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Research Article

Antiproliferative activity of a new derivative from the class of N-glycoside of indolo[2,3-a]pyrrolo[3,4-c]carbazoles

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Abstract

Introduction: The creation of highly effective original anticancer drugs remains an urgent direction of scientific research in tumor therapy. One of the promising groups in this regard is indolocarbazoles and their derivatives, which are capable of initiating various pathways of tumor cell death. The aim of the study was to evaluate an antiproliferative activity of a new, Russian derivative of N-glycoside substituted indolocarbazole 6-amino-12-(α-L-arabinopyranosyl) indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione (LCS-1208) on models of transplantable tumors of mice and on human tumors in *Balb/c* nude mice.

Materials and methods: Indolocarbazole sensitivity to LCS-1208 was assessed on transplantable tumors of mice – lymphatic leukemia L-1210, cervical carcinoma (CC-5), and colon adenocarcinoma (CAC) by five-fold intraperitoneal administration (ip) of the LCS-1208 substance in single doses of 50, 75, 100 mg/kg. Investigation into the effectiveness of the LCS-1208 lyo dosage form was performed on subcutaneous xenografts of human colon cancer SW620 by an intravenous administration (iv). The antitumor effect was evaluated by the tumor growth inhibition (TGI) and an increase in life span (ILS) of the treated animals as compared with the control ones. Evaluation of specific antitumor activity on xenografts was performed according to the tumor/control (T/C%) criterion (maximum criterion T/C≤42%).

Results and discussion: According to the results of the study, the most sensitive to the action of the LCS-1208 substance in the case of an ip administration of a total dose of 375 mg/kg were CAC with TGI=97–62%, p≤0.001 up to 16 days after the treatment, and ILS=36% (criteria for TGI≥70% and ILS≥25%). On xenografts of a human colon cancer SW620, the effectiveness of the LCS-1208 lyo drug dosage form within the range of total doses from 50 to 150 mg/kg in case of iv to *Balb/c* nude mice was set at T/C = 35–2% (criterion T/C<42%).

Conclusion: The presented results suggest possible effectiveness of LCS-1208 in treatment of colon malignant tumors of humans.

Keywords

indolocarbazoles, transplantable tumors of mice, subcutaneous xenografts, tumor growth inhibition.

Introduction

Indolocarbazoles and their derivatives constitute a wide class of natural and synthetic compounds with various types of biological activity, including antitumor activity, and this allows us to consider these compounds as potential antitumor agents. Drugs from the class of indolocarbazoles have attracted the attention of researchers by their ability to interact with several intracellular targets and the ability to induce different cell death paths. Among the representatives of this class, there are the compounds that cause damage to DNA structure by means of intercalation, and that are inhibitors of topoisomerases controlling the processes of DNA replication, transcription and repair, as well as inhibitors of kinases, in particular, CDK-1 kinase and protein kinase C involved in transmission of intracellular proliferative signals (Civenni et al. 2016; Lafayette et al. 2017; Zenkov et al. 2020).

So far, an extensive database on the antiproliferative activity of indolocarbazoles with various chemical structures has been compiled, which makes it possible to consider such substances as promising for carrying out further study (Caruso et al. 2019; Zenkov et al. 2021). Indolocarbazole derivatives containing glycoside substituents attached to the pharmacophore via nitrogen atoms are of special interest (Sánchez et al. 2006; Wada et al. 2007; Kiseleva et al. 2019). Among the biologically active N-glycosides of indolocarbazoles, the alkaloid staurosporine, an effective protein kinase C inhibitor, is well known (Tanramluk et al. 2009). The natural antibiotic rebeccamycin and its water-soluble derivative becatecarin have the properties of topoisomerase I inhibitors (Issa et al. 2019). The manifestation of high antiproliferative activity in such compounds as rebeccamycin and staurosporine determined the search for effective antitumor drugs among their synthetic analogues and low molecular weight derivatives with lower toxic properties. The representatives of this class: midostaurin (Li et al. 2022), enzastaurin (Sadeghi et al. 2021), lestaurtinib (Kangussu-Marcolino and Singh 2022), becatecarin (Schwandt et al. 2012), and edotecarin (Buzun et al. 2020) are undergoing clinical trials. The introduction of new antitumor agents from N-glycosides of indolocarbazoles class into clinical practice is also extremely important for overcoming the drug resistance of tumor cells to treatment.

At the N.N. Blokhin National Medical Research Center of Oncology (NMRCO), a method was developed to synthesize indolo[2,3-a]pyrrolo[3-4-c] carbazole derivative (LCS-1208). This substance is almost insoluble in water and in most organic solvents, which was a major problem when developing the dosage form. As a result of the performed investigations, a lyophilized dosage form: "LCS-1208, 9 mg, lyophilizate for preparation of injection solution" (LCS-1208 lyo) was created and protected by the patent of the Russian Federation (Lantsova et al. 2014; Gulyakin et al. 2021). In the course of studying the effectiveness of the dosage form of LCS-1208 lyo usage, a method to treat human colon cancer SW620 was patented and proposed in the experiment.

The aim of the study was to evaluate the antiproliferative activity of a new Russian N-glycoside substituted indolocarbazole derivative LCS-1208 on transplanted mice tumor models and on human tumors in *Balb/c* nude mice.

Materials and methods

Animals

The work was performed on 24 sexually mature female BDF_1-hybrid mice $F_1(C_{57}Bl/_6j$ *x DBA/2*), 24 female *CBA/Lac* and 26 *Balb/c* mice strains, 38 male *Balb/c* nude mice, weighing 20–22 g. Before treatment, the animals were divided into groups. The number of animals in the control groups was 8–10 mice, and in the test groups – 7–10 animals. Ethics Committee Minutes No. 04P of September 18, 2020.

Mice tumor models include lymphocytic leukemia L-1210, cervical carcinoma (CC-5), and colon adenocarcinoma (CAC). During the experiments, 2–5th *in vivo* passages were used. Transplantation was performed according to a standard technique (Treshchalina et al. 2012; Treshalina 2017).

L-1210 cells were transplanted into female BDF_1 hybrids via the intraperitoneal administration (ip), 10^6 cells per mouse in 0.3 ml of nutrient medium 199. During the experiment, CC-5 was inoculated to *CBA* female mice, and CAC – to *Balb/c* female mice. During the transplantation, inoculation of tumor cells was performed subcutaneously into the right axillary region of each mouse by 50 mg of tumor suspension in nutrient medium 199 at a dilution of 1:10 (5×10^6 cells). The treatment was started 24 h after transplantation of L-1210 cells and 48 h after in the case of CC-5 and CAC (Sof'ina et al. 1980). The start of treatment corresponded to the intensive reproduction time of tumor cells, which ensures their being in the most chemotherapy-sensitive state (Polin et al. 2011).

The CAC and CC-5 tumor strains of mice were generated at the N.N. Blokhin NMRCO. CAC arose in 1971 from a subcutaneous syngeneic transplant of embryo colon in a *Balb/c* mouse. CC-5 was induced by methylcholanthrene in the CBA mice' cervix subcutaneous autotransplant in 1970 (Sof'ina et al. 1980).

During the experiments on immunodeficient mice, a transplantable human colon cancer strain SW620, grown in the form of subcutaneous xenografts, was used (Treshchalina 2012). Each Balb/c nude mouse were injected subcutaneously 0.2 ml of a 20% suspension (40 mg of tumor tissue). Treatment was started 48 h after transplantation.

The test compound – 6-amino-12-(α-L-arabinopyranosyl)indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione (LCS-1208 substance) was dissolved in dimethyl sulfoxide (DMSO) and diluted with saline up to 10% concentration of DMSO. In the experiments on mice with L-1210, CC-5 and CAC, we used a 5-day

regimen with an interval of 24 h of ip administration of the substance LCS-1208 in effective doses of 50, 75 and 100 mg/kg, which were found in the previous experiments (Kiseleva et al. 2015).

The dosage form – LCS-1208 lyo – is an amorphous orange powder containing 98% of the main active ingredient in a vial. Before use, the content of the vial was rehydrated with water for injection to form a homogenous orange solution.

A study of the antitumor activity of LCS-1208 lyo was carried out on SW620 with two-fold intravenous administrations (iv) of 25, 50 and 75 mg/kg doses to *Balb/c* nude mice with an interval of 96 h.

Evaluation of the antitumor effect

The efficacy of treating mice with CC-5, CAC and L-1210 was evaluated according to the standard criteria: tumor growth inhibition (TGI, %) and an increase in life span (ILS, %). The evaluation of specific antitumor activity on xenografts of human tumors was carried out according to the TGI index calculated by the ratio of the average volumes of tumor nodes in the treated and control groups of mice, T/C% ("treatment/control"), taking into account that in the control group T/C=100% and using the maximum criterion T/C≤42% for experiments with developed tumors.

The degree of tumor growth inhibition (TGI and T/C) were calculated by formulas (1, 2):

$$TGI\% = \left(\frac{Vc - Vt}{Vc}\right) \times 100\%, \quad (1)$$

$$\frac{T}{C}\% = \left(\frac{Vt}{Vc}\right) \times 100\%, \quad (2)$$

where Vc and Vt are the average volumes of tumors (mm^3) in the control and treatment groups, which for each solid tumor was defined as a number obtained by multiplication of sizes of three perpendicular diameters of the tumor node. Tumor volume was measured at different periods of time after treatment.

ILS of the treated animals in comparison with the animals from the control group was calculated by formula (3):

$$ILS\% = \left(\frac{ALSt - ALSc}{ALSc}\right) \times 100\%, \quad (3)$$

where ALSt and ALSc are the average life spans (days) in the treatment and control groups of animals.

Doses causing TGI≥70% lasting at least 7 days after completion of the treatment and ILS≥25% for animals with solid tumors and ILS≥75% for the animals with lymphocytic leukemia were considered to be effective (Treshchalina et al. 2012).

Evaluation of treatment tolerance

During the experiments, follow-up of the animals was continued until their death. The tolerability of the LCS-1208 was judged by the state and behavior of the mice. The toxicity of the studied regimens and doses of the LCS-1208 substance and LCS-1208 lyo was evaluated by the time of death of the treated animals in comparison with the death of the animals in the control group, as well as by a decrease in their body weight (≥20%) and spleen mass (indirect signs of general, hematological and immunological toxicity) (Teicher and Andrews 2004; Treshchalina et al. 2012).

Statistical evaluation of the results was performed by using the IBM SPSS Statistics 21 package (license number 20130626-3), followed by comparing the separate groups with one another according to the Tukey's test, and using Excel software when calculating the Fisher criterion. Differences between the compared groups were considered statistically significant at $p < 0.05$.

Results and discussion

Study of the effectiveness of the LCS-1208 substance in mice on lymphocytic leukemia L-1210

The study of the effectiveness of LCS-1208 on lymphocytic leukemia L-1210 was performed with five-fold ip administrations of the LCS-1208 substance in single doses of 50 and 75 mg/kg (total doses 250 and 375 mg/kg) (Table 1). The antitumor effect of the LCS-1208 substance is shown in the form of ILS = 43% ($p = 0.001$ in relation to the control) and ILS = 47% ($p < 0.001$ in relation to the control), for the studied doses, respectively.

In the test groups, the differences between the total doses of 250 mg/kg and 350 mg/kg were insignificant ($p = 0.925$), indicating an equal but insufficient antitumor activity of the LCS-1208 substance on lymphocytic leukemia L-1210 (criterion of effectiveness for animals with lymphocytic leukemia is ILS ≥ 75%).

Table 1. Antitumor Effect of the LCS-1208 Substance When Intraperitoneal Administration to Mice With L-1210

Dose, mg/kg	Total dose, mg/kg	Increase of life span, %	P in relation to the control group
50	250	43	0.001
75	375	47	<0.001

Study of the effectiveness of LCS-1208 substance in mice with CC-5

The LCS-1208 substance with five-fold ip administrations in single doses of 75 and 100 mg/kg (total doses of 375 and 500 mg/kg) generated positive, but not high inhibition of tumor growth in CC-5 with respect to the control group: TGI = 97–52% ($p \leq 0.015$) and TGI=98–59% ($p < 0.001$), respectively, within 7 days (Table 2, Fig. 1).

A single dose of 75 mg/kg (total dose of 375 mg/kg) caused the death of mice in 29% of cases on the 20th day after treatment with an average life span of control mice of 43 (39–54) days. Increasing a single dose to 100 mg kg

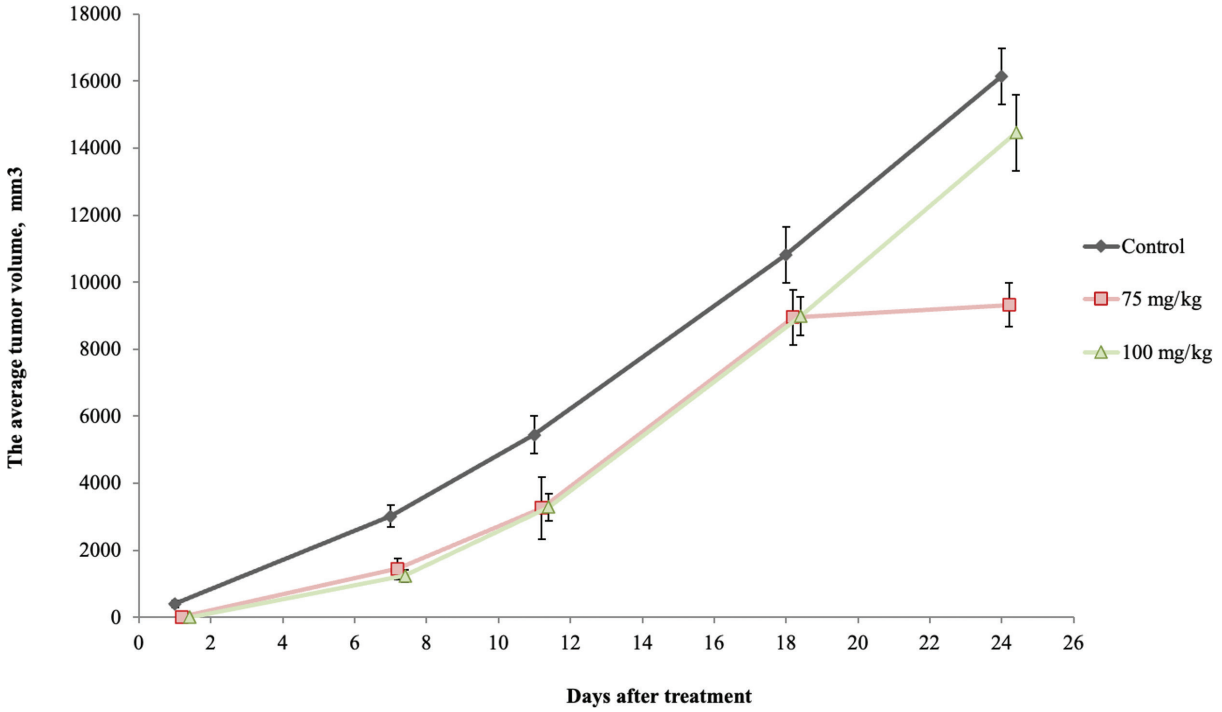

Figure 1. The dependence of the tumor size of cervical cancer CC-5 in mice *CBA/Lac* on a dose of the LCS-1208 substance after five-fold intraperitoneal administrations in comparison with the control.

Table 2. Antitumor Effect of the LCS-1208 Substance When Intraperitoneal Administration to Mice with CC-5

Dose, mg/kg	Total dose, mg/kg	Tumor growth inhibition (TGI) %					Toxicity death, %
		Days after treatment					
		1	7	11	18	24	
75	375	97	52	20	17	42	29
100	500	98	59	39	17	10	43

(total dose of 500 mg/kg) led to the death of mice in 43% of cases at the earlier periods of follow-up (on the 15th day after the end of treatment). Signs of toxicity were expressed as a slight decrease in body weight of the animal by 10% of the initial weight. At autopsy, there were no differences in the weight of the spleen in comparison with the control group of animals.

Study of the effectiveness of the LCS-1208 substance on CAC of mice

When using the LCS-1208 substance a in single dose of 75 mg/kg (total dose of 375 mg/kg), in comparison with the control group, a reliable prolonged inhibition of primary subcutaneous tumor node growth within 16 days after the end of the treatment (TGI = 97–62%, $p \leq 0.001$), and ILS of mice with CAC by 36% was observed (Table 3, Fig. 2).

An increase in a single dose of LCS-1208 to 100 mg/kg (total dose of 500 mg/kg) reliably increased the inhibition of CAC growth by 99–78% ($p \leq 0.001$) within 16 days of follow-up in comparison with the control group, but led to death of 50% mice on the 3rd-5th days after the end of treatment. Signs of toxicity were expressed in a decreased motor activity, a decreased body weight of animals by 46% when compared to their initial weight, as well as in significantly decreased (2–3 times) spleen mass at autopsy in comparison with the spleen mass in mice from the control group.

Table 3. Antitumor Effect of the LCS-1208 Substance When Intraperitoneal Administration to Mice With CAC

Dose, mg/kg	Total dose, mg/kg	Tumor growth inhibition (TGI), %				Increase in life span (ILS), %	Toxicity death, %
		Days after treatment					
		1	7	11	16		
75	375	97	97	86	62	36	0
100	500	99	99	94	78	13	50

Study of the effectiveness of LCS-1208 lyo on subcutaneous xenografts of human colon cancer SW620 *in vivo*

The antitumor activity of LCS-1208 lyo was studied on human tumors in *Balb/c* nude mice in the conditions of an optimal application regimen under the control of tolerance.

The results of the study showed that subcutaneous xenografts of colon cancer SW620 without treatment have dynamics of steady growth. Tumor nodes grow quite quickly and show an 8-time increase within 12 days after transplantation, from Vav=328±148 mm^3 to Vav=2697±414 mm^3. Against this background, all the doses of the LCS-1208 lyo were highly effective and gave a significant, reliable antitumor effect, showing an increase

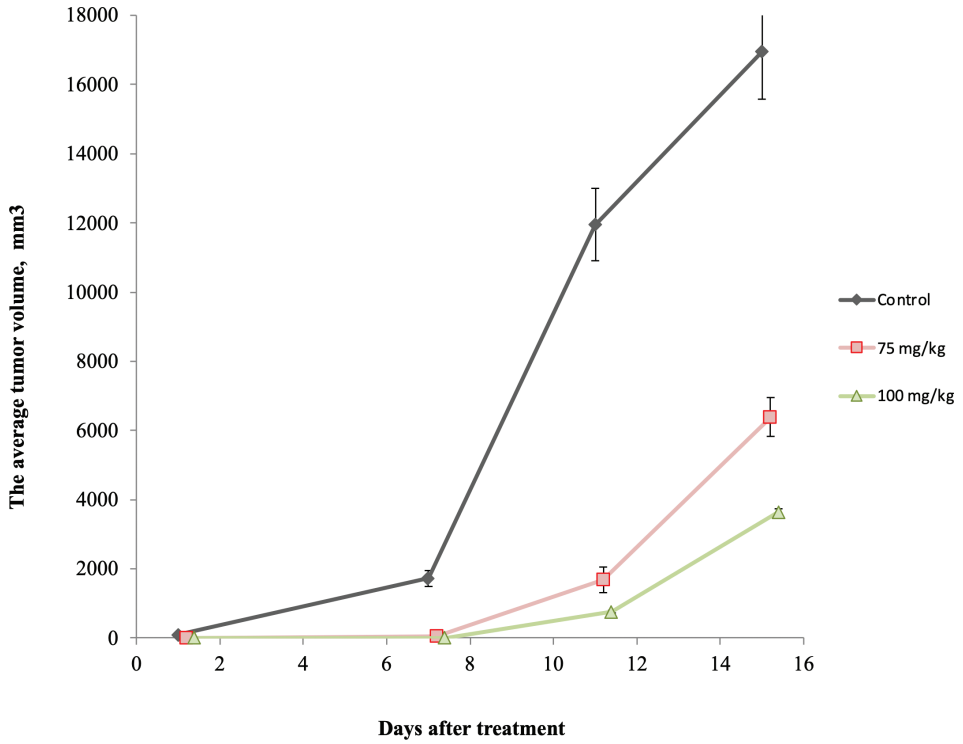

Figure 2. The dependence of the tumor size of CAC in *Balb/c* mice on a dose of the LCS-1208 substance after five-fold intraperitoneal administrations in comparison with the control.

Table 4. Study of the Efficacy of LCS-1208 Lyo on Subcutaneous Xenographs of Human Colon Cancer SW620 in Vivo With Double (after 96 h) Intravenous Administration

No. mice, indicators	Average tumor volumes and performance indicators at various times after treatment											
	1st day				6th day				12th day			
	TGC	Single dose (total dose), mg/kg			TGC	Single dose (total dose), mg/kg			TGC	Single dose (total dose), mg/kg		
		25(50)	50(100)	75(150)		25(50)	50(100)	75(150)		25(50)	50(100)	75(150)
1	333	53	105	78	843	243	53	29	2209	878	148	68
2	359	89	64	145	973	199	50	62	2157	599	98	81
3	401	156	62	29	1926	357	56	25	3012	701	85	death
4	573	121	44	136	1657	157	55	78	3406	545	93	death
5	407	218	72	29	1225	117	95	14	2745	237	250	68
6	295	8	106	41	1398	231	102	23	2840	330	228	59
7	124	59	116	132	1578	249	85	65	2723	348	108	75
8	131	170	181	30	1450	94	101	15	2484	617	215	90
9	–	166	40	108	–	59	46	32	–	715	182	65
10	–	117	131	19	–	53	141	27	–	150	133	21
Vav	328	116	92	75	1381	176	78	34	2697	512	154	66
S	148	64	44	51	358	98	32	23	414	235	61	21
T/C%	100	35	28	23	100	13	6	2	100	19	6	2
Ttest	–	0.00000002	0.0002	0.0006	–	0.000002	0.000001	0.00004	–	0.00004	0.0004	0.00005

Note: TGC – tumor growth control; Vav – the average volume of the tumor; S – standard deviation.

in a level and degree of significance within 12 days after the end of treatment (Table 4). Thus, on the 1st, 6th, 12th days, at a single dose of 25 mg/kg (total dose of 50 mg/kg) – T/C=35%; 13%; 19%, respectively. At a single dose of 50 mg/kg (total dose of 100 mg/kg) – T/C=28%; 6%; 6%, respectively. At a single dose of 75 mg/kg (total dose of 150 mg/kg) – T/C=23%; 2%; 2%, respectively.

However, using a 75 mg/kg single dose (total dose of 150 mg/kg) on the 12th day of treatment led to the death of 20% of mice from toxicity, which was expressed in a decreased motor activity and decreased body weight of animals by 15% compared to the initial one. In preliminary studies, the LCS-1208 lyo in a dose of 150 mg/kg with a slow stream iv infusion to mice with SW620 caused the death of animals from toxicity one day after the administration.

In the framework of this study, in tolerated doses of 25, 50 and 75 mg/kg (total doses of 50, 100 and 150 mg/

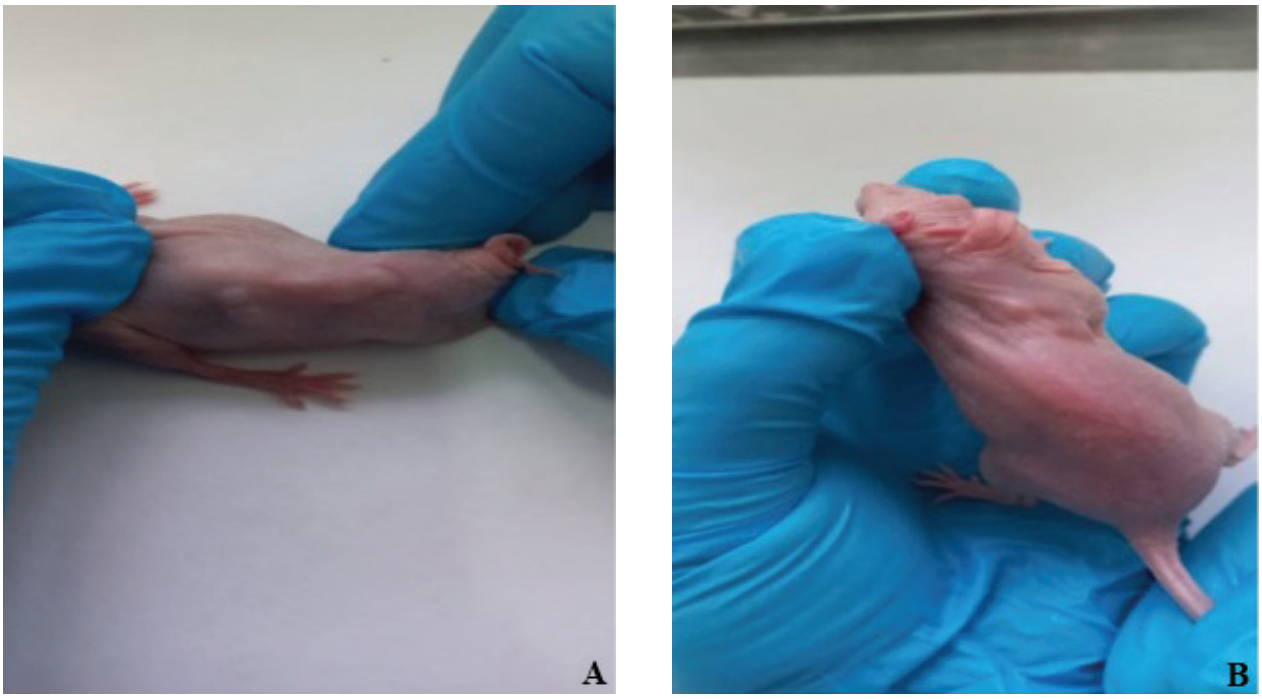

Figure 3. Balb/c nude mice with subcutaneous xenografts of human colon cancer SW620 on the 4th day after the end of treatment **A**. Control; **B**. LCS-1208 lyo.

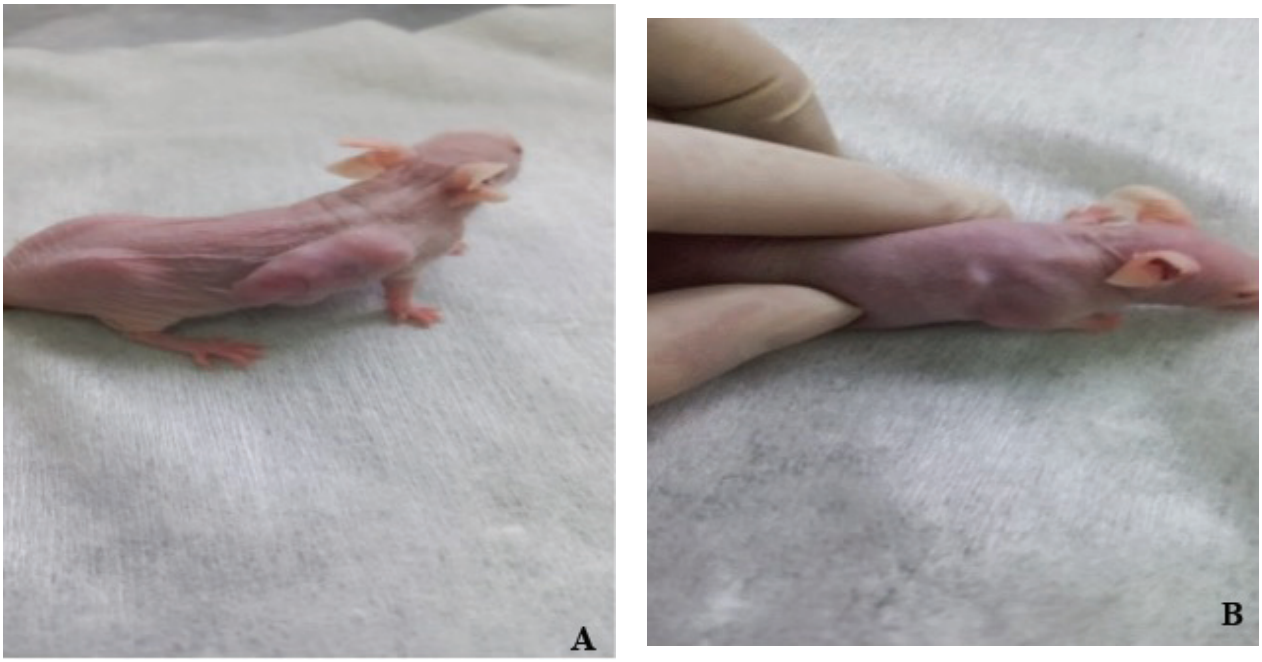

Figure 4. Balb/c nude mice with subcutaneous xenografts of human colon cancer SW620 on the 16th day after the end of treatment **A**. Control; **B**. LCS-1208 lyo.

kg, respectively), the condition and behavior of the mice during 20 days was satisfactory, with no evidence of any side effects. The comparative dynamics of tumor growth, evaluated visually in these animals on the 12th and 20th days after treatment, showed that the tumor growth in the LCS-1208 lyo group almost stabilized, since the tumor sizes were smaller than the tumor sizes in mice from the control group, measured on 12th day after the end of the treatment. *Balb/c* nude mice with subcutaneous xenografts of human colon cancer SW620 are shown on the 4th day (Fig. 3) and 16th day (Fig. 4) after the end of treatment in comparison with the control.

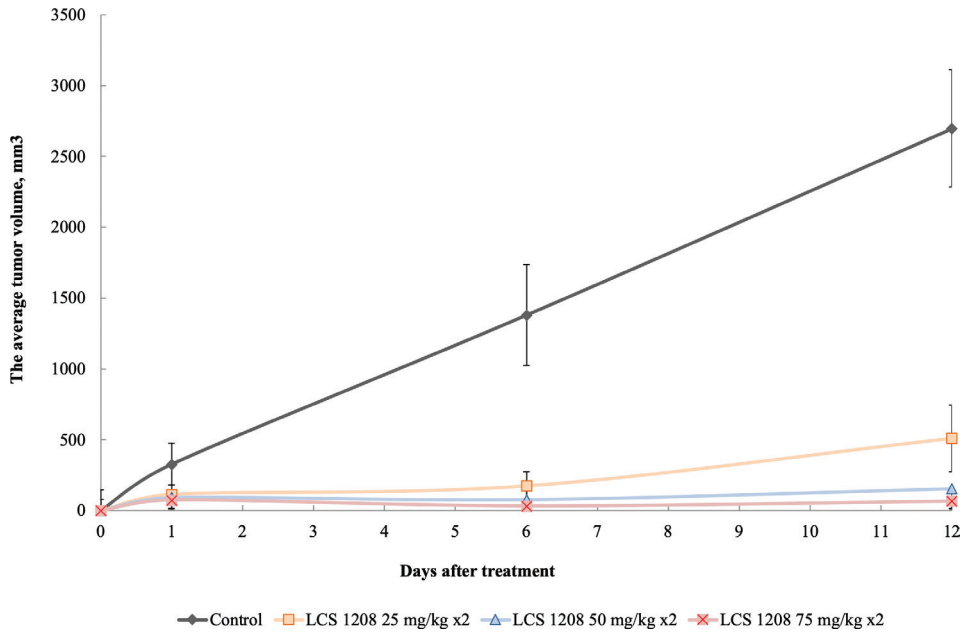

Figure 5. The growth dynamics of SW620 under the action of the LCS-1208 lyo in the range of single doses of 25, 50 and 75 mg/kg with a double intravenou administration with an interval of 96 h.

Conclusion

A necessary condition when developing and studying new drugs is a careful selection of sensitive models in the experiment and special attention to the ratio of risk and effectiveness under the control of treatment tolerance.

As a result of the study of sensitivity of transplanted tumors of mice with L-1210, CC-5 and CAC to the LCS-1208 substance, a high antitumor activity of the LCS-1208 substance with respect to CAC was shown with a 5-fold ip administration of a single dose of 75 mg/kg (total dose of 375 mg/kg). Administration of a total dose of 500 mg/kg resulted in the death of mice.

The antitumor effect of the LCS-1208 substance on CC-5 was noted; however, in the indicated doses and regimen of administration, there was observed the death of animals from toxicity (29–43%).

The LCS-1208 substance in the studied doses with a 5-fold ip administration to mice with lymphocytic leukemia L-1210 did not cause toxicity. The life span of animals was 43–47%, which turned out to be lower than the criterion of effectiveness.

High results of LCS-1208 substance antitumor effect on mice with CAC with indices of TGI=97–62% up to 16 days after treatment and ILS=36% became the basis for a further study of the effectiveness of LCS-1208 lyo dosage form on subcutaneously transplanted xenografts. In our studies performed on xenografts of human colon cancer SW620, the LCS-1208 lyo in the range of total doses from 50 to 150 mg/kg with iv administration to *Balb/c* nude mice showed effectiveness in inhibiting tumor growth T/C=35–2% (T/C criterion<42%). The obtained results demonstrate the high activity of all the studied doses of the LCS-1208 lyo drug substance with direct dependence of an antitumor effect on a total dose (Fig. 5).

The results of this study are comparable with the data obtained by other authors during investigation of the antiproliferative activity of various representatives of the indolocarbazole class. For example, Ciomei M. et al. (2006) studied the antitumor effect of edotecarin when used alone or in combination with 5-fluorouracil, irinotecan, cisplatin, oxaliplatin and the multi-target tyrosine kinase inhibitor SU11248 on the model of human colon cancer xenograft HCT-116. In all the studies, edotecarin was active both as monotherapy and in combination with other antitumor agents (Ciomei et al. 2006). Another analogue of rebeccamycin – NB-506 – in a dose of 300 mg/m^2 inhibited the growth of human colon cancer tumor cells HCT-116 and LS-180, grown as subcutaneous xenografts in immunodeficient mice (Delgado et al. 2018).

Indolocarbazole from staurosporine derivatives CEP-7055 inhibited the growth of subcutaneously transplanted colon cancerxenografts HT-29 and HCT-116 by 50–90% (Ruggeri et al. 2003). CEP-7055 in combination with irinotecan and, to a lesser extent, with oxaliplatin, showed a decrease in primary metastases of colon and liver carcinoma than in case of monotherapy with irinotecan or oxaliplatin (Jones-Bolin et al. 2006). However, further research was discontinued, as CEP-7055 showed no activity during phase I clinical trials (Williams 2008).

The data presented indicate the need to continue preclinical studies of the LCS-1208 lyo drug, and suggest the effectiveness of its use for treatment of malignant tumors of colon in humans.

Conflict of interest

The authors declare no competing interests.

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