PRECLINICAL STUDIES OF THE ANTITUMOR ACTIVITY AND SAFETY OF THE BC1 TEST AGENT (DONOVIT-VS)

REPORT SUMMARY

The experience of using traditional methods of treatment, such as surgery, radiation therapy or cytotoxic chemotherapy, has shown their limited capabilities and extremely low efficiency in the treatment of locally advanced and disseminated forms of malignant neoplasms. At the same time, the low efficiency of cytotoxic therapy (both radiation and chemotherapy) is due to the low selectivity of the antitumor effect and high toxicity to normal and, as a rule, vital organs and tissues. Adverse reactions that occur against the background of cytotoxic therapy significantly limit the effectiveness of the treatment, worsen the quality of life of cancer patients, and sometimes even pose a direct threat to their lives.

The rapid development of the fundamental sciences of wildlife, which has expanded not only our understanding of the mechanisms of the onset and development of malignant tumors, but also the arsenal of modern methods of scientific and clinical research, has made it possible to develop fundamentally new strategies for the treatment of cancer patients. Such new strategies include anticancer antiangiogenic therapy (AAT). It is based on the dependence of the growth and progression of malignant tumors on the degree of their vascularization [1].

AAT is fundamentally different from cytotoxic therapy both in terms of cellular targets and in terms of the goals that it faces. The central cellular target of AAT is not a tumor cell, but an endothelial cell, as the main structural unit of the vascular network [2]. And the main goal of AAT is not to kill the endothelial cell, but to inhibit its proliferation and/or migration and/or differentiation. The goals of AAT and its targets determine, on the one hand, the need for its long duration (metronomic introduction), on the other hand, the possibility of using low doses of antiangiogenic drugs (due to both the high bioavailability of angiogenesis inhibitors and the high sensitivity of endothelial cells to their action). The latter determines the low toxicity (cytotoxicity) of AAT in relation to normal organs and tissues.

In the last decade, there has been an active search for biologically active agents that can provide effective inhibition of tumor angiogenesis. It is known that aconitine belongs to the group of alkaloids with a pronounced activity in relation to the cell membrane. It is a classic inhibitor of sodium channels, which play an important role in cell survival and their functional activity. This is especially true for such normal cells as endothelial cells, whose electrokinetic characteristics are decisive in the process of vascular morphogenesis. In this regard, it can be expected that aconitine-containing biologically active substances may exhibit the ability to inhibit tumor angiogenesis. Such potential antitumor agents with an antiangiogenic mechanism of action include the Donovit-VS (further in the text - BC1 test agent), a brief description of the results of preclinical studies of antitumor activity and safety of which are presented below.

1. PRE-CLINICAL STUDIES OF SPECIFIC OF THE ANTITUMOR ACTIVITY OF THE BC1 TEST AGENT

The purpose of the study was to study the specific antitumor activity of the BC1 test agent in relation to transplanted experimental tumor strains of various genesis and biological properties.

Research objectives:

1. Determine the maximum tolerated dose (MTD) for mice and rats in experiments on the study of acute toxicity of the BC1 test agent.
2. Prove the adequacy of using the content of aconitine for dosing the test agent BC1.
3. To study the antitumor effect of BC1 against leukemic (ascites) strains of experimental tumors (L1210, ascites variant of Ehrlich's carcinoma).
4. To study the antitumor effect of BC1 against solid experimental tumor models (Lewis lung carcinoma, sarcoma 180, B16 melanoma, solid Ehrlich carcinoma, Guerin's carcinoma, glioma).
5. To investigate the dependence of the antitumor effect of BC1 on the dose.
6. To study the mechanism of antitumor activity of the BC1 test agent.
7. To study the antimetastatic effect of the BC1 test agent (LLC, LLC/R9, B16 melanoma).
8. Conduct a comparative study of the antitumor activity of BC1 and reference antitumor drugs with an antiangiogenic mechanism of action (cyclophosphamide and polyclonal antibodies to endothelial cell growth factor - anti-VEGF).
9. To study the antitumor activity of the BC1 test agent against cis-DDP-resistant tumors (LLC/R9, resistant variant of Guerin's carcinoma).
10. To study the effectiveness of the antitumor effect of BC1 in combination with cis-DDP.

Object of study

Pharmacological agent test agent BC1, in the form of tablets "Donovit-VS" composition:

Name of components	Composition in grams	
	1	2
1. Wrestler root extract containing a sum of aconite alkaloids including aconitine	0,00001	0,00000025
2. Milk sugar	0,29699	0,2969975
3. Calcium stearate	0,003	0,003
Tablet weight	0,3	0,3

For preclinical studies, both specific antitumor activity and toxicity, tablets were crushed into powder and dissolved in distilled water.

Route of administration BC1

The proposed antiangiogenic mechanism of the antitumor effect of the BC1 test agent determined the choice of its administration scheme, which is typical for antitumor antiangiogenic drugs (long-term metronomic administration). In this regard, in all studies, 0.4 ml (for mice) or 4 ml (for rats) of an aqueous solution of the BC1 test agent was administered to animals orally by with the help of a probe daily 5 times a week, starting from the second day after tumor inoculation. The duration of therapy with the agent depended on the biological properties of the tumor models and was 2 or 3 weeks.

Experimental animals and tumor models

The studies were carried out on animals from the vivarium of the Institute of Experimental Pathology, Oncology and Radiobiology named after A.I. R.E. Kavetsky National Academy of Sciences of Ukraine using transplanted strains of experimental tumors from the National Bank of Cell Lines (Table 1).

Table 1. Experimental tumor strains and animal lines used in preclinical studies of the specific antitumor activity of BC1

Tumor strains		Grafting method	Animal species	Animal Lines
Name	**Designation**			
Lymphoid leukemia	L1210	intraperitoneally	Mice	DBF_1
Ehrlich carcinoma		subcutaneous	Mice	White non-linear
		intraperitoneally	Mice	White non-linear
Lewis lung carcinoma (original)	LLC	i/m	Mice	C57/Bl
		i/v		
Lewis lung carcinoma (variant resistant to cis-DDP with angiogenesis-dependent growth)	LLC/R9	i/m	Mice	C57/Bl
		i/v		
Soft Tissue Sarcoma	S180	i/m	Mice	White non-linear
Melanoma	B16	i/m	Mice	C57/Bl
Guerin's carcinoma (original version)		subcutaneous	Rats	Wistar
Guerin's carcinoma (cis-DDP resistant variant)		subcutaneous	Rats	Wistar
Glioma		i/cerebrally	Rats	Wistar

Definition of MTD

Studies of the survival of mice in an acute experiment as a function of the dose of BC1 (calculated from the content of aconitine) did not reveal the effect of the sex of the animals, as well as the line of mice on the nature of this dependence. MTD for mice

averaged 1.8±0.1 μg of aconitine per gram of mouse weight. MTD for rats calculated from MTD for mice using the formula:

$$MTDrat = \frac{MTD\text{mice} * K\text{mice}}{Krat}(mg/kg)$$

where: K_{mice}, K_{rat} - conversion factors for mice and rats, respectively, equal to 3.0 (for mice weighing 20 g) and 6.5 (for rats weighing 200 g) [3.4], amounted to 0.85±0.06 mg/kg , which is not statistically different from the MTD value obtained experimentally in experiments on acute toxicity of BC1 for rats (0.97±0.07 mg/kg). This fact indicates the correctness of using formulas for recalculating BC1 doses from one type of biological object to another, which seems to be very important for determining therapeutic doses for clinical trials.

Dosing BC1

In the framework of preclinical studies, dosing of the multicomponent test agent BC1 was carried out according to the content of aconitine in it. To prove the adequacy of using aconitine as the main pharmacologically active ingredient of the BC1 test agent, comparative studies of the effect of chemically pure aconitine (Sigma, USA) and BC1 (aconitine equidoses) were carried out

- survival of C57/Bl mice in acute toxicity experiments
- on the growth of Lewis lung carcinoma (LLC)

The studies performed have shown that the dependence of animal death on the dose of the BC1 test agent (calculated from the content of aconitine) does not differ significantly from that for pure aconitine. At the same time, there are no statistically significant differences in MTD, which for BC1 amounted to 1.8 ± 0.1 μg of aconitine per gram of mouse weight, and for chemically pure aconitine - 1.7 ± 0.3 μg / g of animal weight.

Antitumor activity of test agent BC1

Studies have shown that test agent BC1 exhibits pronounced antitumor activity against solid tumors with angiogenesis-dependent growth: solid variant of Ehrlich carcinoma, variant of Lewis carcinoma LLC / R9 (with its characteristic dependence of growth on the degree of vascularization), sarcoma 180, carcinoma Guerin, glioma) (Table 2). At the same time, the antitumor effect, which for most models is more than 70%, was maintained for at least a week after the end of therapy, or increased (as was the case with sarcoma 180). It is important to note that the antitumor effect of BC1 is dose-dependent. Its maximum values are observed at relatively high total doses of BC1 equal to MTD/2, as well as at extremely low doses (more than 20 times lower than MTD), which indicates the existence of two different mechanisms of the antitumor effect of BC1. At low total doses, BC1 exhibits rather an antiangiogenic antitumor effect, since at these doses it is effective against tumors with a pronounced dependence of growth on neovascularization processes. In total doses of the order of MTD

(MTD/2), BC1 has an antivascular effect (cytotoxic on endothelial cells) rather than a direct cytotoxic effect on tumor cells. This is indicated by the results of a comparative study of the antitumor effect of BC1 at a dose of MTD/2 against ascitic and solid tumors of Ehrlich's carcinoma, as well as the absence of an antitumor effect of BC1 against lymphoid leukemia L1210.

Mechanism of antitumor activity of test agent BC1

The assumptions made on the basis of a comparative analysis of the antitumor effect of BC1 in relation to transplantable tumor strains (including those of the same genesis but different types of growth) about the ability of BC1 to inhibit vascular growth were fully confirmed when studying the nature of the effect of this agent on endothelial and tumor cells. Thus, it was shown that the sensitivity of endothelial cells of the MAEC line to the cytotoxic/cytostatic effect of BC1 is statistically significantly higher than that of tumor cells (Table 3). Moreover, the effectiveness of the cytotoxic / cytostatic effect of BC1 on actively proliferating endothelial cells is an order of magnitude higher than on non-proliferating endothelial cells, which indicates the selectivity of the antiangiogenic effect of BC1 (the ability to stimulate the death of actively proliferating endothelial cells or inhibit their proliferation) compared to its cytotoxic effect as on mature endotheliocytes (antivascular effect), and on tumor cells (direct antitumor effect).

Table 2. Antitumor and antimetastatic effects of BC1 on transplanted tumors

Tumor strains		Grafting method	Total dose	Antitumor effect (% inhibition)	Antimetastatic effect (% braking)		Lifespan increase (%)
Name	Designation				Number of metastases	Metastasis volume	
Lymphoid leukemia	L1210	intraperitoneally	MTD/2	-	-	-	0,0
Ehrlich carcinoma		subcutaneous	MTD/2	77,0	-	-	-
		intraperitoneally	MTD/2	-	-	-	0,0
Lewis lung carcinoma (original)	LLC	i/m	MTD/2	36,0	67,8	37,2	73,0
			MTD/30	0,0	0,0	0,0	-
			MTD/60	0,0	0,0	0,0	-
		i/v	MTD/200	-	0,0	0,0	-
Lewis lung carcinoma (cis-DDP resistant variant with angiogenesis-dependent growth)	LLC/R9	i/m	MTD/2	77,3	0,0	0,0	-
			MTD/20	0,0	0,0	0,0	-
			MTD/60	76,6	35,5	0,0	-
			MTD/100	68,0	46,0	69,3	-
			MTD/200	70,7	88,0	93,0	-
		i/v	MTD/200	-	92,2	78,6	-
Soft tissue sarcoma	S180	i/m	MTD/2	60,0	-	-	-

Melanoma	B16	i/m	MTD/60	0,0	0,0	52,0	-
			MTD/200	0,0	0,0	0,0	-
Guerin's carcinoma (original strain)		subcutaneous	MTD/20	77,0	-	-	-
			MTD/100	69,5	-	-	-
Guerin's carcinoma (cis-DDP resistant)		subcutaneous	MTD/20	0,0	-	-	-
			MTD/100	0,0	-	-	-
Glioma		i/cerebrally	MTD	-	-	-	19,2

Table 3. IC_{50} value for BC1 (concentration of agent that provides 50% inhibition of cell growth) in relation to tumor (LLC, LLC/R9) and endothelial cells (MAEC)

Cell Line	**IC_{50} (µg/ml)**
LLC	23,5± 2,1
LLC/R9	13,3 ± 0,9
MAEC (actively proliferating)	0.95 ± 0.06
MAEC (not proliferating)	8.7 ± 2.1

Studies have also shown that at extremely low non-cytotoxic concentrations of the BC1 test agent, it exhibits a proapoptotic effect on actively proliferating endothelial cells and leads to an inversion of the surface charge of these cells (Table 4), without affecting tumor cells. It is known that in the process of formation of new vessels (vascular morphogenesis), a positively charged endothelial cell forms a tight contact with a negatively charged myocyte, forming an excitable cell pair. The change in the sign of the surface charge of the endotheliocyte under the action of the test agent BC1 prevents the morphogenesis of the vessel, which leads to the inhibition of neovascularization processes. In this case, BC1 behaves as a direct inhibitor of tumor angiogenesis.

Table 4. Effect of BC1 on the characteristics of MAEC cells

BC1 concentration (µg/ml)	**Surface charge density (C/m^2)**	**Apoptotic cell count (%)**
0,0	+6,85 ± 0,80	6,1± 0,13
0,04 (IC_{50}/20)	-8,49 ± 0,82	7,73 ± 0,18
0,08 (IC_{50}/10)	+3,41 ± 0,33	5,65 ± 0,64

Thus, BC1 can inhibit tumor growth by implementing two different mechanisms depending on the dose (or concentration) (the existence of two mechanisms was assumed based on a comparative analysis of the results of the study of the antitumor effect of BC1 as a function of its dose). At relatively high concentrations (or doses) that

are not cytotoxic with respect to tumor cells, BC1 implements an antivascular mechanism of antitumor activity. At low concentrations (subtoxic or nontoxic in relation to actively proliferating endothelial cells), BC1 is able to exhibit a direct antiangiogenic antitumor effect.

Antimetastatic effect of test agent BC1

It is known that the main strategic goal of antiangiogenic antitumor therapy is the inhibition of the process of metastasis. Therefore, a study of the antimetastatic effect of BC1 was carried out. The conducted studies confirmed the ability of the BC1 test agent to inhibit metastasis, both spontaneous and experimental (Table 2). To a greater extent, this is manifested in the ability of BC1 to inhibit the growth of metastases, which is manifested in a decrease in the volume of metastatic lesions. This property of BC1 is manifested even in relation to B16 melanoma, despite the fact that BC1 does not affect the growth of the primary melanoma tumor and the number of metastases in the lungs. A significant antimetastatic effect causes an increase in the lifespan of mice with Lewis carcinoma by 73% against the background of a weak antitumor effect. Our studies have shown that the decrease in the volume of metastatic lesions in this case is due to a decrease in the proportion of metastases in the vascular growth phase (metastases with a diameter of more than 1 mm).

Comparative study of the antitumor activity of BC1 and reference antitumor drugs with an antiangiogenic mechanism of action (cyclophosphamide and polyclonal antibodies to endothelial cell growth factor - anti-VEGF).

Cyclophosphamide in the anti-angiogenic therapy mode (metronome oral administration at a total dose of 200 mg/kg) was chosen as one of the reference drugs. 6] Our studies have shown that the antiangiogenic effect of cyclophosphamide in this mode is based on its ability to significantly inhibit the production of proangiogenic factors by tumor cells (not a direct antiangiogenic effect).

Table 5. Antitumor effect of the BC1 test agent, cyclophosphamide (CP), and mouse anti-VEGF antibodies against the original (LLC) and cis-DDP-resistant variant (LLC/R9) of Lewis lung carcinoma

Antitumor effect	**LLC**			**LLC/R9**		
	BC1 0,9 mg/kg	**CP 200,0 mg/кг**	**Anti-VEGF 20 ng/mouse**	**BC1 0,9 mg/кг**	**CP 200,0 mg/кг**	**Anti-VEGF 20 ng/mouse**
Inhibition of primary tumor growth (%)	36,0	0,0	0,0	77,3	47,0	18,0

A comparative study of the antitumor effect of cyclophosphamide and BC1 with their metronomic oral administration at a total dose of MTD/2 in relation to LLC and

LLC/R9 showed that the BC1 test agent has a more pronounced inhibitory effect on the growth of the primary tumor than cyclophosphamide (Table 5).

A similar result was obtained in a comparative study of the effect of polyclonal mouse antibodies to VEGF (a mouse analogue of Bevacizumab) and the BC1 test agent. True, it should be noted that the dose of antibodies used in the studies was 2-3 times lower than the therapeutic one. On the other hand (as can be seen from Table 2), BC1 at a dose 100 times lower than MTD/2 causes a very high antitumor effect against LLC/R9.

Thus, BC1 exhibits a more pronounced antitumor effect compared to known antitumor drugs.

Antitumor activity of test agent BC1 against cis-DDP-resistant tumors (LLC/R9, resistant variant of Guerin's carcinoma)

The conducted studies did not reveal the effectiveness of BC1 against cis-DDP resistant Guerin's carcinoma in rats (Table 2). The effectiveness of this test agent against the cis-DDP resistant variant of Lewis lung carcinoma is most likely associated not with the drug resistance of these cells, but with the high dependence of the growth rate of this tumor on tumor angiogenesis processes.

Antitumor efficacy of BC1 in combination with cis-DDP

It is known that anti-angiogenic therapy cannot be radical. Therefore, in clinical practice, antiangiogenic therapy is carried out in combination with traditional antitumor therapy.

In order to study the effectiveness of BC1 in combination therapy, studies were conducted on the antitumor and antimetastatic effects of a combination of BC1 (15 oral metronomic injections at a total dose of 1.8 * 10-2 mg/kg) and cis-DDP (6 intravenous injections at a total therapeutic dose 7.2 mg/kg). As can be seen from Table 6, the effectiveness of combination therapy with two agents is significantly greater than each separately, due to their additive antitumor and antimetastatic effects.

Table 6. Antitumor activity of the BC1 test agent and the antitumor drug cis-DDP against the cis-DDP-resistant variant of Lewis lung carcinoma (LLC/R9) in monotherapy and when used together

Antitumor effect (%)	BC1	Cis-DDP	BC1+ Cis-DDP
Inhibition of primary tumor growth	68,0	0,0	60,0

Reducing the number of metastases	46,0	48,7	69,7
Reducing the volume of metastases	69,3	66,5	91,3

CONCLUSIONS

Thus, preclinical studies of the specific pharmacological activity of the BC1 test agent revealed its ability to inhibit the growth of three types of solid tumors in two types of animals by more than 70% and significantly inhibit the process of metastasis, reducing both the number of metastases by more than 80% and the volume of metastatic lesions. up to 90%. Metronomic administration of BC1 causes an increase in the life span of animals with a solid tumor by 73%. The antitumor and antimetastatic effect is dose-dependent and is due to the implementation of two mechanisms of action: antivascular (at total doses of the order of MTD/2) and antiangiogenic (at total doses less than MTD/20). The antiangiogenic mechanism of action of BC1 determines its antitumor and antimetastatic effects only in relation to malignant neoplasms with angiogenesis-dependent growth. At the same time, BC1 exhibits a more significant antitumor and antimetastatic effect compared to traditional antitumor drugs with an antiangiogenic mechanism of action (such as cyclophosphamide and avastin). BC1 is also effective when combined with cis-DDP.

Literature

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2. PRECLINICAL SAFETY STUDIES TEST-AGENT VS1 (DONOVIT-VS)

BC1 acute toxicity study in mice (male and female) and rats

The purpose of the research is to quantify the relationship between a single dose of the test agent and the survival of animals and to study the toxicity of BC1 to normal organs and tissues.

Research objectives

1. Quantification of single lethal doses of test agent BC1 - LD_{50} and LD_{10} (MTD) in mice and rats.
2. Study of the toxic effect of BC1 on normal organs and tissues after a single injection of the test agent.

Research structure

To study the toxic properties of BC1 in mice in an acute experiment, nine groups of animals (for females) and seven for males were used. When studying acute toxicity in rats, 6 groups of animals were formed. Animals of the control groups were administered water orally, and animals of the experimental groups were given an aqueous solution of BC1 in progressively decreasing doses. The duration of observation is 14 days.

Toxicometric observations were carried out for 14 days. Morphological studies (liver, kidney, spleen, lung, stomach, small intestine, heart tissues) were performed in all dead animals. After 14 days, the remaining animals underwent biochemical, morphological studies, cytological studies of the bone marrow and peripheral blood.

Research results

As a result of the research, it was found that the toxic manifestations of BC1 (regardless of the type of animals and gender) were of a pronounced dose-dependent nature. It should be emphasized that the death of animals was observed only for 5-60 minutes after the administration of the drug. 1 hour after the administration of BC1 and throughout the entire observation period (14 days), no deaths of rats and mice were noted, which indicates a low cumulative toxicity of this agent.

An analysis of the dynamics of death of animals made it possible to determine the dose characteristics (by the content of aconitine) of the lethal toxicity of the BC1 agent Table 2.1.

Table 2.1. Dose indicators of lethal toxicity BC1

Animal species	LD_{90} (mkg/g)	LD_{50} (mkg/g)	MTD (mkg/g)
Mice of the C57Bl/6 line (females)	5,9 ± 0,9	2,9 ± 0,53	1,8 ± 0,4

Mice of C57Bl/6 line (males)	4,2 ± 1,4	2,2 ± 0,6	1,4 ± 0,35
Rats of the Wistar line (females)	3,45±0,34	1,56±0,11	0,95±0,07

It should be noted that the prediction of MTD for rats made on the basis of MTD for mice (within the same sex) using the species conversion formula almost completely coincided with the data obtained in an acute experiment, which gives reason to use these formulas when determining the range of toxic and therapeutic doses for clinical research.

Studies have shown that BC1 exhibits toxicity in lethal and sublethal doses mainly in the first minutes after a single injection. In this case, there is a violation of the motor and respiratory activity of animals. Morphologically, hemorrhages are observed in all the studied organs, which indicates significant changes in the permeability of the vascular network induced by BC1. The nature of changes in the hemopoiesis system in this case correlated with the observed pattern of hemorrhages. The most significant changes are expressed in thrombocytosis (regardless of the animal species). The nature and magnitude of the toxic effect of BC1 on white and red blood cells depends on the type of animal and differs in rats and mice. At the same time, no significant toxic effect of BC1 on the bone marrow of animals of both species was recorded. Two weeks later, clinical manifestations of BC1 toxicity in surviving animals at high doses were not detected. In the tissues, against the background of regenerative processes, dystrophic changes still persist.

At non-toxic doses, the effect of BC1 on normal organs and tissues was either absent or minimal.

Immunotoxicity

The aim of the study was to study the toxic effect of the test agent BC1 on the immune system of mice.

The objectives of the study included the assessment of the toxic effect of BC1 on

- B - cellular immunity (humoral response);
- T-cell immunity (cellular reactions);
- functional activity of macrophages (factor of nonspecific resistance of the organism);
- functional activity of natural killer cells of the spleen of mice (factor of nonspecific resistance of the organism);
- mass and cellularity of lymphoid organs, including bone marrow cellularity (activity of factors that take part in specific immunity).

Research structure

All mice were divided into 3 groups: one control and two experimental (6 animals in each group). Animals of two experimental groups for 5 days were orally administered drug BC1 dissolved in 0.5 ml of water in doses: MTD/20 and MTD/2, respectively.

Animals of the control group received water according to a similar scheme of administration.

Research results

It was shown that the test agent BC1 in total doses of MTD/4 and 2.5*MTD has no immunotoxic effect on the humoral and cellular parts of the mouse immune system. There are also no toxic manifestations of BC1 in relation to central and peripheral organs and cells of the immune system. No immunotoxic effect of BC1 on factors of nonspecific immunity was found. At a high dose (MTD/2), BC1 exhibits a mild ability to slightly inhibit the level of functional activity of peritoneal macrophages.

At a low dose equal to MTD/4 (corresponding to the upper limit of the therapeutic dose), BC1 is able to have a stimulating effect on the immune system, enhancing T cell immunity.

Cumulative and chronic toxicity of BC1 in C57/Bl mice

The aim of the study was to study the cumulative and chronic toxicity of the BC1 test agent in C57/Bl mice.

The objectives of the study included:

- Toxicometric studies (including the dynamics of changes in the weight of animals and the mass of internal organs
- Biochemical studies of the functional activity of the kidneys and liver.
- Morphological studies (liver, kidney, spleen, lung, small intestine and heart tissues of experimental animals).
- Studies of the effect of BC1 on the system of hematopoiesis (bone marrow of the epiphysis of the femur and peripheral blood of animals).

Morphological, biochemical, toxicometric studies and studies of the effect of BC1 on hematopoiesis were carried out at the end of the administration of BC1 in half of the animals of each group and 4 weeks after the end of the administration of the agent to analyze the rehabilitation processes in the remaining mice. At the specified time, the animals were sacrificed under light ether anesthesia by decapitation.

Research structure

To study the cumulative properties of the BC1 agent and chronic toxicity, 4 groups of animals were formed, 14 animals each. C0 (control), mice treated per os with water for injection; C1, C2, C3 - mice treated per os with BC1 at a daily dose of MTD/100, MTD/30, MTD/10, respectively. BC1 was administered to mice in a volume of 0.4 ml by gavage daily 5 times a week for 4 months. The total doses of BC1 received by animals during the chronic toxicity studies were 0.9*MTD, 3*MTD, 9*MTD. It should be emphasized that the total therapeutic dose of BC1 does not exceed MTD/2.

Research results

Studies have shown that BC1 is characterized by a low level of cumulation - long-term (4 months) administration of BC1 at a total dose of 9*MTD, which is 90 times higher than the total therapeutic dose (not exceeding MTD/10) and more than 30 times higher than LD50, does not causes death of animals.

In a therapeutic dose, BC1 does not have a toxic effect on all the studied systems and tissues of the animal body.

The observed morphological changes in the structure of parenchymal organs at high total doses of BC1 were not accompanied by functional changes in them, but were reduced mainly to signs of a violation of the functional viability of blood capillaries, basement membranes and connective tissue layers that limit the structural elements of organs, which manifested itself in the formation of hydrated spaces around these structures, diapedesis of erythrocytes, thinning of the walls of blood capillaries. This nature of the side effects of BC1 is directly related to the mechanism of its antitumor action aimed at inhibiting angiogenesis processes, and is reversible: after 4 weeks, there is a preferential normalization of the morphological structure of organs and tissues.

At high doses, BC1 has a weak toxic effect on the hematopoietic system. So at high doses, there is a tendency to increase the number of leukocytes as the dose of BC1 increases and a significant (statistically significant) redistribution of leukocyte populations towards a decrease in the number of lymphocytes and an increase in the number of neutrophils. The number of erythrocytes in the peripheral blood and erythroid cells in the bone marrow also increases, which is most likely due to a compensatory reaction of the hematopoietic system in response to BC1-induced diapedesis of erythrocytes. The observed side effects of BC1 in relation to hematopoiesis are completely reversible and disappear within 4 weeks of the recovery period.

BC1 in none of the studied doses has a toxic effect on the functional activity of the kidneys, and does not affect the activity of the main liver enzymes.

Chronic toxicity study of agent BC1 on Vietnamese lop-bellied pigs

The purpose of the study was to study the toxicity of BC1 after its repeated oral administration in the framework of a chronic experiment on pigs.

The objectives of the study included:

- Clinical observations.
- Hematological studies
- Biochemical studies of peripheral blood.
- Cardiology studies (electrocardiography).
- Morphological studies (liver, kidney, spleen, lung, stomach, pancreas, small intestine, adrenal and heart tissues of experimental animals).

• Studies of the effect of BC1 on the system of hematopoiesis (bone marrow of the epiphysis of the femur and peripheral blood of animals).

Research structure

The studies were carried out on female pigs of the Vietnamese fold-bellied breed at the age of 2 months, weighing from 5.6 to 7.1 kg. 2 groups of animals were formed: control - 2 individuals and experimental - 3 individuals. Experimental animals received BC1 with food daily, 5 times a week for 20 weeks. Control animals received only food.

The total dose of BC1 for BC1 therapy was close to the upper limit of the therapeutic dose and was equal to $P*MTD_{pigs}/12$ (P is the weight of animals in kg), which by the end of therapy for each animal averaged 4.2±0.2 grams of BC1 (or 0.53 mg according to the content of aconitine). MTD_{pigs} was determined by the conversion formula. When calculating the daily dose of administered BC1, we proceeded from the total dose, taking into account the duration of administration and the changing weight of animals.

Research results

Long-term oral administration of BC1 to Vietnamese fold-bellied pigs did not cause changes in the behavior of animals and biochemical parameters that would indicate the toxic effect of the agent. The conducted studies also indicate the absence of BC1 cardiotoxicity. No hemotoxic effect of BC1 was detected. The observed trend towards a decrease in more mature erythroid cells with a simultaneous increase in the number of megakaryocytes in animals of the experimental group is most likely associated with the mechanism of action of BC1, which (dose-dependently) can lead to an increase in vascular permeability with subsequent release of erythrocytes into the intercellular space. This phenomenon was clearly pronounced in the acute experiment at high doses of BC1, and to a small extent also manifested itself in the chronic one. The release of erythrocytes into the intercellular space was recorded in some animals of the experimental group on histological preparations of the liver.

It should be emphasized that the histological analysis of the gastric and small intestine mucosa after long-term oral administration of BC1 did not reveal any signs of irritating effect of the test agent.

Thus, the BC1 test agent does not show toxicity when chronically administered to animals. Moreover, attention is drawn to the positive effect of BC1 on the urinary system, which is manifested in a decrease in the level of creatinine and urea in animals treated with BC1 (compared to control pigs).