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Antitumor Effects of GANU and Other Nitrosourea Derivatives Against Transplantable Leukemias and Solid Tumors in Mice

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Summary. *The antitumor effects of GANU have been examined in a panel of mouse tumors for which data appear to be lacking in the literature. GANU has significant activity against P388 leukemia and TLX5 lymphoma, and also against the solid tumors B16 melanoma and Lewis lung carcinoma; pulmonary metastases of this tumor are particularly sensitive to the effects of GANU. The effects of GANU on TLX5 lymphoma and Lewis lung carcinoma are less pronounced than those of BCNU and CCNU, as already reported for L1210 leukemia. In contrast with other results obtained with this tumor, chlorozotocin has a less pronounced effect than GANU, and virtually none in lung metastases of Lewis lung carcinoma.*

Introduction

Chloroethyl nitrosoureas are a class of antitumor drugs with established clinical antitumor activity against several human malignancies [2–4]. On the other hand, these drugs exert delayed and cumulative bone marrow toxicity, which seriously limits their clinical applications [4, 11]. Numerous chloroethyl nitrosourea derivatives have been synthesized and examined for antitumor activity against transplantable animal tumors; 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (chlorozotocin) and 1-(β-D-glucopyranosyl)-3-(2-chloroethyl)-3-nitrosourea (GANU) are two water-soluble compounds, characterized by the presence in their molecule of a glucose carrier, which appear of particular interest since they cause reduced myelosuppression in mice [1, 4]. Relatively few reports on the antitumor activity of GANU have appeared in the literature compared with chlorozotocin and other nitrosoureas. The aim of the present investigation was therefore to examine the effects of GANU on a broader panel of tumors for which data appear to be lacking in the literature, such as TLX5 lymphoma and P388 leukemias and the solid tumors B16 melanoma and Lewis lung carcinoma in mice. For Lewis lung carcinoma, the differential effects of drug treatment on primary SC tumor growth and on the formation of spontaneous pulmonary metastases have been evaluated; the effects of GANU have been determined in comparison with those of BCNU, CCNU, and chlorozotocin.

Materials and Methods

DrugTreatment. 1,3-Bis(2-chloroethyl)-1-nitrosourea(BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (chlorozotocin) were kindly supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda MD, USA; 1-(β-D-glucopyranosyl)-3-(2-chloroethyl)-3-nitrosourea (GANU) was provided by Schering SpA, Milan, Italy. The drugs were administered IP in volumes of 0.1 ml/10 g animal weight. BCNU and CCNU were suspended in olive oil; chlorozotocin and GANU were freshly dissolved in isotonic NaCl solution.

Tumor Transplantation and Evaluation. The tumor lines were originally provided by the National Cancer Institute, USA, with the exception of TLX5 lymphoma, which was obtained from the Chester Beatty Research Institute, London, England. The implantation of the tumors and the evaluation of the effects of drug treatment was carried out following the protocols of the National Cancer Institute USA [5] and as already described [6, 7, 15]. The tumor lines have been maintained and propagated in CBA/LAC mice for TLX5 lymphoma, in DBA/2 mice for P388 leukemia, and in C57BL/6 mice for B16 melanoma and Lewis lung carcinoma; BDF1 hybrids were used in tests for antitumor activity (CBA/LAC mice were used for TLX5 lymphoma).

Results and Discussion

The results obtained in our tests of the antitumor effects of GANU and a comparison with other nitrosoureas are reported below; the highest doses used for each treatment schedule were the maximum tolerated doses, since the next doses higher cause more than 50% toxic deaths [5]. Data reported in Tables 1 and 2 show that GANU is active in increasing the lifespan of mice bearing P388 leukemia and TLX5 lymphoma. The survival time is significantly increased over a broad range of doses, and some cures (20%) are observed at optimal dosages. On TLX5 lymphoma, the range of active doses of GANU is similar to that of BCNU, whereas CCNU and chlorozotocin are active over narrower dose ranges; however, the increase in lifespan caused by GANU at the optimal dosage is less pronounced than that obtained with the other nitrosoureas. The 100% cure rate observed at the maximum tolerated dose of CCNU is noteworthy.

GANU also appears to be active in solid mouse tumors, as indicated by the data displayed in Tables 3 and 4. The survival time and tumor growth in mice bearing SC B16 melanoma are significantly increased and reduced at dose levels two and one, respectively. Lewis lung carcinoma is also responsive to the

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Table 1. Effects of GANU on the survival time of mice bearing P388 leukemia

Dose (mg/kg)	Survival time		Animals cured/ treated[b]
	days	% T/C[a]	
0	13.8 ± 0.29	–	0/10
0.94	20.2 ± 0.66	146	
1.88	22.2 ± 0.58	161	
3.75	24.2 ± 0.49	175	
7.5	26.8 ± 2.06	194	1/5
15	35.0 ± 3.24	254	1/5

Each value is the mean ± SE obtained in groups of five tumor-bearing mice (10 controls) treated IP 24 h after tumor implantation (10^6 cells/mouse IP). All the reported values obtained in treated groups are significantly different from the control value (Student-Newmann-Keuls test [16]: $P = 0.05$)

[a] % T/C is the percent ratio of the average of each treated group to controls

[b] Animals surviving after 3 months

Table 2. Effects of the tested nitrosourea derivatives on the survival time of mice bearing TLX5 lymphoma

Compound	Dose (mg/kg)	Survival time		Animals cured/ treated[b]
		Days	% T/C[a]	
–	–	9.67 ± 0.21	–	0/19
BCNU	1.19	12.2 ± 0.37	126	
	2.38	13.4 ± 0.24	139	
	4.75	16.3 ± 1.03	169	1/5
	9.5	15.2 ± 0.49	157	
	19	18.2 ± 1.07	188	
CCNU	6.2	12.8 ± 0.49	132	
	12.5	13.4 ± 0.24	139	
	25	16.5 ± 0.65	171	1/5
	50	120	1,240	5/5
Chlorozotocin	3.12	12.2 ± 0.20	126	
	6.25	14.3 ± 0.63	148	1/4
	12.5	17.8 ± 1.16	184	
GANU	0.95	12.0 ± 0.00	124	
	1.88	13.5 ± 0.34	140	
	3.75	14.3 ± 0.61	148	
	7.5	14.7 ± 1.15	152	
	15	12.6 ± 2.42	130	1/6

Each value is the mean ± SE obtained in groups of four to six tumor-bearing mice (19 controls) treated IP 24 h after tumor implantation (10^5 cells/mouse IP). All the reported values are significantly different from the control value (Student-Newmann-Keuls test [16]: $P = 0.05$)

[a] % T/C is the percent ratio of the average of each treated group to controls

[b] Animals surviving after 3 months

Table 3. Effects of GANU on primary tumor growth and on the survival time of mice bearing SC B16 melanoma

Dose (mg/kg/day)	Primary tumor weight (mg)[a, b]	Survival time (days)[b]
0	1,200 ± 158	24.7 ± 1.37
0.63	1,025 ± 261	27.0 ± 2.63
1.25	1,033 ± 206	28.1 ± 2.04*
2.5	435 ± 55*	32.6 ± 1.50*

Each value is the mean ± SE obtained in groups of 10 tumor-bearing mice treated IP daily on days 1–9 after tumor implantation (100 mm^3 of tumor fragments/mouse SC)

[a] Primary tumor weight was determined on day 14

[b] An asterisk denotes a significant difference from controls (Student-Newmann-Keuls test [16]: $P = 0.05$)

effects of GANU, since the growth of the SC primary tumor is significantly reduced at the maximum tolerated dose; spontaneous pulmonary metastases of this tumor appear very sensitive to the effects of GANU, and their number and weight is significantly reduced at the three dose levels tested. In Lewis lung carcinoma, chlorozotocin is approximately as active as GANU in SC primary tumors, whereas it is practically ineffective in lung metastases. BCNU, and particularly CCNU, display a more pronounced activity than GANU and chlorozotocin, both in SC tumors and in pulmonary metastases.

It is thus seen that GANU displays significant antitumor activity in the tumor panel presently employed, in addition to the activity already reported against L1210 leukemia [1, 4, 9, 12], ascitic sarcoma 180 and hepatoma AH-130, Walker carcinosarcoma-256, CCMT spontaneous mammary adenocarcinoma [9], and xenografts of MX-1 mammary adenocarcinoma in nude mice [10]. The antitumor activity observed for GANU in the present set of experiments appears significantly less pronounced than that of BCNU and particularly CCNU, in agreement with detailed structure-activity studies performed in L1210 leukemia [8, 14]. In contrast with the results obtained in these studies [8, 14], the activity of GANU in TLX5 lymphoma and Lewis lung carcinoma seems more pronounced than that of chlorozotocin; the high sensitivity of Lewis lung carcinoma pulmonary metastases to GANU appears particularly interesting.

The data available so far indicate that the antitumor activity exerted by maximum tolerated doses of GANU against the transplantable rodent tumors used is less pronounced than that of BCNU and CCNU, in spite of the reduced hematological toxicity of GANU. Moreover, a phase I study of GANU indicates a dose-dependent hematological toxicity in man [13]. Additional clinical studies, together with experimental investigations on the mode of action, may eventually indicate whether the use of GANU against selected tumors is advantageous, in terms of therapeutic index, in comparison with other nitrosoureas.

Table 4. Effects of the tested nitrosourea derivatives on SC tumor growth and on the formation of spontaneous pulmonary metastases in mice bearing SC Lewis lung carcinoma

Compound	Dose (mg/kg/day)	Primary tumor weight (mg)[b, c]	Lung metastases[b, c]		
			Number	Weight	Animals free
–	–	1,355 ± 88	29.0 ± 2.4	100.2 ± 13.7	0/51
BCNU	1.38	1,314 ± 279	32.2 ± 3.8	89.4 ± 18.0	0/8
	2.75	751 ± 81*	6.9 ± 0.9*	9.2 ± 2.4*	1/16
	5.5	459 ± 34*	3	5.3	7/8
CCNU	4.13	894 ± 134*	1.6 ± 0.2*	0.1 ± 0.0*	4/8
	8.25	0	0	0	16/16
	16.5	0	0	0	16/16
Chlorozotocin[a]	0.88	1,762 ± 329	27.6 ± 3.3	97.9 ± 26.7	0/16
	1.75	979 ± 120	25.9 ± 2.9	54.9 ± 11.2*	0/16
	3.5	638 ± 34*	27.4 ± 8.1	63.8 ± 28.7	1/8
GANU	0.62	1,137 ± 88	18.7 ± 2.5*	60.2 ± 14.1*	0/10
	1.25	977 ± 82	17.2 ± 2.5*	47.3 ± 8.9*	0/10
	2.5	509 ± 94*	11.5 ± 2.8*	29.9 ± 6.2*	3/10

Each value is the mean ± SE obtained in groups of eight to sixteen tumor-bearing mice (51 controls) treated IP daily on days 1–14 after tumor implantation (100 mm^3 of tumor fragments/mouse SC)

[a] Administered in olive oil

[b] Primary tumor weight was determined on day 14 and lung metastases on day 21

[c] An asterisk denotes a significant difference from controls (Student-Newmann-Keuls test [16]: $P = 0.05$)

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