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PDSTP Is the First Drug in Class to Treat Coronavirus Infection

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Abstract—The results of a comprehensive study are presented on the development and creation of an original small PDSTP molecule, able to prevent the SARS-CoV-2 coronavirus infection from binding to the host cell. The PDSTP molecule was designed to electrostatically interact with heparan sulfate proteoglycans on the cell surface, and coronaviruses, particularly SARS-CoV-2, use this mechanism as the first stage of interaction with the cell. By blocking this process, it is possible to stop the life cycle of the virus, thus leading to its death. The drug candidate PDSTP, with its unique mechanism of action, is characterized by a very low toxicity and a high safety profile and demonstrates good efficacy in animal experiments.

Keywords: coronavirus, SARS-CoV-2, heparan sulfate proteoglycan, drug development, PDSTP

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Despite numerous studies in the field of virology, the problems of viral respiratory infections, especially those associated with various coronaviruses, have recently become exceptionally important in the context of the SARS and MERS epidemics and the pandemic caused by the SARS-CoV-2 virus. Coronavirus infections are particularly dangerous since they cause severe viral pneumonia associated with significant, often fatal, lung damage.

The human coronavirus was first isolated in 1965. Later, coronaviruses attracted the attention of researchers when an outbreak of atypical pneumonia, or severe acute respiratory syndrome (SARS), was recorded in China in 2002–2003. The disease was caused by the SARS-CoV virus and spread to other countries: a total of 8273 people fell ill, and 775 died (mortality rate was 9.6%). The MERS-CoV virus became the causative agent of the Middle East respiratory syndrome (MERS), the first cases of which were reported in 2012. In 2015, an outbreak of the Middle East respiratory syndrome occurred in South Korea; 183 people fell ill, and 33 died. In December 2019 in China, an outbreak of pneumonia caused by the SARS-CoV-2 virus began, which in 2020 grew into a pandemic that swept the whole world. A significant number of people have died from the pneumonia caused by this coronavirus. Obviously, pandemics of respiratory viral infections associated with coronaviruses plagued humanity in the past, and there is no reason to believe that this will not happen in the future.

The biology of coronaviruses inevitably guarantees the emergence of new pandemic strains, their timing, genome variability, and antigenic properties being unpredictable. In other words, epidemics and pandemics of new respiratory coronavirus infections will always begin in the absence of drugs for their specific immune prevention and therapy. The latter predetermines the need for an early search for and development of pathogenetic agents and methods for the prevention/treatment of respiratory viral infections, based on the characteristics of the biology of coronaviruses. In addition, it is known that immunity after an illness caused by coronaviruses is short lived and, as a rule, does not protect against re-infection, which necessitates the creation of broad-spectrum antiviral drugs that directly protect the body from viral damage.

Of great importance are problems of the participation of glycans in viral adhesion to host cells and their replication. This knowledge can be used to design and develop a broad-spectrum antiviral drug against both known and yet unidentified viruses. Such drugs can become a reserve tool for the pathogenetic therapy of future coronavirus infections. Note that a number of human pathogenic viruses [1], including herpes

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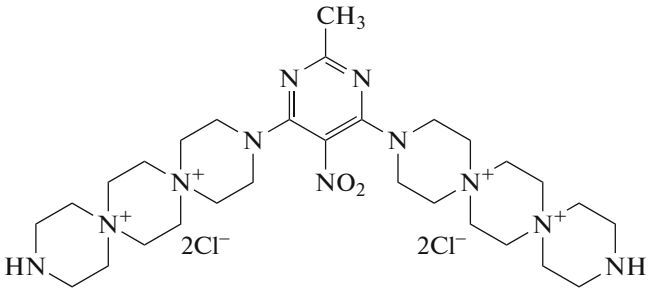

Fig. 1. PDSTP molecule structure.

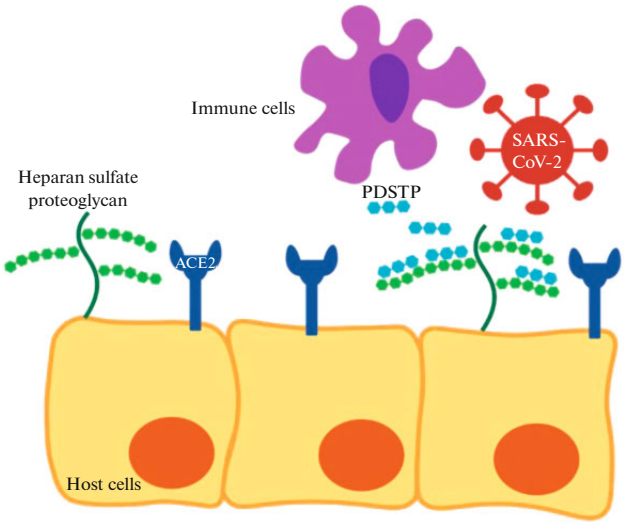

Fig. 2. PDSTP mechanism of action.

viruses types 1 and 2 [2], human papillomavirus [3], cytomegalovirus [4], some types of HIV, respiratory syncytial virus, and coronaviruses [5–7], use a common heparan sulfate–dependent mechanism of attachment to the host cell wall. Hence, blockers of communication between viruses and body cells play an important role in the treatment of viral diseases.

The mechanism of influence on the process of virus invasion into the host cell, based on blocking the adsorption of the virus to the target cell due to the specific blocking of heparan sulfate proteoglycans, is one of the most promising trends in the search for new antiviral compounds. The aim of our study was to find a new drug for the treatment of coronavirus infections, including that caused by the SARS-CoV-2 virus. As a result, the high antiviral activity of (di)dispirotripiperazine pyrimidines was recorded. The di(dispirotripiperazine) pyrimidine derivative PDSTP, designed as a result of work on target-directed drug discovery, can specifically block heparan sulfate proteoglycans located on the cell wall and thus prevent the specific adsorption of viruses to host cells (Fig. 1). This process can be described as blocking the adhesion of the virus to the host cell. Note that the PDSTP drug candidate interacts precisely with the proteoglycans of the host cell, which ensures the breadth and universality of its antiviral action [8].

The mechanism of action of PDSTP [9] seems to be due to the specific property of this compound to bind to heparan sulfate proteoglycans, which leads to a dramatic decrease in the number of virus replications. Attachment of the substance under study is antagonized by heparin, which ensures normal cell interaction with heparin and, as a consequence, the insignificant toxicity of PDSTP (Fig. 2). The target of PDSTP is represented by two sulfate groups located in adjacent saccharide residues. For example, for GlcA2S-GlcNS6S, GlcA2S-GlcNS3S, IdoA2S-GlcNAc6S, IdoA2S-GlcNH23SS6S, IdoA2S-GlcNS6S, and IdoA2SGlcNS3S, a good electrostatic interaction between the negative charge on the sulfate group and the positively charged atoms of nitro-di(dispirotripiperazine) pyrimidines is shown. A similar interaction can also occur with the carbonyl group of the octasaccharide ΔUA-GlcNSIdoUA2S-GlcNAc-UA2S-GlcNS-IdoUA2S-GlcNH23S, which is a necessary site of heparan sulfates for the penetration of the coronavirus into the host cell. Thus, PDSTP blocks key functional groups of heparan sulfate proteoglycans, preventing viral replication and providing high antiviral activity. There are currently no drugs with a similar mechanism of action.

To evaluate the efficacy of PDSTP, we reproduced a SARS-CoV-2 infection model in Syrian hamsters. The virus was titrated in a culture of Vero (B) cells by the number of plaque-forming units and administered to animals intranasally in the amount of 26 μL per hamster at a dose of 4×10^4 $TCID_{50}$. Several groups of animals of the same litter were formed: group I, intact animals (positive control); group II, animals infected with SARS-CoV-2 (negative control); and group III, animals treated with PDSTP at a daily dose of 15 mg/kg intraperitoneally for 5 days after infection from days 3–7 of the infectious process against the background of its manifest signs (treatment group).

During the observation of infected animals, we assessed the manifestations of the symptoms of the disease (sneezing, nasal discharge), the frequency of which did not differ significantly. Animals lost weight. They were taken out of the experiment on the seventh day. At autopsy, the lungs and spleen were removed and weighed, and the specific weight was calculated as a percentage of body weight. The right lung was placed in Petri dishes with saline and subjected to diaphanoscopy to count the number of tissue indurations and foci of hyperemia with hemorrhages in the parenchyma.

It was found that the use of the PDSTP drug prevents the loss of body weight in animals the indicators of which were significantly different from those of the infected control group. In the lungs of infected individuals, viral pneumonia developed with multiple foci of uneven compaction with fuzzy borders of various

sizes, but usually not very large and tending to fuse, especially in the lower parts of the lungs. There were spots on the surface of the lungs of various colors: from light gray and grayish pink to light red and brown. The areas of the lung with a change in color on the section had a granular surface and bulged slightly above the surrounding tissue. In the negative control group, there were practically no areas of normal tissue of a light pink color. The lungs looked compacted, tight–elastic, and edematous. The incision surface was motley, with uneven blood filling. Practically no liquid was squeezed out from the compacted areas during cutting. According to the gravimetric parameters of the organs, PDSTP significantly influenced the formation of viral pneumonia, demonstrating significant differences between the treated animals and the negative control group.

In infected animals, the specific mass of the spleen decreased, apparently reflecting the emerging immunodeficient state. Therapeutic use of the PDSTP drug candidate made it possible to maintain the organ parameters at the level of intact values. It was also noted that PDSTP reduced the number of indurations in the lung tissues, as well as the number of foci of hyperemia and hemorrhage. In the group of animals treated with PDSTP, this effect is visually noticeable and is confirmed by the counting results. In this group, in the lung tissue, there are single foci of compaction with fairly clear boundaries of various sizes, but usually not very large and without fusion. The areas of the lungs with a changed color on the section had a granular surface and did not bulge above the surrounding tissue. Most of the surface had a normal light pink or light red appearance. The lungs looked somewhat compacted, loose–elastic, and slightly edematous. The cut surface was of uniform color and blood filling. When cutting, the liquid was practically not squeezed out.

Safety studies of the drug candidate PDSTP have shown that it has low toxicity and is safe for animals in both single and multiple doses. We have designed, synthesized, and studied the antiviral activity of PDSTP, which has a unique and universal mechanism of antiviral action against SARS-CoV-2. The mechanism of its action provides for a low probability of developing resistance in viruses, a high activity of PDSTP to mutated strains, and a high level of safety.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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