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REVIEW

Molecular Targets in the Chemotherapy of Coronavirus Infection

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Abstract—In the pathogenesis of the infectious process in the respiratory tract by SARS, MERS, and COVID-19 coronaviruses, two stages can be distinguished: early (etiotropic) and late (pathogenetic) ones. In the first stage, when the virus multiplication and accumulation are prevalent under insufficient host immune response, the use of chemotherapeutic agents blocking the reproduction of the virus is reasonable to suppress the development of the disease. This article considers six major chemotherapeutic classes aimed at certain viral targets: inhibitors of viral RNA polymerase, inhibitors of viral protease Mpro, inhibitors of proteolytic activation of viral protein S allowing virus entry into the target cell, inhibitors of virus uncoating in cellular endosomes, compounds of exogenous interferons, and compounds of natural and recombinant virus-neutralizing antibodies. In the second stage, when the multiplication of the virus decreases and threatening pathological processes of excessive inflammation, acute respiratory distress syndrome, pulmonary edema, hypoxia, and secondary bacterial pneumonia and sepsis events develop, a pathogenetic therapeutic approach including extracorporeal blood oxygenation, detoxification, and anti-inflammatory and anti-bacterial therapy seems to be the most effective way for the patient's recovery.

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INTRODUCTION

The family Coronaviridae is comprised of numerous viruses infecting human and diverse animals including farm livestock and wild animals (cats, dogs, bats, cows, camels, pigs, birds, etc.). It consists of two virus subfamilies (Letovirinae and Orthocoronavirinae) including five genera and around 40 virus species [1]. The subfamily Orthocoronavirinae that contains human coronaviruses consists of four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Coronaviruses (CoV) affect various organs and tissues and act as pathogens causing a broad range of diseases including severe human respiratory infection called atypical pneumonia. Usually, viruses of this family induce acute infection manifested by signs of inflammation featured with properties of cytokine storm syndrome [2, 3].

Coronaviruses are enclosed by a lipid envelope (enveloped viruses) and carry genomic positive-sense RNA, which is translated by host ribosomes and guides synthesis of viral proteins as well as sub-genomic RNAs and subsequent replication of the viral genome and assembly of viral particles [1, 4]. Depending on species, coronavirus genomic RNA consists of $25\text{-}30 \cdot 10^3$ nucleotides and bears 22-29 viral genes encoding relevant proteins, four of which (N, S, M, E) play the major structural role in viral particles (Table). Moreover, several accessory viral proteins functioning as ion channels (viroporins) may also be found in virions [5].

Great interest in Coronaviridae has now been raised due to emergence of the dangerous type of human pneumonia caused by the novel *Betacoronavirus* strain SARS-CoV-2 [4]. This strain turned out to be close to bat SARS-like coronavirus as well as those inducing SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome), which caused in 2003 and 2012, severe pneumonia outbreaks in humans, referred as atypical pneumonia. Such infections did not induce a wide pandemic spread but showed a threatening pattern due to high mortality rate reaching up to 9.6-35.5% [2, 36]. Hence, the threat of the emerging

Abbreviations: CoV, coronaviruses; MERS, Middle East Respiratory Syndrome; SARS, Severe Acute Respiratory Syndrome.

Betacoronavirus genes, related proteins and their inhibitors

Gene[1)]	Protein (domain) and size (a.a.)	Found in virion	Protein function	Viral protein inhibitor[2)]	Reference
1ab[3)]	(7096)	–	–	–	–
nsp1	(180)	–	degradation of cell mRNAs, suppressed IFN	–	–
nsp2	transmembrane protein (638)	–	activated NFKβ, augmented inflammation	–	–
nsp3	transmembrane protein PLpro[4)] (1945)	–	cysteine protease, downmodulated p53 and IFN induction	ritonavir/ lopinavir	[6-9]
nsp4	(500)	–	DMV formation	–	–
nsp5	Mpro (306)	–	cysteine protease, downmodulated IFN	ritonavir/ lopinavir	[6-9]
nsp6	(290)	–	restricted autophagy, DMV formation	–	–
nsp7	(83)	–	cofactor nsp8, nsp12	–	–
nsp8	(198)	–	cofactor nsp7, nsp12	–	–
nsp9	(113)	–	dimerization and RNA binding	–	–
nsp10	(139)	–	platform for nsp12, nsp14	–	–
nsp11	(13)	–	(?)[5)]	–	–
nsp12	Pol (932)	–	RNA-dependent RNA polymerase	ribavirin	[10-12]
				favipiravir	[13]
				remdesivir	[6, 14, 15]
nsp13	(601)	–	RNA helicase, 5′-phosphatase	–	–
nsp14	(527)	–	3′→5′ exoribonuclease	–	–
nsp15	(346)	–	endoribonuclease, dsRNA sensor	–	–
nsp16	(298)	–	2-O-MTase, MDA5 sensor, IFN pathway inhibitor	–	–
S	envelope protein (spike protein) (1278)	+	virus entry into target cells	protease inhibitors	[16-22][6)]
				fusion-inhibiting peptides	[23][7)]
				chloroquine	[24-26]
				antibodies	[27-32]
3a	(275)	(?)[5)]	augmented cytokine response and inflammation via NLRP3	–	–
E	envelope protein (viroporin) (75)	+	ion channel, regulates virion assembly	chloroquine	[24-26]
				antibodies	[27-32]
M	viral matrix protein (222)	+	links viral envelope and nucleocapsid	–	–

Table (Contd.)

Gene[1)]	Protein (domain) and size (a.a.)	Found in virion	Protein function	Viral protein inhibitor[2)]	Reference
6	(61)	(?)	–	–	–
7a/b	transmembrane protein (121/43)	+	enhances viral yield	–	–
8a	transmembrane protein (121)	(?)	ubiquitinates IRF3, lowers IFN production	–	–
9b	(38)	(?)	–	–	–
N	nucleocapsid protein (419)	+	forms internal nucleocapsid together with viral RNA	–	–

Comments.
[1)] Genes and relevant protein names (or domains) in virus SARS-CoV-2 listed in order starting from the 5′-end in genomic RNA [5]. *GenBank* data were used to determine the size of the protein (the number of amino acid residues) *(ac.n. YP-009725301.1)*.
[2)] Classes of inhibitor agents with identified mode of action are shown.
[3)] Functions for proteins nsp1-nsp16 (proteolytic products derived from polyprotein 1ab) are considered elsewhere [5, 33, 34].
[4)] SARS-CoV-2 lacks in protein nsp3 one of two papain-like protease domains but preserves ubiquitin-like domains [35].
[5)] A question mark (?) denotes gene products with unidentified function (no data).
[6)] Protease inhibitors (camostat, aprotinin, lutevirin, etc.) indirectly suppress S protein-driven entry by inhibiting its proteolytic cleavage into active subunits S→S1/S2.
[7)] Fusion-inhibiting oligopeptides targeting S protein upon entry into host cells [23].

coronavirus pandemic corroborates the need to develop high-efficacy pharmaceuticals against coronaviruses, refining principles for using available antivirals and development of pathogenetic approaches to the treatment of disease.

Currently, there may be highlighted six essential chemical classes of drugs acting on diverse viral targets able to block coronavirus replication and suppress the development of disease. Such drug classes were designed based upon current knowledge about coronavirus replication and the pathogenetic mechanisms underlying coronavirus infection, and include: (1) viral polymerase inhibitors; (2) inhibitors of the viral protease Mpro, which participates in generation of active viral polymerase; (3) inhibitors of cell proteases involved in activation of CoV S protein that drives virus entry into target cells; (4) endosomal inhibitors of virus deproteinization; (5) preparations containing recombinant interferons α2 and β1; (6) preparations containing antiviral antibodies.

VIRAL POLYMERASE INHIBITORS

Viral polymerase is a standard therapeutic target, and its blockade inhibits replication of the viral genome and thus suppression of replication of the virus. By now, there are diverse multi-specific RNA polymerase inhibitors acting on various viruses due to marked structural and functional similarities of this enzyme existing among different viruses [37]. Ribavirin (furanosyl carboxamide) is among inhibitors of this type because it exhibits high activity against diverse viruses [10] (including coronaviruses) at concentration of 10-25 nM (IC_{50}), with selectivity index of more than 100 [10-12]. Because SARS, MERS, and COVID-19 (coronavirus disease 2019) mainly develop in the respiratory tract cells, ribavirin aerosol inhalations that could create effective antiviral activity at non-toxic concentrations in the airway epithelial layer and thus might serve as the most appropriate drug formulation. This is based on low pulmonary bioavailability shown for oral vs. aerosol ribavirin (1% and more than 70%, respectively) and its subsequent activation via a phosphorylation reaction occurring in the respiratory epithelium [12]. Of note, direct aerosol-delivered action on respiratory epithelium might be most active and efficient at an early stage after the onset of infection, which is accompanied by virus replication at eclipse phase when pathological events of inflammation and edema would have not reached a dangerous level.

Favipiravir and its ribosylated derivatives might be other candidate drugs fighting against coronaviruses [13]; they exhibit high antiviral potential and selectivity index with regard to diverse RNA-bearing viruses [13]. On the other hand, *remdisivir* derived from phosphorylated 1′-cyano substituted adenosine is a broad-spectrum drug displaying high antiviral potential at IC_{50} ranging within 50-70 nM against diverse viruses including coronaviruses [14, 15]. This drug is undergoing the final phase of clinical trials [6].

INHIBITORS OF CORONAVIRUS PROTEASE Mpro

Translation of viral RNA generating a lab polypeptide (MM ~750 kDa) that undergoes autoproteolytic cleavage into 14-16 fragments (nsp1-nsp16), depending on viral type, may function as an active viral polymerase and regulate replication of the viral genome and subsequent synthesis of viral proteins in the first stage of coronavirus replication after entering target cells [1]. It turned out that cleavage of the polyprotein 1ab (pp1ab) is mediated by its own domain 5 (nsp 5) (called protease domain Mpro) exerting specificity of the target proteolytic sites close to picornavirus and HIV proteases [38] and being sensitive to the binary protease inhibitor lopinavir/ritonavir ("Kaletra") [6-9]. In some viruses, two initial breaks within the polyprotein 1ab are performed by protein nsp3 (PLpro), also bearing cysteine protease papain-like domain [35]. Lopinavir/ritonavir simulating proteolytic target sites in viral proteins exhibited pronounced therapeutic effect during SARS and MERS, displaying IC_{50} at the level of 5-20 nM [6, 9]. Structural similarity in protease domain nsp5 from CoVs causing COVID-19, SARS, and MERS allows it to be unequivocally recommend it for treatment of atypical pneumonia during COVID-19 [7, 9]. Use of this drug might be specifically efficient at the initial phase of CoV replication during an early stage of infection.

INHIBITORS OF HOST PROTEASES

Viral S glycoprotein (MM ~150 kDa) drives entry of coronavirus into target cells [1]. For this, CoV S protein undergoes targeted cleavage into two distinct subunits, S1 and S2, within the proteolytic cleavage site (amino acid positions 641-687) [38, 39]. Cell transmembrane-bound protease TMPRSS2 is involved in such activation of S protein [15, 38], whereas inhibitors targeting this protease can suppress cell infection and virus spread at the site of infection [16]. This therapeutic approach might be applied in clinical practice by recommending approved protease inhibitors camostat [16] and aprotinin [17-19].

Moreover, these antiprotease agents exert marked anti-inflammatory activity via inhibiting some pro-inflammatory cytokines and related signaling pathways [19, 40] that may ensure pathogenetic effects to reduce lung inflammation and edema, which might act beneficially during the second phase of the infectious process, wherein pathogenetic mechanisms related to excessive protease activation and subsequent rise in active pro-inflammatory cytokines could exert dominant effects on disease severity [41, 42].

Recent reports from China suggest that along with protease TMPRSS2 [16, 38], cellular proteases furin and endosomal cathepsin L may also be involved in proteolytic activation of S protein resulting in COVID-19 [16, 39, 42]. If a role for furin assumingly activating SARS-CoV-2 would be confirmed, it might then be recommended to use natural plant-derived inhibitors [20] as well as synthetic oligopeptide inhibitors [21, 22] as antivirals.

INHIBITORS OF VIRAL CELL ENTRY

Chloroquine has been widely used in medical practice for treatment of malaria. This drug exhibits pronounced activity against coronaviruses in cell culture and animal models [24]. The mechanism of its action against multiple viruses including *Betacoronaviruses* is mediated by elevated acidic pH value inside cell endosomes that interferes with pH-dependent conformational transition of viral fusion proteins (coronavirus S protein) into their active state thus resulting in retarded virus deproteinization (viral uncoating) inside cell endosomes and prevents further infection of target cells; additionally, this drug may alter glycosylation of cell receptors including ACE2 used by SARS-CoV and SARS-CoV-2 for entry [25]. Based on this platform, chloroquine was recommended for treatment of COVID-19, and it demonstrated positive effects in some patients in China [26].

COMPOUNDS CONTAINING SPECIFIC ANTIVIRAL ANTIBODIES

This approach to therapy of coronavirus infection implies administration of antibodies able to neutralize infectious properties of this virus. In addition, inoculation of antibodies might also be used for early disease prevention called passive immunization. Two essential opportunities are available for using antiviral antibodies [28-30]: (i) design and generation of tailored virus-neutralizing antibodies (or their active antiviral domains) by using gene engineering and biotechnology. Such preparations specific to coronaviruses including SARS-CoV-2 have not yet been created [27]; (ii) a specific antiviral immunoglobulin preparation obtained via a more traditional and simpler technique from convalescent subjects who recovered after coronavirus infection including COVID-19 or from animals vaccinated with SARS-CoV-2 or its components [31, 32]. The first observations have been reported of successfully administered antiviral immunoglobulins purified from convalescent subjects with MERS and COVID-19, which were used for treatment of atypical pneumonia in China during 2020 SARS-CoV-2 outbreak [43].

Preparations containing antiviral antibodies should be used with some caution, because coronavirus infectiveness to immune cells was noted to be augmented by some types of artificial antibodies targeting CoV S protein [44]. Fortunately, such antibodies have not been identified yet in sera from convalescent subjects [31]. Moreover, successful

administration of such preparations should require that convalescent serum immunoglobulins would contain high titer (1/80) of anti-CoV antibodies assessed by HI test [32].

INTERFERON PREPARATIONS

Preparations containing human recombinant interferon α2 and β1 classes were used in therapy of closely-related infections caused by SARS-CoV, MERS-CoV, and SARS-CoV-2 [45, 46]. It was found that interferon β1 exerted slight curative effects, whereas interferon α2 revealed no activity, but the most prominent activity was observed after using interferon β1 in combination with ribavirin [6, 11, 46]. While choosing a treatment strategy, it should be taken into consideration that interferon preparations may exhibit peak efficacy solely at early disease stages, when host reaction has not yet been augmented too much or culminated [2, 47, 48]. Moreover, use of exogenous interferon-based preparations would hardly be rational at later disease stages due to the high quantity of endogenous interferons produced in response to acute coronavirus infection. Finally, inoculation of exogenous interferon-based preparations at late stages of infection by further elevating pre-set high level of endogenous interferons could promote cytokine storm syndrome and inflammation at the site of infection, thereby deteriorating the disease course [3, 48].

COMBINATION THERAPY

The combined use of several drugs (pharmaceuticals) acting on various phases of the virus reproductive cycle or disease pathogenesis called a combination therapy was also administered to treat coronavirus infection. A positive curative effect was observed while administering ribavirin, interferon β1, and lopinavir/ritonavir [6, 11, 46]. This approach allows: (i) improving therapeutic efficacy, (ii) reducing drug dosing, (iii) preventing emergence of dangerous viral mutants with augmented virulence. It is known that a risk of developing viral mutants in an infected host is minimized when applying combination therapy [49].

RELATIONSHIP OF ETIOTROPIC AND PATHOGENETIC EVENTS IN THERAPY OF VIRAL DISEASES

Two major stages can be distinguished within the timeframe of developing acute viral disease. The early (etiotropic) phase dominated by virus replication is called the exponential phase of virus propagation and accumulation (day 7-10 from the onset of viral infection). It is characterized by emergence of developing virus-specific defense reaction to infected host cells (production of immunoglobulins, antigen-specific T and B cell clones, and interferons) and general inflammatory response due to cytokines and chemokines synthesized at the site of infection. Later, growth of the virus declines and this results in developing a pathogenetic phase associated with formation of pathologic mechanisms critically affecting disease outcome as well as posing a threat of potential complications. Among the latter are exuberant inflammation, developing acute respiratory distress syndrome, lung edema and hypoxia, as well as emergence of infections caused by pathogenic microbes and sepsis [2, 3, 50].

Owing to a two-phase pathogenesis in viral diseases, it is reasonable to build a proper therapeutic strategy. In particular, specific antivirals should be available in the therapeutic arsenal and dominate in treatment during the etiotropic phase. In the case of coronavirus it can be referred to as the phase for use of lopinavir/ritonavir, aerosol ribavirin inhalation, injection of virus-specific antibodies and inhibitors of host proteases, and interferon preparations (particularly interferon β1, see above the corresponding sections). On the contrary, the pathogenetic phase should rely on a therapeutic strategy aimed at restriction or relief of pathological life-threating mechanisms by taking into consideration patient condition and severity of pathological events, mainly to eliminate intoxication, reduce lung edema, and improving blood oxygenation to compensate for lung failure, mostly by extracorporeal membrane oxygenation of blood (ECMO) for avoiding rupture of swollen lungs in case of involuntary inhaled oxygen therapy. In addition, anti-inflammatory drugs should be also used during this phase to recover respiratory function by paying special attention to antibacterial therapy to prevent emerging secondary bacterial pneumonia and concomitant sepsis [51, 52].

On the other hand, it might not seem very reasonable to use antivirals during a pathogenetic phase of infection for two main reasons: (i) specific antiviral antibodies as well as B and T cell clones inhibiting virus growth and removing host infected cells have been already formed by the onset of this phase [3, 32, 48], and (ii) avoiding use of such drugs would contribute to lowering their toxic side-effects on developing mature immune response that includes specific antibodies, T and B cell clones, as well as interferon response. However, it should be noted that coronavirus disease might potentially be exacerbated due to virus evolution and continuous replacement of the initial parental virus in a single host. Such a phenomenon was described for *Betacoronavirus*, so that a more virulent within-patient virus strain may emerge during disease progression that could markedly aggravate it and pose a threat to the patient's (or animal's) life [53-55]. Hence, in the case of developing impaired immune response and signs of residual viral infection, administration of antivirals should be continued to lower a risk of developing higher virulence viral

mutants. It seems important that a combination therapy affecting various targets in viral growth should be appropriate to use even at early the phase of infection in order to efficiently prevent emergence of highly virulent viral mutants.

Betacoronavirus, including SARS-CoV-2, elicits infection of the respiratory tract often ending with the development of lung edema, severe hypoxia, and sepsis. Two phases referred as etiotropic and pathogenetic can be highlighted in disease pathogenesis. During the first stage, virus growth and accumulation dominate, which is accompanied by appearance of initial pathological disturbances in the respiratory tract. However, during the second stage virus propagation declines, but pathological events mainly manifested as excessive inflammation and lung edema develop as a secondary consequence of virus-induced cytopathic effects. Whereas in the first stage it is justified to use pharmaceuticals and their combinations (aerosol ribavirin inhalation, lopinavir/ritonavir, protease inhibitors, interferon compounds, antiviral antibodies) aimed at suppressing diverse targets during virus propagation, during the second disease stage it might be important and reasonable to rely on administration of pathogenetic drugs to restrict life-threatening events resulting in marked inflammation, intoxication, hypoxia, secondary pneumonia, and sepsis.

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