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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

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Summary. The cytotoxicity of a new compound, sarcosinamide chloroethylnitrosourea (SarCNU), was compared with that of the clinically available *bis*-chloroethylnitrosourea (BCNU) in 13 primary human gliomas and in 3 human glioma cell lines using the Human Tumor Cloning Assay (HTCA). At concentrations ≤ 16 μg/ml, SarCNU reduced the growth to $\leq 30\%$ of control in 11 of 13 primary gliomas. At similar concentrations, BCNU produced a comparable cytotoxic effect in 6 out of 13 specimens. At concentrations ≤ 16 μg/ml, BCNU reduced colony growth to $\leq 30\%$ of control in all three glioma cell lines and SarCNU produced the same effect in only one glioma cell line. A recently described statistical model [10], which employs the LD_{50} dose of new agents in mice, was used to estimate the achievable peak plasma concentration (PPC) of SarCNU. The calculated PPC for SarCNU was found to be 14.8 μg/ml compared with 2 μg/ml for BCNU. A reevaluation of the cytotoxic activities of SarCNU and BCNU at concentrations approximating their respective PPCs revealed that SarCNU reduced the growth to $\leq 30\%$ of control in one cell line at a concentration below its PPC. In contrast, BCNU exhibited similar toxicity in each cell line only at concentrations exceeding its PPC of 2 μg/ml. In the case of the primary gliomas, SarCNU was active ($\leq 30\%$ of control) in ten tumors at concentrations ≤ 14.8 μg/ml, whereas BCNU was active in only one glioma at a concentration ≤ 2 μg/ml. The results suggest that SarCNU should be more active than BCNU against human gliomas, provided that the statistical model used has correctly estimated the PPC of SarCNU.

Introduction

High-grade gliomas are inevitably fatal, with an average survival of 6–12 months following surgery. One of the most effective chemotherapeutic agents for malignant gliomas is *bis*-chloroethylnitrosourea (BCNU) [4, 14]. A major drawback to treatment with this drug is that it results in delayed, cumulative myelosuppression [3]. Furthermore, treatment consisting of BCNU and radiotherapy is not superior to radiotherapy alone [15]. The development of novel agents that would be more active against gliomas at less myelotoxic doses would be useful.

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SarCNU is a new chloroethylnitrosourea with a sarcosinamide moiety (Fig. 1) [12]. BCNU was compared with SarCNU in the Human Tumor Cloning Assay (HTCA) for in vitro cytotoxicity and in the CFU-C assay for in vitro myelotoxicity [7, 8]. At concentrations of 1–3 μg/ml, which reflect the range of the clinically achievable dose of BCNU, SarCNU was found to be more active with four primary glioma specimens in the HTCA and less myelosuppressive with normal human bone marrow in the CFU-C assay than BCNU. Since the peak plasma concentration (PPC) of SarCNU is not known, the relevance of its in vitro activity with respect to its clinical activity could not be assessed. A recently described statistical model may facilitate the choice of suitable concentrations of novel anticancer compounds for in vitro testing [10]. The model is based on a demonstrated correlation between the LD_{50} of a given agent in normal mice and its clinically achievable PPC in man. We have applied this relationship to obtain the predicted PPC values of SarCNU based on the LD_{50} value in mice. This permitted us to carry out a retrospective analysis of the activity of SarCNU and BCNU at concentrations similar to their respective PPCs in the HTCA with a total of 13 gliomas. We also carried out a similar comparison with three human glioma cell lines.

Materials and methods

Drugs. SarCNU was kindly provided by Dr. T. Suami, Keio University, Japan. BCNU was supplied by the Drug Development Branch, National Cancer Institute (Bethesda, Md).

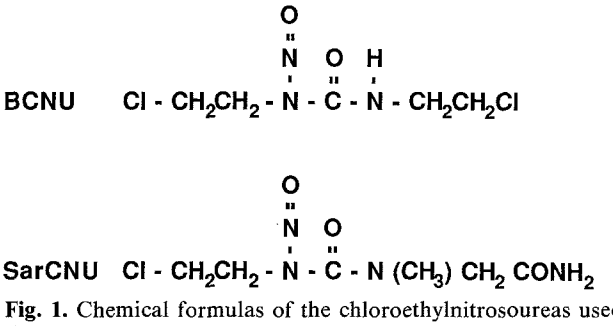

Fig. 1. Chemical formulas of the chloroethylnitrosoureas used in this study. The molecular weights of BCNU and SarCNU are 214 and 223, respectively. The LD_{50} dose in normal BDF_1 mice is approximately 35 mg/kg for BCNU and 392 mg/kg for SarCNU

Fresh human tumor cells. Primary glioma specimens were obtained from previously nontreated patients undergoing surgery for biopsy of these lesions. Single-cell suspensions of tumor cells were obtained using mechanical techniques [7].

Cell lines. The cell lines used were derived from previously nontreated human glioma biopsies. SKI-1 cells were a gift from Dr. J. Shapiro, Cornell University, New York. SK-MG-1 and SK-MG-4 cells were kindly provided by Dr. G. Cairncross, Western University, Ontario. All cell lines were grown and maintained in McCoy's 5A medium supplemented with 10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, NY) and 4 μg/ml gentamycin (Schering, Pointe Claire, Quebec) in a humidified 5% CO_2 atmosphere at 37°C. All cell lines were found to be free of mycoplasma with the Hoechst Stain Kit (Flow Lab, Mississauga, Ontario).

Clonogenic assay. The cytotoxicities of BCNU and SarCNU were determined in parallel in the in vitro HTCA as previously described [5, 7, 9, 13]. Briefly, exponentially growing cells or suspensions of primary gliomas were exposed to graded concentrations of BCNU or SarCNU for 1 h, then plated in semisolid, nutrient-rich media. Under these conditions, cells which are viable have given rise to colonies of 40 or more cells [6]. In the experiments with primary glioma specimens, the underlayers also contained 40 ng/ml of epidermal growth factor (Sigma). The cytotoxicity of BCNU and SarCNU was expressed as the percentage of control (six plates per control and six plates for each drug concentration), which is defined as the ratio of the number of colonies of treated cells to the number of colonies of nontreated cells multiplied by one hundred. The chloroethylnitrosoureas were considered active at concentrations resulting in a reduction in survival levels $\leq 30\%$ of control. These concentrations are designated as effective dose 30 (ED_{30}). With primary tumor specimens, it has previously been demonstrated that anticancer agents that reduce tumor colony growth to $\leq 30\%$ of control in the HTCA result in complete or partial responses in the majority (60%) of patients [13].

Statistical analysis. An analysis of variance with intergroup comparison by least significant differences was used to evaluate the data in HTCA [11].

Calculation of PPC values for SarCNU and BCNU. Peak plasma concentrations for SarCNU were obtained from a recently described regression equation [10], defined as: $\log (\text{PPC}) = -0.788 + (0.755 \times \log (LD_{50}))$, where LD_{50} is expressed in mg/kg and the PPC is obtained in μg/ml.

Results

1. Estimation of the PPC values for SarCNU and BCNU

SarCNU, whose LD_{50} in normal BDF_1 mice is 392 mg/kg [12], has been estimated to have a PPC value of 14.8 μg/ml [10]. The PPC of BCNU has previously been reported to be 2 μg/ml [2].

2. Cytotoxicity of SarCNU and BCNU with primary glioma specimens

A total of 52 glioma specimens were obtained from patients who had not received chemotherapy. Thirteen specimens grew sufficiently (≤ 30 colonies per plate) without excessive clumping to permit testing by the HTCA. The concentrations of BCNU and SarCNU used were in the range of 1–16 μg/ml. The ED_{30} values for SarCNU and BCNU were determined from the survival curves for the primary gliomas (% control vs μg/ml chloroethylnitrosourea) and are summarized in Table 1. Six of the glioma specimens were very small; therefore, a limited number of concentrations of each drug was used. In those cases, the same concentrations of each chloroethylnitrosourea were used. At 1–16 μg/ml, SarCNU reduced colony growth to $\leq 30\%$ of control in 11 of the 13 glioma specimens, whereas at similar concentrations BCNU produced an equivalent result in only 6 of the 13 samples. SarCNU was significantly ($P < 0.05$) more active at 1–3 μg/ml in six gliomas, whereas the two compounds had similar antitumor activity in five specimens at 1–3 μg/ml. BCNU was significantly more active than SarCNU in only two gliomas. The cytotoxicity of BCNU at concentrations ≤ 2.0 μg/ml and of SarCNU at concentrations ≤ 14.8 μg/ml was next examined. At these concentrations, SarCNU reduced colony growth to $\leq 30\%$ of control in ten specimens, whereas BCNU reduced the growth of only one glioma to $\leq 30\%$ of control.

3. Cytotoxicity of SarCNU and BCNU in cultured glioma cells

The dose response curves for SKI-1, SK-MG-4, and SK-MG-1 cells are shown in Figs. 2, 3, and 4, respectively. In each glioma cell line, the dose response curve for SarCNU demonstrates a change in slope at higher drug concentrations, which is not observed with BCNU. The concentrations reducing the colony growth to 30% of control (ED_{30}) are presented in Table 2. When the PPC values are not

Table 1. Estimation of ED_{30} for BCNU and SarCNU in thirteen primary glioma specimens

Glioma specimen[a]	1	2	3	4	5	6	7	8	9	10	11	12	13
ED_{30}[b] (BCNU μg/ml)	>4	3	12	>8	2.7	>8	>8	>8	3	>3	2.4	<1	>16
ED_{30}[b] (SarCNU μg/ml)	<1	1.8	<3	6	8	>8	1.5	2.2	9.8	3	15	<1	>16

[a] Because of the small size of some tumor specimens (tumors 1, 4, 6, 7, 8, and 10), only a limited number of concentrations of each drug was used

[b] The ED_{30} is the concentration that reduces the number of colonies to 30% of control values. The ED_{30} values were estimated from the dose response curves (% control vs μg/ml SarCNU or BCNU) obtained in the HTCA

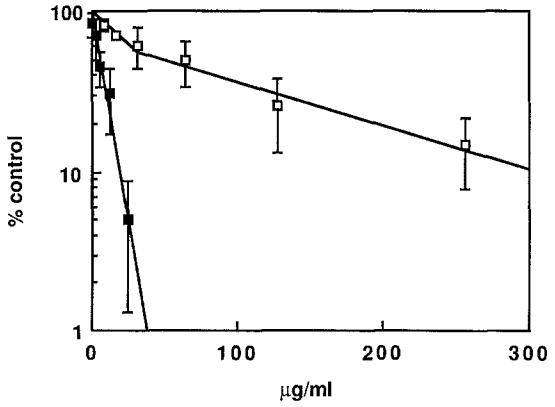

Fig. 2. Cytotoxic activity of SarCNU and BCNU in SKI-1 cells in the HTCA. The percentage of control (the percentage of control colonies) was plotted against the concentrations in µg/ml of BCNU [■] or SarCNU [□]. Each point represents the mean value of three or more independent experiments. The error bars represent the standard error of the mean

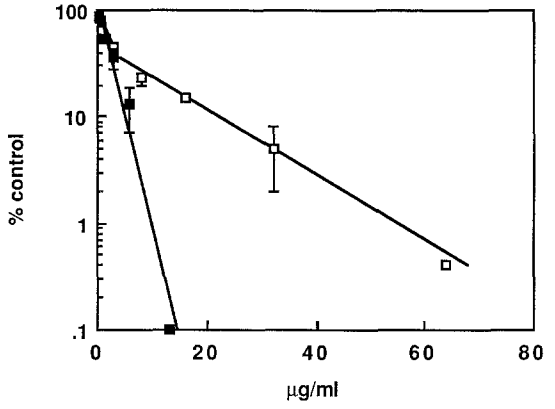

Fig. 3. Cytotoxic activity of SarCNU and BCNU in SK-MG-1 cells in the HTCA. The percentage of control (the percentage of control colonies) was plotted against the concentrations in µg/ml of BCNU [■] or SarCNU [□]. Each point represents the mean value of three or more independent experiments. The error bars represent the standard error of the mean

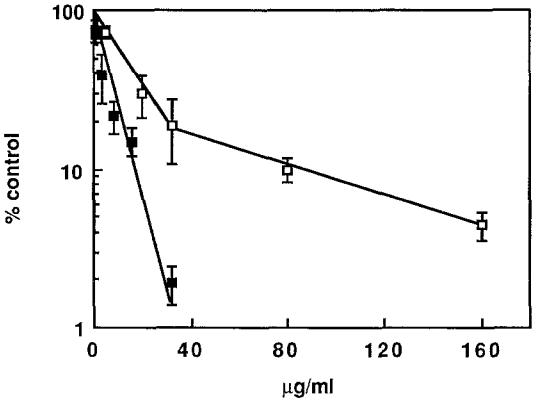

Fig. 4. Cytotoxic activity of SarCNU and BCNU in SK-MG-4 cells in the HTCA. The percentage of control (the percentage of control colonies) was plotted against the concentrations in µg/ml of BCNU [■] or SarCNU [□]. Each point represents the mean value of three or more independent experiments. The error bars represent the standard error of the mean

Table 2. Estimation of ED_{30} for BCNU and SarCNU in human glioma cell lines

Cell line	ED_{30}[a] BCNU	ED_{30} SarCNU
SKI-1	10.7 µg/ml	133 µg/ml
SK-MG-1	3.2 µg/ml	6.6 µg/ml
SK-MG-4	7.4 µg/ml	21.8 µg/ml

[a] The ED_{30} values were estimated from the dose response curves (% control vs µg/ml SarCNU or BCNU obtained in the HTCA)

considered, BCNU is more active than SarCNU in all three glioma cell lines (Figs. 2–4). However, evaluation of the data at the PPC reveals that at 14.8 µg/ml SarCNU reduces colony growth to <30% of control in SK-MG-1 cells, whereas at 2 µg/ml BCNU does not reduce colony growth to ≤30% of control in any of the glioma cell lines.

Discussion

A recently developed statistical regression model that estimates PPC values for new agents [10] allowed us to estimate the PPC for SarCNU and to assess its cytotoxicity at potentially relevant clinical concentrations. The PPC value generated from this model is supported by the in vitro CFU-C data in which SarCNU was 8-fold less toxic than BCNU to WBC progenitors in normal human bone marrow [7]. At concentrations below its estimated PPC, SarCNU was cytotoxic to the majority of the glioma specimens and to one human glioma cell line. In contrast, BCNU was toxic to only one glioma specimen and inactive in the three human glioma cell lines at concentrations below its PPC.

The survival curves obtained for BCNU in each glioma cell line are similar to those previously described [1, 16]. In contrast, the survival curves described with SarCNU change slope at higher drug concentrations in each cell line. Such phenomena may sometimes represent saturation of a carrier-mediated transport mechanism. We are presently investigating the mode of uptake of radiolabelled sarcosinamide in order to determine whether there is a carrier-mediated transport system for this amino acid amide in glioma cells.

The results obtained in the in vitro HTCA with primary glioma specimens and with glioma cell lines suggest that at clinically relevant concentrations, SarCNU is more active than BCNU. If the predicted PPC for SarCNU is truly reflective of significant differences between SarCNU and BCNU, then this new chloroethylnitrosourea may be more effective in the treatment of malignant gliomas.

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