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EVALUATION OF ANTITUMOR ACTIVITY OF BENZIMIDAZOLE DERIVATIVE ON MODELS OF EXPERIMENTAL TUMORS

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The effect of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on the growth and metastasis of transplanted allografts of melanoma B16 and epidermoid lung carcinoma Lewis with intraperitoneal administration was investigated. Antitumor effect was established in relation to primary epidermoid carcinoma of Lewis lung and its metastases to the lungs, as well as in relation to metastatic lung damage for melanoma B16 mice.

Keywords: experimental B16 melanoma, epidermoid Lewis lung carcinoma, dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole, anticancer activity, antimetastatic activity, intraperitoneal administration.

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Introduction. Despite the wide range of modern drugs for medical cancer therapy, their high toxicity necessitates the search for anticancer agents that selectively suppress or inhibit the growth of tumor cells.

Over the past decades, many benzimidazole derivatives have been reported to have pronounced anticancer activity due to their structural similarity to naturally occurring nucleotides (El Rashedy AA, 2013). Benzimidazole-based drugs have been developed and introduced into clinical practice for cancer chemotherapy. Thus, Veliparib (ABT-888), a benzimidazole-based drug, was assigned the status of an orphan drug for non-small cell lung cancer by the FDA in 2016 (https://adisinsight.springer.com/drugs/800028802). Its mechanism of action is to inhibit poly (ADP-ribose) polymerase (PARP) -1 and -2, thereby inhibiting DNA repair and potentiating the cytotoxicity of DNA damaging agents (Penning TD, 2009).

Recent studies have shown the activity of various substituted benzimidazole derivatives with their antiproliferative value against various cancer cell lines such as HCT116, MCF7, HeLa, HepG2, A549 and A431. (Tahlan S, 2019). Anticancer and antimetastatic effect of a benzimidazole derivative dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole was reported (Komarova EF, 2017; RF Patent 2016). Intragastric administration of the pharmacological substance dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole to mice caused minor inhibition (48.7%) on the growth of experimental B16 melanoma at a dose of 220 mg/kg, without affecting the survival of animals with tumors. However, a pronounced inhibiting effect of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on metastatic nodes in the lung was revealed (the metastasis inhibition index was 75.5%).

The purpose of this study was to analyze the effect of intraperitoneal administration of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on the growth and metastasis of experimental transplantable tumors.

Material and methods. The study included 96 female C57Bl6j mice weighing 18-20 g (the protocol of the bioethical commission of Rostov Research Institute of Oncology No. 18 from 10.09.2015). Animals were obtained from the vivarium of the Andreevka branch of the Scientific Center of Biomedical Technologies (Moscow region). All manipulations with animals and their withdrawal from the experiment were carried out in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

The experiment used two strains of transplantable murine tumors: B16 melanoma (EG1) and epidermoid Lewis lung carcinoma (LLC; EG2). The solution of the studied substance in the physiological solution for the administration to animals was prepared ex tempore and injected intraperitoneally once a day for 10

days, 48 hours after subcutaneous tumor transplantation to animals in the single doses were 6.15 (subgroup 1), 30.75 (subgroup 2) and 61.5 (subgroup 3) mg/kg (1/40, 1/8 and 1/4 of LD50, respectively). Control groups (CG1 for B16 melanoma and CG2 for LLC) included animals with tumors who received intraperitoneal injections of physiological saline in similar doses and regimens, instead of the studied solution.

Some of the animals in all groups were euthanized in a CO2 chamber on day 25 after tumor inoculation, and necropsy was performed. The anticancer and antimetastatic activity of the substance was studied in accordance with the Guidelines for the experimental (preclinical) study of new pharmacological substances of the Federal Service for Surveillance in Healthcare and Social Development of the Russian Federation (Mironov, 2013) and the Order of the Ministry of Health of the Russian Federation No. 199n from 01.04.2016.

Anticancer and antimetastatic activity of the studied substance was evaluated by standard parameters: tumor volume, survival, and the number of metastatic nodes. The survival improvement (T/C, %), tumor growth inhibition (TGI, %), and metastasis inhibition index (MII, %) were calculated.

Table 1 presents the experiment design.

Statistical analysis of results was performed using the STATISTICA 12.0 program (StatSoft Inc., USA). The results were checked for a normal distribution by the Shapiro-Wilk and Kolmogorov-Smirnov tests. The significance of differences between the means of the independent samples was assessed using the Student's t-test. Differences were considered significant at $p<0.05$.

Results. The study of an effect of RU-185 intraperitoneal administration on the growth and metastasis of experimental B16 melanoma showed influence on the survival of animals depending on the dose (Table 2). According to the quantitative criteria for the substance activity, intraperitoneal administration of a dose of 30.75 mg/kg improved the survival of animals with B16 melanoma (T/C = 151.3%), and other doses did not significantly affect this indicator.

Intraperitoneal administration of RU-185 did not cause a pronounced inhibiting effect on the growth of subcutaneous B16 melanoma (Table 2). On the 7th day after the end of treatment in subgroups 1 (6.15 mg/kg) and 2 (30.75 mg/kg) EGI, a slight decrease in the volume of the primary tumor node was found, which maintained by day 14 only in subgroup 2 (TGI=23.6%).

Intraperitoneal administration RU-185 changed the studied parameters of anticancer effect on experimental lung carcinoma depending on the substance dose (Table 3). Significant improvement of survival and decreased tumor sizes were registered in EG 2, subgroup 2 (T/C=171.6%). The volumes of the prima-

Table 1

Design of the experiment

Main indices	Animal groups							
	EG1			EG2			CG1	CG2
	1	2	3	1	2	3		
Number of animals in the group AC/AM	12 6/6	12 6/6	12 6/6	12 6/6	12 6/6	12 6/6	12 6/6	12 6/6
Administered substances	dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole						physiological saline	
Single doses, mg/kg	6.15	30.75	61.5	6.15	30.75	61.5		
Injected volumes	0.3 mL/day							
Period of administration	10 days							
Rout of administration	intraperitoneal							

Note: EG1 – experimental group with transplanted B16 melanoma, EG2 – experimental group with transplanted epidermoid Lewis lung carcinoma, CG1 and CG2 – control groups of animals with transplanted B16 melanoma and epidermoid Lewis lung carcinoma, respectively, AC -evaluation of anticancer effect, AM - evaluation of antimetastatic effect.

Table 2

Effect of intraperitoneal dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on B16 melanoma growth dynamics

Single dose, mg/kg	T/C, %	Tumor volume (cm3), M±m (TGI,%)		
		days after the end of treatment		
		1	7	14
6.15	126.9	4.3 [3.1-5.1]	26.9 [16.3-37.5] (15.9)	51.3 [50.5-60.9]
30.75	151.3[1.2]	3.8 [2.6-6.0] (7.3)	26.1 [15.7-37.2] (16.1)	35.9 [35.2-46.5] (23.6)
61.5	84.7	3.9 [1.4-5.3] (4.9)	29.9 [18.9-34.1] (3.5)	43.1 [35.3-54.1] (4.5)
Controls	0	4.1 [1.5-5.6]	31.1 [20.9-37.6]	47.1 [34.9-55.2]

Note: 1 – differences were significant compared to controls ($p<0.05$); 2 - differences were significant compared to subgroups of the experimental group ($p<0.05$). Differences were determined using the Student's t-test.

Table 3

Effect of intraperitoneal dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on LLC growth dynamics

Single dose, mg/kg	T/C, %	Tumor volume (cm^3), M±m (TGI, %)		
		days after the end of treatment		
		1	7	14
6.15	83.3	26.4 [21.9-34.6]	66.9 [53.9-71.1] (12.8)	91.1 [85.6-95.2] (12.1)
30.75	171.6[1.2]	8.1 [6.9-9.1][1.2] (56.2)	37.1 [31.7-45.6][1.2] (51.8)	56.1 [51.2-60.3][1.2] (45.9)
61.5	110.7	15.6 [11.3-19.9] (14.8)	51.4 [46.8-57.1][2] (32.9)	82.1 [73.8-90.6] (20.8)
Controls	0	18.3 [13.9-24.2]	76.7 [73.1-84.2]	103.6 [90.5-121.4]

Table 4

Effect of intraperitoneal dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole on metastasis of B16 melanoma and LLC

Indices		EG1	EG2
Number of metastases per 1 animal	6.15	5.6±1.0[1]	13.1±1.0[1]
	30.75	4.6±0.6[1.2]	10.6±0.6[1.2]
	61.5	12.7±2.9	23.7±0.9
	Controls	14.2±6.2	27.6±1.2
MII, %	6.15	60.2±4.3[2]	63.1±5.1[2]
	30.75	74.5±4.8[2]	79.6±5.6[2]
	61.5	15.8±4.1	16.9±3.9

Note: 1 – differences were significant compared to controls ($p<0.05$); 2 – differences were significant compared to subgroups of the experimental group ($p<0.05$).

ry subcutaneous tumor nodes were 2.3 times lower already on the 1st day after the end of treatment, compared to the control ($p<0.05$), and this effect persisted up to the 14th day (the tumor volume was decreased by 2.1 and 1.9 times on days 7 and 14, respectively, at $p<0.05$).

The next stage of the study was to assess the antimetastatic effect of intraperitoneal dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole against the studied experimental tumors (Table 4).

The substance administration in EG1 reduced the number of metastases in subgroups 1 and 2 by 2.5 and 3.0 times, respectively ($p<0.05$), compared to controls, and did not influence the number of metastases in subgroup 3. The frequency of metastasis in the subgroups was high: 60.4% in subgroup 1 and 74.5% in subgroup 2. Similar results on antimetastatic activity were obtained in EG2. The number of metastases in subgroups 1 and 2, EG2, was decreased by 2.1 and 2.6 times, respectively ($p<0.05$), compared to controls, and did not differ from the number in controls in subgroup 3. MII was higher in EG2 than in EG1 and was 63.1 and 79.6 for subgroups 1 and 2, respectively.

The results demonstrated that intraperitoneal administration of RU-185 at a total dose of 307.5 mg/kg had the inhibitory effect on the growth of the primary epidermoid Lewis lung carcinoma and did not affect the growth of the primary B16 melanoma in mice. However, a significant antitumor effect of intraperitoneal dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole at a total dose of 61.5 mg/kg and 307.5 mg/kg was revealed relative to metastasis of both studied strains, since the number of metastases and the frequency of metastases in the lungs decreased.

Our earlier research of anticancer activity of intraperitoneal RU-185 showed its pronounced inhibitory effect against lung metastases from B16 melanoma without any effect on the growth of the primary subcutaneous B 16 melanoma, and an inhibition of subcutaneously transplanted epidermoid Lewis lung carcinoma (Комарова 2017; Комарова 2021-вторая и патент). The results of this and previous studies on the anticancer activity of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole suggest that the mechanism of its antitumor action is associated with metabolic changes in the lung tissue. Tissue metabolism of various tumors plays an important role for their growth and development (Кит 2015,2014). The growth of a metastatic tumor in the lungs of rats was reported to be accompanied by an increasing imbalance of the main metabolic systems: free radical, hydrolytic, and kallikrein-kinin ones, and a change in the hormonal tissue status, which contributes to the malignant progression in the lung tissue (Комарова - диссертация). However, identification of pathogenetic factors involved in the antimetastatic action of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole showed in this experiment requires further research.

Conclusion. Intraperitoneal administration of dihydrobromide-2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo-[1,2-a] benzimidazole showed its anticancer effect against primary epidermoid Lewis lung carcinoma and its metastases to the lungs, as well as against lung metastases from B16 melanoma in mice.

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THE LEVEL OF STEROID HORMONES IN THE BODY OF WRESTLERS AT DIFFERENT PERIODS OF THE TRAINING CYCLE IN THE CONDITIONS OF THE NORTH

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The aim: to assess the level of steroid hormones in the Yakut freestyle wrestlers in the pre-competitive and recovery period in the spring season. The levels of testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate and cortisol in the blood serum of highly qualified athletes and university students who go in for physical education at least 2 times a week were studied. The lower testosterone content in wrestlers revealed before the competition indicates the intensity of training loads and psycho-emotional stress. A slight increase in testosterone levels after 7-10 days and its excess of the basal level after 30 days after the competition indicates the adequacy of recovery. The level of cortisol is characterized by a significantly lower content before the competition, and a continuing decrease after the competition in the second period (p=0.027; p=0.003) than in the control group. A similar character of changes in the level is also observed in relation to DHEAS. Levels of all steroid hormones 30 days after the competition, exceeding their pre-competition levels, indicates adaptation to stress and shows the recovery of the body. The growth of the T/K index in athletes 7-10 years after the competition indicates the beginning of recovery processes in the body. A higher level of testosterone, cortisol and DHEAS (p = 0.003) - the hormone of the precursor of these hormones one month after the competition indicates a moderate activation of the pituitary-adrenal system to balance the processes of anabolism and catabolism.

Keywords: steroid hormones, cortisol, testosterone, DHEA, wrestlers, training cycle period.

In modern sports, growing physical activity increases neuropsychic stress. Their joint influence with climatic and environmental factors of the North on the body of athletes causes the summation of stress effects that activate metabolic processes. In such conditions of training, an adequate process of adaptation to physical loads becomes relevant for maintaining the performance of athletes and achieving high results.

An important role in maintaining homeostasis in the process of adaptation to physical activity is played by the endocrine system, which regulates anabolic and catabolic processes in the body, where sex hormones play an important role.

Anabolic processes mean the processes of synthesis of substances necessary for the organs and systems of the body. Regenerative processes and anabolism of muscle tissue are dependent on the level of hormones, including testosterone. Its main role is to induce the synthesis of contractile proteins in muscles undergoing strenuous exercise. Also, during competition, testosterone may be needed to mobilize functionality [17].

In parallel, catabolic processes occur in the body - the breakdown of cells and tissues, the decomposition of complex structures with the release of a large amount of energy. Strong physical loads during training and competitive periods can become catabolic stress, causing changes in the hormonal status of the body [12]. An excessive increase in the level of cortisol causes the breakdown of muscle cells, disrupts the delivery of amino acids to them, thereby reducing the athlete's performance. It is known that the nature of hormonal changes depends on the level of fitness of the body, load parameters and the duration of the recovery period [1, 6].

Excessive activation of the pituitary-adrenal system can cause disruption in the functioning of organs, imbalance of various systems and depletion of the functional reserves of the body. Therefore, it is necessary to evaluate the effect of physical activity on the body in different periods of the training cycle [4].

The purpose of this study was to assess the level of steroid hormones in Yakut freestyle wrestlers in the pre-competitive and recovery period.

Material and research methods. The study was conducted in the spring period from March to April 2019. A total of 40 young men aged 17 to 23 were examined. The first group consisted of 18 freestyle wrestlers, candidates for master of sports (CMS) of the Republican School and the College of the Olympic Reserve. The average age was Me-18 (18; 19). The second group included 22 NEFU students. M.K. Ammosov, who go in for physical education at least twice a week. The average age was Me-19 (18; 22).

Blood sampling from all subjects was carried out in the morning hours (8-10 hours) on an empty stomach from the cubital vein into a vacutainer with a coagulation activator, in a state of relative muscle rest. The athletes were examined at different periods of the training cycle, the

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