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 SarCNU 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 monoeponential 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 ± 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 Cmax 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 ± 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 · BSF: body surface area · CENU: chloroethylnitrosourea · CL: total plasma clearance · Cmax: 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 · t1/2, i: half-life of the initial disposition phase · t1/2, abs: half-life of the apparent absorption phase · t1/2, z: half-life of the terminal disposition phase · tlag: absorption lag time · tmax: time of the peak plasma concentration · V1: apparent volume of distribution of the central compartment · V2: total body apparent volume of distribution
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 uptake2 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 physiochemical 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

![Chemical structure of SarCNU](image)

Fig. 1 Chemical structure of SarCNU

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.

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/2F1 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 methoxyflurane 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 washout period of 35 days 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