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PATTERNS OF FORMATION OF CORONAVIRUS INFECTION CAUSED BY SARS - COV -2 AND WAYS TO OVERCOME THEM WITH THE HELP OF A NEW DRUG BASED ON G. LUCIDUM AND ALHADAYA

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ABSTRACT

This article provides a method for correcting coronavirus infection caused by SARS - CoV -2 using a new combination drug based on G. Lucidum and Alhadaya.

Key words: coronavirus, G. Lucidum, Alhadaya, SARS - CoV-2, experiment, nirmatrelvir / ritonavir.

INTRODUCTION

The SARS - CoV -2 virus, related to betacoronaviruses, was first discovered in the Chinese city of Wuhan in 2019 and quickly spread throughout the world. As is known, the virus enters the body through the entrance gate. Introduction into the cell occurs in the presence of receptors for angiotensin -converting enzyme 2 (angiotensin - converting enzyme 2) and TMPRSS 2 (transmembrane protease serine 2), which are expressed on alveolocytes Type II, cells of the small intestine, endothelium of arteries and veins. The mechanism is the enzymatic cleavage of an amino acid from angiotensin II, and the reaction product has a vasoconstrictor effect in acute respiratory distress syndrome (ARDS). Of great importance in the mechanism of damage is a change in the balance of membrane-bound and soluble forms of angiotensin -converting enzyme 2, resulting in a deficiency in the protective action of the enzyme at the tissue level [6, 7]

The key point in the interaction of SARS - CoV -2 with angiotensin - converting enzyme 2 is the enzymatic cleavage of the S protein in the region of the receptor-binding motif (located on the receptor-binding domain) interacts with the extracellular domain of angiotensin -converting enzyme 2 with a high degree of affinity. However, the primary interaction of the virus with the target cell begins with heparan sulfate proteoglycan, which ensures contact between the internal structures of the cell and the components of the extracellular space. Based on this phenomenon, we created a drug based on G. Lucidum and Alhadaya. In previous articles [1, 2, 3, 4] , I pointed out the uniqueness of this compound, but did not fully explain the mechanism of interaction G. Lucidum and Alkhadaya on the course and prognosis of coronavirus infection caused by SARS - CoV -2 were not provided , and therefore we consider pathogenetically justifying the use of a new drug against coronavirus infection and consider it relevant to conduct a study.

The aim of the study. To study the patterns of formation of coronavirus infection and ways to overcome them using a new combination drug based on G. Lucidum and Alhadaya.

Materials and methods of research. To achieve this goal, 200 mature rats of both sexes weighing 250 g were taken. The maintenance of animals, surgical interventions and withdrawal from the experiment were carried out on the basis of the ethical principles declared by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes. The animals were kept in a vivarium with free access to food and water and a natural cycle of day and night. The experiments were carried out under conditions of spontaneous breathing and an ambient temperature of 24-25°C. Virus isolation was carried out in vitro cell culture from a virus-containing sample of clinical material (nasopharyngeal swab). The FLiRT strain was used for intranasal infection of laboratory animals.

All rats (n =200) were divided into 3 groups. Group 1 (n =60, control) – rats infected with coronavirus infection, treated with remdesevir ; Group 2 (n =60, comparison group) – rats infected with coronavirus infection, treated with nirmatrelvir / ritonavir [5]; Group 3 (n =60, main) – rats infected with coronavirus infection, treated with a new drug based on Ganoderma Lucidum and Alhadaya.

Remdesevir a traditional drug, used at a dosage of 20 mg/kg body weight. Nirmatrelvir / ritonavir drug was used at a dosage of 30 mg/kg body weight. Ganoderma Lucidum and Alkhadaya were used at a dosage of 10 mg/kg body weight. All drugs were administered using a probe into the stomach of the animal under study.

Research methods. Biochemical, microbiological and statistical research methods were used.

Biochemical research methods. A general clinical blood test was carried out: the level of hemoglobin, leukocytes, platelets, erythrocytes, leukocyte formula. The level of ferritin, C-reactive protein, creatinine, ALT, AST, LDH activity was also determined, and the level of D - dimer was determined.

Clinical blood test parameters were studied on an automatic hematology analyzer DxH 800 from Becman Coulter (USA). Biochemical blood analysis was carried out using a biochemical analyzer AU-480 Becman Coulter (USA). The coagulogram was performed using an automatic hemostasis analyzer (coagulometer) ACL TOP 300 from Instrumentation Laboratory Co. (USA).

Microbiological research methods. Microbiological studies were carried out (bacteriological culture of sputum, BAL fluid, blood), isolated microorganisms were studied using molecular genetic methods, including determination of class I integrons, RAPD typing, MLVA, genomic sequencing . RAPD typing was performed in combination with the determination of class I integrons of 155 strains (carbapenem-resistant *K. pneumoniae* and *A. baumannii*. A genome- wide sequencing of 18 strains *of K. pneumoniae,* 4 strains *of A. baumannii.*

Clinical isolates were obtained by plating clinical material (BALF, sputum) on 5% blood agar, followed by species identification using MALDI-TOF mass spectrometry (Bruker Daltonics, Germany). The sensitivity of microorganism strains was assessed using the minimum inhibitory concentration (MIC) method in accordance with the recommendations of the European Committee for Testing the Susceptibility of Microorganisms to Antibiotics (European Committee on Antimicrobial Susceptibility Testing, EUCAST).

Statistical research methods. Comparison of two groups when analyzing indicators measured on a quantitative scale and having a normal distribution was carried out using the parametric Student t-test for independent groups. In case of

heterogeneity of data, comparison of two groups was performed using the nonparametric Mann –Whitney test.

Research results. After infection with coronavirus infection, a blood test was performed in rats, where an increased value of C-reactive protein (average value - 126.55 ± 72.34 mg/l), ferritin (732.99 ± 465.96 µg/l), and LDH activity were observed (604.17 ± 253.46 U /l). Among the verified pathogens, *K. pneumoniae predominated* (30.8%) and *A. baumanii* (23.1%). Other pathogens included *Pseudomonas aeruginosa*, *Enterococcus faecilis*, *S. aureus*, *Escherichia coli*, *Streptococcus mitis*. Analysis of the main markers of bacterial infection (levels of leukocytes and neutrophils, procalcitonin) revealed low sensitivity and specificity as markers of the development of bacterial complications.

Table 1.

Hemogram parameters before correction in rats after coronavirus infection.

Hemogram indicators	All rats (n=200)
Leukocytes	9.41 ± 3.94
Red blood cells	4.44 ± 0.54
Hemoglobin	132.2 ± 17.27
Platelets	206.66 ± 71.41
Neutrophils	7.81 ± 3.66
Lymphocytes	1.06 ± 0.52
Monocytes	0.56 ± 0.38

In the biochemical analysis of the blood of rats, an increase in inflammatory blood markers was noted: CRP, LDH, ferritin (Table 2).

Table 2.

Levels of inflammatory blood markers before correction in rats after coronavirus infection.

Indicators	All rats (n=200)
LDH, U / l	604.17 ± 253.46
C-reactive protein, mg/l	126.55 ± 72.34
Ferritin , µg / l	733.0 ± 465.96

In the coagulogram, attention was drawn to an increase in D- dimer in 62.5% of rats: an increase in its level to 1000 ng / ml was observed in 15% of rats, from 1000 to 5000 ng / ml –in 32.5%, from 5000 to 15000 ng / ml –in 3.5%, >15000 ng /ml –in 11.5% of rats.

After correction, the following laboratory parameters were observed in rats of all groups (Table 3).

Table 3.

Hemogram indicators after correction in rats that suffered coronavirus infection.

Indicators	Main group (n=60)	Comparison group (n=60)	Control group (n=60)	Credibility
Leukocytes	8.49 ± 3.22	8.74 ± 4.11	9.22 ± 2.87	$p < 0.05$
Neutrophils	6.69 ± 2.91	6.94 ± 3.04	7.35 ± 3.87	$p < 0.05$
Lymphocytes	0.87 ± 0.23	0.96 ± 0.31	1.02 ± 0.38	$p < 0.01$
Platelets	186.35 ± 67.27	193.92 ± 78.35	202.36 ± 80.14	$p > 0.05$
C-reactive protein	100.94 ± 65.38	111.37 ± 71.24	121.04 ± 71.96	$p > 0.05$
LDH	546.93 ± 211.06	585.5 ± 233.15	594.68 ± 241.47	$p < 0.05$
Ferritin	704.38 ± 347.15	712.5 ± 388.03	722.12 ± 400.72	$p < 0.05$

After correction, normalization of hemological parameters was observed in almost all groups, however, in the main group (G Lucidum and Alkhadaya) these indicators practically decreased to normal ($p < 0.05$).

To identify the characteristics of the microbiome, targeted metagenomic sequencing of the 16S rRNA gene on the Illumina platform MiSeq, according to the Illumina protocol (USA) with subsequent assessment of alpha and beta diversity , also assessed the association of individual microbiome components with the risk of severe COVID-19. Alpha diversity was assessed using the Shannon index; the significance of differences was assessed using the Kraskes-Wallis test (Fig. 1).

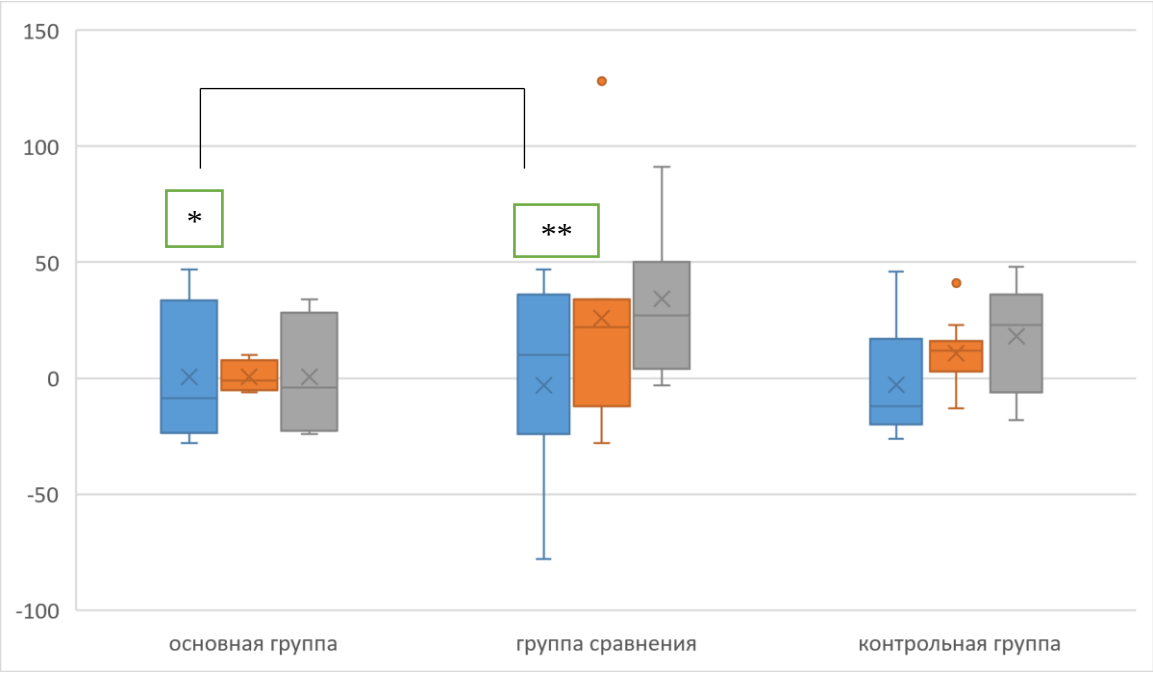

Fig. 1. Alpha diversity of the microbiota of the upper respiratory tract (according to the Shannon index) in rats that have suffered coronavirus infection.

* -Kraskes-Wallis test

Beta diversity was assessed using the “unweighted UniFrac” method, and PERMANOVA analysis was performed to assess the significance of differences between samples of study groups (Figure 2). To compare the representation of individual microorganisms in the structure of microbiomes, one-way analysis using the Mann-Whitney test was used.

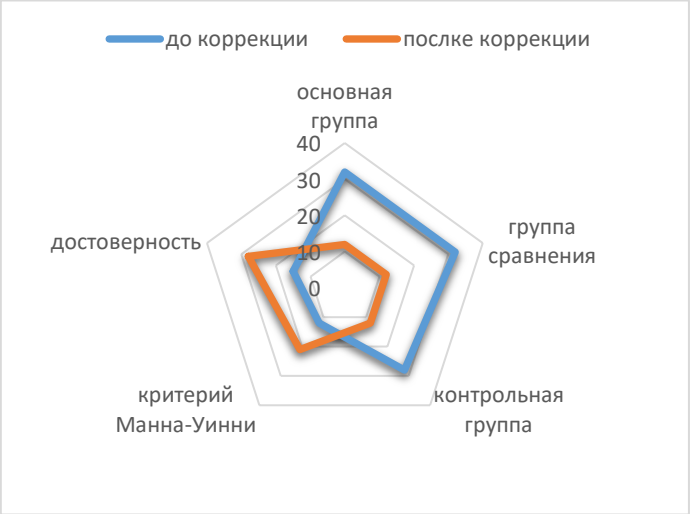

Fig. 2. Beta diversity of the microbiota of the upper respiratory tract in rats exposed to coronavirus infection.

Table 4 presents an analysis of the microbiome before and after drug correction in rats that had suffered coronavirus infection.

Table 4.

Components of the pharyngeal microbiome after correction with drugs in rats that suffered coronavirus infection.

Taxonomic indicator	About the main group	Comparison group	Control group	reliability
Staphylococcus aureus	abs	abs	+	0
K. pneumoniae	abs	abs	+	95% CI = 4.08-8.36
A. baumannii	abs	abs	+	OR = 0.9322147
Pseudomonas aeruginosa	abs	+	+	U test (Mann-Whinney) = 0.9100458

It should be noted that the severe course of COVID-19 is associated not only with a qualitative change in the composition of the microbiome, but also with a change in the representation of certain groups of microorganisms, in particular a decrease in unit weight or the absence *of Akkermansia* pharynx in the microbiome structure *spp., Sneathia spp., Lactobacillus spp.* a decrease in the representation of lactobacilli, with more frequent detection of representatives of the genera *Megasphaera spp.*

Table 5.

Some statistical indicators of coronavirus infection

Indicators	main group	Control group
χ_2 (Pearson test)	0.9185001	0.8210034
U test (Mann-Whinney test)	0.9008417	0.8521073
H test (Kraskes-Wallis test)	0.9341006	0.8438502
W -test Shapiro- Wilk test	0.9120318	0.8230184
95% CI	3.0-6.3	2.4-7.9
OS	0.9572104	0.8207383
- Holm-Bonferroni amendment	0.9130047	0.8018369
r (Spearman correlation analysis)	0.9310082	0.7810284
Hardy-Weinberg equilibrium	0.9013862	0.8109375
Kolmogorov-Smirnov homogeneity criterion	0.9420084	0.8201745
Wilkonson's T test	0.9321025	0.8610043
McNamara M-criterion	0.9400139	0.9331009
Yates Amendment	0.9128459	0.7833214

Discussions. As is known, sequencing of the SARS genome CoV -2 has shown that the main receptor is angiotensin -converting enzyme-2. So, G. Lucidum and Alkhadaya block this receptor through the transmembrane glycoprotein CD 147, which is involved in intercellular recognition, and the neuropilin-1 protein, the product of the NRP 1 gene. The normal mechanism of virus penetration into the cell through the spike protein S, with the help of which the virus clings to angiotensin -converting protein, is disrupted. enzyme 2 on the surface of the cell and penetrates it. Since the virus has a mechanism for suppressing the response (evasion), then G. Lucidum and Alhadaya increases the number of T-regulator effector cells and dendritic cells. The immune response is initially controlled by interferon, IL -21, TGF β, which leads to a switch in the synthesis of antibodies to IgG 1 and IgA 1. The heavy molecule TGF β 1 is released. But with the help of G. Lucidum and Alhadaya, cells migrate into the lung tissue, since this particular drug is specific to protein S or RPD. Since in the human genome among the genes HLA (human leukocytes antigen) there are several combinations of weak genes and the cause of COVID -19 may depend on the ability of the complexes to retain viral proteins, then a new drug based on G. Lucidum and Alkhadaya increases the strength of binding to the virus through the reaction of the immune system, the viral load decreases or even disappears, which leads to the stabilization of the immune system, negating the risk of complications. Also, the new drug can block the B1 and B2 receptors of bradykinin, thereby preventing the development of

acute respiratory distress syndrome. When the new drug interacts with the lungs, we found that the synthesis of hyaluronan synthase 2 decreases or even stops by reducing pro-inflammatory cytokines (IL -1β and TNFα) by macrophages, increasing the area of gas exchange. The number of platelets also increases, which reduces the risk of coagulopathy, and the direct effect of the virus on megakaryocyte cells is eliminated, which is explained by the classical Virchow triad. The concentration of von Willebrand factor, thrombomodulin, and tissue plasminogen activator decreases after using a new drug.

Conclusions. The use of a new drug based on G. Lucidum and Alkhadaya, based on an understanding of the pathogenesis of coronavirus infection caused by SARS-CoV-2 , will allow us to monitor the course and prognosis of the disease, as well as develop new effective drugs for the treatment of COVID -19 and measures to prevent the development of post-Covid syndrome.

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