ABSTRACT

to the thesis of Tuyskanova Moldir Serzhankyzy for the degree of Doctor of Philosophy (PhD) in the specialty "8D05105 Biotechnology"
on the theme: "Development of an animal model to assess the immunogenicity of vaccines against coronavirus infection COVID-19"

General characterization of the work

This dissertation focuses on the investigation of the properties of the COVID-19 virus. It includes the isolation of the virus and the examination of various animal cell cultures' sensitivity to SARS-CoV-2. The study also explores the manifestation of coronavirus infection disease pathogenesis in different types of laboratory animals. The objective is to develop a biological model for evaluating the efficacy of potential vaccines or antiviral treatments. Additionally, this research aims to select the most effective method of causing coronavirus infection in laboratory animals and assess the immunogenicity of vaccines against the infection.

Relevance of the research topic

In the first half of the previous century, the Coronaviridae family's initial representatives were identified. While the virus posed significant challenges to veterinary medicine, the scientific community did not consider it a major issue for epidemics. It only became a concern in 2002 with the discovery of severe acute respiratory syndrome coronavirus (SARS-CoV) in humans. The virus's natural reservoir was found to be bats (Chiroptera, Microchiroptera), which silently carried the virus, spreading it through saliva, urine, and feces. These infected smaller mammals, which are a common food source in Southeast Asian countries. The global fatality rate for the epidemic caused by SARS-CoV was 9.6%.

A new challenge emerged for humanity in early December 2019 with another coronavirus outbreak. This began in the central Chinese province of Hubei and had the potential to cause severe primary viral pneumonia in humans. The isolated cause was identified as a member of the Coronaviridae family. The genome of this agent was found to be 50% homologous to MERS-CoV, 79% to SARS-CoV, and 88% to BtRsCoV. Owing to peculiarities in genome structure, it was named severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), the virus causing the infectious disease COVID-19, with a wide range of clinical manifestations.

The disease has now become a global pandemic. This pandemic is inflicting harm on global health and the economy. Presently, efforts are underway globally to develop a vaccine against coronavirus type 2 infection. Suitable biological animal models offering consistent and reproducible results are needed for preclinical vaccine trials.

Study Objectives

Seeking a suitable animal model and obtaining a control strain of the SARS-CoV-2 virus is crucial for evaluating the immunogenicity and efficacy of COVID-19 vaccines. This involves several key steps, including the isolation of the COVID-19 coronavirus infection virus and the study of the sensitivity of different animal cell cultures to the SARS-CoV-2 virus. Additionally, the virus must be adapted in cell

culture, and its pathogenicity must be studied on different animals. Obtaining a control (challenge) virus with a standard infectious titer is also essential. The search for a biological animal model to evaluate the immunogenicity of vaccines against COVID-19 coronavirus infection is a critical part of this process. Furthermore, it is necessary to select an effective administration method of infection capable of inducing coronavirus infection in laboratory animals, determine the infecting doses (ID50) of the virulent virus capable of causing disease in infected animals, and evaluate the immunogenicity of vaccines against coronavirus infection.

Object of study

SARS-CoV-2 virus, different species of laboratory animals, vaccine against coronavirus infection.

Methods of research

The dissertation research utilized both classical and modern techniques from virology, biotechnology, and immunology.

The scientific novelty of the study

The SARS-CoV-2 virus, which is of considerable epidemiological significance in the Republic of Kazakhstan, was successfully isolated and cultured in a lab setting. Its biological characteristics were examined, and the virus itself was stored in the pathogen depository of the Research Institute for Biological Safety Problems. Here, it is utilized in the creation of diagnostic tools and preventatives for the coronavirus infection. Additionally, it serves as a control virus when evaluating the effectiveness of vaccines and antivirals.

A biological animal model has been created to assess the effectiveness of vaccines and antiviral drugs, replicating the pathogenesis of COVID-19 disease with clinical features similar to those in humans.

The safety and effectiveness of "QazVac", the first domestically produced inactivated vaccine against coronavirus, was initially assessed using a biological animal model established during these studies.

Theoretical and practical significance of the research

The theoretical value of this research lies in examining the SARS-CoV-2 virus's pathogenicity in various animal species, thereby enhancing our understanding of COVID-19 pathogenesis. The practical significance involves creating an animal model to evaluate the efficacy of biological and antiviral treatments for this disease.

Main provisions for defense

The SARS-CoV-2 coronavirus was isolated from clinical samples collected from patients in Almaty city, Republic of Kazakhstan, who fell ill with the new COVID-19 infection. The virus's identity was confirmed through molecular genetic techniques using Vero cell cultures. The cultural characteristics of the SARS-CoV-2 virus were examined using various cell cultures to determine the optimal cultivation parameters.

We searched to identify animals sensitive to the SARS-CoV-2 virus that can be used to create a biological model. This model will be used to study the virus's pathogenicity in hamsters, ferrets, and cats and to identify symptoms similar to those in humans. Different animal infection methods were tested, revealing that intranasal

inoculation was more effective. The safety and protective efficacy of the first domestic QazCovid-in® vaccine against coronavirus was assessed using a biological animal model.

Main research results and conclusions

The experimental data collected during our study led us to the following conclusions:

1. The virus was isolated from clinical samples of patients infected with a novel coronavirus, specifically using Vero cell culture. Molecular genetic methods confirmed the isolated sample as a member of the coronavirus family, specifically the SARS-CoV-2 strand. An isolated epidemic SARS-CoV-2 virus case was cataloged and placed in the repository of the Research Institute for Biological Safety Problems under the tentative name "SARS-CoV-2/KZ_Almaty04.2020".

2. The susceptibility of various animal cell cultures to the SARS-CoV-2 virus was investigated. It was discovered that cells from monkeys and pigs demonstrated greater sensitivity to the virus compared to other cells.

3. A study on the biological and physicochemical properties of the SARS-CoV-2 virus was conducted, adapting it to a selected biological system through blind passaging. The findings revealed that the virus's cytopathogenicity stabilizes after five passages, causing a cytopathic effect (CPE) after 24 h at a certain level of infection. This leads to total cell monolayer degeneration within 72–96 h. Notably, the cytopathogenic impact of the pathogen remained consistent across all analyzed passage levels.

4. The pathogenicity of the SARS-CoV-2 virus was examined across various animal species, including mice, puppies, guinea pigs, rats, piglets, kittens, ferrets, and Syrian hamsters. The studies found that the virus did not impact the mice, puppies, guinea pigs, rats, or piglets, whereas Syrian hamsters, ferrets, and kittens were susceptible to infection. Upon intranasal infection with the "SARS-CoV-2/KZ_Almaty04.2020" strain, these susceptible species exhibited clinical manifestations of COVID-19, which included a lack of appetite, weight loss, diarrhea, and temperature variation (hyperthermia and hypothermia). This infection also prompted viral replication and induced an inflammatory reaction in the lungs.

5. An animal model was chosen to evaluate the immunogenicity of vaccines. It was found that Syrian hamsters are particularly susceptible to COVID-19. The "SARS-CoV-2/KZ_Almaty04.2020" strain triggers a severe physiological response in these hamsters, causing symptoms such as reduced appetite, weight loss, diarrhea, and variable body temperature when it is administered intranasally.

6. A virulent strain of the SARS-CoV-2 virus was standardized through 20 serial passages in the lungs of Syrian hamsters. This procedure demonstrated a gradual increase in virus titer to 105.50 TCID50/mL at the 9th passage. Subsequent passages of the COVID-19 pathogen through a susceptible biological model led to a drop in virus titer. By the 17th passage, the cell culture no longer revealed active viruses.

7. The most effective method for inducing coronavirus infection in hamsters was determined. Hamsters were exposed to the virus by three common methods: intranasal, intravenous, and subcutaneous. The study found that intranasal

administration was the most successful in inducing coronavirus infection compared to the other methods. We selected infecting doses of virulent virus capable of causing disease in 50% of infected animals. We tested seven different virus doses on hamsters. An infectious dose of 1000 TCID proved optimal, affecting 50% of the test subjects.

8. The protective effectiveness of the developed Kazakhstan vaccine, QazCovid-in®, was evaluated using hamsters as a biological model. Studies confirmed the absolute safety of the vaccine for Syrian hamsters. In the evaluation of protective efficacy, the vaccine, with an immunizing dose of 5 μg/dose of a specific antigen, successfully shielded the animals from a wild virus at a dose of 104.5 TCID50/mL.

The thesis goal was successfully achieved, with all tasks being completed. The work includes experimental data that has been statistically validated and holds significant value, surpassing the first criterion threshold ($p<0.05$).

Relation of dissertation work with scientific program plan

This dissertation focused on the development of a COVID-19 vaccine and was conducted as part of the scientific and technical program (IRN No. 64356/PCF-MON-RK-OT-20). The work, which spanned from 2020 to 2022, received targeted funding from the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan.

Publications

The dissertation work resulted in the publication of 11 scientific articles. These include two articles in journals recognized by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Higher Education and Science of the Republic of Kazakhstan and 3 articles in journals indexed in the Web of Science and Scopus databases (Q1, Q4). An additional article was published in another journal. Four posters and theses were also presented at international conferences. One patent application related to the work was submitted to the National Intellectual Property Organization.

Structure and scope of the dissertation

The dissertation comprises an introduction, literature review, materials and methods section, results, and conclusion, along with 202 references. Spanning 114 pages, it encompasses 18 tables, 25 figures, and 3 appendices.