ANNOTATION

for the dissertation of Saken Khaidarov submitted for the degree of Doctor of Philosophy (PhD) under the educational program "8D05110 – Virology" on the topic: "Study of the Antiviral Activity of Drugs Against the SARS-CoV-2 Virus *in vitro*"

General Description of the Dissertation Research

The dissertation aims to provide a comprehensive study of the antiviral activity of modern pharmacological drugs against SARS-CoV-2 in Vero E6 cell culture and in an experimental model using non-transgenic laboratory mice.

During the study, the SARS-CoV-2/KAZ/KAZ/B.1.1/2021 strain (Alpha variant) was isolated and subjected to molecular-genetic characterisation, along with morphological and genetic analyses. To assess antiviral activity, the drugs tenofovir alafenamide (TAF), favipiravir, ribavirin, and dexamethasone were used.

Safe cytotoxic concentrations of the compounds studied and the ranges of inhibitory concentrations (IC_{10}–IC_{100}) were determined. A quantitative assessment of the drugs' effects on SARS-CoV-2 replication was performed using real-time PCR (RT-qPCR). A statistically significant reduction in viral load was observed with tenofovir alafenamide, indicating its pronounced antiviral activity *in vitro*.

A comparative analysis of the RdRP gene (ORF1b/NSP12) of the Alpha and Wuhan strains confirmed a high degree of homology and the preservation of targets for antiviral therapy.

Additionally, the effect of recombinant cytokine IL-33 on the activation of the innate immune response in laboratory mice was studied, allowing an evaluation of this protein's potential as an immunomodulatory agent in coronavirus infection.

The results have practical significance for developing new COVID-19 therapy regimens and for creating experimental models to test antiviral drugs.

Relevance of the Dissertation

The COVID-19 pandemic, which began in 2020, revealed the global healthcare system's high vulnerability. The spread of SARS-CoV-2 was accompanied by significant losses and exposed the lack of effective treatments. Despite advances in vaccine development, the need for directly acting antivirals remains a key challenge in modern medicine.

Of particular importance is the study of the antiviral activity of existing broad-spectrum pharmacological agents such as **tenofovir alafenamide (TAF), favipiravir,** and **ribavirin**. The dissertation focuses on experimental investigation of their cytotoxicity and efficacy in the Vero E6 cell culture, as well as on the

interaction of these drugs with SARS-CoV-2 variants isolated in Kazakhstan, including the Alpha strain (B.1.1.7).

Research Aim

The dissertation aims to conduct a comprehensive in vitro investigation of the antiviral activity and cytotoxicity of several drugs against SARS-CoV-2 and to identify possible mechanisms of action at the level of viral molecular replication.

Research Objectives

1. Isolate and perform molecular-genetic identification of SARS-CoV-2 strains circulating in the Republic of Kazakhstan.
2. Conduct a comparative analysis of the nucleotide sequences of the **RdRP** gene (ORF1b/NSP12) between the Wuhan and Alpha strains.
3. Determine the cytotoxic concentrations (CC_{50}, IC_{10}–IC_{100}) of the drugs under study using **CCK8** and **MTT** methods in the Vero E6 cell line.
4. Assess the antiviral effect of **TAF** and other agents in reducing viral load and inhibiting SARS-CoV-2 replication.
5. Study the effect of recombinant **IL-33** on the innate immune response *in vivo* in experiments on WT mice.
6. Develop recommendations on the use of the drugs studied for antiviral therapy.

Objects of the Study

- Recombinant avian influenza virus;
- Protective proteins of **Mycobacterium bovis**;
- A vector vaccine against bovine tuberculosis.

Research Methods

The objects of research are the SARS-CoV-2 virus (Wuhan and Alpha strains, B.1.1.7) and antiviral drugs (tenofovir alafenamide, favipiravir, ribavirin, dexamethasone).
The **research focuses on the mechanisms of antiviral action of the specified drugs, their effects** on viral replication, and the viability of Vero E6 cells *in vitro*.

Scientific Novelty of the Research

For the first time in Kazakhstan, a comprehensive comparison of the antiviral activity of four drugs against a local SARS-CoV-2 strain (B.1.1.7, Alpha) has been carried out.

New scientific results obtained:

- Safe concentration ranges for **TAF** and **TDF** with low cytotoxicity have been established.
- 99% inhibition of viral replication at optimal **TAF** concentrations has been demonstrated;
- A high degree of homology of the **RdRP** gene between the Alpha and Wuhan strains has been identified.
- The immunomodulatory effect of **IL-33** in model animals has been shown for the first time.
- The mechanism of **RdRP** inhibition and lethal mutagenesis has been experimentally confirmed.

Scientific and Practical Significance

The results obtained are essential for developing new treatment regimens for COVID-19 and other RNA-virus infections. The defined dosage ranges and mechanisms of action of **TAF** can serve as a scientific basis for clinical studies. The developed **Vero E6** cell model is recommended as a standard platform for evaluating antiviral drug efficacy.

Main Provisions Submitted for Defense

1. **Tenofovir alafenamide** exhibits pronounced antiviral activity against SARS-CoV-2 with low cytotoxicity.
2. The conservatism of the **RdRP** gene allows it to be considered a universal therapeutic target.
3. **IL-33** enhances the innate immune response and contributes to reducing viral load *in vivo*.
4. The developed *in vitro* model provides a reproducible assessment of the efficacy and toxicity of antiviral compounds.

Main Research Results and Conclusions

As a result of the study, the following main results were obtained:

1. The **SARS-CoV-2/KAZ/KAZ/B.1.1/2021 strain (Alpha variant) circulating in the Republic of Kazakhstan was isolated and characterised**. Molecular-genetic analysis was performed, including determination of the RdRP (ORF1b/NSP12) nucleotide sequence and comparison with the Wuhan-Hu-1 reference strain. A high degree of homology was observed, confirming the stability of the viral genome's target regions.

2. The **morphological properties** of SARS-CoV-2 were studied using electron microscopy, confirming the typical coronavirus virion structure and determining its size range (80–120 nm).
3. The **cytotoxicity** of four antiviral drugs—tenofovir alafenamide (TAF), favipiravir, ribavirin, and dexamethasone—was evaluated in the Vero E6 cell line using CCK8 and MTT assays. Safe concentrations (CC_{50}) were determined and selectivity indices (SI) were calculated, allowing the establishment of optimal dose ranges for further experiments.
4. The **antiviral activity** of the drugs *in vitro* was investigated. Tenofovir alafenamide showed a pronounced inhibitory effect on SARS-CoV-2 replication, reducing viral load by **99%** compared with control. Favipiravir and ribavirin showed moderate efficacy, while dexamethasone did not exhibit a direct antiviral effect but reduced the virus's cytopathic action due to its anti-inflammatory properties.
5. An **experimental protocol** for evaluating antiviral activity *in vitro* was developed and tested, including determination of **IC_{10}–IC_{100}** and construction of dose–response curves, ensuring a reproducible assessment of the effects of the compounds studied on SARS-CoV-2 replication.
6. **In vivo experiments** were conducted on non-transgenic (WT) mice to study the immunomodulatory effect of recombinant **IL-33**. It was established that IL-33 promotes activation of the innate immune response and increases levels of antiviral cytokines, confirming its potential as an adjuvant therapeutic agent.
7. A **model of interaction** between the viral **RdRP** gene and the active substance of tenofovir alafenamide was proposed, explaining suppression of replication through blockade of viral RNA-dependent RNA polymerase activity.

Connection to the Plan of Main Scientific Works

The dissertation was carried out in accordance with the plan of research works of the Institute of Biological and Medical Research and constitutes part of the grant-funded project AP09260672 on the topic: "Study of the antiviral activity of tenofovir alafenamide and other pharmacological drugs against the SARS-CoV-2 virus *in vitro* and *in vivo*" (2021–2023), funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

The studies presented in the dissertation fully correspond to the directions and objectives of the project, including:

1. isolation and characterisation of SARS-CoV-2 strains circulating in the Republic of Kazakhstan;
2. Investigation of the antiviral activity of medicinal compounds in the Vero E6 cell culture.
3. Determination of safe concentrations, selectivity indices, and mechanisms of action of tenofovir alafenamide;

4. Experimental evaluation of the immunomodulatory effect of IL-33 in model animals (in China in 2024).

The results obtained form part of the fulfilment of the project's planned tasks and have contributed to the development of the national scientific base for studying mechanisms of antiviral drug action and for identifying effective means of COVID-19 therapy.

Publications

Twelve scientific works have been published based on the dissertation, including **2** articles in journals indexed in **Web of Science** and **Scopus**, **5** papers in journals recommended by the CCSES RK, and **5** conference abstracts at international conferences.

Scope and Structure of the Dissertation

The work comprises **140 pages** and includes an introduction, literature review, materials and methods, results and discussion, conclusion, a list of references (**131** sources), **9** tables, **48** figures, and **5** appendices.

Approbation of Results

The main results were presented at international conferences:
• Modern Scientific Technology (Sweden, 2023);
• 3rd International Conference on Virology and Infectious Diseases (COVID-19) (Dubai, 2022);
• International Scientific Conference (Warsaw, 2023);
• Asfen Forum 2024 (Almaty, Kazakhstan).

The work is closely connected with **clinical practice**, as its results aim to identify and scientifically substantiate effective therapeutic agents for the treatment of COVID-19. The experimental data obtained on the antiviral activity and safety of **TAF** can be used to develop antiviral therapy regimens and included in recommendations for clinical trials.