Recent antiviral strategies against human coronavirus-related respiratory illnesses

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Purpose of review

The main purpose of this review is to summarize the current research (2006–2007) concerning the development of novel anticoronaviral strategies and compounds.

Recent findings

Recent research led to the identification of several novel agents inhibiting coronaviral replication. The most promising compounds include carbohydrate-binding agents, neutralizing antibodies and drugs targeting a coronaviral envelope protein.

Summary

Although initial outbreaks of coronavirus that causes severe acute respiratory syndrome (SARS-CoV) were controlled by public health measures, the development of vaccines and antiviral agents for SARS-CoV is essential for improving control and treatment of future outbreaks. Four years after the SARS-CoV epidemic, several compounds with an anticoronaviral activity have been identified.

Keywords

antiviral drugs, antiviral strategies, coronaviruses

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Introduction

Coronaviridae is the family of enveloped viruses with a nonsegmented, positive-stranded RNA genome of about 30 kb. To date, five human coronaviruses (HCoV) have been described: HCoV-229E and HCoV-OC43 were identified in the late 1960s, severe acute respiratory syndrome-associated coronavirus (SARS-CoV) emerged in 2003 and two others, HCoV-NL63 and HCoV-HKU1, have been identified recently, in 2004 and 2005, respectively [1–8]. Although coronaviruses have been recognized as human pathogens for about 50 years, no effective treatment strategy has been approved. This drawback became evident during the SARS-CoV outbreak and triggered numerous studies. Despite that, 4 years after the outbreak, we are still lacking an effective, commercially available drug.

Coronavirus-related respiratory illness

Coronaviruses are known to cause a variety of severe diseases in birds and mammals [9]. Human coronaviruses are mainly associated with respiratory tract illnesses, although some reports also suggest an association with an enteric infection [10]. HCoV-229E and HCoV-OC43 related disease usually appears as relatively mild and self-limiting respiratory tract illnesses, being more severe in elderly or immunocompromised patients [11]. HCoV-NL63 infection is related to acute respiratory dysfunction in infected individuals, and the virus was identified as the major pathogen responsible for croup in young children [12,13]. Infection with HCoV- HKU1 is mostly associated with bronchiolitis and pneumonia [2,14].

The fifth human coronavirus – SARS-CoV – is an etiologic factor of the severe acute respiratory syndrome (SARS) and causes severe lung disorder, leading in some cases to systemic infection and eventually death in about 10% of cases [3–5]. There is no straightforward explanation for the high pathogenicity of SARS-CoV, which is in stark contrast to other members of this family.

Anticoronaviral strategies

The 5′ two thirds of the genome encode one large polyprotein (pp1ab), consisting of several domains with enzymatic activities required for viral replication. The polyprotein during the posttranslational processes undergoes an autocatalytic cleavage, resulting in generation of several functional proteins. The remaining open reading frames (ORFs) in the 3′ part of the genome encode several structural proteins: spike (S), envelope (E), membrane (M), nucleocapsid (N) and additional nonstructural proteins varying in number and position for different species. Additionally, the genomes of the group 2 coronaviruses contain the hemagglutinin esterase gene [15,16].

Virtually all of these genes and the encoded proteins may constitute a target for a therapy. The major difficulties

encountered are related to genetic variability of targets within the population and their similarity to human proteins. The perfect drug target for an anticoronaviral therapy should be relatively conserved and unique for the virus. Although several potential candidates have been suggested within recent years, there is no specific anticoronaviral drug yet approved for clinical use.

Targeting the genome

A term RNA interference (RNAi) refers to sequence-specific silencing of gene expression, an ancient evolutionary conserved process based on specific recognition of double-stranded RNA by the cellular Dicer protein complex. This results in shearing of the dsRNA template and generation of short 21–25 nt dsRNA molecules. These short RNA fragments are further incorporated as a template into the RNA-induced silencing complex (RISC) that has an ability to cleave target mRNAs and share sequence identity with the template [17–21].

The technique to deliver the exogenous small interfering (si)RNA molecules, which can be directly incorporated into the RISC complex, is being used, among others, to specifically silence viral gene expression. To facilitate the design and development of highly effective siRNA molecules targeting viral genes, Naito *et al.* [22•] designed an siVirus software, a web-based antiviral siRNA design engine for highly divergent viral sequences.

Recently, He *et al.* [23•] presented the synergistic effect of multiple siRNAs directed to various genes of SARS-CoV. Application of several siRNAs targeting various regions of the genome is believed to limit the chance of generation of escape mutants. Pyrc *et al.* [24•] identified two synthetic siRNAs targeting the S gene and potently inhibiting HCoV-NL63 replication *in vitro*.

Another approach employs adenoviral vectors to deliver the short hairpin RNA (shRNA) targeting SARS-CoV. The major advantages of such a system rely on the possibility of including several shRNA sequences in a single adenoviral vector and the lack of additional transfection agent [25•].

Viral infection

During a productive coronavirus infection, at the first step of the replication cycle, protein S attaches to its specific receptor on the host cell surface and subsequently undergoes a series of conformational changes, facilitating the fusion process of viral and cellular membranes [26]. Human coronaviruses are using several surface molecules during the entry process. HCoV-OC43 uses *O*-acetylated sialic acid [27], HCoV-229E employs CD13 (aminopeptidase N) [28], whereas HCoV-NL63 and SARS-CoV engage angiotensin-converting enzyme 2 (ACE2) [29,30] to enter the host cell. Several groups reported the inhibition of virus entry using techniques varying from blocking the highly glycosylated viral proteins with plant lectins to locking the structural shift in the S protein with synthetic peptides.

Carbohydrate-binding agents

The carbohydrate-binding agents (CBA) are a group of compounds binding sugar moieties. CBAs were shown to interfere with the viral entry process and inhibit the replication of several viruses, by interaction with highly *N*-glycosylated viral proteins [31]. Recently, novel compounds were recognized and some insight into the mechanism of action has been gained.

Van der Meer *et al.* [32••] evaluated plant lectins [*Hippeastrum* hybrid agglutinin (HHA), *Galanthus nivalis* agglutinin (GNA), *Cymbidium* sp. agglutinin and *Urtica dioica* agglutinin (UDA)] as well as nonplant derived pradimicin-A (PRM-A; mannose-binding nonpeptidic antibiotic) and cyanovirin-N [CV-N; α(1,2) mannose-specific procaryotic lectin] as potential antiviral agents. Viruses tested in this study represented several groups of viruses belonging to the order of Nidovirales (murine hepatitis virus, feline infectious peritonitis virus, feline coronavirus, infectious bronchitis virus, transmissible gastroenteritis virus, Berne virus and equine arteritis virus). All these agents offered a high anticoronaviral activity. Plant lectins and pradimicin A interact with coronaviral envelope proteins, not only during cell attachment process causing steric hindrance but also during fusion and exocytosis or viral egress from the cell. The antiviral potential of these compounds is highly dependent on the host cell type and the level of glycosylation. Presented low in-vivo toxicity and high in-vitro efficacy is encouraging to continue the exploration of these compounds as antivirals [33••].

Neutralizing antibodies

Coronavirus entry at the level of attachment may also be hindered by specific monoclonal or polyclonal antibodies directed to the coronaviral proteins [34–36].

Zhou *et al.* [37,38•] reported an activity of equine antibodies, reducing infection with SARS-CoV in aged mice. In a therapeutic setting, treatment with anti-SARS-CoV F(ab′)2 decreased viral load in the lungs several thousand fold. Subsequently, this antibody has been tested in Syrian hamster, Chinese hamster, rat and macaque models, and protected animals from SARS-CoV infection [39,40].

The immunogenicity of nonhuman-derived antibodies may result in their rapid clearance and reduce their

efficacy [41]. To prevent such a decrease in antibody efficacy, Kang *et al.* [42•] constructed a human antibody library by the phage display technique and used the S protein of SARS-CoV as the target to screen the phage antibody library. Authors identified monoclonal antibodies specific for the S protein of SARS-CoV that completely inhibited virus activity, with an absence of cytopathic effect for 7 days.

Although the monoclonal antibodies exhibit a high antiviral potential, viruses may easily evolve into antibody-resistant variants. Utilizing the human immunoglobulin transgenic mouse, XenoMouse, Coughlin *et al.* [43•] produced several fully human SARS-CoV-S-protein-specific antibodies with a high neutralizing potential. These monoclonal antibodies could be used as a cocktail, simultaneously targeting several neutralizing epitopes and preventing emergence of escape mutants. Similarly, ter Meulen *et al.* [44] presented two monoclonal antibodies targeting two different epitopes within the receptor-binding domain of S protein of SARS-CoV. A combination of these two neutralizing antibodies (CR3014 and CR3022) has the potential to control SARS-CoV infection with no escape mutants observed in the laboratory settings.

Pyrc *et al.* [24•] anticipated the usage of commercially available intravenous immunoglobulins (IVIG), isolated from healthy volunteers, as the potent inhibitors against HCoV-NL63. IVIG was able to inhibit viral infections at therapeutic concentration. Such an approach has already been proposed for respiratory syncytial virus (RSV), but its potential to limit infection with other coronaviruses has not yet been determined.

Fusion inhibitors

The fusion of the coronaviral and cellular membrane, depending on the species, may occur directly on the cell surface [45] or require endocytosis [46]. The major prerequirement for an effective fusion is a structural switch within the S protein, accompanied by the association of two heptad repeat regions (HR1 and HR2) and most likely exposition of a fusion peptide [47]. It was previously reported that interference with HR1/HR2 association results in the inhibition of coronavirus infection [24•,48]. Synthetic peptides homologous to heptad repeat regions were recognized as potent inhibitors of fusion by competitive binding to these regions. The specific inhibition of heptad repeat regions association by a steric hindrance may also be achieved by using specific antibodies. Tripet *et al.* [49] reported that antibodies directed to HR2 inhibited SARS-CoV replication *in vitro*. The mechanistic analysis of the process also proved that the exposition of the heptad repeat region is the key factor, and that indeed heptad repeat-specific antibodies inhibited infection at the stage of the virus entry into the host cell.

It is assumed that as a result of the structural switch, the fusion peptide is exposed and facilitates the membrane fusion. Sainz *et al.* [50] identified several regions of the S protein characterized by high hydrophobicity with high predisposition to interact with lipid membranes. Development of synthetic analogous peptides led to the identification of highly efficient SARS-CoV inhibitors.

Coronaviral enzymes

Coronavirus replication process employs several proteins with enzymatic activity encoded by viral RNA. These proteins are unique for coronaviruses and thereby may be used as targets for potential therapy. The best studied coronaviral enzymes are two proteases (PL^{pro} and M^{pro}) processing pp1a and pp1ab, and the majority of reports present efficient block of coronaviral replication with synthetic or natural compounds inhibiting M^{pro} (for review see [51]). Unfortunately, for other viral proteins such as nsp14 (ExoN) or nsp15 (NendoU), no specific antiviral agents have been identified so far.

Main protease inhibitors

Viral M^{pro} is a highly conserved protein required for maturation of functional proteins and therefore constitutes a key target for the design of anticoronaviral agents [52]. Several recent studies show novel classes of agents inhibiting M^{pro} activity. The majority of identified compounds interact directly with an active site of the enzyme and these include stable benzotriazole esters [53•], tetrapeptide phthalhydrazide ketones, pyridinyl esters and their analogues [54•], peptidomimetic inhibitors [55•], TG-0205221 [56•], coumarin derivative esculetin-4-carboxylic acid ethyl ester from the tropical marine sponge *Axinella corrugata* [57•], series of isatin derivatives [58•], peptide aldehydes [59•] or quercetin-3-b-galactoside [60•]. The majority of the described compounds have been identified using the molecular docking studies, and frequently their ability to inhibit virus replication has not been verified experimentally. In this context, it is worth noting that several novel algorithms for molecular docking have been developed and employed in the discovery of potential inhibitors [61,62]. These algorithms may be successfully used for novel drug development during potential future epidemics.

Interestingly, one study by Wei *et al.* [63•] describes the identification of the M^{pro} inhibitor not interacting with an active center, but with the N-terminal region of the M^{pro}, interfering with the protein dimerization.

Helicase inhibitors

The coronaviral helicase is a motor protein, using the energy derived from nucleotide triphosphate hydrolysis to separate long stretches of double-stranded nucleic acids [64]. Helicase represents a promising target for antiviral therapy because of its pivotal role in viral replication and high conservancy. In spite of this, the development of selective coronavirus helicase inhibitors is considerably difficult, due to a large number of cellular helicases and thereby drug-related cytotoxicity. Earlier, the adamantane-derived bananins were shown to be effective inhibitors of both the ATPase and helicase activities of the SARS-CoV helicase [65]. Recently, employing molecular docking studies, Hoffmann *et al.* [66] identified compounds bearing two phosphonic acid moieties or phosphates located at the distal ends of a molecule, which bind to the catalytic site with higher affinity than ATP. Unfortunately, no biological assays have been performed so far to confirm these findings. Another report presents development of several bismuth-based compounds (bismuth nitrilotriacetate, bismuth nitrate, bismuth tricysteine complex and ranitidine bismuth citrate) inhibiting the ATPase activity and replication of SARS-CoV at micromolar concentrations.

Inhibition of ion channel formation

The coronaviral E protein is a small structural protein that forms the selective ion channels in the lipid bilayer [67]. The exact functions of the coronavirus E protein are still not elucidated but the E protein has been shown to be vital for coronavirus replication, being involved in virus assembly and morphogenesis. Wilson *et al.* [68•] identified hexamethylene amiloride as a potent inhibitor of several coronaviruses. The antiviral potential of this compound has been previously described and is related to hexamethylene amiloride-mediated inhibition of ion channel formation. This compound is currently in use for the clinical treatment of influenza A infections [69,70].

Host proteins involved in coronavirus replication

Although coronaviruses carry in their genome proteins essential for their replication, they also require cellular proteins. Recently, some studies have shown that specific interference with cellular proteins can result in the coronavirus replication arrest. de Lang *et al.* [71•] have demonstrated that the downregulation of ACE2 with IL-4 and IFN-γ resulted in inhibition of SARS-CoV replication, whereas Raaben *et al.* [72•] presented inhibition of murine hepatitis virus with cyclooxygenase inhibitors. Although such an approach is attractive because it limits the chance of resistance development by the virus and has been used with some success during SARS-CoV epidemics [73], extensive studies on potential cytotoxicity of these compounds have to be performed.

Conclusion

Despite the lethal potential of the *Coronaviridae* family members residing in animals, the danger was neglected for many years [74,75]. SARS-CoV emergence and the epidemic gave us a warning that we should study more carefully the pathogens residing within the animal population. At present, even though great progress has been made in the past 4 years, we still lack an approved drug or efficient therapy strategy. In the authors' opinion, current research on antiviral drug discovery should focus on development and validation of existing compounds. The most promising for a standard use are the CBAs, especially plant-derived lectins, and the siRNAs targeting the coronaviral genome, as both can be administered orally. The first group of compounds is characterized by broad specificity and low cytotoxicity. The latter may be potentially prepared as an siRNA cocktail, targeting broad range of respiratory viruses, delivered either as naked RNA or in, for example, adenoviral vectors. The successful inhibition of virus replication and arrest of disease progression in macaques further suggest high antiviral potential of these treatment strategies [76,77]. Another treatment strategy for potentially emerging coronaviruses would be the employment of passive immunity. This approach may provide us the precious time for the preparation of more specific anti-CoV agents such as antiviral drugs and vaccines.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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