ABSTRACT

for the dissertation for Doctor of Philosophy (PhD) degree on the specialty "8D05101-Biology" of

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on the theme "Study of myelo- and lymphocytopoiesis-stimulating activity of synthetic nitrogenous compounds in secondary myelodepressive conditions"

General description of the dissertation. The dissertation is devoted to the study of changes in the cell level in secondary cyclophosphamide-induced myelopoiesis- and lymphocytopoiesis-depressive states and the search for active compounds among newly synthesized nitrogenous compounds that are effective for restoring the cell level in the peripheral blood and lymphomyeloid organs in secondary cyclophosphamide-induced myelopoiesis- and lymphocytopoiesis-depressive states.

Relevance of the research topic. The President of the Republic of Kazakhstan Kassym-Jomart Tokayev updated and outlined the acute problem of the advancement of pharmacological development of own medicals, their introduction into pharmaceutical production with the growth of the pharmaceutical market in the country. One of the pressing problems is the development of drugs with myelopoiesis- and lymphocytopoiesis-stimulating activity. This direction is new, not developed, and is relevant due to the increasing percentage of sick people with secondary forms of myelopoiesis and lymphocytopoiesis depressive conditions. Interest in these drugs has also increased due to their use in the technology of producing oncology vaccines in vitro. Also, the range of drugs presented in therapeutic practice is very limited and each group of drugs has a spectrum of severe side effects.

The purpose of the research. The purpose of the work was to study changes in the level of cells in secondary cyclophosphamide-induced myelopoiesis- and lymphocytopoiesis-depressive states and to search for active compounds among newly synthesized nitrogenous compounds that are effective in restoring the level of cells in the peripheral blood and lymphomyeloid organs in secondary cyclophosphamide-induced myelopoiesis- and lymphocytopoiesis-depressive states.

Research objectives:

1. Research of secondary cyclophosphamide-induced T-lymphocyte depressive states.
2. Research of secondary cyclophosphamide-induced B-lymphocyte depressive states.
3. Isolation by primary screening from newly synthesized nitrogenous compounds with bispidine, dienone, pyrazolopyridine, piperazine, and piperidine structural cores and from liquid trimecaine ionic substances of compounds with high hemostimulating activity.
4. Analysis of the activity of newly synthesized compounds BIV and TIC in restoring the level of T-lymphocytes in the organs of the lymph-myeloid complex against the backdrop of cyclophosphamide-induced T-lymphocyte depression.
5. Analysis of the activity of newly synthesized compounds BIV and TIC in restoring the level of B-lymphocytes in the organs of the lymph-myeloid complex against the backdrop of cyclophosphamide-induced B-lymphocyte depression.
6. Study of the effect of BIV and TIC compounds on bone marrow, splenocytic regenerative myelocytopoiesis in cyclophosphamide-induced myelodepression.

The research objects. The study received 40 compounds under the code BIV: 32 compounds with a bispidine, dienone, pyrazolopyridine, piperazine, and piperidine core in the structure (Research

Institute of Chemical Sciences named after A.B. Bekturov) and 8 compounds were synthesized (KBTU).

The objects were white outbred albino rats from the biological clinic of Al-Farabi Kazakh National University; mice of the *C57BL6/J* line purchased from Charles River Laboratory (USA) and bred at the Kazakh Scientific Center for Quarantine and Zoonotic Infections named after Aikimbaeva.

The following biological material was used: peripheral blood of white outbred albino rats; bone marrow, thymus, and spleen of *C57BL6/J* mice; immunocytofluorometric labels: APC-CD117, APC-CD11b, PE-Ly-6C, PE-Ly6G, PerCP-Ter119+/CD71, APC-B220/CD45R, PE-CD43, PerCP-CD19, PE-MHCII, PE-CD40, PE -CD3e, FITC-CD4, PerCP-CD8a, PerCP-CD25, PE-FoxP3, PerCP-CD28, FITC-CD44. Subpopulations of cells in the bone marrow, thymus and spleen were studied: $CD117^+$ – hematopoietic stem cells; $CD11b^+Ly\text{-}6C^+Ly6G^+$ – monocyte-granulocyte leukocytes; $Ter119^+/CD71^+$ – erythroid cells; $B220/CD45R^+CD43^+CD19^+$ – Pro-B-I-, Pro-B-II- lymphocytes; $B220/CD45R^+CD43^-CD19^+$ – Pre-B-I-, Pre-B-II- and transient B-lymphocytes; $B220/CD45R^+CD19^+$ – immature B-lymphocytes; $B220/CD45R^+CD19^{+/mid}$ – MZB- and FO-B-lymphocytes; $B220/CD45R^+CD19^+MHCII^+$–$B_{act}$-lymphocytes; $B220/CD45R^+CD19^+CD40^+$ – B_{mem} lymphocytes; $CD43^+CD3e^+CD19^-$–Pre-T – lymphocytes; $CD3e^+CD4^+$ – Th-lymphocytes; $CD3e^+CD8a^+$ – CTL-lymphocytes; $CD4^+CD25^+$–Th_{act} lymphocytes; $CD4^+CD25^+FoxP3^+$ – T_{reg} lymphocytes; $CD28^+CD8a^+CD44^+$ – T_{mem}-lymphocytes.

Research methods. To carry out primary screening: a method for studying hemostimulating activity on a model of cyclophosphamide-induced hemodepression (hematological analyzer "MicroCC-20 Plus" (China)), cytological control of a peripheral blood smear using the Giemsa method (microscope Micromed MP-3). To carry out secondary screening: a method for assessing the cellularity of organs of the lymph-myeloid complex, immunocytofluorometric method (FACSCalibur cytofluorometric analyzer (BD Biosciences, USA)).

The scientific novelty of the research. Based on the studies conducted, it was established for the first time that cyclophosphamide caused a significant decrease in the level of reactive primary and recirculating T_{mem}-lymphocytes in the bone marrow (36.91%) and spleen (28%), which may negatively affect the reactivity and usefulness of the secondary T-cell immune response. Cyclophosphamide caused a 2-fold more significant decrease in the level of $FoxP3^+$ T_{reg} lymphocytes (48.08%) in the spleen than in the thymus (19.13%). A significant decrease in the level of $FoxP3^+$ T_{reg} cells caused a significant increase in the level of CTL-lymphocytes (by 32.87%) in the spleen, which can cause severe autoimmune diseases and uncontrolled immune reactions, including sepsis. A compensatory population of the thymus with recirculating Th_{act} lymphocytes (by 132.05%) was also observed, which can lead to hyperreactivity of the immune system. Regarding B-lymphocytopoiesis, cyclophosphamide also caused negative consequences. It caused almost complete devastation of germinal centers from reactive cells: MHC class II^+-B_{act}-lymphocytes (by 74.83%) and B_{mem}-lymphocytes (by 68.05%) in the spleen, which can cause a dangerous leveling of the activity of humoral immunity.

For the first time, primary screening of 32 newly synthesized azaheterocyclic compounds and 8 trimecaine ionic liquid substances was carried out, identifying four compounds with an average level and two compounds with a high level of hemostimulating activity.

It was established for the first time that compound BIV (complex 5-benzyl-7-(*o*-fluorobenzylidene)-2,3-bis(*o*-fluorophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-pyrazolo[4,3-*c*]pyridine with β-cyclodextrin) had pronounced myelo- and lymphocytopoiesis-stimulating activity, exceeding the activity of the drug Methyluracil. Compound BIV showed unique immunomodulatory activity in restoring the level of T-lymphocyte subpopulations: increasing the level of Th_{act}-, $FoxP3^+T_{reg}$-, T_{mem}-lymphocytes to physiological norms; reducing the elevated level of CTL-lymphocytes to the physiological norm. Compound BIV effectively restored the level of B-lymphocyte cells: Pre-B-I-, Pre-B-II-, Immature B-, Transient B-, FO-B-, MZB-, MHC class II^+ B_{act}-, B_{mem}-lymphocytes and moderately restored the level of erythrocyte, granulocyte and monocytic cells.

For the first time, it was established that the compound TIC (ionic liquid N,N-diethyl-2-(mesitylamino)-N-propargyl-oxoethanamonium bromide) had high myelo-, T- and B-lymphocytopoiesis-stimulating activity, significantly exceeding the activity of the drug Methyluracil.

Scientific and practical significance. The thesis contributes to fundamental immunology in the field of immunosuppressive conditions. The study of myelo- and lymphocytopoiesis-stimulating activity in nitrogen-containing compounds TIC and BIV contributes to fundamental pharmacology in the area of leukopoiesis-stimulating drugs.

The results of the dissertation work have practical potential. 6 patents for inventions were Received from the Ministry of Justice of the Republic of Kazakhstan National Institute of Intellectual Property.

Compound BIV has potential as a drug for the treatment of secondary cyclophosphamide-induced myelo- and lymphocytodepressive conditions, as it has unique T-lymphocyte modulatory activity.

The TIC compound has promise as a drug for the treatment of secondary cyclophosphamide-induced myelo- and B-lymphocyte depressive conditions. The TIC compound has the potential to be developed as a compound that can effectively stimulate an increase in the level of T_{mem}-memory cells and CTL cytotoxic lymphocytes, which is of great importance in the development of in vitro oncology vaccines used for cancer therapy.

The main provisions for the defence:

1. It has been established that cyclophosphamide causes a significant decrease in $FoxP3^{+}T_{reg}$ - regulatory lymphocytes in lymphomyeloid organs, because of which the level of CTL-cytotoxic lymphocytes increases. Cyclophosphamide also caused a decrease in reactive primary and recirculating T-costimulated memory cells and a compensatory population of the thymus with recirculating Th_{act} - activated helpers.

2. Cyclophosphamide causes a critical decrease in MHC II^{+} - activated B-lymphocytes and recirculating B_{mem} - memory lymphocytes in the germinal centers of the spleen.

3. Through primary pharmacological screening for hematopoiesis-stimulating activity, two active compounds BIV and TIC were isolated from 40 newly synthesized nitrogenous azaheterocyclic compounds.

4. It was established that the complex 5-benzyl-7-(*o*-fluorobenzylidene)-2,3-bis(*o*-fluorophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-pyrazolo[4,3-*c*]pyridine with β-cyclodextrin (BIV) has unique immunomodulatory activity against T lymphocytes. It can increase the level of Th_{act}-active helpers, T_{mem}-memory cells, and $FoxP3^{+}T_{reg}$ regulatory lymphocytes to the physiological norm, which causes a decrease in the level of CTL-cytotoxic lymphocytes to the physiological norm. Also, the BIV compound effectively restored the level of B-lymphocytes, erythrocytes, granulocytes, and monocytes.

5. It has been established that the ionic liquid N,N-diethyl-2-(mesitylamino)-N-propargyl-oxoethanamonium bromide (TIC) exceeds the activity of the BIV compound and the drug Methyluracil and can quickly restore the level of all T- and B-lymphocyte subpopulations of cells, erythrocytes, granulocytes and monocytes.

Main results and conclusions of the research:

1. Cyclophosphamide had a significant effect on T- lymphocytopoiesis, causing (more than 40%) decrease in the level of c-kit lymphocytes, Pre-T lymphocytes (by 42%), and recirculating T_{mem}-lymphocytes (by 37%) in the bone marrow. In the spleen, it led to a significant decrease (more than 20%) in the total level of T-lymphocytes, including recirculating T_{mem}-lymphocytes (by 28%) and $FoxP3^{+}T_{reg}$-lymphocytes (by 48%), combined with a significant increase in the level of CTL-lymphocytes (by 33%). In the thymus caused a profound inhibition of T-lymphopoiesis, expressed in a significant decrease in the level of immature T-lymphocytes (by 83%), while moderately increasing the level of Th_{act}-activated helper cells (by 32%).

2. Cyclophosphamide had a significant effect on B-lymphocytopoiesis, causing a great decrease (by 34 – 54%) in the levels of various subpopulations of actively proliferating B-lymphocytes in the

bone marrow and spleen (Pro-B-I- and Pro-B-II-, Pre-B -I-, Pre-B-II- and immature B-, transient B-, FO-B and MZB), causing a significant decrease in the level (by 68 –75%) of various subpopulations of mature B lymphocytes (MHC-II activated B- and B_{mem}-lymphocytes) in spleen.

3. As a result of the initial screening of 32 newly synthesized nitrogenous azaheterocyclic compounds with bispidine, dienone, pyrazolopiperidine, piperazine, piperidine structural nuclei and 8 liquid trimecaine ionic substances, 2 compounds (BIV and TIC) with high hemostimulating activity against the backdrop of cyclophosphamide-induced hemodepression were selected. Compounds BIV and TIC were more active than the reference drug Methyluracil.

4. Against the backdrop of cyclophosphamide-induced myelosuppression, BIV and TIC drugs had a stimulating effect on subpopulations of T-lymphocytes (Th_{act}-, $FoxP3^+$ T_{reg}-, T_{mem}-lymphocytes), increasing their level to physiological norms, and reducing the elevated level of CTL - cytotoxic T-lymphocytes to the physiological norm.

Against the backdrop of cyclophosphamide-induced myelodepression, the drug TIC had a stimulating effect on subpopulations of T-lymphocytes (Th_{act}-, $FoxP3^+T_{reg}$-, T_{mem}-lymphocytes), increasing their level to physiological norms and causing an increase in the level of CTL - cytotoxic T-lymphocytes.

5. Against the backdrop of cyclophosphamide-induced myelodepression, the complex 5-benzyl-7-(*o*-fluorobenzylidene)-2,3-bis(*o*-fluorophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-pyrazolo [4,3-*c*]pyridine with β-cyclodextrin (BIV-190 (BIV)) showed intense B-lymphopoiesis-stimulating activity, significantly exceeding the activity of methyluracil, which was expressed in the effective restoration of the levels of various subpopulations of B-lymphocytes: Pre-B-I -, Pre-B-II-, immature B-, transient B-, FO-B, MZB, MHC-II activated B- and B_{mem}-lymphocytes.

Against the backdrop of cyclophosphamide-induced myelodepression, the ionic liquid N,N-diethyl-2-(mesitylamino)-N-propargyl-oxoethanamonium bromide (BIV-119 (TIC)) had more significant B-lymphocytopoiesis-stimulating activity than the drug BIV.

6. Against the backdrop of cyclophosphamide-induced myelosuppression, BIV and TIC compounds had significant myelopoiesis-stimulating activity, restoring the level of erythrocytes, granulocytes and monocytes in the bone marrow and spleen to physiological norms. Compounds BIV and TIC were more active than Methyluracil.

Personal contribution of the author. All the main results described in the dissertation were carried out and collected by the author. The doctoral student fully implemented all the assigned tasks, collected data, carried out statistical processing of data, constructed graphs, drawings, described and discussed the results obtained with conclusions.

Connection between research and scientific project. This dissertation work was carried out within the framework of research grant AP08856051 “Directed modification of polyfunctional azaheterocycles – piperidine and piperazine derivatives into new bioactive molecular systems for agriculture and/or medicine” and AP08857345 “Bifunctional ionic compounds: synthesis and application in medicinal chemistry and Agriculture" within the framework of grant funding for scientific research of the Ministry of Education and Science of the Republic of Kazakhstan, at the Research Institute of Epidemiology and Microbiology named after. Pasteur (St. Petersburg, Russia), as well as at the Kazakh National University named after al-Farabi.

Approbation of the research. The main results of the dissertation were presented and discussed at the following international scientific conferences:

- “Science and education in the modern world: challenges of the XXI century” VII international scientific and practical conference. (Nur-Sultan, Kazakhstan, October 20-22, 2020);
- “Science, Education, Innovation: Topical Issues and Modern Aspects” international scientific and practical conference. (Tallinn, Estonia, December 16-18, 2020);

- “Trends and prospects for the development of science and education in the context of globalization” international scientific and practical Internet conference. (Pereyaslav, Ukraine, April 30, 2020);

- International scientific and practical conference “Modern problems of biology and biotechnology”, dedicated to the 70th anniversary of Doctor of Biological Sciences, professor, corresponding member of the National Academy of Sciences of the Republic of Kazakhstan Tuleukhanov Sultan Tuleukhanovich. (Almaty, Kazakhstan, May 27, 2021);

- “Farabi Alemi” international scientific conference of students and young scientists. (Almaty, Kazakhstan, April 6-9, 2020);

- “Trends and prospects for the development of science and education in the context of globalization” international scientific and practical Internet conference. (Pereyaslav, Uraina, January 29, 2021);

- “Farabi Alemi” international scientific conference of students and young scientists. (Almaty, Kazakhstan, April 6-8, 2021);

- “2nd Advanced Chemistry World Congress” international scientific conference (Berlin, Germany, June 14-15, 2021);

- “Society and Science: Interconnection” III international scientific and practical conference. (Porto, Portugal, November 16-18, 2023);

- “RW Research World” Research World International Conferencee. (Hamburg, Germany, December 3-4, 2023);

“Asfen.Forum, new generation – 2023” 1st International Forum. (Almaty, Kazakhstan, June 5-6, 2023).

Publications. The main results of the dissertation were published in 24 scientific papers, including 1 article in the International scientific journal *Molecules*, which, according to the *Clarivate Analytics* information database (ISI Web of Science) has an impact factor = 7.143, a percentile in the Scopus database = 0.74 (Q1); 1 article included in the international scientific citation database Scopus: International Forum “Modern Trends in Sustainable Development of Biological Sciences”; 2 articles in journals indexed in Web of Science; 7 articles in domestic scientific periodicals recommended by The Ministry of Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 13 scientific publications were published in the proceedings of domestic and foreign international conferences. Received from the Ministry of Justice of the Republic of Kazakhstan National Institute of Intellectual Property 6 patents for inventions.

Scope and structure of the dissertation. The dissertation is written on 148 pages of text. It includes normative references, definitions, notations and abbreviations, an introduction, a literature review, research materials and methods, research results and discussion, a conclusion, and 316 references. The dissertation contains 36 figures, 26 tables, and 6 appendices.