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ORIGINAL ARTICLE

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Plasma pharmacokinetics and bioavailability of 1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea after intravenous and oral administration to mice and dogs

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Abstract *Purpose*: Chloroethylnitrosoureas are among the most widely used chemotherapeutic agents for the treatment of brain tumors. SarCNU (1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea) is an investigational nitrosourea analogue that has shown greater antitumor activity and a more favorable toxicity profile than 1,3-bis(2-chloroethyl)-1-nitrosourea in preclinical studies. The purpose of the present study was to characterize the plasma pharmacokinetics and oral bioavailability of SarCNU in mice and dogs treated by intravenous infusion and gastric intubation. *Methods*: SarCNU was administered to mice by i.v. injection or orally at doses ranging from 10 to 100 mg/kg. Plasma samples were obtained from groups of five animals at each time-point at intervals ranging from 3 min to 2.5 h after dosing. A group of three male beagle dogs were treated with Sar CNU 10 mg/kg given both by i.v. infusion and orally in a crossover design. The concentration of SarCNU in plasma was measured by high-performance liquid chromatography. *Results*: During the initial 90 min after i.v. injection to mice, SarCNU was eliminated from plasma in a monoexponential manner with a mean half-life of 9.8 ± 0.8 min. The total plasma clearance was 47.3 ± 8.7 ml/min per kg and the apparent volume of distribution was 0.7 ± 0.1 l/kg. SarCNU exhibited linear pharmacokinetic behavior following both i.v. and oral administration of doses ranging from approximately 10 to 100 mg/kg. Peak plasma levels provided by a dose of 100 mg/kg given by the i.v. and oral routes were 142.4 μg/ml (0.5 min) and 27.8 μg/ml (9.8 min), respectively. The mean oral bioavailability of the drug was $57.3 \pm 12.6\%$ in mice. In comparison, the disposition of SarCNU in dogs after rapid i.v. injection was biexponential, with half-lives of 5.4 ± 8.4 min and 40.8 ± 9.0 min for the initial and terminal disposition phases, respectively. Mean values of the total plasma clearance and apparent volume of distribution were 17.8 ± 1.8 ml/min per kg and 1.1 ± 0.3 l/kg, respectively. The C_{max} was 18.5 ± 6.5 μg/ml after i.v. injection and 8.5 ± 0.4 μg/ml after oral administration of a 10 mg/kg dose. Oral bioavailability of the drug in dogs ($71.7 \pm 21.2\%$) was greater than that observed in mice. *Conclusions*: SarCNU exhibited linear and consistent pharmacokinetics in mice and dogs with very good oral bioavailability in both species. These findings support the rationale for evaluating SarCNU given by the oral route of administration in phase I clinical trials.

Keywords Antineoplastic agents · Nitrosoureas · Pharmacokinetics · Preclinical studies

Abbreviations *AUC*: area under plasma concentration-time curve from time zero to infinity · *BCNU*: 1,3-bis (2-chloroethyl)-1-nitrosourea · *BSA*: body surface area · *CENU*: chloroethylnitrosourea · *CL*: total plasma clearance · C_{max}: peak plasma concentration · *CNS*: central nervous system · *F*: absolute bioavailability · *HPLC*: high-performance liquid chromatography · *MRT*: mean residence time · *NCI*: National Cancer Institute · *SarCNU*: 1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea · $t_{1/2,1}$: half-life of the initial disposition phase · $t_{1/2,abs}$: half-life of the apparent absorption phase · $t_{1/2,z}$: half-life of the terminal disposition phase · t_{lag}: absorption lag time · t_{max}: time of the peak plasma concentration · V_1: apparent volume of distribution of the central compartment · V_z: total body apparent volume of distribution

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Introduction

The chloroethylnitrosoureas (CENUs) are the first and one of the most important classes of anticancer agents that have been introduced into the clinic. CENUs are primarily used for the treatment of CNS tumors due to their ability to readily cross the blood-brain barrier. However, these drugs are also commonly employed in the treatment of a wide variety of non-CNS malignancies, including small-cell lung cancer, Hodgkin's disease, non-Hodgkin's lymphomas, multiple myeloma and malignant melanoma. More recently, they have been incorporated into multiagent high-dose chemotherapy regimens with stem cell support in patients with breast cancer, neuroblastoma, glioma, melanoma and sarcomas [23, 24]. Despite their broad antitumor activity, the clinical usefulness of CENUs has been limited by delayed-onset, cumulative myelosuppression and pulmonary toxicity [23, 24]. Therefore, although the most widely used compound in this class, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), was approved for clinical use in the mid 1960s, efforts to develop new analogues with a better therapeutic index have continued to the present day.

SarCNU [1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea] is an investigational nitrosourea analogue distinguished by incorporation of the amido derivative of the amino acid L-sarcosine into the molecule (Fig. 1) [21]. Whereas all clinically available CENUs enter cells by passive diffusion [1, 6, 7], the presence of the sarcosinamide functional group allows SarCNU to penetrate cells via the extraneuronal catecholamine uptake$_2$ transporter [13, 18, 19]. The facilitated uptake of SarCNU enables it to achieve higher intracellular concentrations than BCNU in human glioma cell lines and contributes to its enhanced cytotoxicity [19]. Another important structural feature of SarCNU that is unique to this class of agents is the presence of a methyl group at the N-3 position of the molecule. This not only renders the drug more chemically stable, but presumably precludes the formation of an organic isocyanate as a degradation product, which is thought to be responsible for the undesirable pulmonary toxicity observed during long-term therapy with other CENUs [21, 24, 25].

Consistent with these advantageous physicochemical and pharmacological properties, SarCNU has been shown to be more active than BCNU against primary glioma cells and human glioma cell lines in vitro [14, 17] and against human CNS tumor xenografts in vivo [4, 9]. In addition, SarCNU is less toxic than BCNU to mice [9, 21] and less myelotoxic toward normal human bone marrow in the in vitro colony forming unit assay [14]. The prospect for greater effectiveness in the treatment of malignant gliomas than offered by the chemotherapeutic agents that are presently available featured prominently in the selection of SarCNU for preclinical development by the National Cancer Institute (NCI). The present investigation was therefore undertaken to characterize the plasma pharmacokinetics and oral bioavailability of SarCNU in mice and dogs. Knowledge of the peak drug concentration in plasma and total systemic exposure to the drug provided by therapeutically effective doses against in vivo tumor models was desired to establish pharmacological endpoints for dose escalation during the comprehensive preclinical toxicological assessment and subsequent phase I trials of SarCNU.

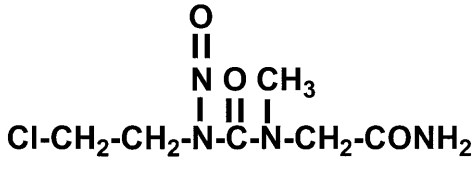

Fig. 1 Chemical structure of SarCNU

Materials and methods

Dosing and sample collection

SarCNU (NSC 364432) was obtained from the Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI (Bethesda, Md.). Dosing solutions of SarCNU for administration to mice were prepared in dimethyl sulfoxide (Sigma, St. Louis, Mo.) such that the intended dose was delivered in a volume of 1.0 µl/g body weight. For disposition studies in dogs, the drug was dissolved in a vehicle composed of 0.05 *M* sodium acetate buffer, pH 5, to deliver the intended dosage in a volume of 1.0 ml/kg body weight. All dosing solutions were used within 45 min of preparation and protected from exposure to light. The drug concentration in each dosing solution was ascertained by HPLC analysis [22].

Male Harlan BALC/c×DBA/2F$_1$ mice (NCI, Frederick, Md.), weighing 20–25 g, were given free access to food and water. Randomly selected animals were treated with single doses of SarCNU, ranging from 10 to 100 mg/kg, by 60-s tail vein injection or gastric intubation. At times that ranged from 3 min to 2.5 h after dosing, groups of five mice were anesthetized with methoxyfluorane and terminally bled by retroorbital puncture using heparinized capillary tubes. The whole blood from each animal was collected in an individual heparin-coated microcentrifuge tube and promptly centrifuged (12,000 *g*, 2 min, 25°C). The plasma was immediately separated, flash frozen, and stored at –70°C until processed for HPLC analysis within 48 h. The time from the beginning of sample collection to freezing the separated plasma never exceeded 5 min; therefore, the extent of drug degradation during these procedures was negligible [22].

Dog studies were performed at the Hazelton Washington Laboratories in Vienna, Va. A group of three male beagle dogs (Hazelton Research Products, Kalamazoo, Mich.) were treated with SarCNU 10 mg/kg given either by i.v. infusion or oral gavage in a crossover design, with a minimum 35-day recovery period between doses. The animals were fully acclimated and given a complete physical examination prior to approval for use by an attending veterinarian. They were subjected to fasting from the evening before to 8 h after receiving the drug. During the study, the age of the dogs ranged from 4 to 6 months, and their mean weight was 10.1 kg (range 8.7 to 12.2 kg). The dogs were given 0.5 mg atropine (Fort Dodge Laboratories, Fort Dodge, Iowa) by intramuscular injection to control salivation before light sedation with i.v. thiamylal sodium (Bio-Centric, St Joseph, Mo.) 1 to 2 h prior to the administration of SarCNU.

A percutaneous 17-gauge i.v. Intrafusor with an 18-gauge 11.4-cm catheter (Sorenson Research, Salt Lake City, Utah) was inserted into the left saphenous vein for administration of the dosing solution. A 17-gauge CVP Intrafusor with an 18-gauge 53.3-cm catheter (Sorenson Research) was inserted percutaneously via the

right saphenous vein for blood sampling. Sterile 0.9% sodium chloride (Abbott Laboratories, Chicago, Ill.), used throughout as a catheter flushing solution, was delivered at about 10 ml/h with a Cormed ML6-8 infusion pump (Dakmed, Buffalo, N.Y.) to maintain patency of the venous catheters. After insertion of a Buster 8F urinary catheter (2×500 mm; A.J. Buck & Sons, Owings, Mills, Md.), the dog was placed in a sling (Alice King Chatham Medical Arts, Los Angeles, Calif.) and permitted to recover from the anesthetic.

Shortly before dosing, an aliquot of blood (10 ml) was collected to provide pretreatment plasma. Subsequently, the dosing solution was delivered from a weighed 65-ml IPR-86 infusion bag (Cardio Medical Products, Rockaway, N.J.) into the venous catheter. The amount of test article administered was determined by weighing the dosing apparatus before and after dosing and calculating the difference. Pharmacokinetic blood specimens (1.5 ml) were drawn into heparin-treated syringes at 18 time-points during the infusion and subsequent 8 h. After the 8-h specimens had been collected, the catheters were removed and the animal was housed in a standard stainless steel cage with a wire mesh floor. Another blood sample was obtained at 24 h postinfusion. Plasma was isolated from the blood samples and stored prior to analysis as indicated above.

Protocols for the pharmacokinetic studies in mice and dogs were approved by the NCI Frederick Cancer Research and Development Center (NCI-FCRDC) Animal Care and Use Committee (Frederick, Md.). All procedures and practices involving research in animals were conducted in facilities accredited by the American Association for Accreditating Laboratory Animal Care in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 85-23).

HPLC analysis of SarCNU

A specific and sensitive HPLC method for assaying SarCNU in plasma was developed for this study [22]. Briefly, to prevent the occurrence of significant drug degradation during workup for analysis, frozen plasma specimens were individually thawed in less than 5 min using an Eppendorf model 5436 Thermomixer (Madison, Wis.) set at 40°C. Immediately thereafter, several aliquots of the sample (50 µl) were pipetted into borosilicate glass centrifuge tubes. The remaining plasma was flash frozen for reanalysis if necessary.

Quantitation was performed by assaying a series of nine plasma standards with SarCNU concentrations ranging from 0.1 to 5.0 µg/ml, together with study specimens on a daily basis. Standard curves were constructed by plotting the peak height ratio of SarCNU to the internal standard, 1-(2-chloroethyl)-3-prolinamide-1-nitrosourea (NCI), against the known drug concentration. Linear least squares regression was performed with a weighting factor of y_{obs}^{-1}, without inclusion of the origin, to determine the slope, *y*-intercept and correlation coefficient of the best fit line. Drug concentrations in study samples were calculated using the results of the regression analysis. Specimens exceeding the upper range of the standard curve were reassayed upon appropriate dilution with drug-free plasma. All samples were initially assayed in duplicate on different days, with additional analyses performed if the replicate determinations deviated from their average by more than 10%. The lowest concentration included in the standard curves, 0.1 µg/ml, was quantified with a precision of 7.8% and an accuracy of 4% of the known concentration, during a period of 8 weeks.

Pharmacokinetic data analysis

Actual doses were calculated from the body weight of the animals on the treatment day, the gravimetrically determined volume of dosing solution delivered, and the assayed concentration of SarCNU in each preparation. The beginning and ending times of the drug input and sample collection intervals were monitored with a digital timer and recorded to the nearest second. Time-points were determined as the difference between the midpoint of the blood collection interval and starting time of dose administration. For the studies in mice, the geometric mean plasma concentration of SarCNU was calculated from the observed concentrations in groups of five animals at each time-point. Pharmacokinetic parameters were estimated by analyzing the geometric mean plasma concentration-time curves of SarCNU for mice and individual plasma profiles were determined in the dogs.

The model-independent equation that best described each plasma profile was identified by weighted nonlinear regression using the WinNonlin version 1.1 software package (Scientific Consulting, Apex, N.C.) as previously described [3]. Equations for drug input by continuous i.v. infusion with either mono- or biexponential first-order disposition were fitted to the observed time courses of the SarCNU plasma concentration following i.v. administration. Experimentally determined plasma profiles of the drug following oral administration were fitted to equations for first-order drug input, with an absorption lag time, and mono- or biexponential first-order disposition. Values of the iterated parameters were used to calculate all pharmacokinetic parameters according to standard equations [3]. The total systemic availability of unchanged drug following oral administration was calculated as $F = CL \cdot AUC_{oral}/dose_{oral}$, using the CL of SarCNU determined in the i.v. dosing studies. Mean values of all pharmacokinetic parameters were calculated as the geometric mean of the individual values estimated by nonlinear regression analysis [12]. Standard deviations for the geometric mean values were estimated by the jackknife method [10].

Plasma protein binding

The binding of SarCNU to mouse plasma proteins at total drug concentrations ranging from approximately 1 to 100 µg/ml was determined by the method of ultrafiltration. Centrifree Micropartition Systems with a M_r 30,000 cut-off YMT membrane (Amicon Division, W.R. Grace & Co., Beverly, Mass.) were prepared for use by washing the membranes three times with 1.0 ml 0.1 *M* potassium phosphate buffer, pH 7.4, facilitated by centrifugation at 1800 *g*. After the final wash, the collection cup was rinsed with distilled water, followed by methanol, then dried in a stream of nitrogen. Frozen mouse plasma, acquired with sodium heparin as the anticoagulant (Harlan Bioproducts for Science, Indianapolis, Ind.), was thawed at ambient temperature and thoroughly mixed. The plasma (600 µl) was pipetted into five microcentrifuge tubes and equilibrated for 15 min in a constant temperature bath maintained at 25°C. A freshly prepared stock solution of SarCNU in dimethyl sulfoxide (6 µl) was then added to each tube and the contents were mixed by vortexing.

After incubating at 25°C for 5 min, two aliquots of 50 µl were withdrawn from each tube for HPLC analysis and 400 µl of the remaining solution was transferred into the reservoir of a micropartition system. An analogous set of samples was prepared using 0.1 *M* potassium phosphate buffer, pH 7.4, instead of plasma, to assess the extent of drug adsorption onto the ultrafiltration membrane. The micropartition systems were centrifuged (1800 *g*, 25°C) until 175–200 µl of ultrafiltrate was collected, which required 4–5 min for plasma samples and 2–3 min for buffer solutions. The ultrafiltrates were assayed in duplicate by HPLC. The mean ± SD of the chromatographic peak height ratio of the drug to internal standard was calculated for each set of five samples. The unbound fraction of drug was determined as the mean peak height ratio of the ultrafiltrates divided by the mean peak height ratio of the same samples prior to ultrafiltration.

Results

Disposition and oral bioavailability of SarCNU in mice

Although soluble in aqueous vehicles, SarCNU was not sufficiently stable to allow a single dosing solution to be

used for an entire day [22]. Since solutions of the drug prepared in DMSO showed no evidence of degradation during 8 h at ambient temperature, the drug was formulated daily in neat DMSO to deliver the intended doses in a volume that could be safely given to mice (1.0 μl/g body weight) [15, 26].

The values of pharmacokinetic parameters for SarCNU administered to mice at four dose levels ranging from approximately 10 to 100 mg/kg by the i.v. and oral routes are summarized in Table 1. Within this dose range, SarCNU exhibited linear pharmacokinetic behavior for both routes of administration. The C_{max} of SarCNU increased linearly ($r = 0.994$) from 12.9 to 142.4 μg/ml as the dose, given by bolus i.v. injection, was escalated from 10.6 to 99.6 mg/kg (Table 1). The AUC was also highly correlated with the dose ($r = 0.998$). As illustrated in Fig. 2 (top), drug disposition was distinctly monoexponential with a $t_{1/2,z}$ of 9.8 ± 0.8 min (geometric mean ± SD, $n = 4$). The MRT of the drug upon i.v. injection was 14.2 ± 1.8 min. Average values of the total plasma clearance and apparent volume of distribution were 47.3 ± 8.7 ml/min per kg and 0.7 ± 0.1 l/kg, respectively.

SarCNU was rapidly absorbed in an apparent first-order manner following oral administration (Fig. 2 bottom). The apparent half-life for absorption was 2.0 ± 0.2 min and peak plasma levels were achieved 9.8 ± 0.7 min ($n = 4$) after dosing. Escalating the dose from 10.1 to 94.3 mg/kg yielded a proportionate increase in the magnitude of C_{max} from 2.9 to 27.8 μg/ml ($r = 0.999$), and of AUC from 95.8 to 1,269 μg·min/ml ($r = 0.992$). The C_{max} of SarCNU upon oral dosing was approximately five times lower than that for the same dose given by bolus i.v. injection. Whereas the SarCNU plasma concentration declined from the peak in a monoexponential fashion, values of its apparent $t_{1/2,z}$ (22.6 ± 4.9 min) and MRT (35.5 ± 6.9 min) were substantially longer than those observed after i.v. dosing. The average systemic availability was $57.3 \pm 12.6\%$ upon oral administration.

Disposition and oral bioavailability of SarCNU in dogs

The disposition of SarCNU in three dogs treated by rapid i.v. injection with an average dose of 9.9 mg/kg was characterized. The geometric means of the pharmacokinetic parameters and derived terms calculated from the estimated values for each animal are summarized in

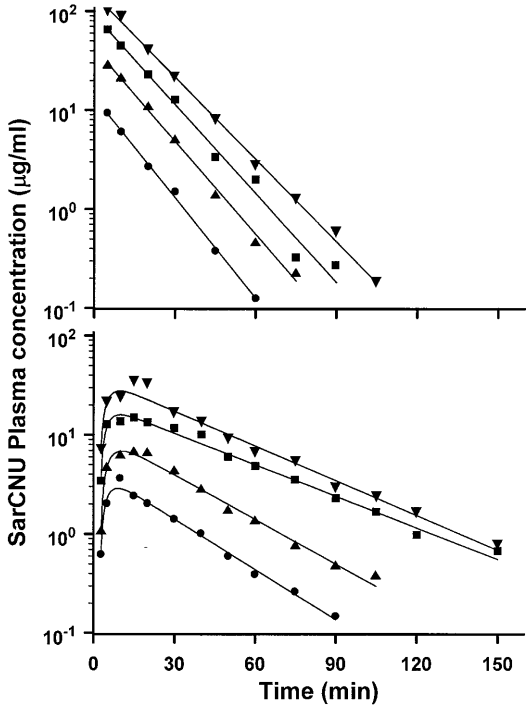

Fig. 2 Plasma concentration-time profiles of SarCNU in mice. Top: Administration by 1-min i.v. injection at doses of 10.6 mg/kg (●), 25.3 mg/kg (▲), 53.6 mg/kg (■), and 99.6 mg/kg (▼). Bottom: Oral administration of 10.1 mg/kg (●), 25.1 mg/kg (▲), 51.5 mg/kg (■), and 94.3 mg/kg (▼). The data points are the geometric means of the observed plasma concentrations of the drug in groups of five mice. Error bars have been omitted for clarity. The solid lines represent the best-fit curves of the experimental data (points) as determined by nonlinear regression analysis

Table 1 Pharmacokinetic parameters for SarCNU determined after i.v. and oral administration to mice

Parameter	Route of administration									
	Intravenous					Oral				
	Dose (mg/kg)				Mean ± SD	Dose (mg/kg)				Mean ± SD
	10.6	25.3	53.6	99.6		10.1	25.1	51.5	94.3	
C_{max} (μg/ml)	12.9	40.8	87.9	142.4	–	2.9	6.9	16.0	27.8	–
t_{max} (min)	–	–	–	–	–	9.0	10.6	9.9	9.8	9.8 ± 0.7
t_{lag} (min)	–	–	–	–	–	2.3	2.4	2.3	2.1	2.3 ± 0.1
$t_{1/2,abs}$ (min)	–	–	–	–	–	1.9	2.3	1.8	1.9	2.0 ± 0.2
$t_{1/2,z}$ (min)	8.9	9.6	10.0	10.9	9.8 ± 0.8	17.8	20.1	28.4	25.7	22.6 ± 4.9
MRT (min)	12.9	13.9	14.5	15.7	14.2 ± 1.8	28.4	32.3	43.5	39.9	35.5 ± 6.9
CL (ml/min/kg)	62.5	43.9	41.4	43.9	47.3 ± 8.7	–	–	–	–	–
V (l/kg)	0.80	0.61	0.60	0.69	0.7 ± 0.1	–	–	–	–	–
AUC (μg·min/ml)	169.7	575.9	1294.3	2267.0	–	95.8	263.2	786.9	1268.5	–
F (%)	–	–	–	–	–	45.5	50.3	73.3	64.4	57.3 ± 12.6

Table 2. The mean C_{max} of SarCNU was 18.5 ± 6.5 g/ml and the mean AUC was 555.4 ± 46.8 g·min/ml. In each case, nonlinear regression analysis revealed that the plasma SarCNU concentration decreased biexponentially (Fig. 3). The $t_{1/2,1}$ was very rapid, with a mean value of 5.4 ± 8.4 min, and the $t_{1/2,z}$ showed a mean value of 40.8 ± 9.0 min. The MRT was 47.4 ± 3.6 min. Average values of the total plasma clearance and of the apparent volume of distribution were 17.8 ± 1.8 ml/min per kg and 1.1 ± 0.3 l/kg, respectively.

As in the mice, SarCNU was rapidly absorbed in an apparent first-order manner upon oral administration to dogs. The plasma profiles were best described by equations for first-order absorption without a lag time and biexponential decay. The mean t_{max} was 10.2 ± 3.0 min, and the average C_{max} achieved was 8.5 ± 0.4 μg/ml, 2.2 times lower than that observed after i.v. dosing. Values of its apparent $t_{1/2,z}$ (34.2 ± 5.4 min) and MRT (55.8 ± 5.4 min) were not very different from those observed after i.v. drug infusion. The oral bioavailability was significantly higher than in mice, with a mean value of 71.7 ± 21.2%.

Plasma protein binding

As shown in Table 3, there were no statistically significant differences between the ultrafilterable fraction of SarCNU upon addition to mouse plasma and pH 7.4 aqueous buffer at concentrations ranging from 1 to 100 μg/ml. This suggests that the systemically circulating drug in mice is almost entirely unbound to plasma proteins.

Discussion

The CENUs represent an important class of anticancer drugs because of their broad spectrum of activity and ability to efficiently traverse the blood-brain barrier. The clinically available CENUs, BCNU and chloroethylcyclohexylnitrosourea (CCNU), enter cells exclusively by passive diffusion [1, 6, 7]. It is plausible that limitations on their therapeutic effectiveness result from an inability to achieve sufficiently higher concentrations within tumor cells than within normal cells of drug-sensitive tissues. This hypothesis provided the rationale for synthesizing a series of L-amino acid amide CENU congeners, based upon the potential of the amino acid moiety to function as a carrier group for selective, facilitated uptake of the drug by neoplastic cells [21]. Among the compounds that have been synthesized, SarCNU is transported into glioma cell lines both by passive and facilitated diffusion mediated by a catecholamine transport system that recognizes sarcosinamide as a carrier [13, 18, 19]. The presence of this carrier is largely restricted to tissues of the nervous system and it is particularly prevalent in glioma cells [16, 18, 19]. This may confer on SarCNU a greater specificity and

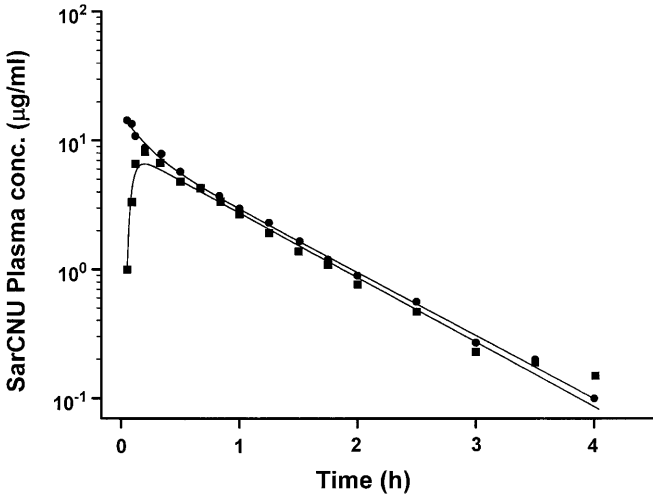

Fig. 3 Plasma concentration-time profiles of SarCNU in dogs. Administration by i.v. bolus injection at a dose of 9.86 mg/kg (●) and oral administration at a dose of 9.94 mg/kg (■). The data points are the geometric means of the observed plasma concentrations of the drug in three dogs. Error bars have been omitted for clarity. The solid lines represent the best-fit curves of the experimental data (points) as determined by nonlinear regression analysis

Table 2 Pharmacokinetic parameters for SarCNU determined after i.v. and oral administration to dogs

Parameter	Route of administration	
	Intravenous	Oral
Dose (mg/kg)	9.9 ± 0.4	9.9 ± 0.5
C_{max} (μg/ml)	18.5 ± 6.5	8.5 ± 0.4
t_{max} (min)	–	10.2 ± 3.0
$t_{1/2,abs}$ (min)	–	2.3 ± 0.8
$t_{1/2,1}$ (min)	5.4 ± 8.4	1.8 ± 1.2
$t_{1/2,z}$ (min)	40.8 ± 9.0	34.2 ± 5.4
MRT (min)	47.4 ± 3.6	55.8 ± 5.4
CL (ml/min/kg)	17.8 ± 1.8	–
V_1 (l/kg)	0.5 ± 0.2	–
V_z (l/kg)	1.1 ± 0.3	–
AUC (μg·min/ml)	555.4 ± 46.8	396.1 ± 78.1
F (%)	–	71.7 ± 21.2

Table 3 Unbound fraction of SarCNU in mouse plasma and pH 7.4 buffer at 25°C

Added drug concentration (μg/ml)	Unbound fraction (mean ± SD, $n = 5$)	
	Mouse plasma	Phosphate buffer[a]
1.1	0.764 ± 0.039	0.841 ± 0.049
2.6	0.730 ± 0.057	0.880 ± 0.048
5.3	0.855 ± 0.075	0.849 ± 0.062
10.5	0.897 ± 0.027	0.867 ± 0.088
26.3	0.856 ± 0.021	0.845 ± 0.046
52.6	0.746 ± 0.112	0.790 ± 0.126
105.3	0.688 ± 0.092	0.719 ± 0.054
Mean	0.791 ± 0.190	0.821 ± 0.195

[a]0.1 *M* potassium phosphate buffer, pH 7.4

cytotoxicity toward brain neoplasms than other CENUs. Consistent with these findings, a pilot study demonstrated that the distribution of radioactivity into the brain of a patient with glioblastoma following systemic [^{11}C]BCNU administration closely paralleled blood flow, as measured by [^{68}Ga]EDTA PET scanning, whereas the administration of [^{11}C]SarCNU resulted in a greater accumulation of radioactivity in the tumor relative to surrounding normal brain tissue [11].

SarCNU also differs from BCNU in being considerably more chemically stable under physiological conditions. Specifically, in comparison to BCNU, its degradation half-life is 6.7-times longer in pH 7.4 aqueous buffer solution (5.5 h) and at least tenfold greater in plasma (2.7 h) at 37°C [8, 21, 22]. All of the CENUs that have been clinically evaluated thus far share a common pathway of decomposition. The immediate degradation products, chloroethyl diazohydroxide and an isocyanate, are both highly reactive species [24, 25]. In contrast, the presence of a methyl group at the N-3 position of SarCNU prevents isocyanate formation and is probably responsible for its enhanced stability [21]. This has considerable therapeutic implications, since the antitumor activity as well as the dose-limiting hematological toxicity of the CENUs are most likely a consequence of DNA alkylation by the chloroethyl carbonium ion generated from the diazohydroxide species [5, 24, 25], whereas macromolecular carbamoylation by the isocyanate may be primarily responsible for the problematic pulmonary toxicity observed during long-term therapy with BCNU [20]. All these distinct characteristics suggest that SarCNU may have an improved therapeutic index as compared to BCNU in the treatment of gliomas.

Preclinical efficacy studies have demonstrated that the antitumor activity of SarCNU is significantly greater than that of BCNU against several human CNS tumor xenograft models when implanted s.c. in mice and that it is also 12-fold less toxic [9]. When SarCNU was given by repeated i.v. injection according to a once-daily for 5 days schedule or an every 4th day times 3 (q4d×3) schedule, the optimally effective doses yielded nine of ten tumor-free survivors, whereas a single dose did not produce any complete regressions. For all dosing regimens that were evaluated, treatment with the optimal dose of BCNU resulted in no tumor-free animals and SarCNU consistently exhibited a greater therapeutic index than BCNU [9]. Oral administration of SarCNU to mice bearing s.c.-implanted glioma tumor xenografts shows excellent activity, with a 100% tumor-free response rate at the MTD of 178 mg/kg q4d×3, while oral BCNU results in a high incidence of toxic deaths and does not produce any tumor regressions [9].

The present investigation was undertaken to characterize the plasma pharmacokinetics and oral bioavailability of SarCNU in mice and dogs. The administration of SarCNU at doses of 10 to 100 mg/kg to mice by the i.v. or oral routes, which were well tolerated, provided peak levels in plasma that ranged from 2.9 to 142.4 μg/ml. These plasma concentrations are well above the concentrations shown to be active against primary glioma cells and glioma cell lines that are sensitive to the cytotoxic effects of SarCNU in vitro [14, 17]. Moreover, even though the MTD in tumor-bearing mice was 167 mg/kg for the i.v. route using the q4d×3 dosing schedule, which produced 90–100% long-term survivors, SarCNU retained high antitumor activity when given at either 66% (111 mg/kg) or 45% (74 mg/kg) of the optimal dose [9]. At the range of doses evaluated in this study, SarCNU exhibited linear pharmacokinetics, with plasma levels decaying in a distinctly monoexponential manner in mice after i.v. and oral administration. The C_{max} of SarCNU was 4.5 to 6 times lower, and the values of its apparent $t_{1/2,z}$ (22.6 vs 9.8 min) and MRT (35.5 vs 14.2 min) were significantly longer when given orally as compared to rapid i.v. injection. The mean oral bioavailability of the drug in mice was $57.3 \pm 12.6\%$.

Pharmacokinetic studies in dogs which were performed at a single dose level of 10 mg/kg in a group of three animals using a crossover design showed that the disposition of SarCNU was clearly biexponential. The C_{max} and AUC achieved in dogs receiving a BSA-normalized dose of 190 mg/m^2 (10 mg/kg) were lower than those in mice treated with a BSA-normalized dose of 150 mg/m^2 (50 mg/kg), both by the i.v. and oral routes. The apparent volume of distribution was 1.8 times greater and the total plasma clearance 2.3 times lower in dogs than in mice. Accordingly, $t_{1/2,z}$ and MRT were significantly longer in dogs. In both species, $t_{1/2,z}$ is considerably shorter than the half-life in plasma for in vitro degradation (2.7 h). This rapid disappearance of SarCNU from the systemic circulation may be due to a combination of partitioning into tissues, as expected for a lipophilic agent, and urinary excretion of a small, nonprotein-bound compound. The observed V_z in dogs (1.1 ± 0.3 l/kg) is consistent with a nonprotein-bound drug which distributes freely to all tissues. As in the mice, SarCNU was rapidly absorbed in the dog in an apparent first-order manner following oral administration with peak plasma levels achieved within 10 min after dosing. The oral bioavailability, however, was significantly greater than that observed in mice, averaging $71.7 \pm 21.2\%$ after the administration of a 10 mg/kg dose.

It has been suggested that absorption of SarCNU is dose-dependent with a decrease in bioavailability at increasing doses. This was based upon a preliminary report that the mean oral bioavailability of SarCNU in male Fisher 344 rats is 96% for a 60 mg/kg dose and 65% for a 100 mg/kg dose [2]. Similarly, the mean oral bioavailability of the drug in beagle dogs is described as decreasing from 81 to 65% when the dose is increased from 6 to 10 mg/kg, respectively [2]. In our study, there was no evidence of dose-dependent absorption of the drug in mice treated with a broader range of doses from 10 to 100 mg/kg.

In summary, the present study served to demonstrate that concentrations of SarCNU that are active against human glioma cells in vitro can be achieved systemically

in mice and dogs without acute toxicity. SarCNU exhibits apparent linear pharmacokinetic behavior in mice when given by either i.v. infusion or gastric intubation at doses ranging from 10 to 100 mg/kg. Oral bioavailability of the drug was 57% in mice and 72% in dogs. On the basis of its predictable pharmacokinetics and good oral bioavailability, coupled with greater antitumor activity and a more favorable toxicity profile than the standard agent BCNU, SarCNU has been advanced into clinical evaluation. Two phase I trials sponsored by the NCI to evaluate SarCNU given orally on a q4d×3 schedule were initiated in 1999. The results of the present study directly supported the feasibility of administering SarCNU by the oral route and will serve to establish whether potentially therapeutic concentrations of the drug can be achieved in humans.

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References

1. Begleiter A, Lam HP, Goldenberg GJ (1977) Mechanism of uptake of nitrosoureas by L5178Y lymphoblasts in vitro. Cancer Res 37:1022
2. Brown AP, Rodvold KA, Tolhurst TA, Tomaszewski JE, Donohue SJ, Levine BS (1998) Oral bioavailability and plasma elimination kinetics of (2-chloroethyl)-3-sarcosinamide-1-nitrosourea (SarCNU) in the rat and dog. Proc Am Assoc Cancer Res 39:525
3. Gabrielsson J, Weiner D (1994) Pharmacokinetic and pharmacodynamic data analysis: concepts and applications. Swedish Pharmaceutical Press, Stockholm, pp 7–114
4. Houchens D, Sheridan M, Nines R, Riblet S, Finfrock M, Trigg N (1989) Glioma and medulloblastoma xenografts as models for brain tumor drug development. In: Wu B-q, Zheng J (eds) Immune-deficient animals in experimental medicine. Karger, Basel, p 157
5. Kaye AH, Laidlaw JD (1992) Chemotherapy of gliomas. Curr Opin Neurol Neurosurg 5:526
6. Lam HY, Talgoy MM, Goldenberg GJ (1980) Uptake and decomposition of chlorozotocin in L5178Y lymphoblasts in vitro. Cancer Res 40:3950
7. Lazarus P, Germina JS, Dufour M, Palmer G, Wallace D, Panasci LC (1983) Comparison of the transport of chlorozotocin and CCNU in L1210 leukemia and murine bone marrow cells in vitro. Cancer Chemother Pharmacol 11:159
8. Levin VA, Hoffman W, Weinkam RJ (1978) Pharmacokinetics of BCNU in man: a preliminary study of 20 patients. Cancer Treat Rep 62:1305
9. Marcantonio D, Panasci LC, Hollingshead MG, Alley MC, Camalier RF, Sausville EA, Dykes DJ, Carter CA, Malspeis L (1997) 2-Chloroethyl-3-sarcosinamide-1-nitrosourea, a novel chloroethylnitrosourea analogue with enhanced antitumor activity against human glioma xenografts. Cancer Res 57:3895
10. Miller RG (1974) The jacknife – a review. Biometrika 61:1
11. Mitsuki S, Diksic M, Conway T, Yamamoto YL, Villemure JG, Feindel W (1991) Pharmacokinetics of ^{11}C-labelled BCNU and SarCNU in gliomas studied by PET. J Neurooncol 10:47
12. Mizuta E, Tsubotani A (1974) Preparation of mean drug concentration-time curves in plasma. A study on the frequency distribution of pharmacokinetic parameters. Chem Pharm Bull 33:1620
13. Noe AJ, Malapetsa A, Panasci LC (1993) Transport of (2-chloroethyl)-3-sarcosinamide-1-nitrosourea in the human glioma cell line SK-MG-1 is mediated by an epinephrine-sensitive carrier system. Mol Pharmacol 44:204
14. Panasci LC, Dufour M, Chevalier L, Isabel G, Lazarus P, McQuillan A, Arbit E, Brem S, Feindel W (1985) Utilization of the HTSCA and CFU-C assay to identify two new 2-chloroethylnitrosourea congeners of amino acid amides with increased in vitro activity against human glioma compared with BCNU. Cancer Chemother Pharmacol 14:156
15. Rosenkrantz HZ, Seay H, Mason M (1963) Dimethyl sulfoxide: its steroid solubility and endocrinologic and pharmacologic-toxicologic characteristics. Cancer Chemother Rep 31:7
16. Schomig E, Russ H, Staudt K, Martel F, Gliese M, Grundemann D (1998) The extraneuronal monoamine transporter exists in human central nervous system glia. Adv Pharmacol 42:356
17. Skalski V, Rivas J, Panasci L, McQuillan A, Feindel W (1988) The cytotoxicity of sarcosinamide chloroethylnitrosourea (SarCNU) and BCNU in primary gliomas and glioma cell lines: analysis of data in reference to theoretical peak plasma concentrations in man. Cancer Chemother Pharmacol 22:137
18. Skalski V, Feindel W, Panasci LC (1989) The cytotoxicity of a 2-chloroethylnitrosourea analog of sarcosinamide in the SK-MG-1 human glioma cell line as a possible indicator for transport. J Neurooncol 7:189
19. Skalski V, Feindel W, Panasci LC (1990) Transport of amino acid amide sarcosinamide and sarcosinamide chloroethylnitrosourea in human glioma SK-MG-1 cells. Cancer Res 50:3062
20. Stahl W, Eisenbrand G (1991) Comparative study on the influence of two 2-chloroethylnitrosoureas with different carbamoylating potential towards glutathione and glutathione-related enzymes in different organs of the rat. Free Radic Res Commun 14:271
21. Suami T, Kato T, Takino H, Hisamatsu T (1982) (2-Chloroethyl)nitrosourea congeners of amino acid amides. J Med Chem 25:829
22. Supko JG, Phillips LR, Malspeis L (1996) Specific high-performance liquid chromatographic assay with ultraviolet detection for the determination of 1-(2-chloroethyl)-3-sarcosinamide-1-nitrosourea in plasma. J Chromatogr B Biomed Appl 677:351
23. Teicher BA (1997) Antitumor alkylating agents. In: DeVita VT, Rosenberg SA (eds) Principles and practice of oncology. Lippincott-Raven, Philadelphia, p 405
24. Tew KD, Colvin M, Chabner BA (1996) Alkylating agents. In: Chabner BA (ed) Cancer chemotherapy and biotherapy: principles and practice. Lippincott-Raven, Philadelphia New York, p 297
25. Weinkam RJ, Lin HS (1982) Chloroethylnitrosourea cancer chemotherapeutic agents. Adv Pharmacol Chemother 19:1
26. Wilson JE, Brown DE, Timmens EK (1965) A toxicological study of dimethyl sulfoxide. Toxicol Appl Pharmacol 7:104