POTENTIAL ANTIVIRAL ACTIVITY OF PROTEASE INHIBITORS AGAINST COVID - 19 / SARS - COV – 2: PROTOCOL OF *IN VITRO* STUDY

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Abstract - Currently, targeted therapeutic agents for the treatment of COVID –19 are still not developed, and effective treatment options remain very limited. The penetration of the COVID - 19 / SARS - CoV – 2 coronavirus into cells depends on the activation of their glycoprotein envelope by virus proteases. Thus, the enzymes are excellent targets for antiviral intervention. However, it is currently still unclear which protease(s) contributes to the spread of the virus in the infected host. Due to the fact that protease inhibitors (aminocaproic and tranexamic acid) have shown their antiviral properties earlier, we assume that these inhibitors will block the virus, thereby preventing the virus from entering the cells of the nasopharyngeal mucosa. In order to assess the potential antiviral activity of aminocaproic and/or tranexamic protease against COVID - 19 / SARS - CoV – 2, we have planned an *in vitro* experimental study on a COVID-19 virus model.

Keywords - COVID – 19, SARS-CoV-2, Protease inhibitors, Antiviral activity.

I. INTRODUCTION

Worldwide, the COVID-19 pandemic has continued to have a negative impact and pressure on the healthcare system since its inception [1, 2], but it is also the cause of serious consequences for the global economy [3]. Currently, targeted therapeutic agents for the treatment of COVID –19 have not yet been developed, and effective treatment options remain very limited [4-7]. This circumstance requires the continuation and improvement of not only anti-epidemic measures against the spread of SARS-CoV-2 [8], but also the development of effective treatment methods is important [9, 10].
Due to their antiproteolytic properties, protease inhibitors show their potential for use in antiviral therapy [11-13], in neurosurgery [14, 15] and in the treatment of malignant neoplasms as a preventive measure against metastasis [16].

The antiproteolytic activity of protease inhibitors by its action can affect the preservation of membrane integrity by neutralizing lysosomal enzymes, acting as a membrane stabilizer and antioxidant, except for its main use as a fibrinolysis inhibitor [14, 15].

However, at present it is still unclear which proteases contribute to the spread of the virus in the infected host [4, 17, 18]. In an earlier study, it was claimed that protease inhibitors (camostat and nafamostat) could be used to inhibit SARS - CoV – 2 replication, but the necessary preclinical and clinical studies were not performed [19].
It is also necessary to take into account the fact that an analysis of the available literature shows that the antiviral activity of protease inhibitors, such as aminocaproic and tranexamic acid, has not yet been studied in the world as a specific protease inhibitor COVID - 19 / SARS - CoV – 2.
Based on this, the ability of protease inhibitors (aminocaproic and tranexamic acid) in inhibiting the activity of COVID - 19 / SARS - CoV – 2.

II. MATERIALS AND METHODS

Ethical issues
The study was approved by the Local Ethics Committee of the S.D. Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan (protocol of the Local Ethics Committee No. 14 (120) of 28.10.2021).

Setting
The study will be conducted on the basis of a laboratory of biological safety level 2 and 3 (Almaty, Kazakhstan).

Characteristics of the SARS - CoV – 2 virus
To study the antiviral activity of drugs in vitro, the HCoV-19/Kazakhstan-KazNAU-NSCEDI-481/2020 strain isolated in 2020 (Wuhan variant with D614G and M153T mutations in protein S) of the SARS - CoV – 2 virus at a dose of 100 TCD50/0.2 cm3 will be used.

Justification of the test system
In the study, use cell culture for coronavirus cultivation. The Vero E6 cell culture (ATCC® CRL 1586™) has been adapted for further research on a model of the virus causing COVID-19.

Storage, maintenance and control of test systems
Storage, reproduction (including environmental conditions), control of viability and growth of Vero E6 cell culture (ATCC® CRL1586™) for coronavirus cultivation is carried out according to the ATCC Product Sheet.

Study of antiviral activity
Antiviral activity of aminocaproic and tranexamic acid in in vitro experiments against the causative agent COVID-19 will be determined by therapeutic, prophylactic and virus inhibitory schemes
The study will consist of 4 phases: 1 – preparation of cell cultures; 2 – determination of cytotoxicity; 3 – determination of antiviral activity (therapeutic, prophylactic and virus inhibitory schemes); 4 – data analysis (Table 1).

№	Procedure	Realization
1	Preparation of Vero E6 cell culture	Preparation of the Vero E6 cell line: defrosting, cell dispersion in culture mattresses. Preparation of Vero E6 cell culture for analysis: cell collection, counting of viable cells and cell sieving into 96-well dies.
2	Determination of cytotoxicity	Determination of cytotoxicity of aminocaproic and tranexamic acid on the Vero E6 cell line using MTT test to determine cell viability and proliferation. Interpretation of the results of cytotoxicity of aminocaproic and tranexamic acid on Vero E6 cell culture.
3	Determination of antiviral activity	-Therapeutic scheme: determination of antiviral activity of aminocaproic and tranexamic acid against coronavirus infection COVID-19/ SARS - CoV – 2; -Preventive scheme: determination of antiviral activity of aminocaproic and tranexamic acid against COVID-19/ SARS - CoV – 2 in Vero E6 cell culture; -Virus inhibitory scheme: determination of antiviral activity of aminocaproic and tranexamic acid against COVID-19/SARS - CoV – 2 on Vero E6 cell culture;
4	Data analysis	Interpretation of cytotoxicity results for Vero E6 cell culture and antiviral activity of aminocaproic and tranexamic acid against COVID-19/SARS - CoV – 2 coronavirus infection.

Table 1. Phases of the study of the antiviral activity of aminocaproic and tranexamic acid against COVID - 19 / SARS - CoV – 2 on the COVID-19 virus model in vitro.

Statistical analysis
The data analysis will be performed after the data for the entire sample has been collected, using the SPSS ® v.20.0 software package. The significance level is established at 0.05.

III. DISCUSSION

The aim of the planned study is to evaluate the potential antiviral activity of aminocaproic and tranexamic acid against COVID - 19 / SARS - CoV – 2 on a COVID-19 virus model *in vitro*.

Interest in the study of this issue was due to the fact that some vaccines against SARS-CoV-2 b have been developed, but efforts are still being made to develop vaccines against SARS-CoV-2 [20] with higher efficacy, and some of them are at various stages of development and clinical trials [21-24]. The viral main protease (Mpro, also called 3C-like protease [3CLpro]) is a therapeutic target [25], and the complex, sequential nature of the virus penetration process provides many acceptable targets for drug development [26].

The penetration of the COVID - 19 / SARS - CoV -2 coronavirus into cells depends on the activation of their glycoprotein envelope by virus proteases [27]. The results of previous studies show that various viruses, including Ebola virus, SARS-CoV, MERS-CoV, and influenza virus use host cell proteases to activate their envelope glycoproteins upon penetration [28-30]. Targeting of host factors involved in virus penetration provides an excellent pathway for the development of such drugs due to the limited number of pathways involved [31, 32]. Thus, protease enzymes are excellent targets for antiviral intervention, which needs to be investigated [27].

In addition, the data obtained on the ability to inhibit COVID - 19 / SARS - CoV – 2 viral proteases can be an excellent starting point for the development of broad-spectrum antiviral drugs.

IV. CONCLUSION

After the study, the results obtained expand the understanding of the potential antiviral activity of aminocaproic and tranexamic acid against COVID-19. If this study gives positive results, therapeutic, prophylactic and virus inhibitory schemes for the use of aminocaproic and tranexamic acid will be developed based on the determination of antiviral activity against COVID-19 in an experiment, which will subsequently justify further clinical studies for the development of antiviral drugs of a wide spectrum of action.

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