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IMPROVING THE TREATMENT OF CORONAVIRUS INFECTION COVID-19

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ABSTRACT

This article provides data on a new method of treating coronavirus infection COVID-19 with a mixture of Ganoderma Lucidum and Alhadai. When performing PCR diagnostics, 110 copies / ml were found in the blood of rats on days 5-6, which indicates high levels of the virus in the respiratory tract. The viral RNA detection rate was 95%. Ganoderma Lucidum contains a large number of amino acids, both essential and non-essential, which bind to the protease domain of ACE 2 or to the S-protein of SARS CoV-2 and prevent the two substances from binding. Thus, there is no excessive accumulation of angiotensin II, which, through the activation of AT1R (angiotensin II receptor type I), leads to acute lung injury.

Key words: coronavirus; PCR test; Ganoderma Lucidum; Alhadaya; type II alveolocytes; SARS CoV-2; acute respiratory distress syndrome.

INTRODUCTION

As you know, coronaviruses are single-stranded RNA viruses with a positive strand. This virus has a round or elliptical shape and a diameter of 40-50 nm. Studies in Wuhan, China had 89% nucleotide identity with SARS-like coronavirus associated with severe acute respiratory syndrome (CoVZXC21), which develops in bats and 11% of SARS-CoV [1].

Results from a retrospective cohort study from March 1 to November 21, 2020 assessing mortality rates in 209 US emergency hospitals, which included 42,604 patients with confirmed SARS-CoV-2 infection, showed higher mortality rates among male patients (12, 5%) compared with female patients (9.6%) [8].

Upon entering the human body, viral RNA replication begins with the synthesis of polyprotein 1a / 1b. Transcription occurs through the replication-transcription complex (RTK), which forms double-membrane vesicles, and through the synthesis of subgenomic uni-guide RNAs (sgRNAs) sequences. Termination of transcription occurs in the regulatory transcription sequences located between the so-called open reading frames (ORF), which work as templates for the production of subgenomic mRNA [3].

In a study by Zheng, J et al [10] that assessed respiratory viral load and serum antibody response in COVID-19 patients, salivary viral load was highest in the first week after symptom onset and then declined over time. Most patients develop antibodies (both IgM and IgG) within 10 days of symptom onset.

Since this infection is new to the human body, the antigen contains only epitopes, the serological prevalence specific to this virus will reflect the cumulative attack rate of this virus [7,9].

Studies by Matthias Götte et al [6] confirmed that SARS-CoV-2 binds to the human host receptor via hACE2 (angiotensin converting enzyme), suggesting that it has the same tissue tropism as the SARS virus. Since hACE2 is mainly expressed in type II alveolocytes (AT2) in the lungs, endothelial cells in blood vessels, epithelial cells of the gastrointestinal tract and hepatocytes, this explains the frequent cases of pneumonia, vasculitis, as well as the detection of viral RNA and antigens in the blood [4.5].

Various drugs are currently available that include antiviral drugs (eg remdesivir), anti-SARS-CoV-2 monoclonal antibodies (eg bamlanivimab / etesevimab, casirivimab / imdevimab), anti-inflammatory drugs (eg dexamethasone), immunomodulators (eg baricitinib , tocilizumab). However, these drugs have side effects on the liver and the search for new drugs is an urgent problem today.

The aim of the study. To assess the effect of a new drug based on Ganoderma Lucidum and Alhadaya on the course of coronavirus infection caused by COVID-19.

Materials and research methods. This study was a randomized, double-blind, placebo-controlled study.

The experiments were carried out on 100 sexually mature rats of both sexes weighing 220-250 g. Keeping animals, surgical interventions and withdrawal from the experiment were carried out on the basis of 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 change of day and night. The experiments were carried out under conditions of spontaneous respiration and an ambient temperature of 24-25 ° C. Virus isolation was performed on viro cell culture from a vaccinated sample of clinical material (nasopharyngeal swab). The replication efficiency of the SARS-Cov-2 virus on the cell culture was assessed by the dynamics of the appearance of cytopathic action and the presence of viral RNA in the analysis of the culture fluid by polymerase chain reaction - PCR.

Rats were intranasally infected with the SARS-Cov-2 strain with a 50% mean tissue culture infectious dose (TCID 50) per 50 μl of inoculum (biological product with live cultures) after intraperitoneal anesthesia using 2.5% sodium thiopental solution.

All animals were divided into equal groups:

Group I - (intact) - (n = 25) absolutely healthy animals

Group II (control) - (n = 25), infection with coronavirus infection COVID-19, placebo treatment - 0.9% saline.

Group III (comparison group) - (n = 25), infection with coronavirus infection COVID-19, treatment with remdesevir (reverse transcriptase inhibitor)

Group IV (main group) - (n = 25), infection with coronavirus infection COVID-19, treatment with a mixture of Ganoderma lucidum (Cordy Gold and Alhadaya

In this work, histological and biochemical research methods were used.

Statistical processing of the material was carried out using parametric and nonparametric methods.

Research results. After infection of rats with an experimentally obtained strain of coronavirus infection SARS-Cov-2, PCR diagnostics were performed to confirm the presence of the virus.

When performing PCR diagnostics, 110 copies / ml were found in the blood of rats on days 5-6, which indicates high levels of the virus in the respiratory tract. The viral RNA detection rate was 95%.

Physical examination of the airways using a probe revealed that almost 100% of the rats had edema and hyperemia of the mucous membrane of the airways. Also, palpation in 90% of rats revealed an increase and thickening of the lymph nodes. Hepatomegaly and splenomegaly were also found in 90% of rats. Also, during physical examination, a decrease in appetite was found in all animals (100%). The experimental animals were lethargic; they practically did not react to the change of day and night. Examination of the conjunctiva of the eyes of laboratory animals revealed edema and hyperemia of the mucous membrane.

Biochemical analysis of blood revealed an increase in creatinine by 35%, which was 71.5 ± 1.4 mmol / L in 80% of infected animals. Electrolytes (K +, Na +, Cl-) were increased by 40% (4.9 ± 0.23, 198.0 ± 2.6, 150.0 ± 3.7 mmol / L). In 90% of the experimental animals, an increase in AST was observed by 45% and amounted to 68 U / L. In 92% of the experimental animals, ALT increased by 25% and was 46 U / L. In 90% of animals, there was an increase in total bilirubin by 45% and amounted to 30 U / L. The D-dimer content increased in all animals (100%) and amounted to 350 ng / L (N <250 ng / L).

The level of C-reactive protein in 90% of laboratory animals increased and ranged from 10 mg / l to 15 mg / l.

Table 1 presents data on the incidence of coronavirus infection.

Table 1. Epidemiological indicators of coronavirus infection COVID-19.

Groups	In the active phase of the disease	Total number of cases	Number of deaths
II	24	25	1
III	23	25	1
III	25	25	2

Histological examination revealed signs of inflammatory cellular infiltrates around the bronchioles and blood vessels, an increase in the number of type II alveolocytes, and massive lesions in the epithelium of the nasal mucosa and trachea were also observed (Fig. 1). Also, diffuse alveolar damage was observed in combination with involvement of the vascular bed of the lungs in the pathological process and alveolar-hemorrhagic syndrome. Also, microangiopathy with thrombosis develops in the vessels of the lungs. Lung changes were macroscopically consistent with the concept of "shock lung".

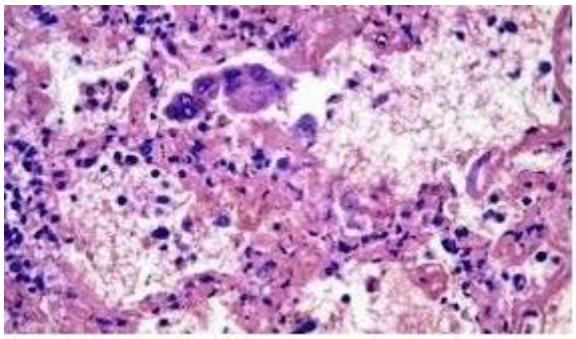

Fig. 1. Histological changes in the mucous membrane of the respiratory tract with coronavirus infection caused by COVID-19. Diffuse alveolar damage (exudative phase); cytotoxic effect of the influenza virus on the cells of the alveolar epithelium with the presence of point eosinophilic inclusions (possibly viral inclusions). Staining with hematoxylin and eosin, x 250.

After the detection of coronavirus infection COVID-19 histological and biochemical methods were introduced to animals of group II 0.9% saline, no positive changes were found.

In animals of group III, after application of remdesevir, 60% of rats showed positive dynamics of treatment. Thus, in 50% of laboratory animals, physical examination of the respiratory tract using a probe revealed a decrease in the degree of edema and not pronounced hyperemia of the mucous membrane of the respiratory tract. Also, palpation revealed a decrease in the size of the lymph nodes in 60% of rats. Also, during physical examination, a normalization of appetite was found in all animals. Physical activity in experimental animals returned to normal. When examining the conjunctiva of the eyes of laboratory animals, 60% showed a decrease in the severity of edema and hyperemia of the mucous membrane. However, 50% of rats still had signs of hepatomegaly and splenomegaly, which was characterized by an increase in biochemical parameters (Table 2).

Table 2. Changes in biochemical parameters before and after using remdesevir.

	n	Before treatment	After treatment	p
Creatinine	23	71,5±1,4 mmol/l	67,8±1,2	<0,05
Electrolytes Na+	20	198,0±2,6 mmol/l	185,0±2,0 mmol/l	<0,05
K^+		4,9±0,23 mmol/l	4,1±0,20 mmol/l	≤0,03
Cl^-		198,0±2,6 mmol/l	175,0±2,1 mmol/l	≤0,05
AST	22	68 Units/l	55 Units/l	
ALT	22	46 Units/l	55 Units/l	
Total bilirubin	20	30 Units/l	22 Units/l	
D-dimer	22	350 ng/l	300 ng/l	
C- reactive protein	23	10 mg/l	8 mg/l	

Thus, 95% CI (confidence interval) in groups III and IV is between 2.4-4.0, which indicates an accurate estimate at $p \leq 0.05$. OR (odds ratio) was 0.9523107 between the use of a new drug based on Ganoderma Lucidum and Alhadaya and the severity of the pathological process in the lungs, $\chi 2$ (Wilkonson test) is 0.93280714, U (Mann-Winie test) is 0.94135082 at $p \leq 0.05$

In animals of group III, after application of remdesevir, there was a partial improvement in biochemical parameters. Since ALT and AST remained high, hepatomegaly and splenomegaly were observed.

Histological examination in animals of group III did not reveal signs of inflammatory cellular infiltrates around the bronchioles and blood vessels, normalization of the number of type II alveolocytes, and also a significant decrease in the lesion in the epithelium of the mucous membrane of the nasal cavity and trachea (Fig. 1). There was also a decrease in the degree of diffuse alveolar damage without involvement of the vascular bed of the lungs in the pathological process.

In animals of group IV, after the use of a new drug based on Ganoderma Lucidum and Alhadai, a positive dynamics of treatment was found. Thus, in 90% of laboratory animals, physical examination of the respiratory tract using a probe revealed the disappearance of edema and hyperemia of the mucous membrane of the respiratory tract. Also, palpation in 90% of rats revealed normalization of the state of the lymph nodes. Also, during physical examination, a normalization of appetite was found in all animals. Physical activity in experimental animals returned to normal. When examining the conjunctiva of the eyes of laboratory animals, 95% showed a decrease in the severity of edema and hyperemia of the mucous membrane. Almost all experimental animals (97%) did not have hepatomegaly and splenomegaly.

Biochemical analysis of blood in animals of group IV revealed a decrease in creatinine by 33%, which was 63.4 ± 1.4 mmol / l in 95% of infected animals. Electrolytes (K +, Na +, Cl-) were reduced by 38% (4.0 ± 0.23, 143.0 ± 2.6, 100.0 ± 3.7 mmol / L). Normalization of AST was observed in 90% of the experimental animals, which was 46 U / L. In 92% of the experimental animals, ALT normalized, which was 46 U / L. In 90% of animals, there was a decrease in total bilirubin by 45% and was 4 U / L. The D-dimer content increased in all animals (100%) and amounted to 250 ng / L.

The level of C-reactive protein in 90% of laboratory animals decreased and amounted to 3 mg / L.

Histological examination in animals of group IV also did not reveal signs of inflammatory cellular infiltrates around the bronchioles and blood vessels, normalization of the number of type II alveolocytes, and, also, the disappearance of

lesions in the epithelium of the mucous membrane of the nasal cavity and trachea. The disappearance of diffuse alveolar damage without involvement of the vascular bed of the lungs in the pathological process was also observed.

Discussion. The use of a new drug based on Ganoderma Lucidum and Alhadaya expands the horizons of treatment for coronavirus infection caused by COVID-19. It is known that angiotensin converting enzyme 2 (ACE2) is a membrane protein of the carboxypeptidase family. In the lungs, ACE2 is expressed in type II alveolocytes and it cleaves angiotensin 2 into angiotensin 1, which activates the MAS receptor. It is also known that angiotensin 1 is an endogenous ligand for the G-protein-coupled Mas receptor, which is expressed on the surface of bronchial smooth muscle cells and alveolar epithelium and suppresses various side effects of angiotensin 2.

Angiotensin-converting enzyme 2 serves as a functional receptor for the entry of the SARS-CoV 2 virus into the target cell. Since the S-protein binds to ACE2 on the cell membrane and attaches to target cells through polar interactions, in the region of the site with protease activity, it has both a vasoconstrictor effect and plays a role in the development of acute respiratory distress syndrome, i.e. the one that provides the separation of 1 amino acid from angiotensin II, the new drug based on Ganoderma Lucidum and Alhadaya blocks the connection of the SARS-CoV 2 S-protein with ACE2 by adding essential amino acids to angiotensin II. Since some amino acids can be replaced in the angiotensin converting enzyme 2 fragment, which binds to the S-protein of SARS CoV-2, to reduce the likelihood of their combining. And since Ganoderma Lucidum contains a complex of amino acids in its composition, they have the greatest efficiency to bind to the protease domain of ACE 2, or to the S-protein SARS CoV-2 and do not allow two substances to bind. Thus, there is no excessive accumulation of angiotensin II, which, through the activation of AT1R (angiotensin II receptor type I), leads to acute lung injury.

Conclusions. Thus, the use of a new drug based on Ganoderma Lucidum and Alhadaya in the treatment of coronavirus infection caused by COVID-19 is justified, since due to the complex of amino acids, there is no excessive accumulation of angiotensin II, which leads to the normalization of biochemical and histological parameters.

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