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Immune reactivity of two biological models to vaccination with inactivated vaccine QazVac against coronavirus infection COVID-19

Balzhan Sh. Myrzakhmetova✉, Gulzhan A. Zhapparova, Karina B. Bissenbayeva, Aizhan S. Toytanova, Moldir S. Tuyskanova, Kuandyk D. Zhugunissov, Lespek B. Kutumbetov

Research Institute for Biological Safety Problems, 080409, Gvardeyskiy, Republic of Kazakhstan

Abstract

Introduction. Specific prevention of a number of infectious diseases has been introduced into the vaccination schedule. The production of immunoprophylactic drugs, in order to establish standard properties, including safety and specific effectiveness, requires strict adherence to manufacturing regulations, and the reliability of the results obtained requires monitoring of these parameters. The specific effectiveness of vaccine preparations is standardized according to the indicators of stimulation of specific antibody response formed in the body of vaccinated model biological objects.

Objective. Determination of the immune reactivity of white mice to vaccination with the QazVac vaccine to establish the possibility of using them as a biological model in assessing the immunogenicity of the vaccine instead of Syrian hamsters.

Materials and methods. The immune reactivity of model animals was assessed by the seroconversion rate, dynamics of antibody titers to the SARS-CoV-2 virus formed in the body after vaccination with the test vaccine. In the case of seropositivity of animals before administration of vaccine or placebo, the level of immune reactivity was calculated by the difference in antibody titers between control and vaccinated animals or by the difference in antibody titers before and after immunization. Specific antibodies were detected and their titer was determined using a neutralization reaction.

Results. The research results showed that the tested biological models had approximately the same immune reactivity to the administration of the QazVac vaccine, confirmed by the level and dynamics of antibody titers. When analyzing the fold increase in antibody titers in comparison to those of control animals, Syrian hamsters were more reactive compared to mice. But SPF white mice were standardized in their lack of the immune reactivity to SARS-CoV-2 virus before the immunization.

Conclusion. The data obtained indicate that the immune reactivity of white mice to the administration of the QazVac vaccine in terms of the rate and dynamics of the formation of virus-neutralizing antibodies is approximately equivalent to the immune reactivity of Syrian hamsters. Before immunization with the vaccine, SPF white mice, in contrast to Syrian hamsters, do not have humoral immunity specific to the SARS-CoV-2 virus. The immune reactivity equivalent to that observed of Syrian hamsters and the absence of antibodies to the SARS-CoV-2 virus at a baseline indicate the superiority of the use of white mice in assessing the immunogenicity of vaccines against COVID-19 and/or obtaining specific factors of humoral immunity.

Keywords: *immune reactivity; SARS-CoV-2 virus; neutralization reaction; vaccine*

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Ethics approval. The authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with Consensus author guidelines for animal use (IAVES 23 July 2010). The research protocol was approved by the Bioethics Committee of the Research Institute for Biological Safety Problems of the Ministry of Health of the Republic of Kazakhstan (Protocol No 2 dated August 14, 2023).

ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
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Иммунная реактивность двух биологических моделей на прививку инактивированной вакциной QazVac против коронавирусной инфекции COVID-19

Мырзахметова Б.Ш.✉, Жаппарова Г.А., Бисенбаева К.Б., Тойтанова С.А., Туысканова М.С., Жугунисов К.Д., Кутумбетов Л.Б.

РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, 080409, пгт. Гвардейский, Республика Казахстан

Резюме

Введение. Специфическая профилактика ряда инфекционных болезней введена в календарь медицинских прививок. Производство иммунопрофилактических препаратов в целях установления стандартных свойств, в том числе по безопасности и специфической эффективности, требует строгого соблюдения регламента изготовления, а достоверность полученных результатов – проведения контроля указанных параметров. Специфическую эффективность вакцинных препаратов стандартизируют по показателям стимуляции гуморальных факторов иммунитета, формируемых в организме привитых модельных биологических объектов.

Цель работы. Определение иммунной реактивности белых мышей на прививку вакциной QazVac для установления возможности их использования в качестве биологической модели в оценке иммуногенности вакцины вместо сирийских хомяков.

Материалы и методы. Оценку иммунной реактивности модельных животных проводили по количеству сероконверсивности, скорости и динамике титров антител на вирус SARS-CoV-2, формируемых в организме после прививки испытуемой вакциной.

Результаты. Результаты исследований показали, что испытуемые биологические модели обладают примерно одинаковой иммунной реактивностью на введение вакцины QazVac, подтверждающим свидетельством которой являлись уровень и динамика титров антител. При анализе кратности увеличения титров антител в сравнении с таковыми контрольных животных, сирийские хомяки обладают сравнительно большей реактивностью. Но белые мыши, свободные от патогенной микрофлоры (СПФ), стандартны по интактности от антител на вирус SARS-CoV-2.

Заключение. Полученные данные свидетельствует о том, что иммунная реактивность белых мышей на введение вакцины QazVac по скорости и динамике формирования вируснейтрализующих антител является практически равнозначной иммунной реактивности сирийских хомяков. В организме белых мышей категории СПФ до прививки вакциной, в отличие от сирийских хомяков, не содержатся факторы гуморального иммунитета, специфичные к вирусу SARS-CoV-2.

Ключевые слова: *иммунная реактивность; вирус SARS-CoV-2; реакция нейтрализации; вакцина*

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

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Introduction

An effective way to prevent infectious diseases and eliminate their epidemic spread, is protecting the immune system with the help of vaccines [1–10]. For example, in order to prevent cases of disease as well as epidemics of measles, poliomyelitis, influenza, etc., immune prophylaxis of people from an early age or in the course of their life against such socially dangerous diseases is carried out in medical practice [11–14]. Specific prophylaxis of a number of infectious diseases is introduced in the immunization schedule. The production of immunoprophylactic preparations, in order to establish standard properties, including safety and specific efficacy, requires strict compliance with manufacturing regulations[1], and the reliability of the obtained results requires control of the mentioned parameters [15, 16]. Specific efficacy of vaccine preparations is standardized by the indicators of stimulation of humoral immunity factors formed in the organism of inoculated model biological subjects.

With the development of the COVID-19 pandemic, various vaccines against this disease have been developed in different countries, some of which are globally recognized, while others are used on a national scale [17–19]. In the Republic of Kazakhstan during the pandemic, imported (Sputnik-V, Russia, SinoVac, China, Pfizer, USA) and domestic (QazVac) vaccines against this pandemic disease were used, the high safety and satisfactory efficacy of which were shown by the results of clinical trials and anti-epidemic data [20–22]. When evaluating the domestic QazVac vaccine efficacy, Syrian hamsters are used as a biological model; they are immunized with the tested series of the vaccine and tested for the content of specific virus-neutralizing antibodies 7 days after the second dose of the preparation [23]. According to the requirements of the Analytical Normative Document (AND)[2] for the quality control of the preparation, blood serum samples from model animals should contain specific antibodies detectable in the neutralization reaction at a titer not lower than 32 at this time point. The use of Syrian hamsters as a laboratory model to evaluate the immunogenic activity of the vaccine was due to the fact that these animals were susceptible to COVID-19 infection [24, 25] and specific antibodies to the SARS-CoV-2 virus were formed in their organism during the post-infection period. From the onset of the coronavirus pandemic to the present, there are a large number of research papers where authors have used various animal species as biological models in the production and control of immunobiological drugs against COVID-19 coronavirus infection [25–35]. Susceptibility to SARS-CoV-2 virus in small rodents (mice, Syrian hamsters) [36–39], carnivores (ferrets, cats, dogs) [40–42] and nonhuman monkeys (green monkey, common marmoset, African baboon, rhesus macaque) was reported by a number of scientists, whose research papers can be found in bioRxiv, Medline and PubMed databases [43–48]. However, experience with Syrian hamsters has shown that they often contain antibodies specific for SARS-CoV-2 virus [49] prior to their use in studies, the presence of which does not provide reliable information on the standardization of the vaccine being tested.

The aim of the study was to determine the immune reactivity of white mice to immunization with the QazVac vaccine in order to establish the possibility of using them as a biological model in assessing the immunogenicity of the vaccine instead of Syrian hamsters.

Materials and methods

Vaccine. QazVac vaccine made from the Omicron-South Africa variant of SARS-CoV-2 virus in three series (series No. 1, No. 2, No. 3) according to the current standard technology was used as a means of immunization.

Virus. The strain of the genetic variant Omicron-South Africa of SARS-CoV-2 virus adapted in Vero cell culture with biological activity of 6.67±0.22 lg TCD_{50}/cm^3 was used for production of the tested vaccine and neutralization reaction.

Cell culture. A WHO-certified Vero cell culture line (WHO) grown in a monolayer in Cell Factory plastic cell factories, mattresses, and plates was used to obtain SARS-CoV-2 virus biomass and to stage the neutralization reaction. Cells were grown in DMEM nutrient medium containing 5–10% fetal bovine serum (FBS). To maintain cell viability, the same nutrient medium was used, but containing 1–2% FBS.

Animals. Clinically healthy golden Syrian hamsters with live weight of 70–80 g and SPF mongrel white mice with live weight of 18–22 g were used as laboratory animals. Animals were kept in the conditions of scientific-experimental biological clinic with biological safety level 2 (ABSL-2), where a sanitary passageway, supply and exhaust ventilation equipped with HEPA filters of fine purification, special cages for keeping animals with autonomous supply of air, feed and water are provided.

Assessment of animal immune reactivity. The immune reactivity of model animals was assessed by the rate of seroconversion, dynamics of antibody titers to SARS-CoV-2 virus formed in the organism after inoculation with the tested vaccine. In case of seropositivity of animals before with the administration of vaccine or placebo, the level of immune reactivity was calculated by the difference of antibody titers between control and vaccinated animals or by the difference of antibody titers before and after vaccine administration. Specific antibodies were detected and their titer was determined by neutralization reaction.

Testing in neutralization reaction. The neutralization reaction was performed on monolayer Vero cell culture prepared in 96-well plastic plates. Double dilutions (1 : 2, 1 : 4, etc.) of the tested blood serum of Syrian hamsters and white mice in the supporting medium and culture suspension of SARS-CoV-2 virus of Omicron

[1]WHO. Coronavirus (COVID-19) Dashboard. Available at: https://covid19.who.int

[2]Analytical regulatory document (AND) for quality control of the inactivated vaccine QazCOVID-in (QazVac). Guardeyskiy; 2021. (in Russian)

variant with a titer of 100 TCD_{50}, taken in equal volume ratios, were used as a reaction mixture. The resulting mixture was incubated at 37 °C for 60 min and added in equal doses to at least 4 wells of a 96-well plate with the test cell culture. As a dose control, the virus suspension was titrated on the same cell culture using its tenfold (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) dilutions in the maintenance medium. For cell culture quality control, at least 4 wells were left without including the reaction mixture and virus, but replacing them with the maintenance medium. Cell culture in plates with neutralization reaction was incubated at 37 °C for 5 days, after which the results of virus cytopathic effect (CPE) were recorded. The absence of CPE in cell culture, when present in control wells with virus dose and absence in wells with cell culture quality control, was considered for virus neutralization or antibody presence, and the presence of CPE, under the specified conditions in the listed controls, was considered for the absence of neutralization and specific antibodies. The highest serum dilution that neutralized virus reproduction in at least 50% of cases was taken as the antibody titer. The antibody titer was given in reciprocal numerical values of twofold dilutions of serum. The titers of virus and serum were calculated according to Reed and Mench [50]. Reliability of the difference of antibody titers formed in model animals was assessed by Student's t-test [51–53].

Immunization of animals. Immunization of model animals was carried out by intramuscular injection of the tested vaccine in a dose of 0.5 ml into the quadriceps muscle of the left hind limb. Before administering the vaccine, the injection site was treated with 70° ethyl alcohol and dried. Instead of the tested vaccine, the control animals were injected with commercial physiological solution of sodium chloride, produced by KhimPharm JSC, Shymkent.

Safety determination. Vaccine safety was assessed by quality control of the vaccine according to the requirements of the Analytical Normative Document (AND) for the preparation, and by injecting two immunizing human doses of the vaccine into the hind limb muscles of ten Syrian hamsters and ten SPF mongrel white mice intramuscularly. The vaccine was considered safe if sterility, endotoxin content, total protein, cellular DNA and pyrogenicity conformed to the normative values established by AND, and all Syrian hamsters and white mice inoculated with a double dose of the preparation survived for 14 days without development of local and general pathology.

Scheme of immunogenicity study. Syrian hamsters and white mice were divided into 5 groups of 20 animals of each species. The first group of animals was inoculated with vaccine series No. 1, the second group with vaccine series No. 2, the third group with vaccine series No. 3, the fourth group with placebo, and the fifth group was left without experimental intervention. Vaccinated and control animals were monitored daily with registration of body temperature and live weight. Before the experiment, blood serum samples were collected from all Syrian hamsters and white mice. Blood samples from Syrian hamsters were collected from the intercostal vein, and from mice from the subcostal vein. On 14, 21, 28, 35 days after vaccine and placebo injection, blood serum was collected from 5 animals of each species, in Syrian hamsters from the intercostal vein and in mice from the cervical vessels by total decapitation. Serum samples were heat-treated at 56 °C for 30 min before examination in neutralization tests.

Statistical processing. Statistical analysis of the results was performed using GraphPad Prism8 software. Reliability of differences between the indicators ($p \leq 0.05$) was determined using the Student's t-test.

The authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with Consensus author guidelines for animal use (IAVES 23 July 2010). The research protocol was approved by the Bioethics Committee of the Research Institute for Biological Safety Problems of the Ministry of Health of the Republic of Kazakhstan (Protocol No 2 dated August 14, 2023).

Results

Controls of the vaccine series used showed that they contained hydrogen ion counts between 7.24–7.31, total protein between 92.765–99.343 μg/0.5, cellular DNA between 37.32–38.94 ng/0.5, endotoxins not more than 0.15 IU/mL and formaldehyde not more than 20 mg/L. Sterility test data by seeding on beef extract broth, beef extract agar, Sabouraud and thioglycol media were negative, indicating purity from contaminating microorganisms.

Syrian hamsters and white mice that received 2 doses of the vaccine intramuscularly remained alive and healthy during 14 days of observation, except for the first day after the injection of the preparation, when reduced mobility of the animals was noted, which was noted for 2–4 h. During the rest of the time, animals of both species vaccinated with two doses of vaccine were active and healthy. No pathologies developed at the vaccine injection site during the observed period.

Based on the results obtained, it is concluded that the three vaccine series tested are safe and suitable for immunogenicity evaluation in model animals and, if necessary, in human volunteers.

All animals inoculated with the vaccine and placebo, as well as kept as pure controls during the entire observation period, which lasted 35 days, remained alive and healthy. The neutralization test results of serum samples collected before the experiment and at subsequent times after vaccine and placebo administration are shown in **Table 1**.

As shown in Table 1, antibodies that neutralize SARS-CoV-2 virus were present in the serum samples of most Syrian hamsters prior to use in the studies. Seropositive animals were present in the two groups used for vaccine inoculation and the placebo group. The mean titers of such antibodies ranged from 3.06 ± 1.7 in the placebo group to 3.6 ± 1.4–8.8 ± 2.8 in groups 1 and 3, respectively. Group 2 animals were completely non-reactive for antibodies neutralizing SARS-CoV-2 virus.

In blood serum samples of all vaccinated animals, irrespective of the series of the vaccine preparation, by the day 14 there was an increase in the titers of antibodies neutralizing SARS-CoV-2 virus from 10.0 ± 3.2 to 26.8 ± 7.84, which exceeded their initial titers 5.3-fold in group 1, 10-fold in group 2 and 3.0-fold in group 3. The mean of antibody titers in the three groups was 18.7 ± 6.12, which is a 4.8-fold increase from the baseline mean titer of 3.9. In the control group (placebo) the multiplicity of antibody increase in this period was insignificant and amounted to 1.7.

In the following terms, further growth of antibody titers was noted in the blood serum of vaccinated animals and by day 21 they ranged from 78.2 ± 25.60 to 96.4 ± 31.35 with their average value for all three groups of 86.2 ± 28.89. By day 28, antibody titers in the groups of vaccinated animals reached maximum values ranging from 102.6 ± 31.35 to 132.5 ± 62.71, with a mean of 115.2 ± 39.87. The mean values of the multiplicity of increase in antibody titers compared to baseline were 22.1 fold by day 21 and 29.5 fold by day 28, and 22.6 and 41.1 fold, respectively, relative to the titers of the control group. On day 35, the rate remained high at 39.84. While in control animals, no increase in antibodies neutralizing SARS-CoV-2 virus was observed during the whole period of observation.

Analysis of individual seropositivity for antibodies to SARS-CoV-2 virus showed that in Syrian hamsters immunized with the vaccine, the seroconversion rate was 100%, i.e. all vaccinated animals formed virus-neutralizing antibodies, the titers of which increased over time.

In studies conducted in a similar manner in parallel on white mice, positive results were also obtained, which are summarized in **Table 2**.

As can be seen from the data in Table 2, no antibodies neutralizing SARS-CoV-2 virus were detected in blood serum samples from white mice, unlike Syrian hamsters, before vaccination. In intact animals of the control group such antibodies appeared in low titers after placebo administration. In animals from experimental groups, immunized with vaccine, antibodies in low titers were detected starting from day 14, with titers that already at this time point exceeded more than 4-fold the level of virus neutralizing antibodies in the control group. In the following days antibody titers in vaccinated mice increased significantly and reached maximum values on the 21st, 28th, 35th days, the average values of which were 99.67 ± 29.61, 113.43 ± 32.03 and 110.46 ± 38.85, respectively. Seropositivity rate among the inoculated animals in all groups amounted to 100%, as well as in Syrian hamsters. Maximum antibody titers were observed on the 28th day, which amounted to 113.43 ± 32.03. On the 21st day after the first immunization the antibody titers in the vaccinated groups were more than 20 times higher than the nonspecific background of virus reproduction delay observed in the control group, and on the 28th day this index was equal to 12.9. Meanwhile, the level of multiplicity of antibody titer increase in the experimental group in comparison with

Table 1. Dynamics of virus-neutralizing antibody titers in blood serum of Syrian hamsters before and after vaccination with QazVac vaccine
Таблица 1. Динамика титров ВНА в сыворотке крови сирийских хомяков до и после прививки вакциной QazVac

Group № Группа	Vaccine series Серия вакцины	Number of animals, abs. Число животных, абс.	Time of serum examination, days Сроки исследования сыворотки крови, сут				
			0	14	21	28	35
1	QazVac, series 1 серия 1	20	3.6 ± 1.4 (10)	19.2 ± 6.4 (5)	96.4 ± 31.35 (5)	132.5 ± 62.71 (5)	114.0 ± 27.3 (5)
2	QazVac, series 2 серия 2	20	0 (10)	10.0 ± 3.2 (5)	78.2 ± 25.60 (5)	102.6 ± 31.35 (5)	79.6 ± 25.97 (5)
3	QazVac, series 3 серия 3	20	8.8 ± 2.8 (10)	26.8 ± 7.84 (5)	84.2 ± 29.73 (5)	110.4 ± 25.60 (5)	105.2 ± 31.4 (5)
Average data for the three groups Средние данные по трем группам		60	3.9 ± 1.9 (30)	18.7 ± 6.12 (15)	86.2 ± 28.89 (15)	115.2 ± 39.87 (15)	99.6 ± 28.22 (15)
4	Placebo – physical solution Плацебо – физ. раствор	20	3.06 ± 1.7 (10)	3.86 ± 1.4 (10)	3.8 ± 1.4 (10)	2.8 ± 0.76 (10)	2.5 ± 0.82 (10)
Multiplicity of the increase in antibody titers in the experimental groups compared to the level of antibody titers in the control group Кратность повышения титров антител в опытных группах от уровня титров антител в контрольной группе			1.27	4.84	22.68	41.14	39.84

Note. Here and in the table. 2: VNA – virus-neutralizing antibodies; antibody titers in inverse multiples of serum dilution; number of animals in parentheses.

Примечание. Здесь и в табл. 2: ВНА – вируснейтрализующие антитела; титры антител в обратных величинах кратности разведения сыворотки крови; в скобках – число животных.

Table 2. Dynamics of virus-neutralizing antibody titers in serum of SPF white mice after inoculation with QazVac vaccine
Таблица 2. Динамика титров ВНА в сыворотке крови белых мышей категории СПФ после прививки вакциной QazVac

Group № Группа	Vaccine series Серия вакцины	Number of animals, abs. Число животных, абс.	Time of serum examination, days Сроки исследования сыворотки крови, сут				
			0	14	21	28	35
1	QazVac, series 1 серия 1	20	0 (20)	4.4 ± 0.96 (5)	89.2 ± 31.4 (5)	104.6 ± 31.4 (5)	98.4 ± 29.4 (5)
2	QazVac, series 2 серия 2	20	0 (20)	8.1 ± 1.71 (5)	112.4 ± 25.7 (5)	129.3 ± 37.11 (5)	119.4 ± 27.8 (5)
3	QazVac, series 3 серия 3	20	0 (20)	5.6 ± 1.19 (5)	97.4 ± 32.9 (5)	106.4 ± 27.6 (5)	113.6 ± 33.6
Average data for the three groups Средние данные по трем группам		60	0 (60)	6.03 ± 1.54 (15)	99.67 ± 29.6 (15)	113.43 ± 32.03 (15)	110.46 ± 38.8 (15)
4	Placebo – physical solution Плацебо – физ. раствор	20	0 (20)	1.4 ± 0.8 (5)	4.8 ± 1.6 (5)	8.8 ± 3.91 (5)	3.6 ± 0.8 (5)
Multiplicity of the increase in antibody titers in the experimental groups compared to the level of antibody titers in the control group Кратность повышения титров антител в опытных группах от уровня титров антител в контрольной группе			0	4.31	20.76	12.89	30.68

the control group by 35 days was extremely high and amounted to 30.7.

The results of neutralization tests showed that both Syrian hamsters and white mice showed vaccine-induced seropositivity in 100% of cases after immunization with QazVac vaccine. Virus neutralizing antibodies in the blood sera of Syrian hamsters and white mice are detected from day 14 after immunization in titers of 18.7 ± 6.12 in Syrian hamsters and 6.03 ± 1.54 in white mice, which reach maximum values during the next 7–21 days. In Syrian hamsters, the maximum antibody titers during this period ranged from 86.2 ± 28.89 to 115.2 ± 39.87, and in white mice from 99.67 ± 29.6 to 113.43 ± 32.03. These data indicate that mongrel white mice have sufficient immune reactivity against the whole-virion antigen of SARS-CoV-2 virus contained in the inactivated vaccine [57, 58]. However, it should be noted that the immune reactivity of Syrian hamsters slightly exceeds such indices of white mice, since in the former the multiplicity of antibody titer increase throughout the study has a greater numerical value. However, this difference was not statistically significant up to day 21. The difference in the multiplicity of antibody titer increase is noted starting from day 28 to day 35 after immunization.

The advantage of SPF mice is that they are guaranteed free of SARS-CoV-2 virus and antibodies to this pathogen before their use in studies. This condition allows obtaining standard results of immunogenicity evaluation of the test drug[3,4] [59].

Discussion

The immunogenic activity of the QazVac vaccine (QazCOVID-in) is assessed by the level of antibodies formed in the body of Syrian hamsters after double inoculation with this preparation [23]. This animal species was taken as a laboratory model to standardize the immunogenic activity of the vaccine due to its relatively high susceptibility to COVID-19 coronavirus infection and sufficient immune reactivity to the causative agent of this disease [24–26]. However, in the process of using these animals, it was noted that antibodies neutralizing SARS-CoV-2 virus in various titers reaching up to 5–6 $\log_2$ were detected in the organism of a number of them (unpublished data). We considered the occurrence of this situation as a consequence of contact of animals with pandemic virus through feed and/or attendants in the process of their breeding and rearing, as they are supplied by private entrepreneurs who keep these animals in conditions that do not provide biological safety from pathogens, including the COVID-19 infection. The lack of intactness of the laboratory model does not allow a positive

[3]Decision of the Bioethics Committee of the Research Institute for Biological Safety Problems of the Ministry of Health of the Republic of Kazakhstan. Protocol № 2; 2023. (in Russian)
[4]GOST 33215–2014. Guide to the care and maintenance of laboratory animals. Rules for equipment of premises and organization of procedures. Interstate standard; 2014. (in Russian)

assessment of the immunogenic activity of the tested preparation, and therefore it is necessary to additionally select animals free of target antibodies. In this case, the process of vaccine standardization may be delayed beyond the regulatory period. In addition, statistical analysis of the influence of neutralizing antibodies in Syrian hamsters, which were present before vaccination, on the subsequent immune reactivity of the animal or antibody production shows that there is a correlation (statistical significance of the difference) between the presence of baseline neutralizing antibodies to SARS-CoV-2 and antibody production [54]. This statistically significant difference was detected at a significance level of $p = 0.05$ with a confidence interval of 0.95 [55]. This setting prevents standardizing the immunogenicity of the drug by the multiplicity of antibody titer increase using seropositive animals.

Therefore, in order to replace Syrian hamsters, mongrel white mice raised under conditions free of pathogenic microflora were used in the trials. The studies were conducted using three vaccine series in parallel on the two model animal species mentioned above.

The results of studies showed that white mice as well as Syrian hamsters were immuno-reactive to inactivated antigen of SARS-CoV-2 virus administered by intramuscular injection in the form of QazVac vaccine sorbed on aluminum oxide hydrate. In animals of both species inoculated with the vaccine, seropositivity of 100% was formed from day 14. The dynamics of antibody titers in white mice closely resembled that in Syrian hamsters. The average numerical values of antibody titers in Syrian hamsters on the 14th day were 3.1 times higher than the level of average antibody titers detected in white mice. However, the multiplicity of antibody titers increase in comparison with the data of the control group in the tested animal species was the same and amounted to 4.31 in white mice and 4.84 in Syrian hamsters. Antibody titers in the following three weeks (21–35 days) in both animal species had the same dynamics of growth up to maximum values by day 28 and with a slight decrease by day 35. The level of antibody titers in Syrian hamsters during these periods ranged from 86.2 ± 28.89 to 115.2 ± 39.87, and in white mice – from 99.67 ± 29.6 to 113.43 ± 32.03, which have no significant differences. The multiplicity of increase in antibody titers compared to the immune background of the control group of animals remained approximately the same in both species at day 21. In the subsequent periods this index in Syrian hamsters (41.14 and 39.84 on days 28 and 35, respectively), significantly exceeded that in white mice (12.89 and 30.68 on 28 and 35 days, respectively), indicating their comparatively greater immune reactivity.

Analysis of the results of comparative tests shows that white mice as well as Syrian hamsters have sufficient immune reactivity to inoculation with inactivated vaccine QazCOVID-in (QazVac) and form specific antibodies against SARS-CoV-2 virus in their organism. The revealed immune reactivity of white mice, despite being comparatively lower than that of Syrian hamsters, is quite sufficient to evaluate the immunogenicity of the tested vaccine. SPF white mice are superior to Syrian hamsters in terms of the absence of baseline antibodies against SARS-CoV-2 virus. Based on these characteristics, SPF mice can be used as a new biological model to assess the immunogenicity of the QazVac vaccine. According to the data on the dynamics of antibody accumulation and titers, the reference point for assessing the immunogenic efficacy of the vaccine can be 21 days after the first immunization instead of 7 days after revaccination, conducted at an interval of 21 days, i.e. 35 days after the first immunization. In this case, the period of assessment of the vaccine immunogenicity will be reduced by 14 days.

Conclusion

1. Immune reactivity of Syrian hamsters and SPF mice to immunizing antigen of inactivated SARS-CoV-2 virus was evaluated using three series of QazVac vaccine against COVID-19 according to the dynamics of virus-neutralizing antibodies.

2. According to the results of the research, SPF mongrel white mice have sufficient immune reactivity to immunization with QazVac vaccine and develop specific antibodies to SARS-CoV-2 virus, as well as Syrian hamsters, in 100% of cases from the 14th day after immunization. The titers of virus-neutralizing antibodies reach maximum values in both species of animals in the period from day 21 to day 35 and range from 86.2 ± 28.89 to 115.2 ± 39.87 in Syrian hamsters and from 99.67 ± 29.60 to 113.43 ± 32.03 in white mice, which have no significant differences.

3. According to the results of the comparative evaluation of the multiplicity of increase in the titers of specific antibodies, the immune reactivity of Syrian hamsters and white mice in the first 21 days seems to be equal, and in the following terms (days 28 to 35) in Syrian hamsters it significantly exceeds the same indicators in white mice.

4. According to the results of studies, neutralizing antibodies against SARS-CoV-2 virus are frequently detected in intact Syrian hamsters, while SPF white mice are free of antibodies to the COVID-19 pathogen. Baseline specific seropositivity in Syrian hamsters has a significant effect on the level of vaccine-induced antibody formation, making questionable the validity of the results obtained in controls.

5. The obtained data indicate that the immune response in white mice to the introduction of QazVac vaccine assessed by the rate and dynamics of formation of virus-neutralizing antibodies is approximately equal to the immune response observed in Syrian hamsters. In contrast to Syrian hamsters, SPF white mice do not contain antibodies to SARS-CoV-2 virus before immunization with the vaccine. Equal immune reactivity to that of Syrian hamsters, and purity from antibodies to SARS-CoV-2 virus show the superiority of using white mice in evaluating the immunogenicity of COVID-19 vaccines. The results allow standardization of vaccine immunogenicity at 21 days after the first vaccination, instead of the current 35 days according to the AND.

6. There is a correlation (statistical significance of the difference) between the presence of SARS-CoV-2 neutralizing factor and antibody production [53, 54]. This statistically significant difference was detected at a significance level of $p = 0.05$ with a confidence interval of 0.95 [55]. The degrees of freedom for Student's t-test with two independent samples are k = 8 [56]. The use of t-test is due to small sample power ($n < 30$, $m < 30$). The difference from the Z-criterion is not significant considering the correction of sample averages such as variance or standard deviation. Using the distribution of parameters for the sample elements within the given ranges, we conclude that the difference is statistically significant with a confidence level of $p = 0.95$.

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Information about the authors:

Balzhan Sh. Myrzakhmetova✉ – candidate of biological sciences, head of the laboratory Especially Dangerous Infectious Diseases, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: balzhan.msh@mail.ru; https://orcid.org/0000-0002-4141-7174

Gulzhan A. Zhapparova – master of biology, senior researcher of the laboratory Especially Dangerous Infectious Diseases, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: gulzhan1003@mail.ru; https://orcid.org/0000-0001-5382-831X

Karina B. Bissenbayeva – master of biology, senior researcher of the laboratory Especially Dangerous Infectious Diseases, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: bisenbayeva.karina@bk.ru; https://orcid.org/0000-0001-5788-6074

Aizhan S. Toytanova – master of biology, senior researcher of the laboratory Especially Dangerous Infectious Diseases, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: aizhana-1308@mail.ru; https://orcid.org/0009-0004-9526-3539

Moldir S. Tuyskanova – master of pedagogical sciences, majoring in biology, junior researcher of the laboratory Collection of microorganisms, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: monica_94@list.ru; https://orcid.org/0000-0001-6565-082X

Kuandyk D. Zhugunissov – PhD, head of the laboratory Collection of microorganisms, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: kuandyk_83@mail.ru; https://orcid.org/0000-0003-4238-5116

Lespek B. Kutumbetov – doctor of veterinary sciences, professor, chief researcher of the laboratory Especially Dangerous Infectious Diseases, Research Institute of Biological Safety Problems, Gvardeyskiy, Republic of Kazakhstan. E-mail: lespek.k@gmail.com; https://orcid.org/0000-0001-8481-0673

Contribution: Myrzakhmetova B.Sh. – study planning, conducting experiments, article design; Zhapparova G.A. – conducting experiments; Bissenbayeva K.B. – conducting experiments; Toytanova A.S. – conducting experiments; Tuyskanova M.S. – conducting experiments; Zhugunissov K.D. – conducting experiments, statistical processing of results; Kutumbetov L.B. – study planning, statistical processing of results, assistance in design of the article.

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Информация об авторах:

Мырзахметова Балжан Шайзадаевна✉ **–** канд. биол. наук, заведующая лабораторией «Особо опасные инфекционные заболевания» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: balzhan.msh@mail.ru; https://orcid.org/0000-0002-4141-7174

Жаппарова Гульжан Амировна – магистр биологии, старший научный сотрудник лаборатории «Особо опасные инфекционные заболевания» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: gulzhan1003@mail.ru; https://orcid.org/0000-0001-5382-831X

Бисенбаева Карина Бисенбаевна – магистр биологии, младший научный сотрудник лаборатории «Особо опасные инфекционные заболевания» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: bisenbayeva.karina@bk.ru; https://orcid.org/0000-0001-5788-6074

Тойтанова Айжан Сейткаримовна – магистр биологии, младший научный сотрудник лаборатории «Особо опасные инфекционные заболевания» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: aizhana-1308@mail.ru; https://orcid.org/0009-0004-9526-3539

Туысканова Молдир Сежанкызы – магистр педагогических наук по специальности биология, младший научный сотрудник лаборатории «Коллекция микроорганизмов» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: monica_94@list.ru; https://orcid.org/0000-0001-6565-082X

Жугунисов Куандык Даулетбаевич – PhD, заведующий лабораторией «Коллекция микроорганизмов» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: kuandyk_83@mail.ru; https://orcid.org/0000-0003-4238-5116

Кутумбетов Леспек Бекболатович – д-р вет. наук, профессор, главный научный сотрудник лаборатории «Особо опасные инфекционные заболевания» РГП «Научно-исследовательский институт проблем биологической безопасности» МЗ РК, пгт. Гвардейский, Республика Казахстан. E-mail: lespek.k@gmail.com; https://orcid.org/0000-0001-8481-0673

Участие авторов: Мырзахметова Б.Ш. – планирование исследования, проведение экспериментов, оформление статьи; Жаппарова Г.А. – проведение экспериментов, оформление статьи; Бисенбаева К.Б. – проведение экспериментов; Тойтанова А.С. – проведение экспериментов; Туысканова М.С. – проведение экспериментов; Жугунисов К.Д. – проведение экспериментов, статистическая обработка результатов; Кутумбетов Л.Б. – планирование исследования, статистическая обработка результатов, помощь в оформлении статьи.

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