УДК 57.083.3:547.474.64:615.273.015.44
https://doi.org/10.20538/1682-0363-2023-4-65-72

Immunomodulatory effect of lithium salt gamma-lactone 2,3-dehydro-L-gulonic acid *in vitro*

Plotnikov E.V.[1, 2], Tretyakova M.S.[1, 3], Krivoshchekov S.V.[4], Kolobovnikova Y.V.[4], Belousov M.V.[1, 4]

[1] *National Research Tomsk Polytechnic University*
30, Lenina Av., Tomsk, 634050, Russian Federation

[2] *Mental Health Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Science*
4, Aleutskaya Str., Tomsk, 634021, Russian Federation

[3] *Cancer Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Sciences*
5, Kooperativny Str., Tomsk, 634009, Russian Federation

[4] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

ABSTRACT

The aim of this work was to study the immunomodulatory effects of lithium salt gamma-lactone of 2,3-dehydro-L-gulonic acid (LiAc) on healthy blood leukocytes and leukemia cells *in vitro*.

Materials and methods. Peripheral blood lymphocytes and neutrophils obtained from healthy donors, as well as THP-1 cells (human monocytic leukemia) were used as test systems. To assess the proliferative activity, lymphocyte blast transformation was used. The antiproliferative effect was studied by the 3H-thymidine incorporation assay. Cytotoxic effects were studied using the Alamar Blue test. The effect on the phagocytic activity was studied using the method for assessing the neutrophil function during bacterial phagocytosis.

Results. LiAc exerted a dose-dependent effect on target cells, including antiproliferative and cytotoxic effects on leukemia cells and a stimulating effect on neutrophils in phagocytosis.

Conclusion. LiAc can be considered as a promising drug with immunomodulatory effects, including a suppressive effect on the proliferative activity of leukemia cells and a stimulating effect on immune mechanisms mediated by neutrophils and macrophages.

Keywords: lithium salts, immunostimulant, neutrophils, lymphocytes, monocytic leukemia, antiproliferative effect, phagocytosis

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Plotnikov E.V., Tretyakova M.S., Krivoshchekov S.V., Kolobovnikova Y.V., Belousov M.V. Immunomodulatory effect of lithium salt gamma-lactone 2,3-dehydro-L-gulonic acid *in vitro*. *Bulletin of Siberian Medicine.* 2023;22(4):65–72. https://doi.org/10.20538/1682-0363-2023-4-65-72.

✉ *Plotnikov Evgeny V.,* plotnikov.e@mail.ru

Иммуномодулирующее действие литиевой соли гамма-лактон 2,3-дегидро-L-гулоновой кислоты *in vitro*

Плотников Е.В.[1, 2], Третьякова М.С.[1, 3], Кривощеков С.В.[4], Колобовникова Ю.В.[4], Белоусов М.В.[1, 4]

[1] *Национальный исследовательский Томский политехнический университет (НИ ТПУ)*
Россия, 634050, г. Томск, пр. Ленина, 30

[2] *Научно-исследовательский институт (НИИ) психического здоровья, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634021, г. Томск, ул. Алеутская, 4

[3] *Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634009, г. Томск, пер. Кооперативный, 5

[4] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель – изучение иммуномодулирующих свойств литиевой соли гамма-лактон 2,3-дегидро-L-гулоновой кислоты (LiAc) на нормальные лейкоциты крови и злокачественные лейкозные клетки *in vitro*.

Материалы и методы. В качестве тест-систем использованы лимфоциты и нейтрофилы периферической крови здоровых доноров, а также злокачественные клетки линии THP-1 (моноцитарная лейкемия человека). Для оценки пролиферативной активности использовалась реакция бластной трансформации лимфоцитов. Изучение антипролиферативного действия выполнено методом включения меченого 3H-тимидина. Цитотоксические эффекты препарата исследованы с помощью аламарового теста. Изучение влияния на фагоцитарную активность выполнено с помощью метода оценки функциональной активности нейтрофилов при фагоцитозе бактерий.

Результаты. LiAc оказал дозозависимое влияние на клетки-мишени, что проявилось в антипролиферативном и цитотоксическом действии в отношении лейкозных клеток и стимулирующем действии в отношении фагоцитирующих нейтрофилов.

Заключение. LiAc может рассматриваться как перспективный препарат, обладающий иммуномодулирующими свойствами, включая супрессивное влияние на пролиферативную активность лейкозных клеток и стимулирующее действие на нейтрофильно-макрофагальное звено иммунитета.

Ключевые слова: соли лития, иммуностимулятор, нейтрофилы, лимфоциты, моноцитарный лейкоз, антипролиферативный эффект, фагоцитоз

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Плотников Е.В., Третьякова М.С., Кривощеков С.В., Колобовникова Ю.В., Белоусов М.В. Иммуномодулирующее действие литиевой соли гамма-лактон 2,3-дегидро-L-гулоновой кислоты *in vitro*. *Бюллетень сибирской медицины.* 2023;22(4):65–72. https://doi.org/10.20538/1682-0363-2023-4-65-72.

INTRODUCTION

The immune system is a complex self-regulating mechanism, providing specific and non-specific protection from both external pathogens and internal threats, including malignant cells. At the same time, an effective immune response implies a balance of immune activation to eliminate the antigen and immunosuppression, which prevents damage to one's own healthy tissues. This balance is ensured by the interaction of macrophages, and regulatory T cells; by the induction of chemokines, cytokines, and antibodies; by the expression of the corresponding receptors and inhibition and proliferation of effector cells.

Thus, normal functioning of the immunity is supported by intrinsic immunostimulatory and immunosuppressive processes [1]. Providing an external regulatory effect on the immune system is a difficult task, which is currently achieved using immunomodulators, i.e. drugs that can stimulate, inhibit or regulate components or alter the functions of the immune system [2]. The compounds with immunostimulatory or immunosuppressive effects may belong to different chemical groups and have different biological targets. Mainly, immunomodulatory effects are realized through the influence on immunocompetent cells, the processes of maturation, migration, cooperation, as well as through the interaction of these cells and their products (cytokines) with the corresponding targets [3].

Many xenobiotics and chemical compounds have unidirectional immunotoxic effects, which is manifested by a negative impact on the functioning of both local and systemic immunity, and induced immunosuppression can lead to an increase in the incidence or severity of infectious diseases and cancer [4]. Therefore, the study of the immunomodulatory properties of promising biologically active compounds is an integral stage in the development of new drugs based on them. A substance acting on cellular immunity *in vitro* will have a complex non-specific effect on the entire immune system, due to high interconnectedness of the components.

A significant number of immunostimulants are currently available as dietary supplements. However, their effect on the actual immune status is very contradictory. Not all immunostimulants have been tested with immunocompetent cells. It is especially difficult to test various herbal stimulants due to high variability of the composition and difficulties of identifying the active agent [5].

Targeted immunostimulation is one of the promising approaches to the treatment of cancer, while non-specific immunostimulation is considered as a maintenance therapy [6]. In general, immunotherapy is the most promising approach to cancer treatment and combines well with chemotherapy and immunostimulants to improve treatment outcomes [7]. Immunomodulators are an important component in the treatment of comorbid pathologies, which improves the prognosis of the primary disease. Recently, it has been shown that immune dysregulation can contribute to tumor progression [8]. Effective immunocorrection requires drugs that have low toxicity and a complex immunomodulatory effect and combine well with known methods of treating tumors, including chemotherapy and radiation therapy.

In this context, lithium preparations stand out due to the presence of a known immunotropic effect. A good example is lithium carbonate, which is widely used in psychiatric practice and is still the mainstay for the treatment of affective disorders, while having pronounced immunostimulatory properties [9, 10]. Lithium has stimulating effects towards hematopoiesis, and, thus, it is a drug for the recovery of the body after radiation exposure [11, 12]. The main molecular target explaining the hematopoietic effect of lithium is the effect on the intracellular enzyme glycogen synthase kinase-3 (GSK-3) [13].

The role and positive effects of lithium in the treatment of cancer patients, including individuals after radiation exposure, have been studied [14]. It is important to note the ability of lithium to activate antiviral immunity [15]. During the coronavirus epidemic of 2020–2021, the effectiveness of lithium drugs in the treatment of COVID-19 was shown [16]. The combination of these data forms a solid basis for an in-depth study of the mechanisms of lithium action and the search for new niches for its use, including the creation of new lithium-containing compounds with complex properties [17].

However, it should be noted that disorders related to oxidative stress play an important role in the pathogenesis of many socially sensitive human pathologies [18–20]. Oxidative stress is a fundamental phenomenon in biology that causes a cascade of reactions [21]. The influence of oxidative stress in autoimmune, mental, cardiovascular diseases, and cancer has been established, therefore, reduction of oxidative stress by antioxidant drugs is clinically justified in many cases [20]. In this context, it is important to obtain and study the properties of new lithium complexes with antioxidant activity towards immunocompetent cells, which reveals certain

prospects for obtaining drugs with combined activity that allow to modulate immunity and reduce oxidative stress in a wide range of pathologies [22–24].

The aim of this work was to study the effect of the lithium salt gamma-lactone of 2,3-dehydro-L-gulonic acid (LiAc) on the proliferative and functional activity of healthy blood leukocytes and leukemia cells, that will provide grounds for developing a promising antioxidant drug with immunomodulatory effects.

MATERIALS AND METHODS

Gamma-lactone of 2,3-dehydro-L-gulonic acid (ascorbic acid) and lithium carbonate (ACS, Sigma-Aldrich, Germany) were used to synthesize the research object. The salt preparation reaction was carried out with stirring and heating up to 40 ºC in deionized water. The reaction product was washed and sterilized with ethanol, then dried. The identity of the compound was confirmed by atomic emission spectroscopy (AES with inductively coupled plasma iCap 6300 Duo), infrared spectroscopy (Agilent Cary), thermogravimetric analysis (thermal analyzer with mass spectroscopy SDQT 600, Thermo Electron Corp.). The elemental analysis found 33% (C), 5.33% (H), 8.1% (Li2O); theoretically calculated values were 33.03% (C), 5.05% (H), 8.21% (Li2O). The water content in the salt was 16.15% (theoretical – 16.51%). The reaction product corresponded to the general formula LiC6H7O6*2H2O. The resulting powder was packed in sealed test tubes and used in experiments.

The effect of the synthesized lithium preparation on human immune blood cells was studied *in vitro* using the lymphocyte blast transformation (LBT). The method is based on the assessment of the transformation and proliferation of lymphocytes when exposed to various antigens and the mitogen phytohemagglutinin (PHA). Lymphocytes were obtained from whole blood samples of three healthy donors. Lymphocytes were isolated by density gradient centrifugation and resuspended in a standard RPMI 1640 medium containing 20% fetal bovine serum, L-glutamine, and streptomycin. Aliquots of 0.1 ml (2×10^6 cells / ml) of the cell mixture were placed in microtest plates. The drug was added to the plate in concentrations of 0.1–0.001 mg / ml with or without PHA. The microtest plates were incubated for 72 hours at 37 °C in an atmosphere of 5% CO_2. Stimulation with PHA, which induces cell proliferation, was used as a positive control. To do this, mononuclear cells (2×10^5 cells per well) in a culture medium were introduced into the wells of a 96-well flat-bottomed plate in the presence of PHA (final concentration – 15 mcg / ml). The cells were also incubated for 72 hours at 37 °C with 5% CO_2.

In the last 24 hours of the cell culture process, 1 μCi of [3H]-thymidine was added to each well of the experimental and control groups. The cells were collected by fiberglass filters (Seaton, Ind. Sterling, VA) and the amount of included [3H]-thymidine was determined using the liquid scintillation β-counter (Delta 300, model 6891, TM Analytic Inc., Netherlands).

The phagocytic activity of neutrophils was evaluated using bacterial phagocytosis [25]. Heparinized venous blood was washed with a medium 199 by centrifugation of samples to determine the phagocytic activity of neutrophils. Gram-positive bacteria *Staphylococcus aureus* – H209 were used as a substrate for phagocytosis. Bacteria were added to the leukocyte suspension. The studied compound in various concentrations (0.1–0.001 mg / ml) was added to the microtest plates. The samples were placed in the incubator for 30 minutes and shaken every 10 minutes. After incubation, the cells were fixed in formalin. Then the samples were centrifuged to make smears with the parameter determining phagocytosis. The smears were stained according to Romanowsky – Giemsa staining. Next, the following parameters were calculated. The percentage of active neutrophils was calculated as the number of neutrophils positive for *S. aureus* in terms of 100 neutrophils. The engulfment capacity of neutrophils was characterized by the phagocytic index, which was calculated as the total number of *S. aureus* cells engulfed by 100 positive neutrophils and divided by 100, which gives the average number of microbes engulfed by one active neutrophil.

In the experiment, the THP-1 monocytic leukemia cell line was used at a concentration of 2×10^5 cells per well of a 96-well plate in a complete culture medium RPMI 1640 containing 10% fetal bovine serum, L-glutamine, and antibiotics. The cells were introduced into the wells of 96-well flat-bottomed plates in 100 mcl of medium. After that, LiAc was added in the dose range from 0.1 mg / ml to 0.001 mg / ml. Then, 1 μCi of [3H]-thymidine was added to each well. The cells were incubated for 24 hours at 37 °C with 5% CO_2. The cells were collected by fiberglass filters (Seaton, Ind. Sterling, VA) and the amount of incorporated [3H]-thymidine was determined using the liquid scintillation β-counter (Delta 300, model 6891, TM Analytic Inc., Netherlands).

THP-1 monocytic leukemia cell lines and healthy human peripheral blood mononuclear cells were used as a biological object for cell cytotoxicity tests *in vitro*. The cytotoxicity of the studied drug was evaluated on cell cultures using the Alamar Blue assay. The cells were cultured in a complete RPMI 1640 medium enriched with fetal bovine serum, L-glutamine, and antibiotics and plated into a 96-well plate with 20,000 cells per well (in 180 mcl of medium). Then the drug was added to each well in the appropriate concentration (in the dose range of 0.1–0.001 mg / ml). Additional wells were used for untreated control (negative control) and control of dead cells (positive control). Then the plate was placed in the incubator with 5% CO_2 and at a temperature of 37 °C. After 48 hours of incubation, 20 mcl of Alamar Blue reagent was added to each well. The plate was placed back in the incubator for 4 hours. Then the optical density was measured at 570 nm (with background subtraction at 620 nm). Cell viability was evaluated by the formula (sample optical density (OD) – positive control OD) / (negative control OD – positive control OD) and expressed as a percentage of the surviving cells.

RESULTS

The study of the intensity of LBT after introduction into the culture of LiAc cells made it possible to establish the presence of a suppressive effect of the drug on lymphoproliferation, depending on the concentration. Thus, a dose of 0.1 mg / ml significantly inhibited cell proliferation by more than 60% compared to the control group (Fig. 1).

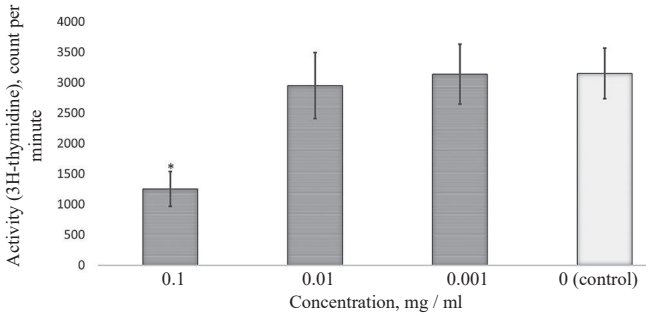

Fig. 1. The effect of LiAc on the lymphocyte PHA-stimulated proliferation (by the incorporation of 3H-thymidine (count per minute)). Here and in Fig. 2, 3, the data are presented as $M \pm SD$. * significant difference from the control, $p \leq 0.05$

The effect of the drug on the functional activity of peripheral blood neutrophilic leukocytes of donors was studied using the model of incomplete phagocytosis. The obtained results regarding the effect of the drug on phagocytic activity are presented in Table.

Table

The effect of LiAc on the phagocytic activity of neutrophils, $M \pm SD$

Parameter	Control	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Percentage of active neutrophils, %	56 ± 3	61 ± 4	67 ± 5*	55 ± 4
Engulfment capacity of neutrophils, bact. units	4.2 ± 0.2	4.9 ± 0.2*	5.1 ± 0.5*	4.2 ± 0.3

Statistically significant differences in the experimental group vs. the control group ($p < 0.05$)

Table 1 shows that after exposure to LiAc, the percentage of active neutrophils capable of phagocytizing staphylococci and the engulfment capacity of neutrophils in the dose range of 0.1–0.01 mg / ml increase by 15–20%.

As can be seen from Table 1, LiAc does not significantly affect phagocytic activity in all dose ranges, but phagocytosis completeness at low concentrations increases slightly. The percentage of active neutrophils increases. The data obtained indicate the absence of an inhibitory effect on phagocytes in this dose range.

The cytotoxic effect of the drug on THP-1 cells was evaluated. The results are shown in Fig. 2.

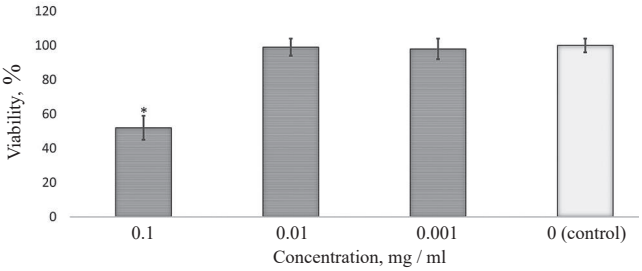

Fig. 2. Cytotoxic effect of LiAc on THP-1 cells

The antiproliferative effect of LiAc on leukemia cells was also evaluated; the results are shown in Fig. 3. Studying bioactivity on the THP-1 cell model made it possible to confirm the presence of the antiproliferative effect of the drug.

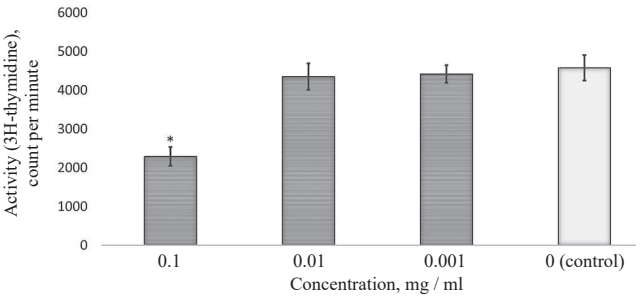

Fig. 3. The effect of LiAc on the proliferative activity of THP-1 cells, by 3H-thymidine incorporation (count per minute)

DISCUSSION

The obtained results revealed a noticeable antiproliferative effect of the drug on PHA-stimulated lymphocytes in the LBT (Fig. 1). The effect on lymphocytes observed at the dose of 0.1 mg / ml was statistically significant. In general, the antiproliferative effect on the mitogen-stimulated lymphocyte population under the influence of the studied drug correlates with the previously shown effect of ascorbic acid [26]. However, it is important to note that a stimulating effect on the activity of phagocytes was not observed. This study revealed a stimulating effect on phagocytosis parameters (Table). This phenomenon characterizes the complex properties of LiAc. At the same time, an increase in several parameters was noted, including the percentage of active neutrophils and the engulfment capacity of neutrophils in the concentration range of 0.1–0.01 mg / ml. No significant effect was recorded at lower doses. Stimulation of phagocytosis parameters may have a certain interpolation to cellular immunity in general.

Based on this, it is possible to predict the presence of a mild immunostimulating effect *in vivo*. These data generally correlate with the literature data on the stimulating effect of lithium on hematopoiesis, and in particular on granulocytopoiesis [27]. Lithium-mediated immunostimulation can be used in cases of leukopenia of various origins [28] and even immunodeficiency [29]. However, when lithium salts are used, the mechanisms of hematopoiesis induction are primarily due to the action of the cation. The anionic component of the salt provides new properties, for example, antioxidant, antihypoxant, and also participates in the regulation of hematopoiesis [30]. At the same time, there are studies showing the cytotoxic effect of antioxidants [31, 32]. *In vitro*, this effect has been proven for LiAc (Fig. 2), when a dose-dependent decrease in the viability of the leukemia cell population was shown. These data were confirmed on other types of tumor cells and via *in vivo* testing in liver cancer [33]. The study by K. Polliredddy et al. shows the inhibitory effect of ascorbate on pancreatic tumors [34].

Our study on the effect of LiAc on the THP-1 cell model with the incorporation of labeled thymidine confirmed the presence of a pronounced antiproliferative effect on actively dividing malignant cells (Fig. 3). Thus, the level of inhibition of malignant cell proliferation by the incorporation of 3H-thymidine 24 hours after exposure to the drug at a concentration of 0.1 mg / ml decreased from 4,579 ± 327 count per minute from the baseline (in the control) to 2,293 ± 241 count per minute (59% of the control values). Lower concentrations of the drug (0.001–0.01 mg / ml) did not have a suppressive effect on the cells ($p \leq 0.05$).

It is obvious that the intracellular mechanisms of the established antiproliferative and cytotoxic action are non-specific to the cell type and are generally due to the ambivalent action characteristic of ascorbates, which exhibit an antioxidant effect only in small concentrations. It should also be borne in mind that high antioxidant parameters of a substance *in vitro* do not always reflect the effects obtained *in vivo* due to the limited assessment methodology [35]. Exposure to ascorbates at high doses leads to a pro-oxidant reaction and an increase in the intracellular level of ROS, primarily hydrogen peroxide [36]. This effect can be considered as the main non-specific component responsible for the antiproliferative effect of the drug. In this case, the dualism of *in vitro* action manifests itself through a dose-dependent decrease in cell viability and proliferation.

CONCLUSION

The drug showed a significant dose-dependent suppressive effect on actively dividing cells. Suppression was noted on tumor lines and on lymphocytes stimulated to divide in LBT. A non-specific suppressive effect of the lithium complex in high concentrations was observed. At the same time, it was shown to have a stimulating effect on neutrophilic leukocytes, which play an important role in the resistance of the body to infections. The data obtained allow to consider the use of LiAc for developing drugs with immunomodulatory effect.

REFERENCES

1. Bartolucci S., Piccirillo C.A. Immune regulation in human health and disease. *eLS*. 2017;2017:1–17. DOI: 10.1002/9780470015902.a0000952.pub2.
2. Markova T.P. Immunotropic drugs and adaptogens. *RMJ*. 2019;8(I):60-64 (in Russ.).
3. Romantsov M.G., Shuldyakova O.G., Kovalenko A.L. Immunomodulators with antiviral activity. *Modern Problems of Science and Education*. 2004;1:29–33 (in Russ.).
4. Bou Zerdan M., Moussa S., Atoui A., Assi H.I. Mechanisms of Immunotoxicity: Stressors and Evaluators. *Int. J. Mol. Sci*. 2021;22(15):8242. DOI: 10.3390/ijms22158242.
5. IRTA. Review of immune stimulator substances/agents that are susceptible of being used as feed additives: mode of action and identification of end-points for efficacy assessment. *EFSA*

Supporting Publications. 2015;12(12):905E. DOI: 10.2903/sp.efsa.2015.EN-905.
6. Gomez-Cadena A., Barreto A., Fioretino S., Jandus C. Immune system activation by natural products and complex fractions: a network pharmacology approach in cancer treatment. *Cell Stress*. 2020;4(7):154–166. DOI: 10.15698/cst2020.07.224.
7. Wang J.S., Wang H.J., Qian H.L. Biological effects of radiation on cancer cells. *Mil. Med. Res*. 2018;5(1):20. DOI: 10.1186/s40779-018-0167-4.
8. Candeias S.M., Gaipl U.S. The Immune system in cancer prevention, development and therapy. *Anticancer Agents Med Chem*. 2016;16(1):101–107. DOI: 10.2174/1871520615666150824153523.
9. Won E., Kim Y.K. An oldie but goodie: lithium in the treatment of bipolar disorder through neuroprotective and neurotrophic mechanisms. *Int. J. Mol. Sci*. 2017;18(12):2679. DOI: 10.3390/ijms18122679.
10. Losenkov I.S., Plotnikov E.V., Epimakhova E.V., Bokhan N.A. Lithium in the psychopharmacology of affective disorders and mechanisms of its effects on cellular physiology. *Zh. Nevrol. Psikhiatr. im. S.S. Korsakova*. 2020;120(11):108–115. DOI: 10.17116/jnevro2020120111108.
11. Ferensztajn-Rochowiak E., Rybakowski J.K. The effect of lithium on hematopoietic, mesenchymal and neural stem cells. *Pharmacological Reports*. 2016;68(2):224–230. DOI: 10.1016/j.pharep.2015.09.005.
12. Hager E.D., Dziambor H., Winkler P., Höhmann D., Macholdt K. Effects of lithium carbonate on hematopoietic cells in patients with persistent neutropenia following chemotherapy or radiotherapy. *J. Trace Elem. Med. Biol*. 2002;16(2):91–97. DOI: 10.1016/s0946-672x(02)80034-7.
13. Kast R.E. How lithium treatment generates neutrophilia by enhancing phosphorylation of GSK-3, increasing HIF-1 levels and how this path is important during engraftment. *Bone Marrow Transplant*. 2008;41(1):23–26. DOI: 10.1038/sj.bmt.1705872.
14. Greenberg D.B., Younger J., Kaufman S.D. Management of lithium in patients with cancer. *Psychosomatics*. 1993;34(5):388–394. DOI: 10.1016/s0033-3182(93)71841-1.
15. Rybakowski J.K. Antiviral, immunomodulatory, and neuroprotective effect of lithium. *J. Integr. Neurosci*. 2022;21(2):68. DOI: 10.31083/j.jin2102068.
16. Qaswal A.B., Suleiman A., Guzu H., Harb T.A., Atiyat B. The Potential Role of Lithium as an Antiviral Agent against SARS-CoV-2 via Membrane Depolarization: Review and Hypothesis. *Scientia Pharmaceutica*. 2021;89(1)*:11. DOI: 10.3390/SCIPHARM89010011.*
17. Plotnikov E., Voronova O., Linert W., Martemianov D., Korotkova E., Dorozhko E. et al. Antioxidant and immunotropic properties of some lithium salts. *Journal of Applied Pharmaceutical Science*. 2016;6(1):086–089. DOI: 10.7324/JAPS.2016.600115.
18. Liguori I., Russo G., Curcio F., Bulli G., Aran L., Della-Morte D. et al. Oxidative stress, aging, and diseases. *Clin. Interv. Aging*. 2018;13:757–772. DOI: 10.2147/cia.s158513.
19. Plotnikov E., Korotkova E., Voronova O., Sazhina N., Petrova E.,Artamonov A. et al. Comparative investigation of antioxidant activity of human serum blood by amperometric, voltammetric and chemiluminescent methods. *Arch. Med. Sci*. 2016;12(5):1071–1076. DOI: 10.5114/aoms.2015.50234.
20. Olefir Y., Romanov B., Kukes V., Sychev D., Prokofiev A., Parfenova O. et al. The role of oxidative stress in the pathogenesis of socially significant human diseases and ways of its drug correction. *Medical News of the North Caucasus*. 2021;16(4):450–455. DOI: 10.14300/mnnc.2021.16109.
21. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol*. 2015; 4: 180-3. DOI:10.1016/j.redox.2015.01.002.
22. Plotnikov E., Korotkova E., Voronova O., Dorozhko E., Bohan N., Plotnikov S. Lithium-based antioxidants: electrochemical properties and influence on immune cells. *Physiology and Pharmacology*. 2015;19(2):107–113.
23. Plotnikov E., Korotkova E., Voronova O. Lithium Salts of krebs cycle substrates as potential normothymic antioxidant agents. *J. Pharm. Bioallied. Sci*. 2018;10(4):240–245. DOI: 10.4103/jpbs.jpbs_140_18.
24. Pham-Huy L.A., He H., Pham-Huy C. Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci*. 2008;4(2):89–96.
25. Novikov D.K., Novikova V.I. Assessment of the immune status. M: Medicine, 1996:240 (in Russ.).
26. Belin S., Kaya F., Duisit G., Giacometti S., Ciccolini J., Fontés M. Antiproliferative effect of ascorbic acid is associated with the inhibition of genes necessary to cell cycle progression. *PLoS One*. 2009;4(2):e4409. DOI: 10.1371/journal.pone.0004409.
27. Joyce R.A. Sequential effects of lithium on haematopoiesis. *British Journal of Haematology*. 1984;56(2):307–321. DOI: 10.1111/j.1365-2141.1984.tb03958.x.
28. Young W. Review of lithium effects on brain and blood. *Cell Transplant*. 2009;18(9):951–975. DOI: 10.3727/096368909x471251.
29. Gallicchio V.S., Hughes N.K., Tse K.F., Ling J., Birch N.J. Effect of lithium in immunodeficiency: improved blood cell formation in mice with decreased hematopoiesis as the result of LP-BM5 MuLV infection. *Antiviral Res*. 1995;26(2):189–202. DOI: 10.1016/0166-3542(94)00075-j.
30. Agathocleous M., Meacham C.E., Burgess R.J., Piskounova E., Zhao Z., Crane G.M. et al. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. *Nature*. 2017;549(7673):476–481. DOI: 10.1038/nature23876.
31. Sotler R., Poljšak B., Dahmane R., Jukić T., Pavan Jukić D., Rotim C. et al. Prooxidant activities of antioxidants and their impact on health. *Acta Clin. Croat*. 2019;58(4):726–736. DOI: 10.20471/acc.2019.58.04.20.
32. Carocho M., Ferreira I.C. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem. Toxicol*. 2013;51:15–25. DOI: 10.1016/j.fct.2012.09.021.
33. Wan J., Zhou J., Fu L., Li Y., Zeng H., Xu X. et al. Ascorbic acid inhibits liver cancer growth and metastasis in vitro and *in vivo*, independent of stemness gene regulation. *Frontiers in Pharmacology*. 2021;12:726015. DOI: 10.3389/fphar.2021.726015.
34. Polireddy K., Dong R., Reed G., Yu J., Chen P., Williamson S.

et al. High dose parenteral ascorbate inhibited pancreatic cancer growth and metastasis: mechanisms and a phase I/IIa study. *Sci. Rep*. 2017;7(1):17188. DOI: 10.1038/s41598-017-17568-8.
35. Kotha R.R., Tareq F.S., Yildiz E., Luthria D.L. Oxidative Stress and Antioxidants—A Critical Review on In Vitro Antioxidant Assays. *Antioxidants*. 2022;11(12):2388. DOI: 10.3390/antiox11122388.
36. Mastrangelo D., Pelosi E., Castelli G., Lo-Coco F., Testa U. Mechanisms of anti-cancer effects of ascorbate: Cytotoxic activity and epigenetic modulation. *Blood Cells Mol. Dis*. 2018;69:57–64. DOI: 10.1016/j.bcmd.2017.09.005.

Authors' contribution

Plotnikov E.V., Tretyakova M.S. – conception and design, carrying out of experiments. Krivoshchekov S.V. – obtaining, purification and quality assessment of LiAc samples, discussion of the results. Belousov M.V., Plotnikov E.V. – analysis of the results, interpretation of the data, drafting of the manuscript and critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

Authors' information

Plotnikov Evgeny V. – Cand. Sci. (Chemistry), Associate Professor, Research School of Chemical and Biomedical Technologies, NR TPU; Senior Researcher, Endogenous Disorders Department, Mental Health Research Institute, Tomsk NRMC, Tomsk, plotnikov.e@mail.ru, https://orcid.org/0000-0002-4374-6422

Tretyakova Maria S. – Post-Graduate Student, Research School of Chemical and Biomedical Technologies, NR TPU; Engineer, Oncoteranostics; Junior Researcher, Laboratory of Tumor Progression Biology, Cancer Research Institute, Tomsk NRMC, Tomsk, trremar@mail.ru, https://orcid.org/0000-0002-5040-931X

Krivoshchekov Sergey V. – Cand. Sci. (Chemistry), Associate Professor, Pharmaceutical Analysis Division, Siberian State Medical University, Head of the Research and Education Laboratory of Chemical and Pharmaceutical Research, Tomsk, ksv_tsu@mail.ru, https://orcid.org/0000-0001-5505-7141

Kolobovnikova Yulia V. – Dr. Sci. (Med.), Associate Professor, Head of the Normal Physiology Division; Professor, Pathophysiology Division, Siberian State Medical University, Tomsk, kolobovnikova.julia@mail.ru, http://orcid.org/0000-0001-7156-2471

Belousov Mikhail V. – Dr. Sci. (Pharmaceut.), Professor, Head of the Pharmaceutical Analysis Division, Siberian State Medical University; Professor at the Research School of Chemical and Biomedical Technologies, NR TPU, Tomsk, mvb63@mail.ru, https://orcid.org/0000-0002-2153-7945

(✉) **Plotnikov Evgeny V.,** plotnikov.e@mail.ru

Received 22.02.2023;
approved after peer review 07.09.2023;
accepted 14.09.2023