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Comparative Analysis of Bioactivity of the Russian-Made Antitumor Substances of the Nitrosourea Group

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We performed an *in vivo* comparative study of activity of three substances of the nitrosourea group produced in Russia. All substances demonstrated high antitumor activity against various solid and leukemic tumors. Aranosa significantly enhanced life duration in mice with leukemia (by 65-194%) and inhibited the growth of solid tumors (by 49-99.6%). Lisomustine and ormustine showed higher activity than aranose. Single administration of lisomustine increased life span of mice (by 22-114%) and resulted in cure of all animals in four models: lymphoblastic leukemia L-1210, lymphocytic leukemia P-388, Lewis lung carcinoma, and cervical cancer RShM-5. After ormustine treatment, full recovery was observed only in groups with lymphocytic leukemia P-388 and cervical cancer RShM-5. These findings attest to higher activity of lisomustine in the studied models.

Key Words: *antitumor activity; nitrosourea-based substances; tumor*

Substances based on nitrosourea (NU) are among the most promising groups of modern antitumor agents in clinical oncology. They are widely used in clinical practice for the treatment of CNS tumors, combined therapy of some solid tumors, and hemoblastoses [9]. Their effects are determined by their ability to cross the blood—brain barrier, differences in the molecular mechanisms of action for substances with similar structure, lipophility, and delayed myelosuppressive effects (5-6 weeks) [5].

The introduction of nitrosourea group into organic substances of various types (aliphatic and cycloaliphatic carbohydrates, heterocycles, and sugars) yielded a variety of active antitumor substances that considerably differ by their therapeutic and toxic effects on various types of tumors and normal tissues [13].

Molecular mechanisms of bioactivity of NU are determined by high reaction activity of their biodegradation products (alkylation and carbamoylation of macromolecules). This results in modification of DNA structure, impairment of transcription and translation, and blockage of reparation systems (mostly due to inhibition of O6-alkylguanine transferase) [15].

Comparative analysis of various NU substances showed that agents with high alkylating activity, high solubility in lipids, and low chemical stability (high reaction activity) demonstrate higher antitumor activity [14].

Similar to the majority of antitumor substances, NU derivatives are characterized by high systemic toxicity. Selectivity can be improved via conjugation of the cytotoxic part of the molecule with various functional groups promoting penetration of NU molecules into tumor cells [8], *e.g.* lisomustine and ormustine carry amino acid residues that are more intensively accumulated by tumor cells than by normal cells. The presence of amino acid residue provides more effective transport of the agents through the blood—brain barrier. The same function performs monosaccharide L-arabinose residue in the structure of aranose (Fig. 1).

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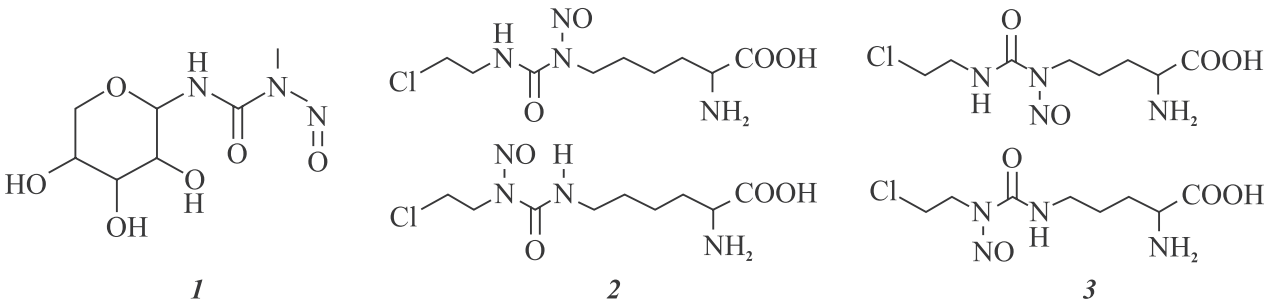

1 *2* *3*

Fig.1. Structural formulae of aranose (*1*), lisomustine (*2*), and ormustine (*3*).

Testing of Russian-produced drugs aranose, lisomustine, and ormustine has been performed at the N. N. Blokhin National Medical Research Center of Oncology since 1970s until now. Based on the results of preclinical [10,11] and clinical studies, aranose and lisomustine are used for the treatment of melanoma and lung cancer. These drugs are well tolerated and exhibit anti-metastatic effects. Another promising agent ormustine exhibits high antitumor activity against Mel Kor human disseminated melanoma and Jurkat human T-cell leukemia cell lines [1]. Comparison of the cytotoxic effects of aranose, lisomustine, and ormustine against various cell lines showed higher activity of ormustine [3].

Here we compared antitumor activity of these drugs of the NU group on mice with transplanted tumors.

MATERIALS AND METHODS

The antitumor activity of three Russian-made preparations of the NU group aranose (lyophilizate for preparing solution for injection, 500 mg; Naukoprofi Branch of the N. N. Blokhin National Medical Research Center of Oncology), lisomustine (lyophilizate for preparing solution for injection, 100 mg), and ormustine (lyophilizate for preparing solution for injection, 125 mg; N. N. Blokhin National Medical Research Center of Oncology) was analyzed.

Antitumor activity was studied on the immunocompetent hybrid (C57Bl/6-DBA/2)F1 mice (18-25 g) with transplanted tumor and leukemia. The animals were kept in an experimental biological laboratory (vivarium) of the N. N. Blokhin National Medical Research Center of Oncology and received water and pelleted food *ad libitum*. All experiments were performed in accordance to the ethical requirements to investigations on biomodels and laboratory animals accepted at the N. N. Blokhin National Medical Research Center of Oncology [2].

The following models were used: L-1210 lymphoblastic leukemia, P-388 lymphocyte leukemia, La hemocytoblastoma, MOPC-406 plasmacytoma, and solid tumors (Ca-755 mammary adenocarcinoma, Akatol colon adenocarcinoma, LLC Lewis lung cancer, B-16 melanoma, RShM-5 cervical cancer, and S180 sarcoma).

Aranose in a single dose of 150-200 mg/kg was intramuscularly injected 5 times every 24 h. Lisomustine in a dose of 175-200 mg/kg was administered intraperitoneally. Ormustine in a dose of 125 mg/kg was injected intravenously.

Solid and ascitic tumors were inoculated to the laboratory animals by the standard method. For transplantation, solid tumor tissues were minced with scissors to homogenous substance, medium 199 was added at a ratio 1:10, and 0.5 ml of this suspension (~50 mg tumor cells) was injected subcutaneously in the right axillary area. During transplantation of ascitic tumors, the mice received 0.3 ml ascitic fluid diluted with medium 199 and containing 10^6 tumor cells. The treatment started 24 h after modeling of the hematopoietic tumors and 48 h after inoculation of solid tumors.

Antitumor activity was estimated by tumor growth inhibition (TGI), life span prolongation (LSP), and cure (% of animals without signs of tumor process within 90 days). TGI was measured every 3-4 days, LSP was calculated for mice living less than 90 days, cure was evaluated after 90 days.

To estimated TGI, tumor volume was calculated by the multiplication of three maximal perpendicular sizes of the tumor node (length, width, and height) in each animal. Then the mean volume of the tumor per group was calculated. The measurements of the tumor volume were performed after completion of treatment course.

TGI was calculated by the formula:

$$TGI=(Vc-Vt)/Vc\times100\%,$$

where Vc and Vt are the mean volume of the tumor (mm^3) in the control and treatment groups, respectively.

LSP was calculated in mice living less than 90 days by the formula:

$$LSP=(Dc-Dt)/Vc\times100\%,$$

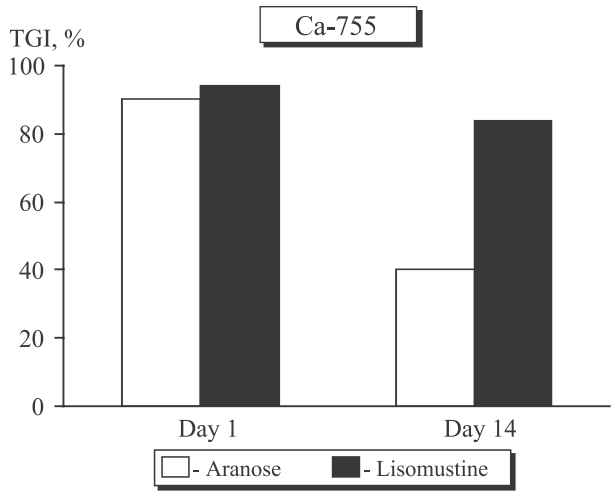

Fig. 2. Changes in TGI in mice with mammary adenocarcinoma Ca-755 on days 1 and 14 after treatment termination [10,11].

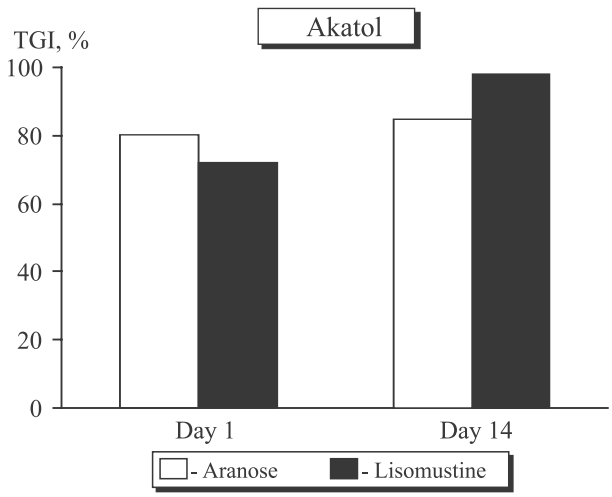

Fig. 3. Changes in TGI in mice with colon adenocarcinoma Akatol at days 1 and 14 after treatment termination [10,11].

where Dc and Dt are the mean life span (days) of mice in the control and treatment groups, respectively.

TGI≥50% and LSP≥50% were taken as the minimum activity criteria.

The groups were formed in order to obtain statistically significant results: control group (no treatment) consisted of 8-12 animals and treatment groups consisted of 6-8 mice.

Statistical analysis was performed using the Fisher and Student's test [4]. Between-group differences were considered significant at $p \leq 0.05$.

RESULTS

In previous studies, activity of Russian-produced made drugs, NU derivatives, was compared with that of foreign medicinal substances. It was found that aranose and lisomustine have higher activity towards plasmacytoma MOPC-406 and spontaneous leukemia in AKR mice in comparison with carmustin [1,3,5]. The therapeutic effect of ormustine was shown to be similar to the effect of mustoforan [6].

We compared antitumor activity of the test drugs against leukemia and solid tumors. Analysis of the spectrum of antitumor activity showed that all drugs are active against hemoblastoses (Table 1). Aranose significantly increased life span in mice with all four leukemia types (65-194%). Single administration of ormustine and lisomustine [7,12,15] not only increased life span (by 22-113%), but also led to complete cure in some animals with L-1210 and P-388 cancer. The efficiency of lisomustine and ormustine against all studied leukemia types except for La hemocytoblastoma was higher than the efficiency of aranose.

All substances significantly inhibited the growth of solid tumors (Table 2). The most pronounced activity was observed in animals with LLC, cervical cancer RShM-5, and sarcoma S180. Lisomustine administration led to complete cure in 80-100% mice with LLC, sarcoma S180, and RShM-5. Activity of ormustine against LLC strain was inferior to that of lisomustine, while in mice B-16 melanoma, TGI and LSP were higher after ormustine treatment. TGI produced by aranose varied from 49 to 99.6% in different types of tumors. This parameter on the model of Akatol colon adenocarcinoma and B-16 melanoma was significantly higher than after the treatment with other drugs. This can be related to reduced carbamylating activity of this agent. However, as distinct from lisomustine and ormustine, aranose treatment did not enhance the life duration of mice.

Less pronounced antitumor activity against some types of the tumors can be related to poor penetration through the blood—brain barrier [5]. Higher activity of lisomustine and ormustine is probably associ-

TABLE 1. Antitumor Activity of NU in Various Leukemia Models

Parameter	Tumor strain			
	L-1210	P-388	La	MOPC-406
Aranose				
LSP, %	143	194	65	103
Lisomustine				
LSP, %			22	113
Cure, %	100	100	100	100
Ormustine				
Cure, %	66.7	100	—	—

Note. "—" not analyzed.

TABLE 2. Antitumor Activity of NU towards Various Types of Solid Tumors

Parameter	Tumor strain					
	Ca-755	Akatol	B-16	LLC	RShM-5	S180
Aranose						
TGI, %	90	80	99.6	52	—	49
Lisomustine						
TGI, %	94	72	97			
LSP, %	67	114	70			
Cure, %				100	100	80
Ormustine						
TGI, %	—	—	93.3	99.9		—
LSP, %	—	—	84	84		—
Cure, %	—	—			100	—

Note. "—" not analyzed. All changes in TGI were significant in comparison with the control ($p \leq 0.05$).

ated with production of alkylating and carbamoylating molecules as well as α-amino acid derivatives during hydrolysis. These substances act as potential antimetabolites. Moreover, chloroethyl group present in these preparations significantly increases the rate of their degradation, which might enhance the therapeutic effects of these agents.

The duration of the antitumor effects is an important factor of the efficiency of antitumor drugs. Comparison of TGI for aranose and lisomustine on days 1 and 14 after treatment termination (Figs. 2, 3) showed that TGI decreased by day 14 [1,11], but the antitumor activity was still present which attested to long-term antitumor effect and efficiency of these substances at various terms of tumor development.

The time when the treatment was started directly affects tumor size; therefore, in the treatment of advanced tumors, substances with high antitumor activity are preferable. According to previous reports [10-12], administration of drugs at the early stages of tumor growth (on day 2 after tumor inoculation) was more effective than administration at the stage of advanced tumor (days 7-9 after tumor inoculation) (Table 3).

The *in vivo* study of the efficiency of drugs based on NU showed broad spectrum of the effects of aranose, lisomustine, and ormustine against various types of leukemia and solid tissues. High TGI on day 14 after treatment termination suggests that the antitumor activity of the test drugs is retained for a long time, while the effects observed after the treatment started at various time points (2 or 7-9 days after tumor inoculation) indicates high inhibiting activity. Lisomustine and ormustine had higher efficiency against most studied types of tumors due to the presence of amino acid residue in the structure. Single administration of lisomustine prolonged the life span in mice (by 22-114%) and led to complete cure in animals with four tumors (L-1210 lymphoblast leukemia, P-388 lymphocyte leukemia, LCC, and RShM-5 cervical cancer). Ormustine induced complete cure only in two studied models (P-388 and RShM-5). These results demonstrate higher activity of lisomustine on the studied models.

TABLE 3. Dependence of TGI (%) on the Time When the Treatment Was Started (according to [10-12])

Substance	Tumor strain	Time when the treatment was started, days	TGI on days 12-14 after treatment termination
Aranose	Akatol	2	77
		7	69
Lisomustine	LLC	2	Cure 100%
		7	Cure 63%
Ormustine	B-16	2	99.3
		9	63

Note. All changes in TGI were significant in comparison with the control ($p \leq 0.05$).

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