Received: 23 May 2025 / Accepted: 11 December 2025 / Published online: 27 February 2026

DOI 10.34689/SH.2026.28.1.014

UDC 616-008.851

PRECLINICAL STUDY OF THE ACUTE TOXICITY OF INTERLEUKIN-1ß ENCAPSULATED INTO THE AUTOLOGOUS ERYTHROCYTE GHOSTS

Kulzhan Berikkhanova[1,3,4]*, https://orcid.org/0000-0002-6371-9210
Gulyash Tanysheva[2], https://orcid.org/0000-0001-9531-5950
Alexandr Gulyayev[1], https://orcid.org/0000-0001-5098-4675
Erlan Taigulov[3], https://orcid.org/0009-0006-2320-864X
Saken Kozhakhmetov[3], https://orcid.org/0000-0002-0075-0376
Nurzhan Bikhanov[4], https://orcid.org/0009-0002-9718-8865
Yernur Zakirov[1], https://orcid.org/0009-0009-6351-7756
Nurzhan Berikkhanov[5], https://orcid.org/0009-0003-6696-2886
Askhat Zhilkaidarov[1,6], https://orcid.org/0009-0004-5266-3655
Yessenhan Sultan[7], https://orcid.org/0009 0009 4593 8661

[1] National Laboratory Astana, Nazarbayev University, Astana, Republic of Kazakhstan;
[2] «Semey Medical University» NCJSC, Semey, Republic of Kazakhstan;
[3] «Astana Medical University» NCJSC, Astana, Republic of Kazakhstan;
[4] University Medical Center, Nazarbayev University, Astana, Republic of Kazakhstan;
[5] City polyclinic №2, Taraz, Republic of Kazakhstan;
[6] City Hospital №1, Astana, Republic of Kazakhstan;
[7] City polyclinic №14, Almaty, Republic of Kazakhstan.

Abstract

Introduction. The study of transport systems for targeted drug delivery is considered to be one of the promising areas of modern experimental and clinical medicine. Preclinical studies confirm the feasibility of creating a cellular system for targeted delivery of interleukin-1β in the body, based on autologous red blood cell ghosts, to improve the outcomes of treatment of purulent-inflammatory soft tissue diseases. The acute toxicity study is a crucial step in the preclinical safety study of autologous erythrocyte ghosts containing interleukin-1β.

Aim. To study the acute toxicity of Interleukin-1β, encapsulated in autologous rat erythrocyte ghosts.

Materials and methods. Interleukin – 1 beta was encapsulated in autologous red blood cells of rats by the method of hypoosmotic hemolysis. A study of the acute toxicity of Interleukin-1β encapsulated in autologous erythrocyte ghosts was conducted on white outbred rats of both sexes, weighing 250-300 g (40 animals in the main group and 50 in the control group). In the control group, the studied drug was administered in free form as an injection solution intravenously once into the femoral vein. In the main group, interleukin-1β encapsulated in autologous erythrocyte ghosts (pharmacocytes) was administered into the femoral vein. The range of administered doses of interleukin-1β was from 0.07 μg/kg to 20 μg/kg. During the 14 days, the rats of the main and control groups were under observation, at a constant (26-28°C) ambient temperature, and were kept with constant access to water and food. The timing of intoxication onset and animal death was recorded, with a detailed description of the observed clinical manifestations. On day 15, all animals from both groups were euthanized under deep anesthesia, performed in compliance with the principles of humane treatment of experimental animals, followed by pathomorphological examination.

Results. The LD50 for the free form of interleukin-1β was established at 15,030 ng/kg. Within the dose range used, it was not possible to determine the LD50 for interleukin-1β encapsulated in autologous red blood cell ghosts, as further dose escalation was technically challenging. Pathomorphological studies showed that administration of free interleukin-1β in the control group animals induced a pronounced inflammatory reaction in the subcutaneous adipose tissue and a moderate inflammatory response in the liver and kidneys. In contrast, administration of interleukin-1β encapsulated in autologous red blood cell ghosts caused only minor degenerative changes in the tissues and was manifested by moderate signs of venous congestion.

Conclusion. The results of the study of acute toxicity of interleukin-1β deposited in autologous red blood cell ghosts prove that pharmacocytes can significantly reduce the toxicity of the drug. We assume that the decreased toxicity of interleukin-1β upon encapsulation in autologous red blood cell ghosts is associated with altered pharmacokinetics.

Keywords: *interleukin-1b, acute toxicity, erythrocyte ghosts, pharmacocytes, targeted drug delivery.*

For citation:

Berikkhanova K., Tanysheva G., Gulyayev A., Taigulov E., Kozhakhmetov S., Bikhanov N., Zakirov Ye., Berikkhanov N., Zhilkaidarov A., Sultan Ye. Preclinical study of the acute toxicity of Interleukin-1ß encapsulated into the autologous erythrocyte ghosts *// Nauka i Zdravookhranenie* [Science & Healthcare]. 2026. Vol.28 (1), pp. 112-121. *doi 10.34689/SH.2026.28.1.014*

Резюме

ДОКЛИНИЧЕСКОЕ ИССЛЕДОВАНИЕ ОСТРОЙ ТОКСИЧНОСТИ ИНТЕРЛЕЙКИНА-1В, ИНКАПСУЛИРОВАННОГО В АУТОЛОГИЧНЫЕ ТЕНИ ЭРИТРОЦИТОВ

Кульжан Берикханова[1,3,4]*, https://orcid.org/0000-0002-6371-9210
Гулияш Танышева[2], https://orcid.org/0000-0001-9531-5950
Александр Гуляев[1], https://orcid.org/0000-0001-5098-4675
Ерлан Тайгулов[3], https://orcid.org/0009-0006-2320-864X
Сакен Кожахметов[3], https://orcid.org/0000-0002-0075-0376
Нуржан Биханов[4], https://orcid.org/0009-0002-9718-8865
Ернур Закиров[1], https://orcid.org/0009-0009-6351-7756
Нуржан Берикханов[5], https://orcid.org/0009-0003-6696-2886
Асхат Жилкайдаров[1,6], https://orcid.org/0009-0004-5266-3655
Есенхан Султан[7], https://orcid.org/0009 0009 4593 8661

[1] ЧУ "National Laboratory Astana", Назарбаев Университет, Астана к., Республика Казахстан;
[2] НАО «Медицинский университет Семей», Семейк к., Республика Казахстан;
[3] НАО «Медицинский университет Астана», Астана к., Республика Казахстан;
[4] КФ «University Medical Center» Назарбаев Университет, Астана к., Республика Казахстан;
[5] Городская поликлиника №2, Тараз к., Республика Казахстан;
[6] Городская больница №1, Астана к., Республика Казахстан;
[7] Городская поликлиника №14, Алматы к., Республика Казахстан.

Введение. Одним из перспективных направлений современной медицины является использование транспортных систем для целенаправленной доставки лекарственных препаратов. Доклинические исследования подтверждают возможность создания клеточной системы для направленной доставки интерлейкин-1β в организме на основе теней аутологичных эритроцитов для улучшения результатов лечения гнойно-воспалительных заболеваний мягких тканей. Исследование острой токсичности является важным этапом доклинического исследования безопасности аутологичных теней эритроцитов, содержащих интерлейкин-1β.

Цель: изучение острой токсичности Интерлейкина-1β, инкапсулированного в аутологичные тени эритроцитов крыс.

Материал и методы: Интерлейкин – 1 бета был инкапсулирован в аутологические эритроциты крыс по методике гипоосмотического гемолиза. Для изучения острой токсичности инкорпорированного препарата использовали белых беспородных крыс обоего пола массой 250-300 г. В экспериментальной группе было 40 голов, в контрольной –50 голов крыс. В контрольной группе интерлейкин-1β вводили в свободной форме в бедренную вену. В основной группе вводили в бедренную вену интерлейкин-1β, инкапсулированный в аутологичные тени эритроцитов (фармакоциты). Диапазон вводимых доз интерлейкина - 1β от 0,07мкг/кг до 20 мкг/кг. В течение 14 дней крысы находились под наблюдением и содержались при постоянной температуре окружающей среды (26-28°С) со свободным доступом к пище и воде. Регистрировали сроки развития интоксикации и гибели животных с подробным описанием наблюдаемой клинической картины. На 15-й день все животные из обеих групп были подвергнуты эвтаназии под глубокой анестезией которую выполняли с соблюдением правил гуманного отношения к экспериментальным животным и затем проводили патоморфологические исследования.

Результаты: LD50 для свободной формы Интерлейкина- 1β установлено на уровне 15030 нг/кг. В пределах использованных доз определить LD50 для Интерлейкина-1β, инкапсулированного в аутологичные тени эритроцитов, не удалось, дальнейшее повышение доз технически было затруднено. Результаты патоморфологических исследований показали, что введение Интерлейкина- 1β в свободной форме у животных контрольной группы вызывало выраженную воспалительную реакцию в подкожно-жировой клетчатке и умеренную воспалительную реакцию в печени и почках. Введение Интерлейкина-1β, инкапсулированного в аутологичные тени эритроцитов, приводило лишь к незначительные дегенеративным реакциям в тканях и проявлялось умеренными признаками венозного застоя.

Выводы: Включение интерлейкина-1 бета в аутологичные тени эритроцитов привело к снижению острой токсичности. Предполагаем, что снижение токсичности Интерлейкина- 1β при инкапсуляции в аутологичные тени эритроцитов обусловлено изменением фармакокинетики.

Ключевые слова: *интерлейкин-1β, острая токсичность, тени эритроцитов, фармакоциты, целенаправленная доставка лекарств.*

Для цитирования:

Берикханова К., Танышева Г., Гуляев А., Тайгулов Е., Кожахметов С., Биханов Н., Закиров Е., Берикханов Н.Жилкайдаров А., Султан Е. Доклиническое исследование острой токсичности Интерлейкина-1β, инкапсулированного в аутологичные тени эритроцитов // Наука и Здравоохранение. 2026. Vol.28 (1), С. 112-121. *doi 10.34689/SH.2026.28.1.014*

Түйіндеме

ЭРИТРОЦИТТЕРДІҢ АУТОЛОГИЯЛЫҚ ҚАБЫҚШАЛАРЫНА ИНКАПСУЛЯЦИЯЛАНҒАН ИНТЕРЛЕЙКИН-1В-НЫҢ ЖЕДЕЛ УЫТТЫЛЫҒЫН КЛИНИКАҒА ДЕЙІНГІ ЗЕРТТЕУ

Кульжан Берикханова[1,3,4*], https://orcid.org/0000-0002-6371-9210
Гулияш Танышева[2], https://orcid.org/0000-0001-9531-5950
Александр Гуляев[1], https://orcid.org/0000-0001-5098-4675
Ерлан Тайгулов[3], https://orcid.org/0009-0006-2320-864X
Сакен Кожахметов[3], https://orcid.org/0000-0002-0075-0376
Нуржан Биханов[4], https://orcid.org/0009-0002-9718-8865
Ернур Закиров[1], https://orcid.org/0009-0009-6351-7756
Нуржан Берикханов[5], https://orcid.org/0009-0003-6696-2886
Асхат Жилкайдаров[1,6], https://orcid.org/0009-0004-5266-3655
Есенхан Султан[7], https://orcid.org/0009-0009-4593-8661

[1] ЖМ "National Laboratory Astana", Назарбаев Университет, Астана қ., Казахстан Республикасы;
[2] «Семей медицина университеті» КеАҚ, Семей қ., Казахстан Республикасы;
[3] «Астана медицина университеті» КеАҚ, Астана қ., Казахстан Республикасы;
[4] КҚ «University Medical Center» Назарбаев Университеті, Астана қ., Казахстан Республикасы;
[5] Қалалық емхана №2, Тараз қ., Казахстан Республикасы;
[6] Қалалық аурухана №1, Астана қ., Казахстан Республикасы;
[7] Қалалық емхана №14, Алматы қ., Казахстан Республикасы.

Кіріспе. Заманауи медицинаның перспективалы бағыттарының бірі дәрілік препараттарды мақсатты жеткізу үшін транспорттық жүйелерді пайдалану болып табылады. Клиникаға дейінгі зерттеулер жұмсақ тіндердің іріңді-қабыну ауруларын емдеу нәтижелерін жақсарту үшін аутологиялық эритроциттердің қабықшалары негізінде денеде интерлейкин-1β-ны мақсатты жеткізу үшін жасуша жүйесін құру мүмкіндігін растайды. Жедел уыттылықты зерттеу интерлейкин-1β бар эритроциттердің аутологиялық қабықшаларының қауіпсіздігін клиникаға дейінгі зерттеудің маңызды кезеңі болып табылады.

Мақсаты: егеуқұйрықтардың эритроциттерінің аутологиялық қабықшаларына инкапсуляцияланған интерлейкин-1β жедел уыттылығын зерттеу болды.

Материал және әдістер: Интерлейкин-1 бета гипоосмотикалық гемолиз әдісі бойынша егеуқұйрықтардың аутологиялық эритроциттеріне инкапсуляцияланды. Жүктелген препараттың жедел уыттылығын зерттеу үшін салмағы 250-300 г болатын екі жыныстағы ақ тұқымсыз егеуқұйрықтар қолданылды. Эксперименттік топта 40 бас, бақылау тобында -50 бас егеуқұйрық болды. Бақылау тобында интерлейкин-1β ерітіндісі феморальды көктамырға таза түрде енгізілді. Негізгі топта эритроциттердің аутологиялық қабықшаларына (фармакоциттер) инкапсуляцияланған интерлейкин-1β феморальды көктамырға енгізілді. Интерлейкин - 1β дозаларының диапазоны 0,07 мкг/кг-нан 20 мкг/кг-ға дейін.14 күн ішінде егеуқұйрықтар бақылауда болды және тамақ пен суға еркін қол жетімді тұрақты қоршаған орта температурасында (26-28°C) болды. Байқалған клиникалық көріністі егжей-тегжейлі сипаттай отырып, уыттылық деңгейі мен жануарлардың өлімінің даму мерзімдері тіркелді. 15-ші күні екі топтағы барлық жануарлар терең анестезиямен эвтанизацияланды және эксперименталды жануарларға адамгершілік қатынас ережелерін сақтай отырып жүргізілді, содан кейін патоморфологиялық зерттеулер жүргізілді.

Нәтижелер: интерлейкин - 1β таза формасы үшін LD50 15030 нг/кг деңгейінде анықталды. Пайдаланылған дозалар шегінде эритроциттердің аутологиялық қабықшаларына инкапсуляцияланған интерлейкин-1β үшін LD50 анықтау мүмкін болмады, дозаны одан әрі арттыру техникалық тұрғыдан қиын болды. Патоморфологиялық зерттеулердің нәтижелері бақылау тобындағы жануарларда интерлейкин - 1β таза түрінде енгізу тері астындағы май тінінде айқын қабыну реакциясын және бауыр мен бүйректе орташа қабыну реакциясын тудырғанын көрсетті. Эритроциттердің аутологиялық қабықшаларына инкапсуляцияланған интерлейкин-1β енгізу тіндерде аз ғана дегенеративті реакцияларға әкелді және веноздық тоқыраудың орташа белгілерімен көрінді.

Қорытынды: интерлейкин-1β-ны эритроциттердің аутологиялық қабықшаларына жүктеу жедел уыттылықтың төмендеуіне әкелді. Эритроциттердің аутологиялық қабықшаларына инкапсуляция кезінде интерлейкин-1β уыттылығының төмендеуі фармакокинетиканың өзгеруіне байланысты деп есептейміз.

Түйінді сөздер: *интерлейкин-1β, жедел уыттылық, эритроциттердің қабықшалары, фармакоциттер, дәрі-дәрмектерді мақсатты жеткізу.*

Дәйексөз үшін:

Берикханова К., Танышева Г., Гуляев А., Тайгулов Е., Кожахметов С., Биханов Н., Закиров Е., Берикханов Н., Жилкайдаров А., Султан Е. Эритроциттердің аутологиялық қабықшаларына инкапсуляцияланған интерлейкин-1β-ның жедел уыттылығын клиникаға дейінгі зерттеу // Ғылым және Денсаулық сақтау. 2026. Vol.28 (1), Б. 112-121. *doi 10.34689/SH.2026.28.1.014*

Introduction

The study of transport systems for targeted drug delivery is considered to be one of the promising areas of modern experimental and clinical medicine [41,24,22,12]. Targeted drug delivery not only increases the effectiveness of therapy by creating optimal concentrations of the drug directly at the pathological site, but also significantly reduces the risk of systemic side effects. Among the most promising delivery systems are cellular carriers, including erythrocyte ghosts and their nanoderivatives, which exhibit biocompatibility, prolonged circulation time, and the ability to protect encapsulated drugs from rapid degradation. These approaches open up opportunities for personalized therapy, improved treatment outcomes, and reduced drug burden on the patient's body, making them highly relevant for further preclinical and clinical studies [30,19,18,29,17,34,38]. The development of innovative approaches in the pathogenetic therapy of pyoinflammatory diseases through the targeted delivery of immunomodulators to the inflammatory site is of great interest for practical medicine [1,24,22,12]. We have developed a transport system based on autologous erythrocyte ghosts for targeted cytokine delivery in the body to improve the results of treatment of pyoinflammatory diseases of soft tissues [14,15,32].

The use of blood cells to create systems for targeted drug delivery is undoubtedly the simplest, most clinically accessible, and logically justified option for addressing this problem. Targeted transport of drugs deposited in autologous erythrocytes provides a substantial increase in treatment efficacy and a significant reduction in drug toxicity by enhancing bioavailability and enabling drug accumulation directly at the site of the infectious process, bypassing excessive systemic distribution [15,37].

Drug delivery using erythrocyte ghosts is supported by the physiological characteristics of the inflammatory response. Purulent-inflammatory sites are known to exhibit enhanced blood supply and a high density of macrophages. When autologous erythrocyte-based carriers loaded with drugs are introduced into the body, they are preferentially taken up by cells of the mononuclear phagocyte system. Within the inflammatory focus, these erythrocyte pharmacocytes undergo phagocytosis, leading to the controlled release of the encapsulated drug and the establishment of a sustained, locally elevated drug concentration. This outcome unattainable with conventional drug administration methods [15].

The introduction of erythrocyte pharmacocyte–based targeted drug delivery technology into clinical practice opens prospects for a significant increase in the effectiveness of therapy in the treatment of severe surgical infections. Targeted drug delivery not only provides a pronounced anti-inflammatory effect at the site of purulent inflammation but also reduces overall toxicity through selective distribution and reduced daily dosage. The results of conducted studies have confirmed the possibility of substantially lowering overall toxicity and drug burden compared with traditional treatment methods. Furthermore, this technology promotes the rational use of drugs, reduces their consumption, and thus yields significant economic and social benefits. Conducted experimental and clinical trials have demonstrated the feasibility of using erythrocyte ghosts containing deposited antibiotics or cytokines to enhance the treatment outcomes of surgical infections. It has been shown that the use of erythrocyte pharmacocytes enables the formation of consistently high concentrations of the drug both in blood serum and in the wound site for a prolonged period following a single dose—an effect fundamentally different from conventional intravenous administration or local tissue saturation with the same drug [14].

The acute toxicity study is a crucial step in the preclinical safety study of autologous erythrocyte ghosts containing interleukin-1β [11,36].

Aim. To study the acute toxicity of Interleukin-1β, encapsulated in autologous erythrocyte ghosts.

Materials and methods

Interleukin – 1 beta (Betaleukin, lyophilizate for solution preparation 0.5 μg/mL, Federal State Unitary Enterprise "State Scientific Research Institute of Pure Biochemistry", Russian Federation). A total of 90 albino Wistar rats weighing 250–350 grams were housed under standard controlled conditions at the National Biotechnology Center's vivarium. The environmental conditions included a temperature of 22 ± 2 °C, humidity of 55 ± 5%, and a 12-hour light/dark cycle. The rats were fed ad libitum with unrestricted access to food and water. The experimental work was performed in accordance with the legislation of the Republic of Kazakhstan, Recommendation №33 of the Board of the Eurasian Economic Commission dated November 14, 2023 «About the Guidelines for working with laboratory (experimental) animals during preclinical (non-clinical) studies» and the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes, as well as the provisions of Directive 2010/63/EU of the European Parliament and Council of the European Union of September 22, 2010 on animal welfare used for scientific purposes.

The use of experimental animals was approved by the Local Ethical Committee of PI "National Laboratory Astana" (№ 05-2022/21.10.2022). All procedures were carried out with appropriate anesthesia, under isoflurane inhalation anesthesia.

The creation of pharmacocytes and encapsulation of the Interleukin-1β drug in them was done according to the previously described author's method [43].

A study of the acute toxicity of Interleukin-1β encapsulated in autologous erythrocyte ghosts was conducted on white outbred rats of both sexes, weighing 250-300 g (40 animals in the main group and 50 in the control group).

In the control group, the studied drug was administered in free form as an injection solution intravenously once into the femoral vein. The range of administered doses of interleukin-1β was from 0.07 μg/kg to 20 μg/kg.

In the main group, the drug was administered in the form of pharmacocytes intravenously once into the femoral vein.

In all cases, observations were made from the first minutes of changes in the color of exposed areas of the skin. During the entire study period, the rats of the main and

control groups were under observation, at a constant (26-280C) ambient temperature, and were kept with constant access to water and food. Throughout the subsequent period, the animals' behavior and appearance were observed, and any changes that could be attributed to the toxic effect of the drug were recorded. The observation lasted 14 days. The lethal doses were calculated using probit analysis: y = (α + β x), where y is the proportion of dead animals, x is the decimal logarithm of the drug dose expressed in nanograms per kilogram, is the distribution function of the standard normal variable, and α and β are regression coefficients [3]. Regression coefficients were determined using the STATISTICA program.

14 days after the administration of the studied drug, the rats of the main and control groups were withdrawn from the experiment. Histological studies of rat organs revealed pathomorphological changes after administration of drugs at a dose of 0.8 μg/kg. For the pathomorphological examination of organs and tissues, animals were removed from the experiment under inhalation anesthesia by dislocation of the cervical vertebrae. Samples of organs and tissues (liver, heart, kidneys, testicles, brain, spleen, adrenal glands) were collected from each group for pathomorphological examination. Tissue samples were fixed in 10% neutral formalin and alcohol-formol. 6-8 μm thick histosections, prepared on an MPS-2 rotation microscope, were deparaffinized and stained with Mayer's hematoxylin and eosin, picrofuchsin according to Van Gieson, methyl green and pyronin according to Brachet, periodate K and Schiff's reagent according to McManus [2,6].

Statistical processing of the results was made using the Statistica 12 software package. The results obtained are presented as "arithmetic mean ± standard error of the mean". Intergroup differences were assessed using the nonparametric Mann-Whitney U-test. Differences were considered reliable at a significance level of $p < 0.05$ [35,26].

Results

The results of experimental studies on the acute toxicity of interleukin-1β (Betaleukin) in free form and in the form of the drug deposited in autologous erythrocytes (pharmacocytes) are presented in Tables 1 and 2.

Table 1.

Results of acute toxicity determination of interleukin-1β preparation, standard intravenous interleukin-1β preparation (control group)

Dose, ng/kg	Mortality/survival count
70	0/10
700	0/10
3300	1/10
13333	3/7
20000	4/6

The lethal dose level for interleukin-1β in free form is:
y = ((-5.21) +(1.64) x), therefore, DL50=15030 ng/kg
DL16=3690 ng/kg

Table 2.

Results of determination of acute toxicity of the interleukin-1β drug deposited in pharmacocytes (experimental group).

Dose, ng/kg	Mortality/survival count
70	2
700	0/10
3300	0/10
13333	0/10
20000	0/10

As can be seen from the presented data, the maximum technically possible dose under the conditions of this experiment of 20,000 ng/kg does not cause mortality in the experimental animals used. Further increases in doses were technically impossible. Hypothetically, based on the semi-logarithmic curve shown in Figure 1, mortality rates could only be achieved with technically unrealistic doses of the administered drug.

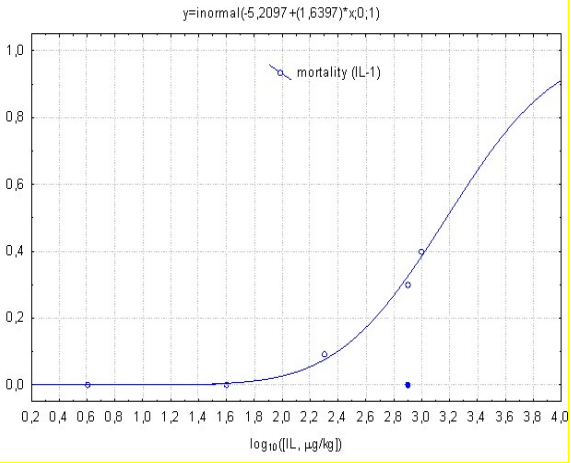


Figure 1. Semi-logarithmic dose-response curve for Interleukin-1 in the form of pharmacocytes

Thus, the results of the study of acute toxicity of the drug interleukin-1β in free form correspond to the data presented in the available literature: 50% lethal doses of interleukin-1β – DL50 = 15030 ng/kg [4,5,7,8,9,10,13,16,20,21,23,25,27,28,31,33, 39,40].

As can be seen, the deposition of interleukin-1β in pharmacocytes leads to a significant decrease in the toxicity of the drug. It is pharmacocytes that provide a decrease in the toxic potential of the cytokine under study.

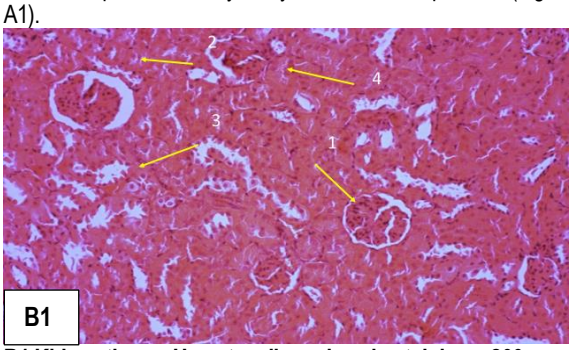
This was confirmed by the results of pathomorphological studies. Histological studies of animal organs after administration of drugs for acute toxicity studies are shown in the following figures (Figure 2).

The results of the histological examination of the organs of rat after the introduction of Betaleukin in free form and the organs of rat after the introduction of Betaleukin in pharmacocytes for the study of acute toxicity are given below..

Results of histological examination of rat organs after administration of Betaleukin in free form.

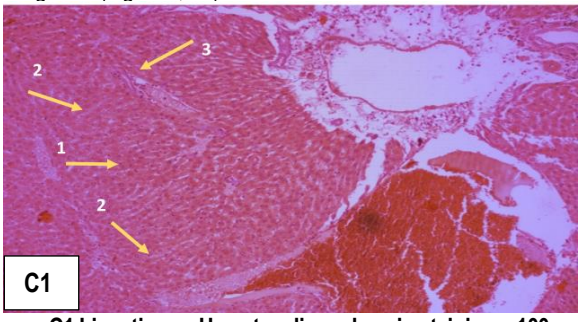
A1 Heart tissue. Hematoxylin and eosin staining. x200.
1- cardiomyocytes; 2- hemorrhage zones.

The structure is not damaged. Preserved transverse striation is observed. There are extensive hemorrhage fields in the form of accumulation of erythrocytes and hemosiderin in the stroma and in the intermuscular spaces. There are small hemorrhages and moderate stromal edema between the muscle fibers. Cardiomyocytes with signs of protein hyaline-droplet dystrophy. The vessels of the interstitial tissue are full-blooded, in single vessels there is separation of erythrocytes from blood plasma. (Figure 2, A1).



B1 Kidney tissue. Hematoxylin and eosin staining. x200.
1 - renal corpuscle. 2 - proximal tubule. 3 - distal tubule.
4 - lobulated glomerulus.

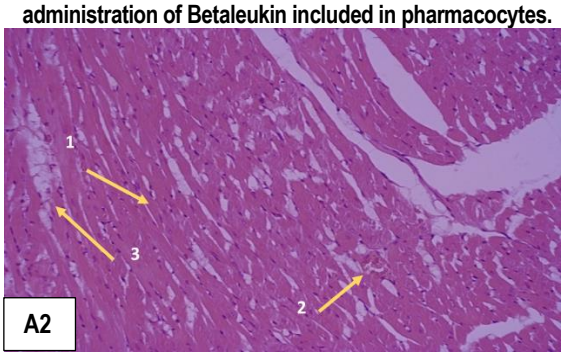
Interstitial edema, vascular and interstitial tissue congestion is observed. The cortex and medulla are preserved. Vascular glomeruli with moderate vascular loop congestion are found in the cortex. Some vascular loops form lobule-like structures. The epithelium of the proximal and distal tubules shows signs of protein hyaline droplet dystrophy. The vessels of the interstitial tissue are congested (Figure 2, B1).



C1 Liver tissue. Hematoxylin and eosin staining. x100.
Hepatocytes. 2 - sinusoids. 3 - portal tract.

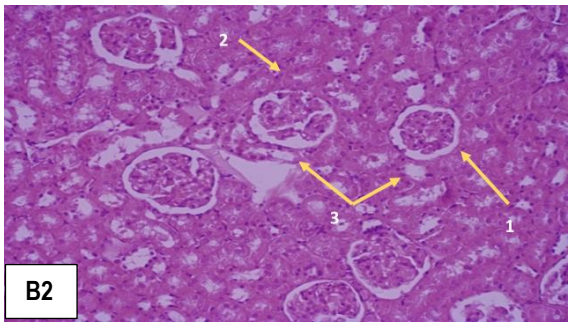
The preserved zone of liver tissue is represented by sharply dilated deformed portal tracts infiltrated with lymphocytes, histiocytes, and an admixture of neutrophilic leukocytes. Portal tracts are deformed and sclerotic. Proliferation of bile ducts. Hepatocytes in liver lobules with signs of hyaline-droplet dystrophy. In the sinusoids, there are clusters of erythrocytes and stellate reticuloendotheliocytes (Figure 2, C1).

Results of histological examination of rat organs after administration of Betaleukin included in pharmacocytes.



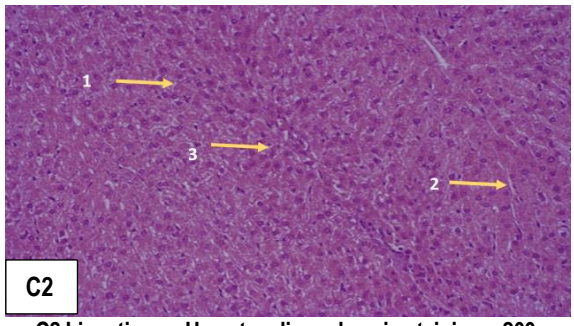
A2 Heart tissue. Hematoxylin and eosin staining. x200.
1- cardiomyocytes; 2- isolated minor hemorrhage areas;
3- interstitial tissue vessels.

The tissue structure is preserved. Transverse striation of myocytes is observed. Cardiomyocytes with signs of protein hyaline-droplet dystrophy. Mild intermuscular edema. Interstitial tissue vessels are moderately full-blooded. Minor hemorrhages are observed in isolated areas in the stroma and intermuscular spaces (Figure 2, A2).



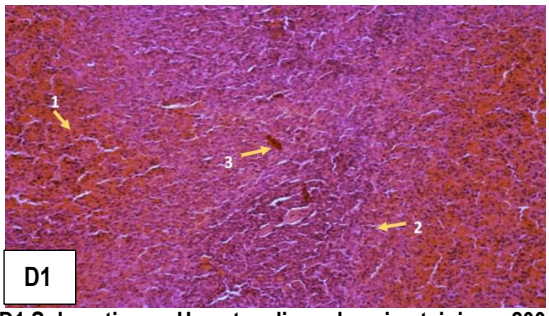
B2 Kidney tissue. Hematoxylin and eosin staining. x200.
1 - renal corpuscle. 2 - proximal tubules. 3 - distal tubules.

The cortical and cerebral layers are preserved. Vascular glomeruli with moderate congestion of vascular loops are found in the cortical layer. Vascular loops of the glomeruli are moderately full-blooded, with minor mesangial proliferation. Epithelium of proximal and distal tubules with hyaline-droplet dystrophy (Figure 2, B2).



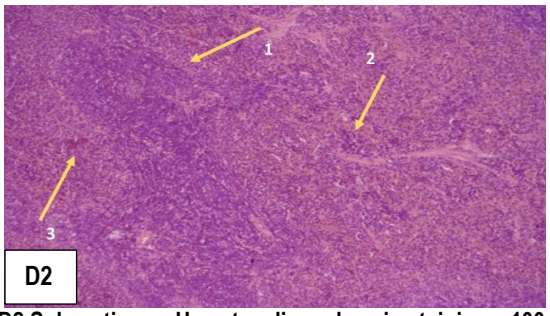
C2 Liver tissue. Hematoxylin and eosin staining. x200.
1 - Hepatocytes. 2 - sinusoids. 3- portal tract.

The structure of the liver tissue is not damaged. The vessels of the portal tracts and central veins are full-blooded. In the portal tracts, proliferation of bile ducts is observed. Hepatocytes in the liver lobules are with signs of protein hyaline-drop dystrophy. In the lumen of the sinusoids, there is a slight accumulation of erythrocytes and low-active stellate reticuloendotheliocytes are found (Figure 2, C2).



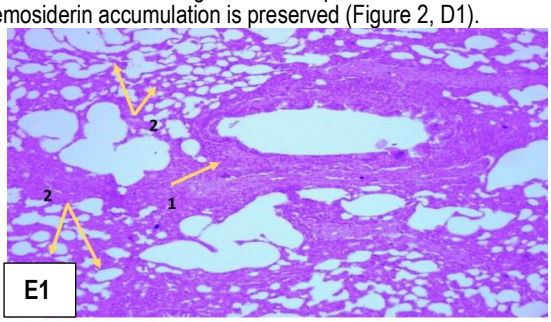
D1 Spleen tissue. Hematoxylin and eosin staining. x200. 1 - interstitial tissue vessels. 2 - follicles. 3 - hemosiderin accumulation.

Interstitial tissue vessels are full-blooded. Preserved follicles are found. Moderate congestion of spleen tissue with brown hemosiderin accumulation is preserved (Figure 2, D1).



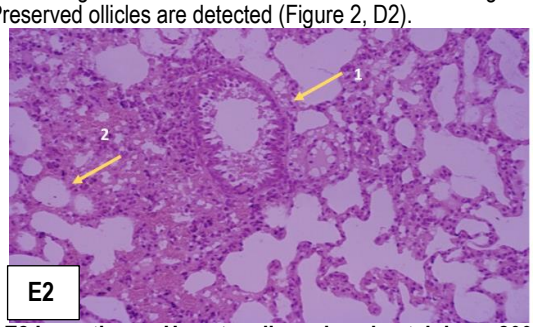
D2 Spleen tissue. Hematoxylin and eosin staining. x100. 1-follicles. 2-interstitial tissue vessels. 3-hemorrhage foci.

Spleen tissue is with venous congestion, with focal hemorrhages. Interstitial tissue vessels are congested. Preserved ollicles are detected (Figure 2, D2).



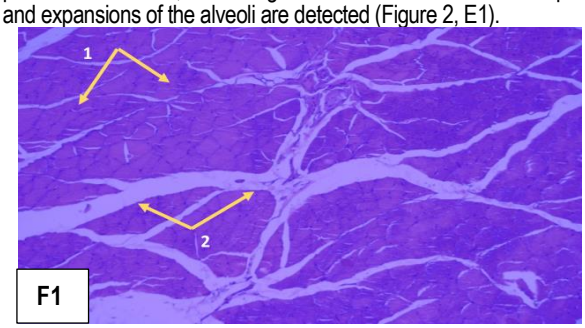
E1 Lung tissue. Hematoxylin and eosin staining. x100. 1 - bronch. 2 - alveoli.

The structure is damaged. Microscopically, exudate with a large accumulation of lymphocytes, histiocytes and a moderate number of neutrophilic leukocytes is determined in the respiratory part and bronchi. Peribronchial changes predominate: thickening of the peribronchial tissue, narrowing of the bronchial lumen. Collapses and expansions of the alveoli are detected (Figure 2, E1).



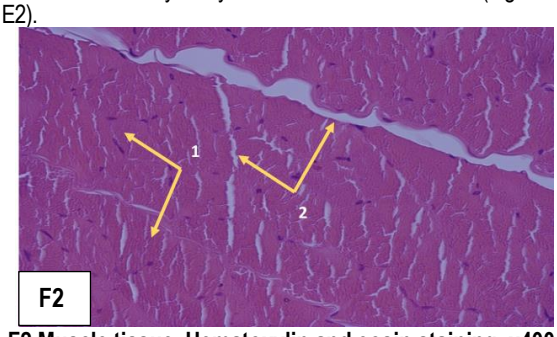
E2 Lung tissue. Hematoxylin and eosin staining. x200. 1 - bronch. 2 - alveoli.

The structure is not damaged. Moderately thickened interalveolar septa are determined. Focal - emphysematous swelling of the alveoli. Pronounced lymphollicles around the bronchi. In the arteries, blood plasma proteins are determined and there is an accumulation of erythrocytes closer to the arterial wall (Figure 2, E2).



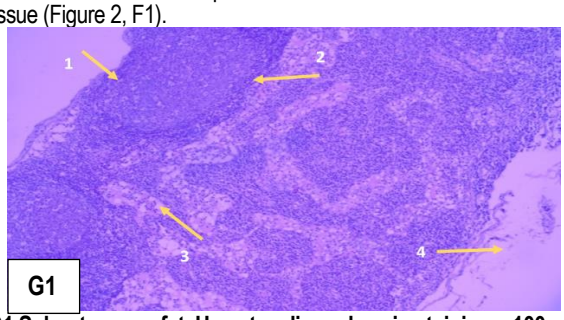
F1 Thigh muscles. Hematoxylin and eosin staining. x100. 1 - skeletal muscle. 2 - intermuscular tissue edema.

The structure of the skeletal muscle is preserved with signs of transverse striation with pronounced edema of the intermuscular tissue (Figure 2, F1).



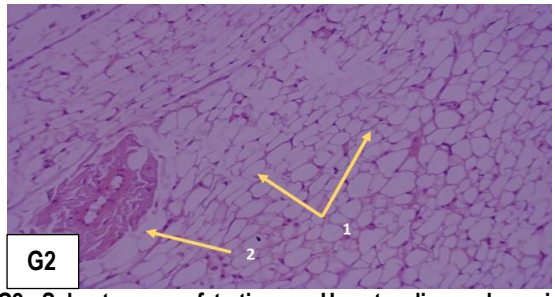
F2 Muscle tissue. Hematoxylin and eosin staining. x400. 1 - skeletal muscle. 2 - intermuscular tissue.

Skeletal muscle with transverse striation with signs of protein-hyaline droplet dystrophy of myocytes (Figure 2, F2).



G1 Subcutaneous fat. Hematoxylin and eosin staining. x100. 1 - epidermis; 2 - basal layer; 3 - dermis; 4 - subcutaneous fat.

The epidermal tissue is represented by fragments of multilayered squamous epithelium with proliferation of the basal layer of cells. Under the squamous epithelium in the thickness of the subcutaneous fat there is focal inflammatory infiltration of lymphocytes, histiocytes and neutrophilic leukocytes.



G2 Subcutaneous fat tissue. Hematoxylin and eosin staining. x200. 1 – fat vacuoles; 2 – interstitial tissue.

Subcutaneous fat tissue is represented by preserved fat vacuoles and interstitial tissue.

Figure 2. Results of histological studies of animal organs.

Histological examination of animal organs after administration of the drugs at 0.8 μg/kg revealed the following:

а) A moderate toxic effect is observed when Betaleukin is administered in free form, which manifests itself in a pronounced inflammatory reaction in the subcutaneous fat tissue and a moderate one in the internal organs (Fig. A1 – G1).

в) A lower toxic effect was determined after the introduction of Betaleukin included in pharmacocytes (Fig. A2 – G2), since minor dystrophic processes and moderate signs of venous congestion were observed in the organs.

Discussion

The results of the study of acute toxicity of interleukin-1β deposited in pharmacocytes prove that pharmacocytes can significantly reduce the toxicity of the drug. Thus, if the results of the study of acute toxicity of interleukin-1β in the standard, free form correspond to the data presented in the available literature: 50% lethal doses of interleukin-1β - DL50 = 15030 ng / kg, then the acute toxicity of the drug in the form of pharmacocytes is significantly lower than the toxicity of the free drug. The level of lethal doses could not be determined for technical reasons. The lower toxicity of deposited interleukin-1β is also confirmed by the results of pathomorphological examination. Thus, it was established that according to the data of histological examination of tissues after intravenous administration of DL50% betaleukin in free form in surviving animals (rats) on the 15th day, toxicity is manifested in a pronounced inflammatory reaction in the subcutaneous fat tissue and a moderate one in the liver, kidneys, and heart. In the same situation, when an equivalent dose of this cytokine was administered in the form of pharmacocytes, only minor dystrophic processes and moderate signs of venous congestion were detected. Pharmacocytes evidently reduce the toxic potential of cytokines.

This fact, previously established by us for pharmacocytes containing an antibiotic, allows us to build upon the premise and assert that, as a drug form, pharmacocytes reduce the toxic potential of the medicinal substance deposited in them; this position is new and previously unproven, although assumptions have been made by individual researchers [14,42,32]. The study demonstrates that encapsulation of interleukin-1β in pharmacocytes markedly reduces its acute toxicity compared to the free form, as confirmed by both survival data and histological findings. These results support the concept that pharmacocytes can serve as an effective cellular transport system to minimize drug toxicity and justify further preclinical research toward clinical application.

Conclusion

The data obtained in our experimental conditions on the possibility of reducing the toxicity of interleukin-1β in the case of deposition of this substance in pharmacocytes allow us to confirm the general position on cellular transport systems, and also to continue preclinical studies of pharmacocytes with the prospect of creating a new transport system for the clinic. Thus, erythrocyte pharmacocyte–based targeted drug delivery technology represents an innovative and pathogenetically justified approach that improves the outcomes of surgical infection treatment, reduces toxicity, increases the safety of therapy, and broadens the opportunities of clinical pharmacology in the field of personalized medicine.

Conflict of Interest. *The authors declare that they have no conflict of interest.*

Contribution of authors. *All authors were equally involved in the writing of this article.*

Acknowledgements: *none.*

Funding: *This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan Grant №AP19676272, №AP26102345 and by Nazarbayev University under Collaborative Research Program Grant № 211123CRP1614, A.G.*

Literature:

1.*Арефьева Н.А., Азнабаева Л.Ф., Симбирцев А.С.* Применение беталейкина в лечении больных риносинуситом. Новости оториноларингологии и логопатологии. 2001. № 2(26). С. 175–178.

2.*Кузин М.И., Костюченок Б.М.* Рана и раневая инфекция. М.: Медицина. 1991. С.106.

3.*Плойд Э., Ледерман У., Тюрин Ю.Н.* Справочник по прикладной статистике. В 2-х т.: Пер. с англ. / Под ред. —М.: Финансы и статистика, 1989. - 510 с.: ил.

4.*Гершанович М.Л., Филатова Л.В., Кетлинский С.А., Симбирцев А.С.* Беталейкин (человеческий рекомбинантный интерлейкин-1 бета) – новый эффективный стимулятор и протектор лейкопоэза в условиях комбинированной химиотерапии злокачественных опухолей. Вопросы онкологии. 2000. Т.46, № 3. С. 354–360.

5.*Новиков В.В.* I фаза клинического изучения беталейкина (человеческого рекомбинантного интерлейкина -1 бета) у больных злокачественными новообразованиями. II Российский национальный конгресс "Человек и лекарство". - Москва. - 10-15 апреля 1995.

6. *Покровская М.П., Макаров М.С.* Цитология раневого экссудата как показатель процесса заживления. М.: Медгиз. 1942. С42.

7.*Симбирцев А.С.* Биология семейства интерлейкина-1 человека. Иммунология. 1998. № 3. С. 9–17.

8.*Симбирцев А.С., Попович А.М.* Сфера применения рекомбинантного интерлейкина-1 бета при лечении больных с иммунодефицитными состояниями при травме и сепсисе. Анестезиология и реаниматология. 1996. № 4. С. 76–78.

9.*Симбирцев А.С., Чернушевич И.И.* Рекомбинантный интерлейкин-1 бета (Беталейкин) в лечении хронического гнойного среднего отита. Новости оториноларингологии и логопатологии. 1999. № 3. С. 58–61.

10. *Akash M.S., Rehman K., Chen S.* IL-1Ra and its delivery strategies: inserting the association in perspective. Pharm Res. 2013 Nov;30(11):2951-66. doi: 10.1007/s11095-013-1118-0. Epub 2013 Jun 22. PMID: 23794040 Review.

11. *Akhila J.S., Deepa S., Alwar M.C.* Acute toxicity studies and determination of median lethal dose. Curr Sci. 2007; 93:917–20.

12. *Amritha F., et al.* Multiple cargo deliveries of growth factors and antimicrobial peptide using biodegradable

nanopolymer as a potential wound healing system. Intern. J. Nanomedicine. 2019; 14: 2253–2263.

13. *Bayne E.K. Rupp E.A., Limjuco G. Chin J., Schmidt J.A.* Immunocytochemical detection of interleukin I within stimulated human monocytes. J. Exp. Med. 163: 1267-1280. 1986.

14. Berikkhanova K. et al. Red blood cell ghosts as promising drug carriers to target wound infections. MEDICAL ENGINEERING & PHYSICS Том: 38 Выпуск: 9 SI Стр.: 877-884. 2016. https://doi.org/10.1016/j.medengphy.2016.02.014

15. *Berikkhanova K. et al.* Drug-loaded erythrocytes: Modern approaches for advanced drug delivery for clinical use, Heliyon, Volume 10, Issue 1, 2024, e23451, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2023.e23451.

16. *Boraschi D., Villa L., Ghiara P., Tagliabue A., Mengozzi M., Solito E., Parente L., Silvestri S., Van Damme J., Ghezzi P.* Mechanism of acute toxicity of IL-1 beta in mice. Eur Cytokine Netw. 1991 Jan-Feb;2(1):61-7. PMID: 1873493.

17. *Chin J.S., Madden L., Chew S.Y., Becker D.L.* Drug therapies and delivery mechanisms to treat perturbed skin wound healing. Adv Drug Deliv Rev. 2019 Sep-Oct;149-150:2-18. doi: 10.1016/j.addr.2019.03.006. Epub 2019 Apr 6. PMID: 30959068.

18. *Christian D.A., Hunter C.A.* Particle-mediated delivery of cytokines for immunotherapy. // Immunotherapy. – 2012. – Vol. 4(4). - P.425-441.

19. *Clements A.E.B., Murphy W.L.* Acta Biomater. Injectable biomaterials for delivery of interleukin-1 receptor antagonist: Toward improving its therapeutic effect. 2019 Jul 15;93:123-134. doi: 10.1016/j.actbio.2019.04.051. Epub 2019 Apr 25. PMID: 31029831 Review.

20. *Dinarcllo C.* Multiple biological properties of recombinant human interleukin 1. Immunobiology. 172: 301-315. 1986.

21. *Fibbe W.E., Schaafsma M.R. Falkenburg J.H.F., Villemze R.* The biological activities of interleukin-1. Blut. 59: 147-156. 1989.

22. *Garbayo E., Pascual-Gil S., Rodríguez-Nogales C., Saludas L., Estella-Hermoso de Mendoza A., Blanco-Prieto M.J.* Nanomedicine and drug delivery systems in cancer and regenerative medicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2020 Apr 29: e1637. doi: 10.1002/wnan.1637.

23. *Geneva-Popova M., Murdjeva M.* Study on proinflammatory cytokines (IL-1 beta, IL-6, TNF-alpha) and IL-2 in patients with acute hepatitis B. Folia. Med. (Plovdiv). 1999. - Vol. 41, №1. - P. 78-81.

24. *Jain K.K.* An Overview of Drug Delivery Systems. Methods Mol Biol. 2020; 2059:1-54. doi: 10.1007/978-1-4939-9798-5_1.

25. *Kampschmidt R.F., Jones. T.* Rate of clearance of interleukin-l from the blood of normal and nephrectomized rats. Proc. Soc. Exp. Biol. Med. ISO: 170-173. 1985.

26. *Kjell Brunnström, Marcus Barkowsky* Statistical quality of experience analysis: on planning the sample size and statistical significance testing, Journal of Electronic Imaging 27(5), 053013 (25 September 2018). https://doi.org/10.1117/1.JEI.27.5.053013

27. *Klapproth J., Castell J., Geiger T., Andus T., Heinrich P.C.* Fate and biological action of human recombinant interleukin I in the rat in vivo. Fur. J. Immunol. 19: 1485-1490. 1989.

28. *Lomedico P.T. Gubler U., Hellmann C.P., et al.* Cloning and expression of murine interleukin-l cDNA in Escherichia cotÃ-. Nature (Lond.). 312: 458-462. 1984..

29. *Mingmao Chena et al.* Dynamic covalent constructed self-healing hydrogel for sequential delivery of antibacterial agent and growth factor in wound healing. Chem. Engineering J., V. 373, 2019, P.413-424.

30. *Mullerad J., Cohen S., Benharroch D., Apte R.N.* Local delivery of IL-1 alpha polymeric microspheres for the immunotherapy of an experimental fibrosarcoma. Cancer Investig. 2003, 21, 720–728. [Google Scholar] [CrossRef]

31. *Newton R.C., Uhi J., Covington M. and Back O.* The distribution and clearance of radiolabeled human interleukin-l Â»1in mice. Lymphokine Res..7:207-216, 1988.

32. *Nurgozhin T. et al.* Pharmacokinetics of ceftriaxone included in cellular transport system. 12th Congress of the European-Association-for-Clinical-Pharmacology-and-Therapeutics, SPAIN. 27-30, 2015. European Assoc Clin Pharmacol & Therapeut THERAPEUTICS Том: 37 Выпуск: 8 S. Стр.: E62-E62. AUG 2015. DOI:10.1016/j.clinthera.2015.05.187

33. *Oppenheim. J.J., Kovacs. E.J., Malsushima K. and Durum S.K.,* There is more than one inlerleukin 1. Immunol. Today. 7:45-56, 1986.

34. *Park J.W., Hwang S.R., Yoon I.S.* Advanced growth factor delivery systems in wound management and skin regeneration. Molecules. 2017;22(8):1259.

35. *Rosner B. and Grove D.* Use of the Mann–Whitney U-test for clustered data. Statist. Med., 1999, 18: 1387-1400. https://doi.org/10.1002/(SICI)1097-0258(19990615)18:11<1387::AID-SIM126>3.0.CO;2-V

36. Saganuwan S.A. Toxicity study of drugs and chemicals in animals: An overview. BJVM. 2016 2016 online first. ISSN 1311-1477. [Google Scholar].

37. *Sergazy S., Berikkhanova K., Gulyayev A., et al.* Cell-Based Drug Delivery Systems: Innovative Drug Transporters for Targeted Therapy. Int. J. Mol. Sci. 2025, 26, 8143. https://doi.org/10.3390/ijms26178143

38. *Sergazy S., Zhetkenev S., Shulgau Z., Berikkhanova K., Gulyayev A., et al.* Investigating the Suitability of Mare's Milk-Derived Exosomes as Potential Drug Carriers. Biomolecules 2024, 14, x. https://doi.org/10.3390/xxxxx.

39. *Shoji Kudo, Keiko Mizuno, Yoshikatsu Iirai, Takefumi Shimizu.* Clearance and Tissue Distribution of Recombinant Human Interleukin I β in Rats. Cancer research 50. 5751-5755. September 15. 1990.

40. *Veltri S., Smith J.W.,* 2nd. Interleukin 1 trials in cancer patients: A review of the toxicity, antitumor and hematopoietic effects. Stem Cells 1996, 14, 164–176. [Google Scholar] [CrossRef] [PubMed]

41. *Zhao Y.Z., Du L.N., Lu C.T., Jin Y.G., Ge S.P.* Potential and problems in drug delivery systems. Int J Nanomedicine. 2013; 8:1621-33;

42. *Zhumadilov Zh. et al.* Cytotoxicity reduction of rifampicin by incorporation into red blood cell ghosts. International Journal of Preclinical and Pharmaceutical Research. 2014, Vol. 5(3). -P.147-155. file:///D:/172_147-155(preclinical).pdf

43. *Zhumadilov Zh.Sh., Makarenkova R.V.* Pharmacokinetics of kanamycin during targeted delivery to the liver in erythrocyte ghosts in animals with experimental acute cholecystitis. Antibiot Khimioter. 1990;35(11):37-8. Russian. PMID: 2092638. https://pubmed.ncbi.nlm.nih.gov/2092638/

References: [1-9]

1. Aref'eva N.A., Aznabaeva L.F., Simbirtsev A.S. Primenenie betaleikina v lechenii bol'nykh rinosinusitom [Use of betaleukin in the treatment of patients with rhinosinusitis]. *Novosti otorinolaringologii i logopatologii* [News of Otolaryngology and Speech Pathology]. 2001. № 2(26). pp. 175–178. [in Russian]

2. Kuzin M.I., Kostyuchenok B.M. *Rana i ranevaya infektsiya* [Wound and wound infection]. M.: Meditsina. 1991. p.106. [in Russian]

3. Lloid E., Lederman U., Tyurin Yu.N. *Spravochnik po prikladnoi statistike* [Handbook of applied statistics.]. V 2-kh t.: Per. s angl. / Pod red. - M.: Finansy i statistika, 1989. - 510 p.: il. [in Russian]

4. Gershanovich M.L., Filatova L.V., Ketlinskii S.A., Simbirtsev A.S. Betaleikin (chelovecheskii rekombinantnyi interleikin-1 beta) – novyi effektivnyi stimulyator i protektor leikopoeza v usloviyakh kombinirovannoi khimioterapii zlokachestvennykh opukholei [Betaleukin (human recombinant interleukin-1 beta) – a new effective stimulator and protector of leukopoiesis in conditions of combination chemotherapy of malignant tumors]. *Voprosy onkologii* [Issues of oncology]. 2000. T.46, № 3. pp. 354–360. [in Russian]

5. Novikov B.B. I faza klinicheskogo izucheniya betaleikina (chelovecheskogo rekombinantnogo interleikina -1 beta) u bol'nykh zlokachestvennymi novoobrazovaniyami [Phase I clinical study of betaleukin (human recombinant interleukin-1 beta) in patients with malignant neoplasms]. II Rossiiskii natsional'nyi kongress «Chelovek i lekarstvo» [II Russian National Congress «Man and Medicine»]. - Moskva. - 10-15 aprelya 1995. [in Russian]

6. Pokrovskaya M.P., Makarov M.S. *Tsitologiya ranevogo ekksudata kak pokazatel' protsessa zazhivleniya* [Wound exudate cytology as an indicator of the healing process]. M.: Medgiz 1942. P.42. [in Russian]

7. Simbirtsev A.S. Biologiya semeistva interleikina-1 cheloveka [Biology of the human interleukin-1 family]. *Immunologiya* [Immunology]. 1998. № 3. pp. 9–17. [in Russian]

8. Simbirtsev A.S., Popovich A.M. Sfera primeneniya rekombinantnogo interleikina-1 beta pri lechenii bol'nykh s immunodefitsitnymi sostoyaniyami pri travme i sepsis [Scope of application of recombinant interleukin-1 beta in treatment of patients with immunodeficiency states in trauma and sepsis.]. *Anesteziologiya i reanimatologiya* [Anesthesiology and reanimatology]. 1996. № 4. pp. 76–78. [in Russian]

9. Simbirtsev A.S., Chernushevich I.I. Rekombinantnyi interleikin-1 beta (Betaleikin) v lechenii khronicheskogo gnoinogo srednego otita [Recombinant interleukin-1 beta (Betaleukin) in treatment of chronic suppurative otitis media]. *Novosti otorinolaringologii i logopatologii* [News of otolaryngology and speech pathology]. 1999. № 3. pp. 58–61. [in Russian]

Authors' information

Kulzhan Berikkhanova – MD, Candidate of Medical Sciences, Associate Professor Leading Researcher, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan, e-mail: kberikkhanova@nu.edu.kz, https://orcid.org/0000-0002-6371-9210

Gulyash Tanysheva – MD, Head of Department of Obstetrics and Gynecology, Semey Medical University, Semey, Kazakhstan, e-mail: gulyash1965@mail.ru, https://orcid.org/0000-0001-9531-5950

Alexandr Gulyayev - Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan, agulyayev@nu.edu.kz, https://orcid.org/0000-0001-5098-4675

Erlan Taigulov – MD, Professor, doctor of Medical Sciences, Department of Surgical Diseases, Astana Medical University, Astana, Kazakhstan, e-mail: tajguloverlan208@gmail.com, https://orcid.org/0009-0006-2320-864X

Saken Kozhakhmetov - MD, Candidate of Medical Sciences, Associate Professor, Head of Department of Surgical Diseases, Astana Medical University, Astana, Kazakhstan, e-mail: kozhahmetov.s@amu.kz, https://orcid.org/0000-0002-0075-0376

Nurzhan Bikhanov - MD, Candidate of Medical Sciences, Director of Clinical Academic Department of Surgery, University Medical Center, Nazarbayev University, Astana, Kazakhstan, e-mail: nbikhanov@mail.ru, https://orcid.org/0009-0002-9718-8865

Yernur Zakirov – MD, Researcher, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan, e-mail: yernur.zakirov@nu.edu.kz

Nurzhan Berikkhanov - MD, Head of Surgical Department, City polyclinic №2, Taraz, 080000, Kazakhstan, e-mail: nberikkhanov@mail.ru, https://orcid.org/0009-0003-6696-2886

Askhat Zhilkaidarov - MD, surgeon, City Hospital №1, Astana, Kazakhstan, e-mail: zhilkaidarov.ash@mail.ru, https://orcid.org/0009-0004-5266-3655

Yessenhan Sultan - MD, infectious disease physician, City polyclinic №14, Almaty, 050000, Kazakhstan, e-mail: sensultan@mail.ru. https://orcid.org/0009 0009 4593 8661

Corresponding author:

Kulzhan Berikkhanova – MD, Candidate of Medical Sciences, Associate Professor Leading Researcher, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan,

Address: 53 Kabanbay batyr ave., Astana, 010000, Republic of Kazakhstan

E-mail: kberikkhanova@nu.edu.kz

Tel: +7 771 022 90 35