SARS-COV-2 – THE LATEST GLOBAL THREAT AND THE PROSPECT OF COVID-19 THERAPY WITH THE USE OF CHITOSAN

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Abstract

Epidemics of infectious diseases have always been a threat to humanity and have contributed to increased mortality in the affected areas. This also applies to a new species of coronavirus identified in 2019, SARS-CoV-2, which is responsible for the COVID-19 pandemic. Despite preventive measures implemented all over the world to minimise the spread of the pathogen as well as the development of vaccines, which have been approved for emergency use, the situation is still worrying. Moreover, the problem is exacerbated by the lack of targeted treatments for COVID-19 patients. One possible solution is the using preparations based on natural raw materials, including chitosan. This biopolymer is of great interest due to a number of unique biological properties, among which its antiviral effect is a key feature. Hence, this paper presents the application possibilities of chitosan-based solutions in the prevention and treatment of viral diseases, with particular emphasis on COVID-19.

Keywords: *human coronaviruses, SARS-CoV-2, COVID-19, chitosan*

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1. Introduction

Infectious diseases, caused mainly by viruses, bacteria, fungi, protozoa, prions or parasites, have accompanied humans and other living organisms since the dawn of time. They spread through various routes of transmission: direct contact with an infected person (via food, inhalation transmission, inoculation through damaged skin, sexual contact), contact with wild or domestic animals, from mother to foetus during pregnancy or childbirth (vertical infections), through indirect contact (infected objects) and also via carrier insects. The infectious diseases that can cause an epidemic, and often even a pandemic, include HIV/AIDS; herpes simplex; influenza; measles; chicken pox; shingles; hepatitis A, B and C; and viral haemorrhagic fever [1-3].

Currently, from a medical point of view, infectious diseases caused by coronaviruses are also a problem, although they have been outside of mainstream research in virology and epidemiology for many years. This has been due to the widespread belief that coronaviruses induce symptoms of a mild cold that do not require medical intervention. This view was exacerbated by the lack of specialised diagnostic methods and targeted therapy. At that time, the main techniques for identifying pathogens were multiplication on cell lines and antigenic differentiation using the electron microscope [4-6]. This state of knowledge was maintained until 2003, when, along with the development of laboratory techniques enabling the detection of microorganisms, the highly pathogenic HCoV-SARS virus was identified, and in 2012, HCoV-MERS was found [7, 8].

Numerous studies from recent years indicate that there is a huge group of animal viruses that pose a potential threat to humans due to the possibility of crossing the species barrier. It was, therefore, no great surprise that in December 2019, in the Chinese city of Wuhan, another zoonotic coronavirus appeared – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – causing a respiratory disease called COVID-19 ('Co', *corona*; 'vi', *virus*; and 'd', *disease*) [9, 10].

Despite the use of experimental therapies with vitamins and antiviral drugs as well as vaccines, the COVID-19 pandemic continues, and in some countries, such as India, the situation is very worrying. Therefore, researchers around the world are still looking for optimal therapeutic solutions to mitigate the course of SARS-CoV-2 infection. Due to a number of unique biological properties, chitosan has also recently become extremely popular.

This article aims to characterise the *Coronavirinae* subfamily, focusing on the SARS-CoV-2 virus and the course of the infection, symptoms and treatment methods. In addition, the potential application possibilities of chitosan in the prevention of SARS-CoV-2 infection and the treatment of COVID-19 were reviewed.

2. Characteristics of Coronaviruses

2.1. Taxonomy

Coronaviruses are a large group of viruses belonging to the *Nidovirales* order, the *Coronaviridae* family and the *Coronavirinae* subfamily, among which we distinguish four genera (*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*) and many species capable of infecting animals and humans (HCoVs) (Figure 1) [11].

The type of receptors used during cell penetration and the genetic differences of species have been the basis for distinguishing subgenera (lines) A, B, C and D within each type.

Alpha- and betacoronaviruses infect various species of mammals, including humans, and their main natural reservoir is bats. In humans, the disease usually takes the form of infections of the respiratory tract; in animals, the infection mainly manifests as gastrointestinal inflammation, for example, infection with the transmissible gastroenteritis coronavirus

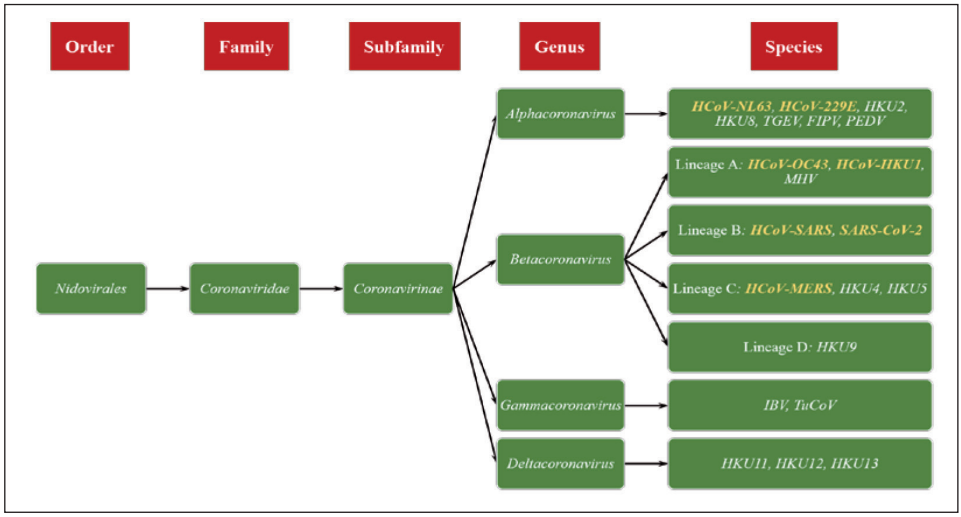

Figure 1. Classification of coronaviruses (yellow font indicates human pathogens). This figure has been adapted from previous studies [6, 12-14]

(TGEV). On the other hand, wild birds are the reservoir of pathogens of the gamma and delta genera. As a result of breaking the species barrier, these viruses have been transferred to domestic birds and to some species of mammals, such as marine mammals [15-17].

For this reason, the problem of coronavirus transmission from one host to another is particularly important in terms of the emergence of new species that are highly pathogenic for humans, as has been the case with HCoV-SARS, HCoV-MERS and SARS-CoV-2 viruses. Currently, it is believed that the above species of microbes come from the bat, but other wild or domestic animals have acted as an intermediate host and have allowed the virus to be transmitted to humans. The most likely route of HCoV-SARS transmission is a palm civet (*Paguma larvata*), in the case of HCoV-MERS the role of the carrier is played by dromedary camels and for SARS-CoV-2 the intermediate host is probably the Malayan pangolin (*Manis javanica*) [18-20].

2.2. Molecular Structure and Genome Organisation

Coronaviruses have been classified as enveloped viruses whose helical genome is composed of non-segmented, single-stranded, positive-sense RNA (+ssRNA). Due to the virion size (80-220 nm) and genome length (26-32 kb), they are among the largest known RNA viruses so far [6, 21].

These pathogens owe their name, first proposed in 1968, to the appearance of a virion, which is a single complete viral particle. The shape of the virion resembles a crown (Figure 2) made of surface protrusions, which are formed by the spike protein (S) responsible for interaction with the receptor on the cell surface. The spikes have the S-1 glycoprotein in their upper parts, which, by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, allows the pathogen to bind to the surface of the host cells. In turn, the type II transmembrane serine protease (TMPRSS2) and the S-2 glycoprotein (located in the lower parts of the spines) allow the virus to enter these cells. In this way, the coronaviruses can attack various cells that contain ACE2 receptors, causing damage to, for example, tissue lining blood vessels and organs such as lungs, heart, kidneys or testes [6, 22].

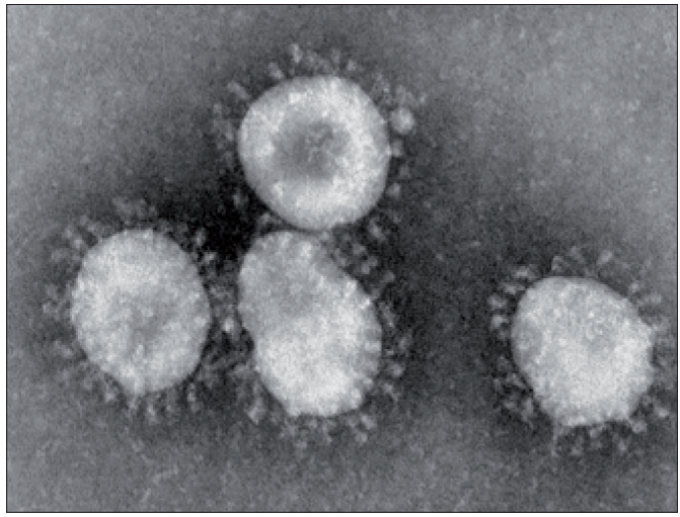

Figure 2. Electron microscopy image of HCoV-229E (Authors: Dr Fred Murphy and Sylvia Whitfield. This photo comes from the Centers for Disease Control and Prevention; Public Health Image Library (PHIL), identification number 10270) [23]

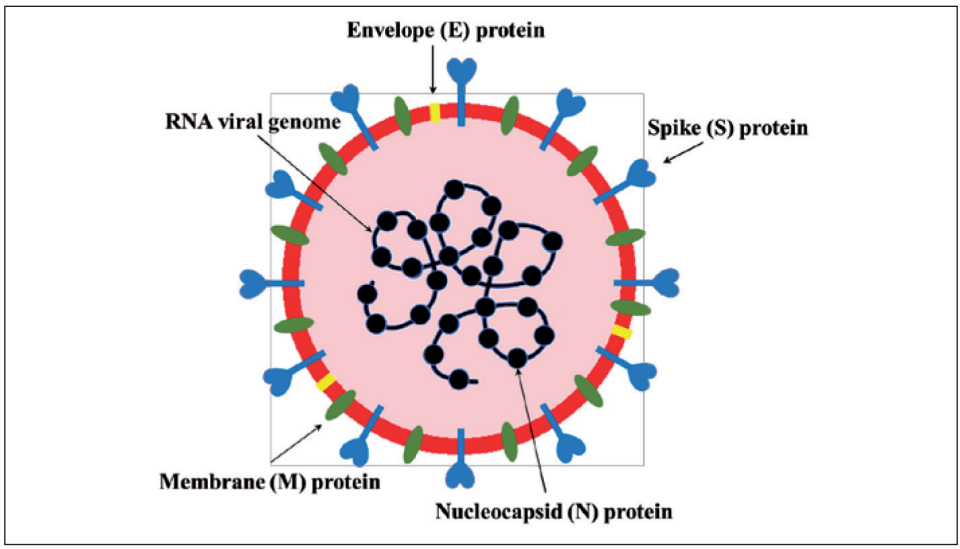

Figure 3. Structure of the coronavirus virion (adapted from [26])

In addition to the S protein, the virion capsid consists of two additional structural proteins: the envelope (E) protein and the membrane (M) protein, as shown in Figure 3.

The E protein is responsible, among others, for the formation of virions, while the M protein is the basic viral matrix protein. In the case of some coronaviruses, the capsid also includes haemagglutinin-acetylesterase (HE) glycoprotein, which, according to the literature, was introduced into the genome of coronavirus ancestors as a result of recombination with the messenger RNA encoding HE of influenza C. The HE protein

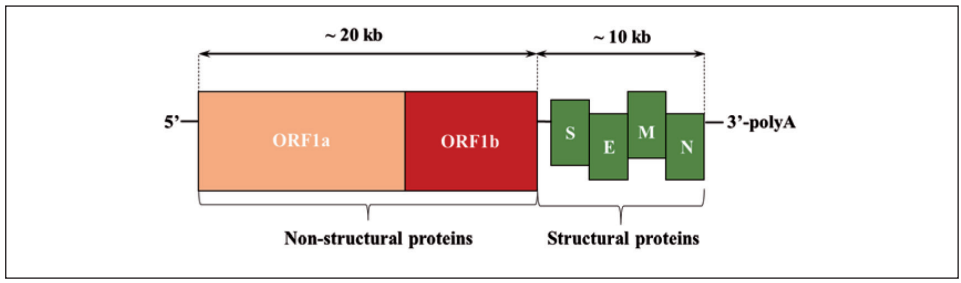

Figure 4. Schematic organisation of the coronavirus genome (adapted from [6])

exhibits haemagglutinin properties, binds sialic acid on the cell surface and shows acetylesterase activity, thanks to which the virus more easily penetrates and spreads on mucous membranes. In addition, the coronavirus genome, together with the nucleocapsid (N) protein, forms a ribonucleoprotein folded into a tight helix [6, 24, 25].

All coronaviruses have a similar genome organisation (Figure 4) with at least six open reading frames (ORFs).

At the 5' end there is a methylated cap and at the 3' end there is a polyadenylated tail (polyA). The main part of the viral RNA (~ 20 kb) is occupied by two frames: ORF1a and ORF1b, translated into two large polyproteins (pp1a, pp1ab), which, as a result of autoproteolysis, are broken down into 15-16 non-structural proteins that are the essence of the replication process. These proteins also play an important role in suppressing host proteins, blocking the immune response and stabilising RNA. The remaining one third of the RNA length (~ 10 kb) is the coding area of the so-called structural proteins (S, E, M and N). Moreover, in some coronavirus species, in addition to the four major structural proteins in the genome, there are sequences encoding additional proteins, such as the HE protein, the 3a/b protein and the 4a/b protein [6, 27, 28].

2.3. Coronavirus Species Capable of Infecting Humans

The first records of human coronavirus infections date back to the beginning of the 1960s. In 1965, scientists identified the HCoV-B814 virus, which was isolated from a child showing symptoms of a mild infection of the upper respiratory tract. Unfortunately, due to the loss of the sample and the lack of specialised research methods, the species affiliation of the isolate was not determined [29].

Over the next 2 years, new isolates were obtained: HCoV-229E (1966) and HCoV-OC43 (1967); they were assigned to alpha- and betacoronaviruses, respectively. To determine the picture of infection with human coronaviruses, studies were carried out on a group of healthy volunteers who were knowingly infected with pathogens. As a result of the observation, only symptoms of a mild cold lasting up to 7 days were noted. In the case of HCoV-229E infection, the most common symptoms are runny nose, mucosal congestion, sore throat, a feeling of weakness and, occasionally, cough, and about 30% of infections are asymptomatic. A similar clinical picture is observed in HCoV-OC43 infections, with cough being more common. However, in children, the elderly and people with reduced immunity, these pathogens can often lead to an exacerbated disease manifested by bronchitis, subglottic laryngitis, and even pneumonia [5, 6, 29].

Human low-pathogenic coronaviruses that have been identified so far also include HCoV-NL63 (alphacoronavirus) and HCoV-HKU1 (betacoronavirus). HCoV-NL63 was first isolated in Amsterdam from a 7-month-old girl with respiratory symptoms, while the

second pathogen was from a 71-year-old male with symptoms of pneumonia who had returned from China (Shenzhen). As a result of many years of observation, it was found that HCoV-NL63 infection is usually mild, with symptoms such as fever, cough, sore throat and rhinitis, and mainly affects children up to 5 years of age and immunocompromised people. Additionally, in over 70% of cases the infection occurs as a co-infection with other pathogens, such as human rhinovirus, enterovirus and parainfluenza viruses. In the case of HCoV-HKU1 infection, similar symptoms are observed, with approximately 50% of patients additionally experiencing febrile seizures [29-32].

The above-mentioned four species of coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) are endemic all over the world, although in the temperate climate in the autumn-winter season there is an increase in infections caused by these pathogens. Moreover, in some regions – for example, Hong Kong – the peak incidence falls during the spring-summer period [15].

In addition to the presence of low-pathogenic coronaviruses, scientists have also identified microbes of an epidemic nature, including HCoV-SARS. The first case of infection with this virus was recorded on 16 November 2002, in a man from Foshan (Guangdong Province, China) who presented with respiratory symptoms and fever. Transmission of the virus was very rapid and during the epidemic (2002-2003), the pathogen made 8,422 people sick in 32 countries and regions, and 919 people died (mortality rate ~10%). The course of virus infection was characterised by two stages. The first phase of the disease comprised increased body temperature, chills, muscle pain, weakness and diarrhoea, which are typical flu-like symptoms. In the second stage, pneumonia and hypoxemia developed [5, 7, 15].

In June 2012, a new species of coronavirus – HCoV-MERS – was detected, and the first reported case was of a man from Saudi Arabia [33]. This time there was no worldwide epidemic, but the threat has not passed – the number of infections and deaths is increasing year by year. By September 2019, according to data from the World Health Organization (WHO), 2,468 cases of infection were recorded (of which 2,077 were in Saudi Arabia), including 851 deaths (mortality rate is ~35%). The clinical picture of the infection is heterogeneous. The disease may be asymptomatic or lead to rapidly progressive pneumonia, and in people with comorbidities (diabetes, obesity, end-stage renal disease) it may even lead to death [15, 34, 35].

The current global problem is SARS-CoV-2.

3. The Latest Threat: SARS-CoV-2

SARS-CoV-2, which causes the disease called COVID-19, is the newest among the seven species of coronavirus identified so far; it is highly pathogenic for humans. The first SARS-CoV-2 virus infection was identified in Wuhan (Hubei Province, China) on 8 December 2019, but the WHO was not notified until 31 December 2019. However, there are reports of dating the first cases of infection with the virus as 17 November 2019 [36, 37].

SARS-CoV-2 has turned out to be a highly contagious virus, and its transmission could have been favoured by resistance to environmental factors and increased migration of people at that time due to the celebration of the New Year in China and travels to this part of the world. For this reason, the further spread of the virus beyond China's borders became only a matter of time, leading to the outbreak of the pandemic, which was announced on 11 March 2020 by the WHO [37].

Initially, outside of China, the most dangerous situation from an epidemic point of view occurred in Italy and Spain, which constituted two of the main sources of the spread of the virus in Europe and in the United States. In Poland, the occurrence of the first

infection is dated to 4 March 2020, and subsequent cases of the disease were observed in many places simultaneously, which indicates an independent transfer of the virus from several countries. According to WHO data, as of 18 June 2021 there were 176,945,596 cases of SARS-CoV-2 infection, including 3,836,828 deaths [38].

3.1. Structure and the SARS-CoV-2 Receptor

The SARS-CoV-2 virus belongs to betacoronaviruses and shows similarity to the previously identified highly pathogenic human virus species HCoV-SARS (79.5%) and HCoV-MERS (55%), yet it has the highest (96%) nucleotide sequence identity with the RaTG13 bat coronavirus [39]. The organisation of the SARS-CoV-2 genome corresponds to the general structure of the coronaviruses presented in sub-chapter 2.2. However, based on comparative modelling studies, the genome of the new pathogen has 17 proteins, including 13 non-structural proteins of wORF1ab (wNsp1, wNsp3, wNsp4, wNsp5, wNsp7, wNsp8, wNsp9, wNsp10, wNsp12, wNsp14, wNsp15 and wNsp16), three structural proteins (wE, wN and wS) and one ORF (wORF7a) [40]. Moreover, in the case of this virus, the presence of the haemagglutinin-esterase gene, which is characteristic mainly of the A-line of betacoronaviruses, has not been identified [39].

Since the SARS-CoV-2 virus was identified, a continuous analysis of its genome has been carried out around the world to track the evolution of the virus and determine how it spreads across continents. At the time of writing this article, four variants are of the greatest concern among the SARS-CoV-2 mutations studied: B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma) and B.1.617.2 (Delta) [41]. Regardless of the variant of the SARS-CoV-2 virus, the S fusion protein (spike protein) plays a key role in the process of its penetration into a sensitive host cell, and the cell membrane receptor is ACE2. The literature data show that a potential cause of a different clinical picture of virus infection in humans may be genetic variation in the ACE2 gene and the degree of its expression in individuals and ethnic groups [37]. Nevertheless, it was found that men are more likely to be infected with the virus and have a more severe course of COVID-19 than women due to the higher concentration of ACE2 in their blood [42].

3.2. Virus Transmission

The SARS-CoV-2 virus is transmitted mainly by droplets and the most common infection occurs through direct contact with its host. As a result of many months of observation, it was found that the greatest risk of virus transmission is in the early stage of the disease. The incubation period of the pathogen ranges from 2 to 14 days before the onset of symptoms, but patients may remain contagious for up to 2 weeks after the symptoms have cleared; hence, the WHO has recommended a period of 14 days of isolation [43, 44].

In addition, infection with the virus is possible through contact with surfaces or objects on which the pathogen is present and its subsequent transfer with the hands to the mouth, nose or eyes. It turns out that the virus can remain stable for a long time on many surfaces, such as copper (4 h), cardboard (24 h), stainless steel (48 h) and plastic (72 h) [45].

It should also be mentioned that there are reports on the possibility of transmitting SARS-CoV-2 via the faecal-oral route, which may be confirmed by the presence of genetic material of the virus in the stool in some people with COVID-19 and gastrointestinal symptoms (nausea, vomiting, diarrhoea) [46, 47].

To minimise the risk of contracting the coronavirus, the generally accepted sanitary and hygiene rules should be followed. It is, therefore, important to wash one's hands frequently with soap or disinfect them with an alcohol-based hand rub, and avoid touching one's nose, mouth and eyes with unwashed hands. The mouth and nose should be covered

with a mask, especially in crowded places; it is essential to keep social distance (at least 2 m), ventilate rooms frequently and limit travel to regions with an unregulated epidemic situation. In the case of contact with people who have been confirmed with COVID-19, one should immediately undergo quarantine and monitor one's health (body temperature and blood saturation levels) [15, 37].

3.3. Symptoms and Course of Infection

The clinical picture of SARS-CoV-2 infection can vary widely, ranging from asymptomatic infection to severe pneumonia, which may result in the patient's death. Common symptoms of the disease include fever > 38°C, weakness, malaise, headache, muscle pain, shortness of breath and dry cough. In addition, unusual symptoms such as loss of taste or sense of smell may appear. Some patients are also diagnosed with nausea, vomiting or diarrhoea. In serious cases, acute respiratory distress syndrome (ARDS) may develop, leading to death.

Children are less likely to be infected than adults, and the course of infection is usually asymptomatic. The SARS-CoV-2 virus is especially dangerous for the elderly and people with comorbidities (diabetes, obesity, hypertension, cardiovascular diseases, cancer, chronic obstructive pulmonary disease, chronic kidney disease, hepatitis B), whose risk of death is greatest.

Based on many months of observations of people infected with the SARS-CoV-2 virus, it has been determined that about 80% of all infections are mild and similar to influenza, and about 6% of patients are in a severe state (respiratory failure and septic shock) [15, 37, 48].

4. Characteristics of Chitosan

Chitosan, an *N*-deacetylated chitin derivative, is made of D-glucosamine and *N*-acetyl-D-glucosamine molecules linearly linked by β-1,4-glycosidic bonds [49]. The deacetylation process is usually carried out with the use of hydrated alkali, mainly sodium hydroxide (NaOH) (Figure 5.). As a result of the hydrolysis reaction, acetyl groups are replaced with

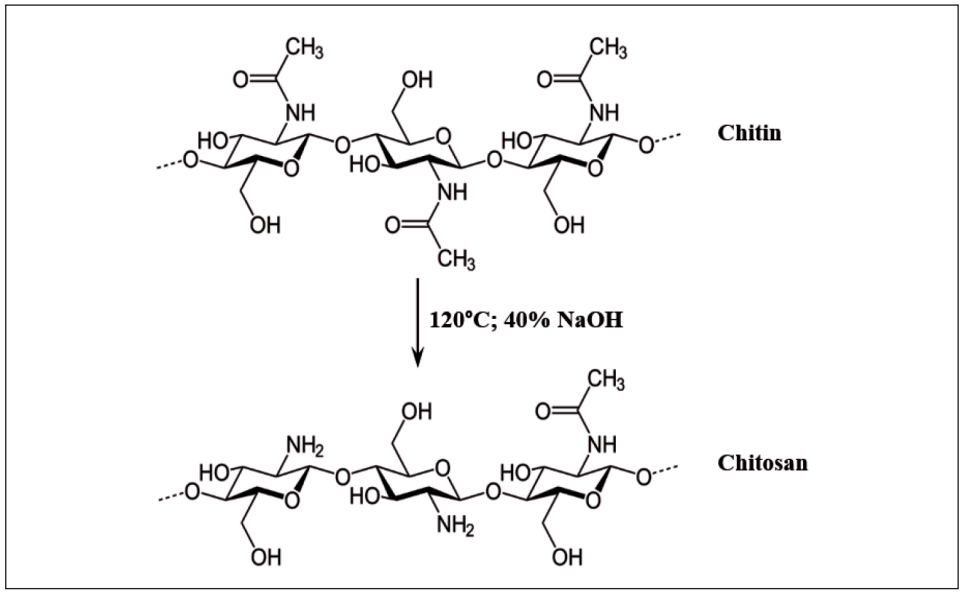

Figure 5. Scheme for obtaining chitosan from chitin

amino groups. The deacetylation degree of chitosan can be regulated by modifying the base concentration, temperature, reaction time and the parameters of the initial polymer (chitin) [50, 51].

Treatment in 40% NaOH at 120°C for 1-3 h yields a product with a deacetylation degree of about 70%. Increasing the reaction time to 48 h makes it possible to achieve a deacetylation degree of even 100%, yet it comes at the cost of a significant decrease in the viscosity of the solution, which is related to the degradation of the polymer chain [52, 53].

An alternative to the above process may be to treat chitin with agents containing concentrated NaOH solutions in the presence of an organic solvent such as 2-propanol, 2-methyl-2-propanol or acetone. However, this method is characterised by low deacetylation efficiency (low deacetylation degree and higher chitosan molecular weight) [53].

As shown in Figure 5, the presence of amino groups in the structure of chitosan results in better solubility than chitin and, therefore, the possibility of wider application in, for example, cosmetology, pharmacology, implantology or biomedical engineering. Due to its acid-base properties, chitosan is soluble in dilute organic acids, such as formic, acetic, citric and lactic acids, and in dilute inorganic acids, such as hydrochloric, perchloric or nitric acids [54].

In terms of biomedical applications, the biological properties of the polymer deserve special attention. Chitosan is primarily characterised by non-toxicity, biodegradability, mucoadhesiveness and bioactivity (acting on living organisms by inhibiting or activating their life processes). The most important of these features is biological activity, including binding pathogenic bacteria or substances necessary for bacterial growth, biocompatibility with living cells, lowering serum cholesterol content and agglutination of red blood cells. Moreover, chitosan has antifungal properties and does not cause allergies or skin irritations [55, 56].

4.1. Antiviral Activity

In addition to the above-mentioned properties of chitosan, its antiviral effect is also important, especially in the context of its use in the prevention and treatment of viral diseases in humans. The antimicrobial effectiveness of the polymer is affected by many factors, including: the source of chitin, the conditions of the deacetylation process (base concentration, temperature and reaction time), the degree of chitosan deacetylation, molecular weight and deproteinisation [57-59]. Additionally, the effectiveness of chitosan can be increased by modifying it, involving the substitution of, for example, a sulpho group in the C-2 (NH_2 group), C-3 (OH group) or C-6 (CH_2OH group) position, which leads to obtaining anionic polymer derivatives [60].

Many years of research conducted in foreign research centres have confirmed the ability of chitosan to induce plant resistance to viral infections, prevent the development of phage infection in infected microbial cultures and inhibit viral infections in animal cells.

It has been shown that the antiviral effect of the polymer in plants is based on inhibiting the spread of viruses and viroids in them and enhancing the immune response to infection. The studies have concerned many species of viruses, including alfalfa mosaic virus (AMV), bean goldish mosaic virus (BGMV), peanut stunt virus (PSV), tobacco necrosis virus (TNV), tobacco mosaic virus (TMV), potato virus X (PVX) and potato spindle tuber viroid (PSTV) [61-63].

On the other hand, in the context of the antiviral effectiveness of chitosan against phage infections, scientists have proved that the activity of a polymer is primarily influenced by the degree of its polymerisation, concentration in nutrient medium and molecular structure. Moreover, the presence of positive charges on the compound molecule influences the antiphage activity to a greater extent than the use of anionic polymer derivatives such as

6-*O*-sulphate or *N*-succinate-6-*O*-sulphate. Overall, research studies suggest that chitosan may limit bacteriophage replication through various mechanisms: reducing the viability of bacterial cells, neutralising the infectivity of mature phage particles in the inoculum and daughter phage particles or blocking replication of the virulent phage [62, 64, 65].

Research on the use of chitosan and its derivatives in combating viral infections in animals is also important. The conducted experiments have concerned several species of animal viruses such as murine norovirus 1 (MNV-1), feline calicivirus F-9 (FCV-F9), foot-and-mouth disease virus (FMDV) and porcine epidemic diarrhoea virus (PEDV). Based on the obtained results, chitosan mainly enhances the functional activity of macrophages and granulocytes, helper cells of the immune system [62, 66-68]. However, the most important aspect from the human point of view is the possibility of using the polymer in the prevention and treatment of human viral infections caused by a wide range of pathogens: DNA viruses (HBV, HPV, HSV), RNA viruses (HCV, RSV, influenza viruses) and retroviruses (HIV).

In 2010, Prego *et al*. [69] were one of the first groups to develop chitosan-based nanoparticles, using the ionic gelation method, to increase resistance to hepatitis B virus (HBV) infection. These systems were used to deliver virus-like particle antigens (rHBsAg). Based on the research, the authors concluded that the nanoparticles allowed for an efficient association of large amounts of antigen (> 60%), which was then released in an active and permanent manner. This solution made it possible to induce several times higher levels of anti-rHBsAg IgG than conventional vaccines, which demonstrates the possibility of using chitosan-based nanoparticles as a potential adjuvant for the administration of subunit antigens to vaccines.

AbdelAllah *et al*. [70] also investigated the possibility of using chitosan-based systems as adjuvants for hepatitis B vaccines. The scientists used several alternative solutions: chitosan (Ch), a system based on chitosan (Ch) and sodium alginate (S) and a composite with alum (Al). The three-component (AlChS) composite showed the strongest effect on the immune response, which was determined based on cytokine levels, rate of seroconversion and anti-HBsAg.

On the other hand, Gao *et al*. [71] attempted to evaluate an antiviral anionic chitosan derivative – 3,6-*O*-sulphated chitosan (36S) – against human papillomavirus (HPV) infection, which plays a key role in the development of cervical cancer. Based on the conducted research, the scientists showed that the system they developed effectively inhibited multiple genital HPV genotypes by targeting the viral capsid protein and the PI3K/Akt/mTOR cell pathway. This proves the possibility of using a chitosan derivative as a next-generation antiviral solution.

Studies on the use of chitosan-based solutions showing antiviral activity against the herpes simplex virus type 1 (HSV 1) have been carried out by Choi *et al*. [72] and Donalisio *et al*. [73]. In 2016, Choi *et al*. [72] tried to create a system composed of chitosan and a heat-inactivated green fluorescent protein expressing HSV (G-HSV). Based on *in vivo* experiments carried out in a mouse model, they found that the use of the polymer in infected animals increased the incidence of T cells, dendritic cells (DC) and natural killer (NK) cells in relation to the control group. These findings indicate that chitosan is a good immune stimulator. On the other hand, Donalisio *et al*. [73] developed chitosan nanospheres (NS) obtained using the nano-emulsion template method, which were loaded with acyclovir, an antiviral drug from the group of nucleoside analogues used primarily in the treatment of herpes simplex virus infections. Based on the biological research carried out on the Vero cell line infected with HSV-1 and HSV-2 strains, the scientists proved that the obtained acyclovir-NS complex showed higher antiviral activity than the use of the pharmaceutical alone. About 30% of this preparation was delivered after 6 h, which indicates that the developed system could be used for the local treatment of HSV infections.

Another important issue is the possibility of using chitosan and its derivatives in the treatment of the infectious disease caused by the hepatitis C virus (HCV). Loutfy *et al*. [74] presented the results of research on curcumin-chitosan (CuCs) nanocomposite as a promising anti-HCV-4a factor in human hepatoma cells Huh7. The developed system ensured 100% inhibition of viral entry and replication, which was confirmed by the HCV core protein expression. The results of the research proved that it is possible to use CuCs as a therapeutic agent in the treatment of hepatitis C.

Not without significance are also studies devoted to the use of the polymer in the prevention and treatment of infections caused by respiratory viruses: respiratory syncytial virus (RSV) and influenza viruses. Iqubal *et al*. [75] attempted to develop an alternative to traditional RSV vaccines. Scientists investigated the possibility of delivering plasmid DNA formulated with chitosan through the nasal mucosa, which they used in infected mice. As a result of the observations, a significant reduction ($p < 0.001$) of the viral load in the lungs of the immunised animals was noted compared to the control group. Mori *et al*. [76] analysed the antiviral effect of composites made of silver nanoparticles (AgNPs) and chitosan (Ch), which they obtained in the form of floc-like powders as a result of synthesis in an aqueous environment. Based on the research carried out to determine the effectiveness of the developed systems in fighting the influenza A H1N1 virus, the scientists found that the antiviral effect of the composite depends mainly on the concentration of AgNPs and their size. In turn, Mann *et al*. [77] investigated the effectiveness of intranasal vaccines with two types of adjuvants: chitosan glutamate (CSN) and *N*,*N*,*N*-trimethylated chitosan (TM-CSN) against the H5N1 avian influenza virus strain. The studies were carried out using a ferret model, which showed the beneficial effects of both types of preparations used. These solutions induced high levels of antibodies, prevented the animals from dying and reduced virus replication. Particularly satisfactory results have been reported for the TM-CSN vaccine, which induced protective immunity against intratracheal challenge, and no pathogen was found in the respiratory tract and brain in ferrets. This proves the feasibility of using chitosan-based vaccines to prevent influenza virus infection.

Zheng *et al*. [78] examined the possibility of using intranasal vaccines. Based on *in vivo* experiments carried out in a mouse model, the scientists found that the use of chitosan in infected animals protects them against infection with the H7N9 virus (highly pathogenic for humans), as well as the H1N1 and H9N2 viruses. They observed increased infiltration of leucocytes in the bronchoalveolar lavage and elevated levels of proinflammatory cytokines in bronchial and lung tissues, which was caused by the activation of the mucosal immune response by the polymer used in the vaccines. These findings specify that the proposed preparations constitute an interesting prophylactic solution in the prevention of infections caused by various strains of the influenza virus.

Retroviruses are an extremely dangerous family of RNA viruses that cause many diseases, including AIDS and some cancers. In 2019, Mobarakeh *et al*. [79] published their research on the use of chitosan nanoparticles in combination with polyethylenimine (PEI) and carboxymethyl dextran (CMD) to introduce anti-HIV siRNA. Based on the conducted biological experiments, the authors proved that nanoparticles significantly reduced the RNA and protein expression of HIV-1 *tat*. These findings indicate the possibility of using this solution in gene therapy, especially against HIV infection.

5. Application Potential of Chitosan in the Prevention and Treatment of COVID-19

The results of the scientific studies discussed in the previous chapter, which confirm the high antiviral activity of chitosan and its derivatives against a wide range of viruses,

including pathogens causing respiratory system infections in humans, constitute the starting point for considering the possibility of using the polymer in the fight against infections caused by SARS-CoV-2. The lack of targeted COVID-19 treatment methods that have been validated by multicentre clinical trials is an additional incentive to conduct more research in this area. It should be emphasised that the advantages of using natural preparations, which include chitosan, are the fewer side effects of the therapy and its high safety.

Over the last year, there have been several publications indicating the possibility of using chitosan in the prevention and treatment of infections caused by the latest betacoronavirus. Chandrasekar *et al*. [80] attempted to evaluate the efficacy of a submucosal, two-dose, heterologous vaccine against the SARS-CoV-2 virus. The preparation was developed with the use of quil-A-loaded chitosan (QAC) nanoparticles as an adjuvant. Based on the research carried out in a mouse model, the researchers showed that the solution led to the development of the neutralising antibody response in the serum and bronchoalveolar lavage of mice. In addition, there were T cells against the virus, with the simultaneous absence of side effects (signs of the respiratory system or lack of appetite). These data underscored the possibility of using the developed preparation as a new generation of protective vaccine.

The application potential of chitosan-based systems as constituent in vaccines against the novel coronavirus has also been investigated by Tatlow *et al*. [81] and Srivastava *et al*. [82]. Tatlow *et al*. [81] developed an inhaled chitosan plasmid DNA vaccine containing the coronavirus S protein DNA sequence for the treatment of COVID-19 patients and for vaccination. The findings showed that the preparation creates a kind of antagonistic environment for the virus, disrupting its ability to bind to the ACE2 receptor on the host cell. Srivastava *et al*. [82] determined the immunogenicity of the receptor binding domain (RBD) of the S glycoprotein of SARS-CoV-2 based on studies performed in a mouse model. The experiments used recombinant RBD antigen and three types of adjuvants: Zn-chitosan (CHT), Alhydrogel – aluminium hydroxide (ALH) and Adju-Phos – aluminium phosphate (ADP). Based on 5 months of observations, the authors noted the best neutralising activity for the preparation containing CHT (after the first immunisation, 80% neutralisation titre [NT80] was ~270, and after the second immunisation it was over 2,400).

Not without significance is also the work of Pyrć *et al*. [83] concerning the use of an antiviral nasal spray preparation based on a chitosan derivative – *N*-palmitoyl-*N*--monomethyl-*N*,*N*-dimethyl-*N*,*N*,*N*-trimethyl-6-*O*-glycolchitosan (GCPQ). Based on biological studies, the scientists determined that this substance prevents the virus from penetrating the nasal epithelial cells, has a long residence time in the nostrils and may be one of the promising ways to reduce the spread of COVID-19. The mechanism of action of the preparation is most likely related to the electrostatic bonding of GCPQ and the pathogen.

Another preventive solution limiting further transmission of the coronavirus was presented by Farzin *et al*. [84]. The authors developed a voltametric genosensor for COVID-19 detection by determining the RNA-dependent RNA polymerase (RdRP) sequence. In this device, silver ions (Ag^+) in the hexathia-18-crown-6 (HT18C6) were used as a redox probe, while a carbon electrode was modified by using quantum dots covered with chitosan and dendrimer. The findings showed that the proposed genosensor has a large detection range and good selectivity, which indicates the potential of this solution in determining the SARS-CoV-2 RdRP sequence in sputum samples.

Considering the antiviral properties of the polymer in question, Mathout *et al*. [85] discussed the validity of the potential use of positively charged chitosan nanofibres obtained by electrospinning as an additive to protective clothing (for example, gowns or masks) for

medical facility employees. Having a positive zeta potential, this solution could allow for electrostatic repulsion of virus particles and consequently reduce its transmission.

In turn, Kalathiya *et al*. [86] identified the presence of a homotrimer cavity formed by three subunits that could affect binding of the viral S protein to the ACE2 receptor ('bouncing spring'). Based on their observations, the authors formulated a hypothesis that the cavity may act as an acceptor for chitosan or macrolides. This discovery is the starting point for the development of a new category of targeted drugs against SARS-CoV2.

Milewska *et al*. [87] presented interesting research on the use of a chitosan derivative – *N*-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC) – as a virus inhibitor. Based on the studies conducted on *in vitro* and *ex vivo* models of human airway epithelia, the scientists showed that their proposed solution effectively blocks infection with both SARS-CoV-2 and HCoV-MERS coronaviruses and shows great potential for use as a targeted antiviral drug.

On the other hand, Alitongbieke *et al*. [88] analysed the ability of β-chitosan (a crystalline polymorphic form) to block the binding interaction between SARS-CoV-2 S-RBD and ACE2 and examined the impact of the polymer on pathogen-induced inflammation. They showed that the proposed compound performed a function similar to antibodies, enabling the neutralisation of SARS-CoV-2 S-RBD and the prevention of its binding with the ACE2 receptor.

Another noteworthy development is the technology developed in 2020 by Bioavanta-Bosti based on chitosan nanoparticles (Novochizol™) for encapsulating active pharmaceutical ingredients that could be delivered to a specific place in the body and then gradually released. Based on preliminary studies, scientists proved that the use of Novochizol™ aerosol preparations would allow for the delivery of a therapeutic dose for up to 3 h [89].

As previously mentioned, the S protein of coronaviruses, including SARS-CoV-2, plays a key role in pathogen penetration into host cells, with the S-1 glycoprotein binding to the ACE2 receptor, which is most abundant in the lungs on type II pneumocytes. This may explain damage to these organs in COVID-19 patients. However, Mironova *et al*. [90] highlighted an additional aspect of the action of the pathogen's surface proteins, indicating their binding to the haemoglobin molecules contained in erythrocytes. This degrades haem (an iron-containing porphyrin), a component of haemoglobin, and releases harmful iron ions to the bloodstream, which in turn induces the production of reactive oxygen species. The consequence of this process may be the development of oxidative stress, contributing to damage to the lungs and other organs. The authors emphasised the potential role of two substances in the treatment of COVID-19 patients, namely dihydroquercetin (one of the strongest antioxidants) and uridine (pyrimidine nucleoside), which preserves the structure of pulmonary alveoli and the air-blood barrier of the lungs in the event of hypoxia.

Based on these findings, we have initiated a discussion of the possibility of using chitosan hydrogels containing uridine 5'-monophosphate disodium salt, which are the subject of our research [91-93], in the treatment of COVID-19. We suggest that the SARS-CoV-2 surface proteins may bind to chitosan, preventing or limiting the destruction of haemoglobin. As a good adsorbent, this polymer can also bind the released iron ions, reducing oxidative stress, and the use of uridine monophosphate would simultaneously protect the pulmonary alveoli.

6. Conclusions and Future Outlook

Coronaviruses, which cause a significant percentage of respiratory tract infections in humans, mainly of mild and self-resolving course, were not the leading subject of research

until the end of the 20th century. It was only the outbreak of the HCoV-SARS epidemic that has prompted scientists to take a closer look at the *Coronaviridae* family.

From medical, social and economic points of view, the current problem is the new betacoronavirus – SARS-CoV-2. Despite the COVID-19 pandemic lasting more than a year and the emergence of many scientific reports on the origin of the virus, its evolution, description of the clinical picture of infection and diagnosis of the disease as well as the introduction of vaccines, the epidemic situation is still worrying. The seriousness of the threat is also exacerbated by the lack of targeted antiviral therapy that has been validated by multicentre clinical trials.

One of the solutions considered by researchers in the field of medicine is the possibility of using chitosan and its derivatives for the prevention and treatment of COVID-19. These polymers are interesting raw materials that can be used for the preparation of, for example, protective vaccines, genosensors as well as self-contained pharmaceutical preparations or drug carriers. Nevertheless, the potential application of the above solutions in common clinical practice requires long-term biological studies to assess their cytotoxic and genotoxic effects on the human body. It should be remembered that the possible toxicity and the ability of the systems proposed in the literature to cross the blood-brain barrier may be one of the main reasons limiting their use in medicine. In addition, it is especially important to monitor the epidemiological situation constantly, bearing in mind that coronaviruses have already proved three times that they pose a serious threat to human health and life, and it can be assumed that SARS-CoV-2 is not the last highly pathogenic representative of the *Coronaviridae* family.

7. References

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