Basic Investigations

ANTITUMOR EFFECT OF SARCNU IN A 0^6-METHYLGUANINE-DNA METHYLTRANSFERASE POSITIVE HUMAN GLIOMA XENOGRAFT MODEL

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ABSTRACT

Objective: To assess whether novel analogue of nitrosoureas, 2-chloroethyl-3-sarcosinamide-1-nitrosourea (SarCNU), has antitumor effect to 0^6-methylguanine-DNA methyltransferase (MGMT) positive tumors *in vivo*. Methods: MGMT positive human glioma cell line SF-767 xenografts in nude mice were treated with SarCNU. The antitumor efficacy of SarCNU was compared with the results of 1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU) treatment with or without 0^6-benzylguanine (0^6-BG) preadministration. Results: Since the SF-767 is MGMT strongly positive, BCNU treatment alone did not result in a satisfactory anticancer effect. As expected, 0^6-BG by depleting MGMT activity, significantly enhanced BCNU antitumor efficacy ($P<0.001$). More interestingly, SarCNU treatment alone had a better antitumor effect than 0^6-BG plus BCNU treatment ($F=51.7$, $P=0.00036$). Conclusion: Since SarCNU enters cells via extraneuronal monoamine transporter (EMT), the enhanced antitumor activity of SarCNU in this MGMT positive human tumor xenograft model may be due to the presence of EMT in SF-767. SarCNU may be used as an alternative treatment for MGMT positive tumors, specifically for tumors expressing EMT.

Key words: 2-chloroethyl-3-sarcosinamide-1-nitrosourea, Chemotherapy, Extraneuronal monoamine transporter, Glioma xenograft, 0^6-methylguanine-DNA methyltransferase

Chemotherapy for malignant brain tumors in addition to surgical resection and radiotherapy remains the foundation of glioma therapy. Unfortunately, the clinical response to a standard chemotherapeutic agent, such as chloroethylnitrosourea (CENU), is unsatisfactory as many gliomas are resistant to CENU. One well-characterized mechanism of drug resistance involves DNA repair protein, 0^6-methylguanine-DNA-methyltransferase (MGMT).[1,2] Accumulating evidence indicates that MGMT-positive tumors are more resistant to CENU than MGMT-negative tumors.[1–4] A novel analogue of nitrosoureas, 2-chloroethyl-3-sarccsinamide-l-nitrosourea (SarCNU) that presently is undergoing phase I clinical trial, as compared to 1, 3-bis(2-chloroethyl)-l-nitrosourea (BCNU), has demonstrated increased anti-effects both *in vitro* and *in vivo*.[5–7] However, the previously reported *in vivo* results were from studies with MGMT negative tumors. In the present investigation, we used an MGMT positive human glioma SF-767 xenograft model to assess whether SarCNU also has a superior antitumor effect on MGMT positive tumors.

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MATERIAIS AND METHODS

Human Glioma Animal Models

Random-bred female or male athymic (NCR nu/nu) mice were housed on sterile bedding in microisolator cages (NASA 1000) with water and food provided *ad libitum*. All animal studies were conducted in Chinese Experimental Animal Association approved facilities. The human tumor xenografts were produced as previously described.[8] Briefly, human glioma SF-767 cells (1.2×10^7 /0.5 ml) were injected subcutaneously (s.c.) in the right axillary region of the mice (male or female) at age 6–8 weeks with an approximate body weight of 20 g. As soon as the tumor reached about 1000 mm^3 in size, the mice were sacrificed, and the tumor fragment approximately 4 mm^3 was used for reimplantation. When the tumors reached around 150 mm^3 in size, the mice were grouped randomly and treatment was initiated.

Treatment

SarCNU (National Cancer Institute, USA) was dissolved in 0.001 mmol/L sodium citrate buffer (pH 4.0), while BCNU (Bristol Laboratories of Canada, Montreal, Canada) was dissolved in 10% ethanol in saline for administration. 0^6-benzylguanine (0^6-BG), a kind gift from Dr. Robert C. Moschel, at NCI-Frederick Cancer Research and Development Center, Frederick), was dissolved in dimethyl sulfoxide. Treatment of SarCNU or BCNU at a dosage of 167 mg/kg or 20 mg/kg respectively, was scheduled as q4d × 3 intraperitoneally (i.p.). The 0^6-BG (100 mg/kg, i.p.), if used, was administered 24 hours before the treatment.

Observation

The tumors were measured in two dimensions twice weekly. The tumor volume was calculated using the formula $(a^2 \times b)/2$ (a: width in mm, b: length in mm).[7] The changes in tumor size for each treated (T) versus control (C) group (%T/C) were calculated by dividing the median treated tumor size by the median control tumor size on each observation day and multiplying by 100. A %T/C value less than 40 was considered active. The log cell kill was calculated as {[(T/C)-Duration of treatment] × 0.301}/doubling time, where the doubling time is the time required for the tumor size to increase from 200 mm^3 to 400 mm^3. T and C were the median days to reach the tumor size of 500 mm^3 for treated and control mice, respectively. A log cell kill of zero indicates that the cell population at the end of treatment was the same as it was at the start of treatment. A log cell kill +6 indicates a 99.9999% reduction in the cell population. Tumor growth delay is expressed as a percentage [%(T-C)/C] by which the treated group is delayed in attaining a specified number of doubling time compared to controls. T and C are the doubling time in days for treated and control group, respectively.

Statistical Analysis

Statistical analysis was done utilizing analysis of variance with intergroup comparisons (Microsoft Excel version 4.0).

RESULTS

There were no drug related animal deaths in the entire experiment. All the treatments, except only the 0^6-BG group, were active even though no complete tumor regressions or tumor free animals were observed. All the animals treated only with BCNU showed a very mild antitumor effect (Figure 1, Table 1). Although the optimal %T/C at day 20 was 32.1 which is considerably effective, the net log cell kill was only -0.0067, indicating the tumor cell population was not reduced by treatment with BCNU alone. As expected, the animals that received 0^6-BG before the BCNU injection, had significantly improved antitumor efficacy (Figure 1, Table 2). Surprisingly, the mice bearing SF-767 tumors that were treated with SarCNU alone had an even better anticancer effect than those of the 0^6-BG plus BCNU group (Figure 1 and Figure 2, Table 2).

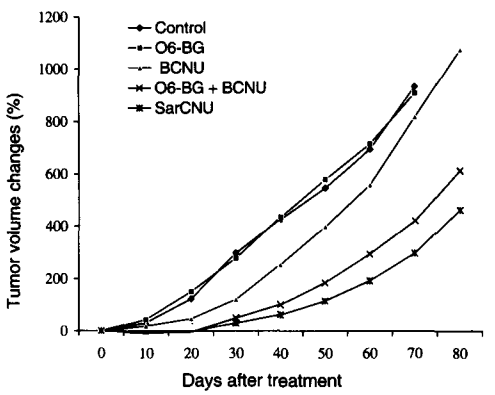

Fig. 1. Tumor size changes of human glioma SF-767 xenografts after treatment.

DISCUSSION

Chloroethylnitrosourea alkylates DNA at the 0^6-position of deoxyguanosine resulting in chloroethyl adduct which further undergoes an intramolecular circularization and then crosslinks DNA, producing a lethal lesion. MGMT can repair alkyladducts formed by

CENU so that prevents further formation of DNA crosslinks thereby decreasing the cytotoxicity of CENU.[2] 0^6-BG is a free base inhibitor which can inactivate MGMT, therefore reverse CENU drug resistance.[9] In this study, as expected, by using 0^6-BG and thereby depleting MGMT activity, BCNU antitumor activity was significantly enhanced. We previously compared SarCNU with BCNU in a MGMT negative human glioma SHG-44 xenograft model.[8] SarCNU at its maximally tolerated dosage (MTD) demonstrated excellent activity against the human glioma cell line SHG-44, in an athymic mice solid tumor model (both s.c. or i.c.). These results have also been confirmed by using SF-295 human glioma s.c. xenograft model.[7] However, SHG-44 and SF-295 cell lines were MGMT negative or MGMT poor. In the present investigation, the SF-767 cell line was MGMT strongly positive,[10] and also demonstrated a mild antitumor efficacy, when it was treated only with SarCNU. The SarCNU antitumor efficacy was even superior to that of BCNU plus 0^6-BG treatment, indicating that SarCNU may present an alternative treatment for MGMT positive tumors.

Table 1. Response of human glioma xenografts to treatment

Xenograft	Treatment*	n	Optimal %T/C (day)	Net log cell kill	Growth delay % (T-C)/C
SF-767	Control	6			
	0^6-BG	6	87 (30)	–0.261	8
	BCNU	6	32.1 (20)	–0.007	20
	0^6-BG + BCNU	6	-7.7 (10)	0.205	101
	SarCNU	6	-9.1 (10)	0.310	150

*BCNU (20 mg/kg) and SarCNU (167 mg/kg) were scheduled as q4d × 3 ip. 0^6-BG (100 mg/kg) was given 24 hours before BCNU or SarCNU administration.

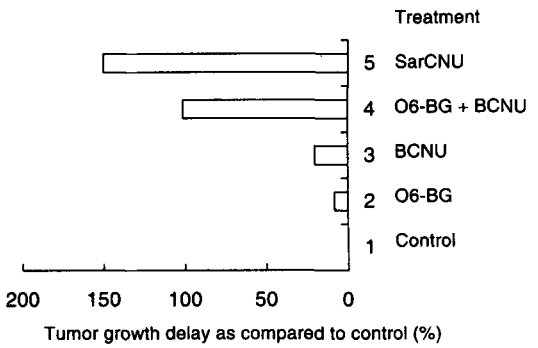

Fig. 2. Tumor growth delay of human glioma SF-767 xenografts after treatment.

Table 2. Comparison of optimal % T/C in different treatment groups in SF-767 xenografts

Comparison	F	P
Control & 0^6-BG	0.3	0.58582269
& BCNU	3.6	0.10499599
& 0^6-BG+BCNU	62.1	0.00022070
& SarCNU	86.1	0.00008854
BCNU & 0^6-BG+BCNU	894.7	0.00000009
& SarCNU	776.5	0.00000014
0^6-BG+BCNU & SarCNU	51.7	0.00036524

SarCNU contains an amino acid amide group, N-methylglycinamide, known as sarcosinamide, which allows the drug to enter cells via the extraneuronal transporter for monoamine transmitters (EMT), i.e. extraneuronal noradrenaline transporter or uptake.[6] Utilizing the relatively SarCNU-resistant SKI-1 human glioma cell line and the SarCNU-sensitive SKMG-1 cell line, it has been demonstrated that SarCNU uptake was more rapid and was saturable in the SKMG-1 cells. Furthermore, the characteristic of SarCNU uptake suggested that the drug uptake was via the EMT.[11] Utilizing reverse-transcription polymerase chain reaction (RT-PCR), we have determined that SKMG-1 is EMT-rich while SKI-1 is EMT-poor with approximately a 14 fold difference.[10] Thus the enhanced cytotoxicity in SKMG-1 may due to its higher EMT expression. The SF-767 cell line used in the present xenograft model is EMT strongly positive. As indicated in this study, SarCNU alone demonstrated significant antitumor efficacy in this xenograft model, suggesting that EMT may also be important in the *in vivo* response to SarCNU. Thus, EMT expression in a tumor may serve as a marker for SarCNU treatment. Fortunately, EMT has been shown to be expressed in various tissues including glial cells and in many human tumor cell lines.[10, 12, 13] We recently have investigated EMT expression by RT-PCR, in 30 human brain tumor specimens and found that the majority of the brain tumors have strong EMT expression, only two samples had no detectable EMT (data not shown). As demonstrated in the present investigation SarCNU shows a superior antitumor efficacy than BCNU plus 0^6-BG in a xenograft model expressing both MGMT

plus 0^6-BG in a xenograft model expressing both MGMT and EMT. It can be further suggested that in EMT expressing tumors, SarCNU may serve as a good chemotherapeutic agent despite the presence of MGMT.

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