Study on erythropoiesis-, leukopoiesis- and thrombocytopoiesis – stimulating activity of compounds BIV-241, BIV-242, BIV-243

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Abstract. The significance of the study is due to the problem of the increase in patients with secondary forms of leukopoiesis-depressive diseases. The development of leukopoiesis-stimulating drugs is necessary. These drugs are used in the treatment of cancer patients to prevent the development of febrile neutropenia, to stimulate the immune system in the postoperative period, to restore immune reactivity in environmentally caused secondary immunodeficiency syndrome. The rate of increase in patients with the secondary form of leukopoiesis-depressive syndrome is due to the increased use of cytostatic drugs. Preclinical work was carried out to study new synthetic compounds BIV-241 (dimethyl ((4-methoxyphenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate), BIV-242 (dimethyl ((3-methoxyphenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate), BIV-243 (dimethyl ((2-fluorophenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate) for leukopoiesis-stimulating activity. The studies were conducted on a cyclophosphamide-induced model of pancytopenia on outbred white rats. Compounds BIV-241 and BIV-242 did not show the desired activity. Compound BIV-243 is of interest for further development, as it had moderate leukopoiesis-, erythrocytopoiesis- and thrombocytopoiesis-stimulating activity.

1 Introduction

The relevance of the study relates to the problem of the increase in patients with secondary forms of leukopoiesis-depressive diseases [1]. According to statistics from the European Academy of Allergy and Clinical Immunology (EAACI), secondary immunodeficiency diseases are the primary cause of cancer and increased mortality among children and working-age adults on the planet [2]. Due to the increasing number of cancer patients, patients with environmentally caused secondary immunodeficiency diseases, as well as the increasingly widespread use of cytostatics in therapeutic practice, there is a growing need for

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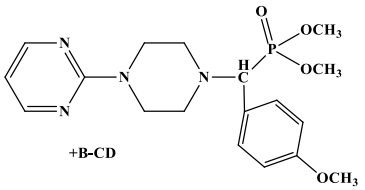
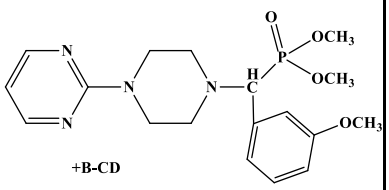
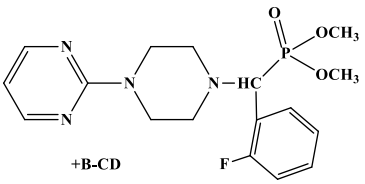
the development of drugs with leukopoiesis-stimulating effects [3]. Drugs that have the ability to stimulate leukopoiesis, such as peptide drugs, cytokines, sodium deoxyribonucleate-based drugs, have various serious side effects [4, 5]. This area is underdeveloped and there is a need to develop new drugs with leukopoiesis-stimulating activity. The purpose of the study was to conduct a preclinical study of compounds with leukopoiesis-, erythrocytopoiesis-, and thrombocytopoiesis-stimulating effects among phosphanate derivatives. The novelty of the work lies in the elucidation of the hemostimulating activity of newly synthesized compounds with a phosphanate core with dimethyl-piperidine-piperazine radicals.

2 Materials and research methods

2.1 Chemical structure of the studied compounds

For the experiment, the compounds BIV-241, BIV-242 and BIV-243 were synthesized at al-Farabi Kazakh National University. Chemical structure of newly synthesized compounds unified under the code BIV (Biologically Interesting Version) is presented in the Table 1.

Table 1. Chemical structure of newly synthesized compounds under the code BIV

No	Code	Structure	Chemical name of the compound
1	BIV-241		**$C_{18}H_{25}N_4O_4P + C_{42}H_{70}O_{35}$** dimethyl ((4-methoxyphenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate
2	BIV-242		**$C_{18}H_{25}N_4O_4P + C_{42}H_{70}O_{35}$** dimethyl ((3-methoxyphenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate
4	BIV-243		**$C_{17}H_{22}FN_4O_3P + C_{42}H_{70}O_{35}$** dimethyl ((2-fluorophenyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methyl)phosphonate

2.2 Design of the experiment

The study of the stimulating effect on erythro-, leuko- and thrombocytopoiesis was carried on 36 healthy adult female white laboratory rats (aged 12-16 weeks; weighting 210-280 g). Animals were received from the vivarium of al-Farabi Kazakh National University. The experiment was designed with consideration of the ethical principles for treatment of laboratory animals [6]. The studies were performed in accordance with the “Rules for

conducting preclinical (nonclinical) studies of biologically active compounds" and "Ethical principles and recommendations for scientific experiments on animals" and the research was approved by the protocol of the Local Ethics Committee of al-Farabi Kazakh National University No. 20226/10 from 12 June, 2023. Table 2 reflects the experimental design of the study.

Table 2. Experimental design

Group	**Number of rats**	**The cyclophosphamide at a dose of 30 mg/kg body weight, dissolved in saline to a total volume of 0.5 ml (intramuscularly administration)**			**the chemical compounds at a dose of 0.4 mg/kg body weight, dissolved in saline to a total volume of 0.5 ml (intramuscularly administration)**		
		days of the experiment			**days of the experiment**		
		1st	**2nd**	**3rd**	**7th**	**9th**	**11th**
BIV – 241 (introduced chemical compound BIV-241)	6	+	+	+	+	+	+
BIV – 242 (introduced chemical compound BIV-242)	6	+	+	+	+	+	+
BIV – 243 (introduced chemical compound BIV-243)	6	+	+	+	+	+	+
Control (introduced chemical compound methyluracil)	6	+	+	+	+	+	+
Placebo (only the saline in a volume of 0.5 ml)	6	+	+	+	+	+	+
Intact	6	-	-	-	-	-	-

Blood samples were collected from rats (on the 15th days of the experiments) under the mild anesthesia with ketamine/xylazine in sterile hematologic tubes VF-052SDK with 2 mL of EDTA (K2).

2.3 Statistic analysis

The obtained data were processed by the methods of mathematical statistics using Microsoft Excel (version 2018) with the development of Student's t-test. Data is presented as mean (M) ± standard deviation (SD). Observed differences were considered statistically significant at $p<0.05$.

Table 3. Peripheral blood parameters

Blood counts	BIV-241	BIV-242	BIV-243	Control group	Placebo group	Intact group
	1	2	3	4	5	6
WBC, $\cdot 10^9$/l	2.7±0.4	5.2±2.1	11.3±0.9	6.2±0.4	4.7 ± 0.3	9.02±0.32
	$P_{1-6} < 0.01$ $P_{1-5} < 0.01$		$P_{3-4} < 0.05$ $P_{3-5} < 0.05$			
LYM, %	52.6±2.8	56.8±0.2	55.9±2.7	60.0±3.9	57.0 ± 1.6	69.9 ± 1.1
NEU, %	33.2±0.9	34.7±0.9	36.5±2.5	32.6±1.6	36.0 ± 9.3	30.0 ± 0.8
MI, %	15.3±2.5	8.7±1.2	8.2±0.2	7.2±0.4	7.0 ± 5.3	0.3 ± 0.1
	$P_{1-4} < 0.01$ $P_{1-5} < 0.01$					
LYM, $\cdot 10^9$/l	1.4±0.1	2.9±1.3	6.5±0.21	3.7± 0.3	2.7 ± 0.8	7.7 ± 1.0
	$P_{1-6} < 0.01$		$P_{3-5} < 0.05$			
NEU, $\cdot 10^9$/l	0.9±0.1	1.8±0.8	4.3±0.6	2.0±0.9	1.7 ± 0.6	3.3 ± 0.7
MON, $\cdot 10^9$/l	0.4±0.1	0.4±0.1	0.8±0.1	0.4±0.0	0.3 ± 0.0	0.03 ± 0.0
RBC, $\cdot 10^{12}$/l	8.4±0.5	7.2±0.1	7.67±0.1	6.1± 0.1	3.6 ± 0.2	7.3 ± 0.2
	$P_{1-5} < 0.05$	$P_{2-5} < 0.05$	$P_{3-5} < 0.05$			
HGB, g/l	141.0±4.5	130.5±8.5	134.0±10.2	122.0± 4.0	91.1 ± 2.6	140.0 ± 6.0
	$P_{1-5} < 0.05$	$P_{2-5} < 0.05$	$P_{3-5} < 0.05$			
HCT, %	36.4±3.2	32.6±1.7	34.7±0.1	22.3± 0.7	20.7 ± 0.3	36.3 ± 0.2
MCV, fl	46.9±2.1	46.7±2.1	47.2±3.1	52.4± 0.4	42.8 ± 0.1	83.6 ± 0.2
MCH, pg	17.9±1.7	18.1±1.4	17.5±2.05	12.7± 0.4	12.2 ± 0.3	18.4 ± 0.1
MCHC, g/l	365.0±18.2	379.5±19.3	359.5±21.1	428.0±9.33	363.6 ±5.00	403.0± 4.0
RDW-CV, %	14.9±0.9	15.8±2.01	14.2±2.03	25.3± 0.5	23.0 ± 0.40	23.6 ± 0.2
PLT, $\cdot 10^9$/L	745.0±56.2	861.5±100.5	898.0±21.0	521.0 ± 135.3	422.0 ±41.3	690.0 ± 166.3
	$P_{1-5} < 0.01$	$P_{2-5} < 0.01$	$P_{3-5} < 0.01$			
PCT, %	0.6±0.1	0.6±0.1	0.7±0.1	0.2± 0.1	0.2 ±0.0	0.372 ± 0.1
MPV, fl	8.4±0.1	8.1±0.1	8.8±0.1	6.1 ± 0.5	5.5 ± 0.1	7.4 ± 0.3
PDW, %	14.5±0.1	12.7±0.1	13.8±0.1	12.2 ± 0.5	11.2 ± 0.2	11.4 ± 0.4

3 Results and discussion

Animals in the intact group had indicators that corresponded to healthy animals (Table 3).

Erythrocyte indicators. The total erythrocyte count was (7.3±0.2)·10^{12}/l of blood with a hemoglobin index (145.1±6.0) g/l of whole blood. Average concentration of hemoglobin in the erythrocyte mass was (403.1±4.0) g/l of blood. The hematocrit and mean erythrocyte volume were (37.4±1.30) % and (82.6±0.3) fl, respectively, within normal limits.

Platelet indicators. The total platelet index, average platelet volume, thrombocrit were (690±166.3)·10^9/l of blood, (7.4±0.30) fl and (0.37±0.08) %, respectively, which were also in within normal limits.

Leukocyte indicators. The total leukocyte count (11.08±0.32)·10^9/l of blood was equal to the minimum acceptable normal value. In the blood leukogram, the relative indicator of lymphocytes, the relative indicator of granulocytes and the relative indicator of monocytes were (69.7±1.1) %, (30.0±0.8) %, (0.2±0.1) %, respectively. The values of lymphocytes,

granulocytes and monocytes were corresponding to the norm (7.7±1.1) 10^9/l of blood, (3.3±0.7) $\cdot 10^9$/l of blood, (0.1±0.0) $\cdot 10^9$/l of blood, respectively.

The administration of the cytostatic drug cyclophosphamide led to a decrease in all peripheral blood parameters by 2.0-2.6 times. Red blood cell count fell 1.95 times to a value of (3.59±0.2) $\cdot 10^{12}$ / l of blood ($p < 0.05$) with a decrease in the level of hemoglobin by 1.53 times to a value of (96.0 ± 2.7) g/l of blood ($p < 0.05$). The average concentration of hemoglobin in the erythrocyte mass decreased insignificantly to the value of (363.6±5.00) g/l of blood, the hematocrit indicator and the volume of erythrocytes decreased to (22.9±1.1) % and (41.8±0.1) fl respectively. It is erythrocytes that mainly make up the volume of blood cells, and the decrease in hematocrit reflected a decrease in the volume of erythrocytes relative to the volume of blood plasma. In the platelet mass, a decrease in the number of platelet cells was also recorded by 1.64 times to a value of (422.0±41.3) $\cdot 10^9$ /l of blood ($p < 0.05$). The average platelet volume and thrombocrit decreased by 1.34 and 1.6 times to (5.5±0.1) fl and (0.2±0.1) % ($p < 0.05$), respectively (Table 3).

Significant changes occurred in the volumes of leukocytes. The total leukocyte count decreased by 2.34 times to a value of (4.7±0.3) $\cdot 10^9$/l of blood ($p < 0.01$). A shift in the blood leukogram occurred to the left with a decrease in agranulocytic leukocytes, namely lymphocytes, and an increase in the relative index of granulocytes. Absolute indicators decreased more significantly than relative ones. The absolute lymphocyte count decreased to (2.7±0.9) $\cdot 10^9$/l of blood by 2.86 times ($p < 0.01$), the absolute granulocyte count decreased to (1.7±0.6) 10^9/l of blood by 1.95 times ($p < 0.01$) (Table 3).

Thus, we obtained a decrease in the proliferative activity of erythro-, thrombocyto- and leukopoiesis. Next, against the background of erythro-, thrombocyto-, and leukopenia, newly synthesized compounds were introduced under the code BIV. Then, based on the results of the blood hemogram (Table 3), the compounds were divided into 2 groups: with no activity, with moderate activity.

Compounds with moderate activity: BIV-243. Compounds that did not have the desired activity: BIV-241, BIV-242.

Compounds with no erythro-, thrombocyto-, leukocyte-stimulating activity: BIV-241 and BIV-242.

For compounds lacking the desired activity, BIV-241 and BIV-242, low leukocyte counts were recorded. No recovery of lymphocyte parameters was observed. High monocytic counts were observed, which was apparently caused by intoxication of the body. Intoxication caused dehydration of the body. There was a false increase in erythrocyte and platelet counts.

Compound BIV-243 showed moderate activity. Despite the high leukocyte count (11.3±0.96)$\cdot 10^9$/l of blood, exceeding that of the intact group (9.02±0.32)$\cdot 10^9$/l of blood, the distribution of the blood leukogram was towards granulocytes. There was a weak recovery of the lymphocyte count and a significant increase in granulocytes. The Harkavi Index increased, which indicated an imbalance in the physiological balance of leukocyte cells in the blood leukogram with a predominance of granulocytes.

In rats, the relative indicator of lymphocytes should significantly prevail over the relative indicator of neutrophils. It is thanks to this ratio that the immunoreactivity of adaptive immunity in the order Rodents is higher than in other animal species. In the case of the BIV-243 compound, the recovery was shifted towards granulocytes, which indicated a violation of the body's immunoreactivity. In the group of compounds BIV-243, the lymphocyte indicator was (55.75±2.75) %, which turned out to be lower than the value of intact animals (69.72±1.10) % and was at the level of the placebo group. The relative granulocyte index was (36.35±2.55) % and was at the level of the placebo group. A fairly high monocyte count was also recorded, which indicated intoxication of the body and tissue necrosis. The body got dehydrated. The false high total erythrocyte count was (7.6 ± 0.1) 10^{12}/l of blood (Table 3).

The hemoglobin level was also high (137.0 ± 10.2) g/l and was close to the blood values of animals from the intact group.

The platelet level of compound BIV-243 was high due to dehydration of the body. The total platelet count was (898.0 ± 21.0) $\cdot 10^9$/l of blood. This indicator significantly exceeded the values of the intact group (690.0 ± 166.3) $\cdot 10^9$/l of blood and the control group (521.0 ± 135.3)$\cdot 10^9$/l of blood ($p<0.05$) (Table 3). Thrombocrit values, platelet distribution width, and mean blood platelet counts were also high.

Compounds BIV-241 and BIV-242 differed from compounds BIV-243 in the presence of a methoxyphenyl radical. Compound BIV-243 was distinguished by the presence of a fluorophenyl radical, which apparently influenced the change in the pharmacodynamic properties of the compound. It is planned to conduct in-depth pharmacological studies to clarify the pharmacological properties of the BIV-243 compound.

4 Conclusion

Against the background of cyclophosphamide-induced pancytopenia, compounds BIV-241 and BIV-242 did not show high hemostimulating activity and caused changes in the blood characteristic of intoxication of the body. Compound BIV-243 showed moderate hematopoiesis-stimulating activity. It did not cause changes in the blood characteristic of compounds with high toxicity. The compound BIV-243 is of interest for further development in modeling the chemical nucleus with other radicals.

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