NSC 743400 displayed 72 h IC₅₀ values of 0.3, 0.56, and 1.2 μM against P388, MCF-7, and HCT-118 cells, respectively [2]. In addition to its effects on Topo I, NSC 743400 may also exert part of its antitumor effect through antiangiogenesis [3]. Whereas camptothecin, topotecan and SN-38 are substrates for the efflux pump ABCG2, and related indenoisoquinoline analogues are substrates for both ABCG2 and MDR1, NSC 743400 is not a substrate for either transporter [2], implying that overexpression of these pumps will not be a likely mechanism of resistance. Because of these favorable pharmacological characteristics, NSC 743400 is a promising anticancer drug candidate.

To guide the clinical development of NSC 743400, preclinical investigations were performed to characterize the pharmacokinetics of this compound in rats and dogs. To this end, LC-MS/MS assays were developed and used to quantitate concentrations of NSC 743400 in the plasma of rats and dogs given single doses of NSC 743400.

Material and Methods

Test Article and Formulation Vehicles

NSC 743400 was obtained through the Developmental Therapeutics Program, National Cancer Institute (HPLC purity 96.9%). Two formulation vehicles were used for intravenous administration. For the preparation in vehicle 1, a 10 mg/mL stock formulation of the agent was prepared in 10 mM citric acid (Fisher Scientific, Fairlawn, NJ) before dilution in 5% dextrose in water (D5W; Hospira, Inc., Austin, TX) for dosing. For vehicle 2, a 2 mg/mL pre-formulated preparation of the agent in water for injection (WFI, pH adjusted to 2.7 with HCl) was diluted in D5W for dosing. Pharmacokinetic studies of oral NSC 743400 in dogs utilized gelatin capsules (Size #12, Torpac, Inc., Fairfield, NJ) containing only test article.

Protein binding

Protein binding was assessed in dog plasma and tissue culture medium with 10% fetal bovine serum, which contained 4,300 and 10,760 ng/mL of NSC 743400. After Centrifree micropartition devices (Millipore, Bedford, MA) with a 30,000 D molecular weight cut-off, proved unacceptable due to non-specific adsorption, protein binding was assessed by rapid equilibrium dialysis with Pierce RED devices (Thermo Fisher Scientific, Rockford, IL) with an 8,000 D molecular weight cut-off. After an incubation period of 24 h at 37 °C against phosphate buffered saline, samples were diluted in control dog plasma and analyzed as detailed below. Experiments were performed in replicates of 4.

Animals

Pilot studies were performed in dogs, followed by confirmatory studies in rats and dogs. All animals were housed in accordance with standards established in *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and by the U.S. Department of Agriculture (USDA) in the Animal Welfare Act (Public Law 99-198). Animal rooms were held within a temperature range of approximately 17-24°C and a relative humidity range of approximately 30-70%. Fluorescent lighting was set to provide 12 hour light-dark cycles. Animals were held in quarantine for approximately 2 weeks prior to dosing, during which time they were observed daily for survival and general health. Prior to randomization and group assignment a physical examination was performed on each animal to ensure its health and suitability as a test subject.

Fischer 344 rats (approximately 8-10 weeks of age; Charles River Laboratories, Inc., Kingston, NY) were individually housed in cages with automatic watering systems. Certified rodent diet was available *ad libitum* throughout the study.

Naïve purebred beagle dogs (approximately 8 to 16 months of age; Ridgman Farms Inc., Mt. Horeb, WI) were individually housed in pens equipped with automatic watering systems *ad libitum*. Dogs had access to a quantity of certified canine diet sufficient to meet nutritional requirements for at least two hours per day. For pilot studies, female beagle dogs (8 to 16 months of age; Bridge Global Pharmaceutical Services, Inc.) were acclimated to laboratory conditions for at least 2 days prior to the first dose.

Study Design and Dosage

Two groups of male and female Fischer 344 rats (12 animals per sex per group) were administered a single intravenous dose of NSC 743400 at dose levels of 12 or 24 mg/m². All doses were administered as a slow bolus via tail vein in vehicle 1 at a dosing volume of 2 mL/kg of body weight. On dosing day, rats were observed for clinical signs of toxicity approximately 1-2 h after dosing and daily thereafter until study termination. In addition, animals were observed twice daily (at least six hours apart) for moribundity/mortality. All rats were euthanized on day 6 (females) or 7 (males) and the carcasses were discarded without necropsy. There were no control or vehicle treated rats in this study.

Pilot dog studies (2 non-fasted female dogs per dose level) were performed by administration of NSC 743400 as a 1-h intravenous infusion at doses of 215, 430, and 646 mg/m² or as a single oral dose of 860 and 1720 mg/m² in gelatin capsules. For the confirmatory study, male and female dogs (3 animals per sex) were part of three experimental groups, with a minimum of 7 days in between consecutive experiments to ensure the washout of the formulation and its effects. These animals were administered a single 1-h intravenous infusion of NSC 743400 prepared at three dose levels and in two different vehicles as follows: doses of 10, 50 or 100 mg/m² formulated in vehicle 1 or a dose of 50 mg/m² in vehicle 2. Doses were administered via a catheter placed in the right saphenous vein at a dosing volume of 2.5 mL/kg of body weight. Dogs were monitored closely during dosing and up to approximately two hours after dosing, and once daily for three days post-infusion. Additionally, during the treatment period, animals were observed twice daily (at least six hours apart) for moribundity/mortality. Neither dog study included concurrent controls, as such, each dog served as its own control with comparisons being made before and after dosing with NSC 743200.

Rationale for Species and Dose Selection

Rats and dogs were used in this study as these species were used for the toxicology studies. These are standard and acceptable species to evaluate safety of test articles prior to their administration in human clinical trials. The doses used for pharmacokinetic studies were based on the results from range-finding and definitive toxicology studies using the same dosing schedule as the proposed clinical trial. In the rat studies, doses of 48 and 60 mg/m², administered once a day for 5 consecutive days, produced severe weight losses of 13-20%, relative to the control animals. The definitive study also revealed a marked decrease in neutrophils (up to 80% decrease, relative to controls) at 48 mg/m². Hence, the maximum tolerated dose (MTD) for this drug was 24 mg/m²/d for rats when given as a slow iv bolus once a day for 5-consecutive days. We therefore used 24 mg/m²/d as the highest rat dose, and a lower dose expected to be at or close to the no observable adverse effect level (NOAEL). The 50 mg/m² dose was chosen for the confirmatory study in dogs because this was the MTD established for dogs in the definitive toxicology study when NSC 743400 was given as a 1-hr iv infusion once a day for 5-consecutive days. The 10 mg/m² dose was included as it was expected to be at or close to the NOAEL. Data from the preliminary studies in dogs with intravenous doses that exceeded the MTD were included as they provided insight into the plasma concentrations associated with the adverse effects. Early preliminary toxicology studies with oral administration of NSC-724998 (the free base of

NSC 743400) to dogs required increasing the dose stepwise until an MTD was observed ($<1600 \text{ mg/m}^2$). As such, this dose was chosen as the high oral dose, and 800 mg/m^2 was chosen as it was expected to be at or close to the NOAEL. The clinical starting dose for NSC 743400 (2.5 mg/m^2) was set at 1/10 of the maximum tolerated dose in the more sensitive species (rat).

Sample Collection

Blood samples for plasma drug level determination were collected from three rats per sex per group via retro-orbital sinus puncture before, and at the following times after dosing: 2, 10, 15, 30 and 60 min and 2, 4, 8, 12, 24, 48 and 72 h and anticoagulated with EDTA. No rat was bled more than three times over a 24-h period.

For the pilot dog study, samples for plasma drug quantitation were collected before, and at 2, 5, 10, 30 min, 1, 2, 4, 8, 12, 24, 28, 32, 36, and 48 h after the end of infusion from each individual dog and processed as described below. Blood samples were also collected for hematology, clinical chemistry and coagulation parameters prior to dosing, and 3, 8 and 15 days post dosing for the pilot dog study. For the confirmatory dog study, samples for plasma drug quantitation were collected from each animal via the jugular or cephalic vein before and at 2 min before the end of the 1-h infusion, and at 5, 10, 15, 30 and 60 min, and 2, 4, 8, 12, 24, 48 and 72 h post-infusion. The EDTA-anticoagulated samples were on ice until plasma preparation, which was stored at -70°C .

Analytical Methods

For NSC 743400 quantitation in rat plasma, a $50 \mu\text{L}$ aliquot was mixed with 0.25 mL of acetonitrile containing 12.5 ng of $[\text{D}_8]$ -NSC 743400 (internal standard, IS). After vortex-mixing for one minute, the sample was centrifuged at approximately 4°C and $8000 \times g$ for 10 min to remove precipitated proteins. The supernatant was diluted with 0.5 mL of water followed by injection.

For NSC 743400 quantitation in dog plasma, a $100 \mu\text{L}$ aliquot was mixed with 1 mL of acetonitrile containing 25 ng of $[\text{D}_8]$ -NSC 743400 IS, followed by centrifuging. The supernatant was transferred to a clean tube and dried under nitrogen flow at room temperature. The dried residue was reconstituted in 0.5 mL of ACN/water/formic acid (20/80/0.1, v/v/v), vortex-mixed and centrifuged again, followed by injection.

Calibration samples were prepared in control rat or dog plasma (Bioreclamation Inc., Westbury, NY) at 1, 2, 5, 10, 50, 100, 500 and 1000 ng/mL , with QC samples at 2.4, 400 and 800 ng/mL . The chromatographic system consisted of a Agilent 1200 HPLC system (Agilent Technologies, Wilmington, DE) with Synergi Polar-RP column ($80\text{\AA}$, $30 \times 2.0 \text{ mm}$, $4 \mu\text{m}$, Phenomenex, Torrance, CA) maintained at 25°C and perfused at a flow rate of 0.30 mL/min . The mobile phase consisted of solvent A: formic acid in water (0.1%, v/v) and Solvent B: formic acid in ACN (0.1%, v/v). The mobile phase gradient consisted of a number of step gradients: after $5 \mu\text{L}$ sample injection, initial conditions with solvent B at 35% were held for 0.01 min, increased to 55% and held constant for 3 min, increased again to 95% and held constant for 3 min, returning to initial conditions for 3 min of re-equilibration. The retention time of NSC 743400 was approximately 1.6 min ($k=0.6$). Mass

spectrometric detection was performed with an API 3000 mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) in the multiple reaction monitoring (MRM) positive ion mode. MRM transitions were m/z 479.3 to 392.2 for NSC 743400 and m/z 487.3 to 392.2 for [D₈]-NSC 743400. Ion spray voltage was -4200 V, ion source temperature was 450 °C, and collision energy was 28 V.

Method validations in rat and dog plasma were performed based on FDA guidance [4], and included assessment of: selectivity, linearity, precision, accuracy, recovery and stability. The accuracy of individual calibrators was in the range of 90 to 110%. Between-run precision for the back-calculated calibrator concentrations was less than 10% CV. Analysis of replicate QC samples resulted in within-run and between-run precision <6% CV and accuracy within 10% of the true value. The agent stability in plasma samples was demonstrated at -70 °C for 1 month; after three freeze-thaw cycles; on bench-top at ambient temperature for 24 h, and in processed samples stored in an autosampler at 4 °C for 24 h.

A similar LC-MS/MS method ranging from 1-500 ng/mL was used to quantify NSC 743400 in dog plasma samples from the pilot studies, with the following substantive differences: 1) standard curves were prepared with NSC 724998 (the free base of NSC 743400), with appropriate corrections for molecular weight; 2) the internal standard was the indenoisoquinoline analog NSC 725776; 3) ethyl acetate was used for sample extraction; 4) the same mobile phases were pumped through a 100 mm column using a modified gradient with a 20 min run time and a 3.9 min retention time ($k=5$) for NSC 743400. Based on replicate QC samples in multiple runs, the accuracy was 98.9 – 101%, and the precision was <6% CV. Any samples with results above the upper limit of quantitation were diluted to within the calibration range with control plasma.

Pharmacokinetic and Statistical Analysis

For rats, pharmacokinetic analysis was performed on average plasma NSC 743400 concentration data with the non-compartmental model for IV-bolus (WinNonlin Professional Edition version 4.1, Pharsight Inc., Mountain View, CA). Area under the plasma concentration-time curve (AUC) from time zero to the last measured concentration (AUC_{0-t}) was estimated by the log trapezoidal rule. AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated using the elimination rate and the last measureable plasma concentration. All PK analyses for individual dogs were done non-compartmentally with WinNonlin, with the agent concentration at the time point of 2 min before the end of the infusion taken as the 0 minute post-infusion result. Pilot dog pharmacokinetic data were analyzed non-compartmentally with PK Solutions 2.0 (Summit Research Services, Montrose, CO).

Statistical analyses (for dogs only) were performed for $t_{1/2}$ (elimination half-life), C_{max} (maximum observed concentration), $AUC_{0-\infty}$, V_z (volume of distribution) and CL (total body clearance) using log-transformed PK parameter data (with the exception of $t_{1/2}$ and T_{max}). For C_{max} and AUC, the data were normalized to the body surface area dose (i.e., mg NSC 743400/m²) 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 ($p < 0.05$).

Results

Acute Toxicities

All rats were described as normal after dosing and during the 6-7 day post dosing observation period.

In the pilot dog study, animals receiving NSC 743400 at 215 or 430 mg/m² survived until study termination. Those receiving 646 mg/m² became moribund on Day 5 and were euthanized the same day. Prior to euthanasia, this dose produced urticaria, and erythema and swelling around the neck, mouth and ears during dosing, and soft and/or mucoid feces, limping on forelimb and hypoactivity between Days 4 and 5. Pancytopenia, hepatocellular injury and renal dysfunction were evident immediately prior to euthanasia based on clinical pathology analysis. Gross pathology observations at necropsy included blood-like fluid in loose stool in the gastrointestinal (GI) tract. Microscopic examination revealed epithelial atrophy and crypt necrosis in the GI tract, and centrilobular hepatocyte vacuolation in the liver. Dogs receiving NSC 743400 at 215-430 mg/m² had milder responses consisting of erythema and edema of the face which presented during or shortly after dosing. The facial swelling and erythema resolved within 2 h post dosing without intervention, and within 15 min of giving diphenhydramine. Other toxicities observed in these animals included swelling at injection site, GI effects (emesis, diarrhea), hypoactivity, and appetite loss. Pancytopenia and hepatocellular damage were also noteworthy at 430 mg/m², based on clinical chemistry analysis. Since these animals survived and were returned to the stock colony, pathology data were not available.

Oral doses also produced GI effects. Soft/mucoid feces was observed at both oral dose levels, whereas diarrhea, emesis and appetite loss were limited to the 1720 mg/m² dose. It is noteworthy that emesis occurred in both animals and on multiple days, however emesis started 2 hours post dosing for one dog, which survived, and on day 2 for the second dog which became moribund on Day 5 and was euthanized the same day. There were no abnormal gross pathology observations for the animal that was euthanized, however, microscopic evaluation of tissues revealed epithelial hyperplasia with atrophy and crypt necrosis in the small intestine, congestion of spleen and thymic involution.

Dogs in the lower-dose cohorts (10-50 mg/m²) of the confirmatory study exhibited no adverse reaction during or after intravenous infusion. One of the 6 dogs dosed at 100 mg/m² had facial swelling during dosing which resolved within 2 h post dosing. No animals died or were sacrificed moribund during the confirmatory studies.

Pharmacokinetics of NSC 743400 in Rats

Plasma NSC 743400 concentration-time profiles following intravenous dosing of 12 and 24 mg/m² to male and female in rats are presented in Figure 2 and associated pharmacokinetic parameters are presented in Table 1. After intravenous bolus administration to rats, NSC

743400 was slowly eliminated, with an estimated $t_{1/2}$ of 2.1 and 3.6 h for males and longer half-lives of 4.2 and 4.6 h for females for the 12 (low) and 24 mg/m² (high) dose groups, respectively. Clearance was higher in males at 51-56 L/hr/m² (males) compared with 29 L/hr/m² (females).

Pharmacokinetics of NSC 743400 in Dogs

Plasma NSC 743400 concentration-time profiles following intravenous dosing to dogs are presented in Figure 3, and pharmacokinetic parameters are provided in Table 2. In the confirmatory studies, NSC 743400 was eliminated relatively slowly, as reflected in the estimated $t_{1/2}$ values ranging between 5.9-14 h, after an initial rapid tissue distribution phase. The increases observed for $t_{1/2}$ for the 100 mg/m² group when compared to the 10 and 50 mg/m² groups were statistically significant, and this trend was continued at the 215-646 mg/m² dose levels studied in the pilot groups. Clearance values varied between groups, between 28-58 L/h/m² in the confirmatory studies, but not as much as $t_{1/2}$ and trended to lower values at higher doses. Peak concentrations (C_{max}) of NSC 743400 increased with dose, but again in a less than proportional manner. No statistically significant differences were observed due to gender.

Plasma profiles after oral administration were very variable without apparent increase of exposure with increasing dose administered (Table 2 and Figure 4). Comparison with the exposures after IV NSC 743400 suggest a variable oral bioavailability of 5-40%.

For the comparison between the groups with the same dose (50 mg/m²) but different vehicles (vehicle 1 vs. vehicle 2), only the changes observed for C_{max} were statistically different, though quantitatively small.

Protein binding in dog plasma and tissue culture medium was 96.1-97.7% and 93.2-98.5%, respectively (Table 3).

Discussion

The present study was performed to characterize the pharmacology of the novel indenoisoquinoline NSC 743400 in rats and dogs, in preparation for first-in-human clinical trials.

The NSC 743400 plasma concentrations achieved in rats and dogs at the doses used are comparable to the IC₅₀ values determined *in vitro*. NSC 743400 displayed 72 h IC₅₀ values of 0.3, 0.56, and 1.2 μM against P388, MCF-7, and HCT-116 cells, respectively [2], which correspond to 154, 288, and 618 ng/mL. In rats, and in the highest doses of the dogs, these concentrations were achieved in plasma. Protein binding data suggest that the free fraction in media is only up to double the free fraction in dog plasma. Therefore, the observed plasma concentrations are pharmacologically relevant, in line with the observations of toxicity in the 215-646 mg/m² dosed dogs. Assessment of protein binding in human plasma has been reported to be in the same range as the binding in dog plasma, at more than 98% [5].

At the 12 and 10 mg/m² dose groups in rats and dogs, respectively, AUCs were comparable in both species, at approximately 230-400 h•ng/mL with CL values in the 30-50 L/h/m² range. Male rats appeared to have double the clearance of female rats, resulting in lower plasma AUC values in males compared to females. This is consistent with the sexually dimorphic expression of liver cytochromes P450 in rodents [6,7].

The observed apparent non-linear behavior of NSC 743400 should be taken into account when conducting dose escalation studies in humans, and has led to real-time pharmacokinetic monitoring of plasma concentrations in ongoing phase I trials [8] ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01794104) Identifier: NCT01794104 and NCT01051635). The BSA-normalized clearance observed in rats and dogs was more than 10-fold higher than human clearance values of 1.54 L/h/m² reported in ongoing human trials, suggesting that human tolerable doses may be much lower assuming equal pharmacodynamics [8]. Although the *in vitro* metabolism of NSC 743400 has been studied and has resulted in the detection of pharmacologically active metabolites [9], the *in vivo* metabolic fate, and the contribution of various metabolic pathways to the total clearance remains unknown. Further studies, elucidating metabolic pathways involved, may lead to understanding of the non-linear pharmacokinetics observed in the current study.

No substantial pharmacokinetic differences were observed between the two dog groups dosed with the agent at the same level using different vehicles. Only C_{max} was statistically significantly different between the two groups, with higher values for the formulation prepared in 10 mM citric acid/D5W than for WFI-HCl/D5W. However, the difference (<18%) was small, and without replicate design may be attributable to between occasion variability.

Our results indicate that the oral capsule administration of NSC 743400 resulted in erratic absorption, and would not be suitable for oral clinical studies. The observed diarrhea, emesis and appetite loss, similar to toxicity observed in dogs after oral dosing of topotecan and 9-nitrocamptothecin[10,11], may also have contributed to suboptimal absorption of the dose administered.

Previous studies [12] reported that a 15 min infusion of NSC 743400 to dogs at doses as low as 10 mg/kg (215 mg/m²) resulted in lethality. In the current dog study, single 1-h infusions did not cause lethality, although at the highest dose (646 mg/m²), animals became moribund at day 5. Obviously, shorter infusions of NSC 743400 produce higher peak plasma concentrations, which may be associated with the greater observed toxicity. If the pharmacokinetics of NSC 743400 are indeed non-linear, a shorter infusion duration would be expected to result in a disproportionately high C_{max}. If the same holds true for humans, shorter infusion schedules in phase I trials may well result in toxicity even at low doses.

In conclusion, NSC 743400 is a promising Topo I-interactive agent that is currently in clinical trials. The preclinical data presented in the current manuscript raise a number of issues that should be addressed in further investigations, such as the metabolic fate and the possibility of non-linear pharmacokinetics of NSC 743400. While these data were not used as the basis for selecting a clinical starting dose, they are essential for comparing with

plasma concentrations in the clinic, given the apparent correlation between high peak concentrations and an increase in adverse effects.

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Figure 1. Chemical structure of NSC 743400

Figure 2. Average plasma NSC 743400 concentration *versus* time profiles following intravenous dosing at 12 and 24 mg/m² to male (M) and female (F) rats

Figure 3. Average plasma NSC 743400 concentration-time profiles following intravenous dosing at a variety of doses (10-646 mg/m²) to male (M) and female (F) dogs

Figure 4. Plasma concentration *versus* time curves of NSC 743400 after administration of 860 (N=2) or 1720 (N=2) mg/m² NSC 743400 as an oral dose to female beagle dogs

Table 1

Plasma pharmacokinetic parameters of NSC 743400 in rats

Route	Dose (mg/m ²)	Vehicle	Sex	n	t _{1/2} (h)	C _{max} (ng/mL)	AUC _{0-∞} (h*ng/mL)	V _Z (L/m ²)	CL (L/h/m ²)	
IV bolus	12	Vehicle 1 I	male	12	2.1	406	232	156	50.9	
			female	12	4.2	301	403	178	29.3	
	24		male	12	3.6	335	421	287	56.0	
			female	12	4.6	568	806	194	29.3	

Values are presented as mean (no statistical comparison).

Vehicle 1: NSC 743400 was first dissolved at 10 mg/mL in 10 mM citric acid before dilution in 5% dextrose in water for dosing

Table 2
Plasma pharmacokinetic parameters of NSC 743400 in dogs

Route	Dose (mg/m ²)	Vehicle	Sex	N	t _{1/2} (h)	C _{max} (ng/mL)	AUC _{0-∞} (h*ng/mL)	VZ (F) (L/m ²)	CL (F) (L/h/m ²)
IV-infusion	10	Vehicle 1 ¹	M	3	5.9 ± 1.9 ^b	106 ± 20 <i>a,b</i>	383 ± 114	225 ± 18 <i>b</i>	28.0 ± 9.7
			F	3	8.0 ± 3.9	75.7 ± 13	262 ± 45	426 ± 152	38.9 ± 6.3
	50		M	3	6.6 ± 1.7 <i>c</i>	333 ± 43 <i>a,d</i>	1501 ± 693	348 ± 105 <i>c</i>	41.1 ± 25
		100	F	3	5.9 ± 2.3	275 ± 43	1110 ± 267	401 ± 199	46.7 ± 9.9
			M	3	9.9 ± 1.9 <i>b,c</i>	461 ± 159 <i>b</i>	1853 ± 621	799 ± 115 <i>b, c</i>	58.2 ± 19
			F	3	14.0 ± 1.4	547 ± 94	2879 ± 801	748 ± 240	36.6 ± 9.9
IV-infusion Pilot	50	Vehicle 2 ²	M	3	7.8 ± 3.6	280 ± 24 <i>d</i>	1443 ± 651	388 ± 25	42.4 ± 26
			F	3	7.9 ± 2.2	257 ± 31	1108 ± 297	542 ± 213	47.2 ± 12
	215		F	1	16.8	772	9652	394	20.8
		Vehicle 1 ¹	F	1	19.6	1094	9929	502	20.2
	430		F	1	13.9	2116	12155	568	33.0
			F	1	18.5	1022	8329	954	48.0
PO Pilot	646		F	1	35.5 [*]	1523	27447	1130	21.8
			F	1	34.7 [*]	2007	39957	720	15.0
	860		F	1	27.8 [*]	483	17217	1692	50.0
		Gelatin Capsules	F	1	91.0 [*]	1.6	19.4	32460	43600
	1720		F	1	11.9	244	6295	9200	274
			F	1	17.6 [*]	180	2335	19920	738

For N>1, values are presented as average ± SD.

¹Vehicle 1: the agent was first dissolved at 10 mg/mL in 10 mM citric acid before dilution in 5% dextrose in water (D5W) for dosing
²Vehicle 2: the agent was first dissolved at 2 mg/mL in water for injection (WFI; pH adjusted to 2.7 with HCl) before dilution in D5W for dosing

Statistically significant differences were as follows:

^a_{low} (10 mg/m²) and mid (50 mg/m²) dose; difference due to dose

^b_{low} and high (100 mg/m²) dose; difference due to dose

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C_{mid} mid and high dose; difference due to dose

d_{mid} mid-vehicle 1 and mid-vehicle 2 ; difference due to vehicle

C_{max} maximum plasma concentration (end of infusion); AUC_{inf} area under the plasma concentration *versus* time curve, extrapolated to infinity.

* estimate is relatively unreliable because of the short time of sampling relative to the estimated half-life (<3:1).

Table 3
Protein binding of NSC 743400 in dog plasma and tissue culture medium containing 10% fetal bovine serum

Concentration (ng/mL)	4,300 % bound	10,760 % bound
Dog plasma (%CV)	96.1 (0.7)	97.7 (0.4)
Tissue culture media (%CV)	93.2 (2.8)	98.5 (0.3)

CV, coefficient of variation