Aprotinin - a new multi-target drug candidate or "magic shotgun" for the therapy of COVID-19

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ABSTRACT

Aprotinin showed high efficacy and safety in a prospective study of combination therapy for hospitalized patients with moderate to severe COVID-19 pneumonia.

INTRODUCTION

In December 2019 in Wuhan, China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the multisystem inflammatory syndrome COVID-19, was first discovered.

The WHO declared on 11 March 2020 the outbreak of COVID-19 a pandemic. As of 19 September 2020, more than 30.5 million cases have been reported in 188 countries and territories, resulting in more than 952,000 deaths; more than 20.8 million people have recovered [1].

Favipiravir (Avifavir) [2-5] and remdesivir (Veklury) [4, 6] were the first known antiviral drugs repurposed for the treatment of COVID-19 and temporarily registered by the Russian Health Ministry [7] and the US FDA [8] respectively.

Timely use of avifavir (<10 days after infection) can, as a rule, prevent the development and transition to a severe form of COVID-19 [5].

On August 28, 2020, the FDA expanded the Emergency Use Authorization of the investigational antiviral Veklury® (remdesivir) to treat all hospitalized patients with COVID-19, in addition to the previous authorization for patients hospitalized with severe COVID-19 [9]. However, the effectiveness of this drug is highly questionable. The first randomized, placebo-controlled trial of remdesivir among patients with COVID-19 was conducted in China, although it was not able to complete enrollment of participants to meaningfully assess effectiveness [10]. Another randomized, double-blind, placebo-controlled clinical study of 237 hospitalized patients with severe COVID-19 also found no clinical benefit of remdesivir versus placebo [10]. A randomized open-label study of 397 hospitalized patients with confirmed SARS-CoV-2 infection and pneumonia showed no significant difference between a 5-day course (200 patients) and a 10-day course (197 patients) of remdesivir. Furthermore, the lack of a placebo-controlled study made it impossible in principle to determine the effectiveness of the study drug. [11]. A randomized, open-label, multicenter study was conducted to evaluate the efficacy and side effects of remdesivir given to 596 hospitalized patients with moderate severity of COVID-19 for 5 or 10 days, compared with standard treatment [12]. According to J. Bloom [13] the conclusions of the authors of this study [12] were a bit strange: "Among patients with moderate COVID-19, those randomized to a 10-day course of remdesivir did not have a statistically significant difference in clinical status compared with standard care at 11 days after initiation of treatment. Patients randomized to a 5-day course of remdesivir had a statistically significant difference in clinical status compared with standard care, but the difference was of uncertain clinical importance ." The abovementioned clinical studies of remdesivir have shown that the effects range from nil to moderate. It is hard to see how hopes that remdesivir might be the silver bullet to defeat COVID-19 will ever be realized [13].

The current treatment of COVID-19 is mostly supportive, and respiratory failure due to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) is the leading cause of death.

Given that COVID-19 poses a serious threat to public health and the economy around the world, it seems advisable to search for new effective drugs for the prevention and therapy of SARS-CoV-2 and COVID-19, the number of which is still extremely limited.

SARS-CoV-2 uses an angiotensin converting enzyme 2 (ACE2) receptor, which is highly expressed in the lungs, kidneys, gastrointestinal tract, liver, vascular endothelial and arterial smooth muscle cells. COVID-19 is a multisystem inflammatory syndrome as all these organs and systems are potential targets for SARS-CoV-2 infection [14-16]. Therefore, the successful treatment of COVID-19 is possible only with the use of the combination therapy, including the use of typical multi-target drugs.

C-reactive protein (CRP) is the one of important biomarkers for predicting the severity of COVID-19.CRP is produced by the liver. Its level rises when there is inflammation in your body. This damage causes inflammation that the body tries to heal by sending a "response team"of proteins called "acute phase reactants". CRP is one of these proteins. CRP is a valuable marker to anticipate the possibility of aggravation of non-severe adult COVID-19 patients, with an optimal threshold value of 26.9 mg/L. Compared with non-severe patients, the aggravated patients had much higher levels of CRP (median 43.8 [12.3–101.9] mg/L vs 12.1 [0.1–91.4] mg/L). Higher plasma CRP level indicated severe COVID-19 pneumonia and longer hospitalization [17, 18].

The concentration of D-dimer in the blood along with CRP is the second important biomarker for predicting the possibility of exacerbation in COVID-19 patients. This is a biomarker of importance in suspected thromboembolism (VTE). Some recent research demonstrates that if a patient with COVID-19 has high D-dimer levels when admitted to hospital, the risk of death is elevated [23]. Increased concentration of fibrinogen and D-dimer in the blood of patients activates hypercoagulation. Fibrinogen is cleaved by thrombin to form a soluble fibrin monomer that non-covalently collects to form a polymer. The latter is covalently cross-linked by factor XIIIa into fibrin polymers to form an insoluble clot, which is further cleaved by plasmin to form fibrin degradation products (FDP). Among these FDPs is the D-dimer, the presence of which is due to the formation and degradation of the fibrin clot in vivo. Elevated levels of crosslinked fibrin degradation products are found in conditions of activated coagulation, including disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT), pulmonary embolism, surgery, cancer, and cirrhosis. Fibrinogen and especially D-dimer are useful tests when evaluating a patient with suspected DIC syndrome. In addition, D-dimer is commonly used to diagnose or rule out thrombotic events such as deep vein thrombosis or pulmonary embolism. [19-22].

Patients infected with COVID-19 requiring orotracheal intubation (OTI) with higher levels of D-dimer have an increased risk of developing pulmonary embolism (PE) [24]. The increasing odds of in-hospital death is associated with d-dimer greater than 1 µg/mL on admission [25]. In the several studies authors have suggested an association between COVID-19 pneumonia and venous thromboembolism (VTE) [26-27]. It was suggested that the prevalence of VTE was 25%, with a sensitivity, specificity and negative predictive value of D-dimer cut-off value of 1.5 µg/mL [27].

The use of low-molecular-weight heparins significantly reduces the number of deaths. However, an unfavorable prognosis is already observed with the progression of the disease, mainly in patients with a severe form of the disease [28].

Hypercoagulability syndrome is directly related to the development of a "cytokine storm", which is a consequence of the simultaneous avalanche-like activation in the blood of COVID-19 patients of multidirectional proteases, the kinin-kallikrein system, and pro- and anti-inflammatory cytokines, primarily the pro-inflammatory cytokines interleukin-1 and interleukin-6. To suppress the cytokine storm, monoclonal inhibitors of interleukin-1 and interleukin-6 are used, but these have a narrowly targeted effect [29]. For the same purpose, steroid hormones are used as immunosuppressants, but they have a narrow range of clinical applications, which is more justified at the onset of the development of a "cytokine storm". Under these conditions, to prevent the progression of systemic microcirculatory thrombosis and an avalanche-like increase in the "cytokine storm", the use of protease inhibitors with a broad spectrum of proteazolytic activity is promising. [30].

In our opinion, aprotinin is an excellent candidate for the therapy of the multisystem inflammatory syndrome COVID-19. Aprotinin is a natural proteinase inhibitor obtained from bovine lung and has a long history of clinical use since the 1960s, as well as a good safety profile [31]. First, aprotinin is a nonspecific inhibitor of the serine proteases - especially trypsin, chymotrypsin, plasmin, and kallikrein. The inhibition of kalikrein leads to inhibition of factor XIIa formation, inhibition of the intrinsic pathway of coagulation, fibrinolysis, and thrombin generation, and to the attenuation of the pro-inflammatory response [32-34]. Second, aprotinin inhibits transmembrane serine protease 2 (TMPRSS2), a host cell protease responsible for cleaving and activating the SARS-CoV-2 S protein. That is, aprotinin inhibits the penetration of SARS-CoV-2 into cells [35-37]. These data led us to hypothesis that aprotinin is a typical multi-target drug or "magic shotgun" [38] and could represent an efficient treatment for moderate and severe forms of COVID-19. Therefore, we aimed to assess

the use of aprotinin in patients who were admitted to hospital for moderate and severe forms of COVID-19 with symptoms indicative of worsening respiratory function.

METHODS

The prospective study "Aprotinin therapy of COVID-19 infection in hospitalized patients with moderate to severe COVID-19 pneumonia" was conducted May 08 - June 27, 2020 at Hospital No. 4 of I.M. Sechenov First Moscow State Medical University. Study Protocol No. 09-20 of 05/07/2020 was approved by the Local Ethics Committee of I.M. Sechenov First Moscow State Medical University 05.07.2020.

The clinical study included 23 patients, comprising 14 men and 9 women with an average age of 60.7 ± 8.3 years. The eligible patients included hospitalized men and non-pregnant women aged 18 years or older who signed the informed consent form. Nine patients (39%) were admitted to hospital on the basis of a positive PCR test, and 14 patients (61%) on the basis of computed tomography and clinical picture data. The study included patients with a moderate (70%) and severe (30%) COVID-19 severity. Of the patients, 20 (87%) had at least one of the predictors of an aggravating prognosis of the disease (PAPD) and the factors of poor prognosis (FPP) for COVID-19. There were 12 patients (52%) over 60 years old, 11 of whom (48%) had PAPD and FPP for COVID-19. The severity of changes in viral pneumonia and the nature of the lesion of the lung parenchyma was observed: in 10 CT-2 patients (43.5%) (zones of compaction of the "ground glass" type with damage to 25-50% of the lung parenchyma); in 11 CT-3 patients (47.8%) (areas of compaction of the "ground glass" type and consolidation with lesions of 50-75% of the lung parenchyma) and in 2 CT-4 patients (8.7%) (areas of compaction of the type "ground glass" and consolidation in combination with reticular changes with lesions of ≥75% of the lung parenchyma).

The patients were more likely to have cardiovascular diseases: ischemic heart disease (IHD), exertional angina FC II, and angina pectoris FC II - 43.2%, angina pectoris FC III - 13.04%. Atherosclerosis of the coronary arteries was diagnosed in 65% of the patients. The incidence of cardiovascular diseases in the patients over 60 years old was 87%. Stenting of the coronary arteries had occurred in 4% of the patients, while another 13% had experienced episodes of atrial fibrillation. Diabetes mellitus type 2 was found in 17.4% of patients, more often in men - 75%. Class 1 and Class 2 obesity was noted in 26% of the patients. Chronic bronchitis and pulmonary

emphysema were suffered by 8.7% of patients, while bronchial asthma was diagnosed in 13% of the patients.

The main clinical manifestations were considered an increase in body temperature above 37.5 ° C, the level of respiratory failure (SpO2 ≤ 95%). Diagnostic criteria were the severity of changes in viral pneumonia and the nature of lesions of the lung parenchyma according to computed tomography.

Among the biochemical signs, C-reactive protein (CRP), D-dimer, lactate dehydrogenase (LDH) – especially distinguished as a sign of the destruction of lung tissue – and the level of lymphopenia were considered as the factors of an unfavorable prognosis according to the multicenter observations [15].

Within 5-7 days, all patients underwent a full range of drug therapy in accordance with the recommendations of the Ministry of Health of the Russian Federation [7]: hydroxychloroquine, azithromycin or mefloquine, azithromycin or lopinavir / ritonavir, recombinant interferon beta-1b, low molecular weight heparins as anticoagulants, supportive care (SOC), non-invasive lung ventilation (NIV), and the therapeutic prone position. After 5-7 days of drug therapy, the patients' condition did not improve. In this regard, all patients were additionally administered aprotinin (Gordox ™). On the first day, 500,000 KIE Gordox ™ (5 ampoules of 10 ml per 250 ml of saline) was injected intravenously, and in the following 3-5 days 1,000,000 KIE Gordox ™ (10 ampoules of 10 ml) per day.

RESULTS

After 5-7 days of the drug therapy recommended by the Ministry of Health of the Russian Federation [7], the patients' condition did not improve. Persistent hyperthermia persisted in 17 patients (74%) and respiratory failure (SpO2 ≤95%) in 16 patients (70%). In all patients, CRP exceeded the norm by 10 times or more, in 7 patients (30%) the level of CRP did not change, in 16 patients (70%) the level of CRP had a persistent tendency to increase. The severity of changes in viral pneumonia in 6 patients (26%) had negative dynamics according to computed tomography in the form of an increase in the area of the lesion and progression of consolidation foci. An increase in the PQ interval according to electrocardiography, or unstable angina pectoris with elements of cardiocoronary spasm and / or atrial fibrillation, against the background of the use of hydroxychloroquine, was recorded in 3 patients (13%), which required a change in the treatment

regimen. Against the background of increasing respiratory failure, 7 patients (30%) were transferred to the intensive care unit (ICU).

As a result of the combined treatment including aprotinin, all patients showed positive dynamics in their clinical picture: the relief of hyperthermia occurred in 2-3 days, hemodynamics stabilized, SpO2 values gradually increased on average from 88% to 96%, and the progression of respiratory failure was stopped in all patients. Respiratory function support was carried out using non-invasive ventilation (NIV) in 16 patients (70%); none of the patients was transferred to mechanical ventilation.

All 7 critically ill patients from the ICU were transferred to the conventional therapy unit after 10 days, and later after 9-71 days were discharged from the hospital.

During treatment with apritinin, all patients showed a significant decrease in CRP parameters: in 4 patients (17.4%), the CRP level decreased by 3.8 times on day 1, and in 6 patients (26.1%), the CRP level decreased on days 2-3 by 2.7 times. The median time to normalization of CRP was 6 days (IQR 6-6).

Against the background of a positive clinical picture with a decrease in hyperthermia, an increase in saturation and a decrease in the CRP level on days 2-3 after treatment with aprotinin, there was a slight increase in D-dimer values in 7 patients (31.8%), which is explained by the activation of thrombolysis under the action of the drug. However, subsequently, the D-dimer level rapidly decreased, and the median time to normalization of the D-dimer CRP was 4.7 days (IQR 3.2-6.8).

Before treatment with aprotinin, 60% of patients had a twofold increase in blood lactate dehydrogenase (LDH> 700 U / L) - the most important marker of cell damage, including pulmonary parenchyma. After the introduction of aprotinin, the LDH level decreased to values less than 340 U / L.

In 3 severe patients (13.6%) with negative dynamics according to computed tomography data and 4 severe patients (18.2%) with CT-3 lung lesion, progression of lung parenchyma lesions was prevented.

There were no deaths among all patients. The length of stay in the hospital was 15.4 days (11-26 days) for 16 patients (70%) in the main group, and 22-79 days for 7 severe patients (30%) (those transferred to the ICU).

DISCUSSION

Results of the prospective study "Aprotinin therapy for hospitalized patients with moderate to severe COVID-19 pneumonia" conducted May 08 - June 27, 2020 at Hospital No. 4 of I.M. Sechenov First Moscow State Medical University, demonstrated the effectiveness and expediency of using aprotinin to prevent the progression of COVID-19 complications, including reducing the manifestations of systemic inflammation. The drug was found to be safe for use, and no allergic reactions to drug administration or clinically significant side effects were observed. Maintaining a sufficient therapeutic concentration of the drug made it possible to reduce systemic inflammation, and to neutralize the adverse effects of the "cytokine storm" and the progression of disease complications. According to the data of computed tomography in dynamics, stabilization was noted, mainly at the CT-2 level, with a decrease in the area of lung-tissue lesions. No lethal outcomes were recorded during the observation period. All patients were discharged from the hospital after treatment.

Conclusion. Aprotinin has demonstrated in the prospective study high potential in combination therapy as an adjunct to standardized COVID-19 therapy. However, for the final verification of the effectiveness and safety of the drug, a full-fledged randomized trial is required, which is scheduled to take place in the coming months.

POTENTIAL CONFLICTS OF INTEREST

The clinical trial was performed at Hospital No. 4 of I,M, Sechenov First Moscow State Medical University.

AUTHORS' CONTRIBUTIONS. TVK, NPM, and MVV were the principal investigators responsible for patient recruitment, study treatment and data collection in accordance with the Protocol. AAI, AAS, VGL, and EVY conceived of this project, proposed a variant of its organization and controlled the progress of its implementation. VGL, and SVP worked out the statistical aspects of the study and analysis of the results. RKK developed a preclinical study of aprotinin and organized its implementation. MAT studied and analyzed the potential market for the combination therapy investigated. AVI was responsible for scientific project coordination and publication editing.

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