JAC antiviral

Journal of Antimicrobial Chemotherapy (2008) **62**, 437–441
doi:10.1093/jac/dkn243
Advance Access publication 18 June 2008

The management of coronavirus infections with particular reference to SARS

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The human coronaviruses (HCoV) OC43 and 229E are common causes of upper respiratory tract infections. Severe diseases were rare, however, until the emergence of the severe acute respiratory syndrome (SARS)-CoV in 2003. Since then, other novel CoV (NL63 and HKU1) have been described, and they have caused respiratory infections worldwide. Potentially exposed laboratory workers or animal handlers with rapidly progressive pneumonia not responding to standard antibacterial coverage must be isolated with contact and droplet, and for specific situations, airborne precautions, till rapid tests of respiratory and faecal samples are negative for SARS-CoV. Generally, the viral loads collected at different anatomical sites correlate with the severity of symptoms and mortality. Shedding of SARS-CoV peaks at day 10 after the onset of symptoms, which theoretically allows ample time for antiviral treatment. The disease is characterized by uncontrolled replication of the virus and a prominent pro-inflammatory response. No randomized controlled trials with a specific anti-coronavirus agent have been conducted with respect to therapy or prophylaxis. Reports using historical matched controls have suggested that treatment with interferon alfacon-1 (a synthetic interferon) combined with steroid, protease inhibitors together with ribavirin, or convalescent plasma containing neutralizing antibody, could be useful. Prophylaxis with interferon or hyperimmune globulin may be considered for unprotected exposure. The role of immunomodulators to decrease excessive inflammation remains elusive. Other non-SARS-CoV infections are generally milder in immunocompetent hosts, and scientific data on antiviral treatment of these viruses are scarce.

Keywords: antiviral therapy, immunomodulators, respiratory tract infections, severe acute respiratory syndrome

Introduction

The order Nidovirales consists of two families of enveloped, positive-sense, single-stranded RNA viruses, the Arteriviridae and the Coronaviridae. Potential human pathogens are found in the two genera of Coronaviridae, *Coronavirus* and *Torovirus*. Human coronaviruses (HCoV) were initially discovered in the mid-1960s as causes of respiratory tract infections, and the two principal pathogens are HCoV-OC43 and HCoV-229E. Interest in coronaviruses was rekindled in 2003 with the global outbreak of severe acute respiratory syndrome (SARS), which resulted in over 8000 cases and a global case-fatality rate of 11%. The aetiological agent of SARS was found to be a novel coronavirus currently named SARS-CoV.[1] Unlike HCoV-OC43 and HCoV-229E, the severity of illness, high mortality rate and the pandemic potential of SARS-CoV prompted a rapid search for effective antiviral therapies. Following the discovery of SARS-CoV, other coronaviruses were subsequently recognized to be common causes of community-acquired respiratory infections, especially HCoV-NL63 and HCoV-HKU1. These two novel agents are only found in humans and cause acute respiratory diseases of lesser severity and mortality than the SARS-CoV, especially in an immunocompetent host. However, more active surveillance has shown that these new viruses can also contribute to severe community-acquired pneumonia.[2]

Transmission of SARS-CoV occurs primarily through large droplets and through contact, with possible airborne transmission in some rare situations. The risk of transmission is reflected in the at-risk populations: wild animal handlers (in southern China), household contacts and healthcare workers. The last group is especially susceptible and accounts for 21% of all global cases. Aerosol-generating procedures are particularly risky. The basic reproduction number of the infection in the early epidemic was 2–4.

At the emergence of SARS in late 2002 and its re-emergence in late 2003, the affected patients were animal handlers, while

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all the sporadic cases afterwards were related to exposed laboratory workers. Therefore, patients with the relevant exposure history who developed rapidly deteriorating pneumonia must be isolated by contact, droplet and if possible airborne precautions, till the serial RT–PCR tests of their respiratory and faecal samples, collected at presentation and at the peak of viral shedding which is 10 days after the onset of the illness, are negative for SARS-CoV. In addition to RT–PCR, antibody detection is another confirmatory test for diagnosing SARS. However, definitive diagnosis by serology requires demonstration of a 4-fold rise in antibody titre between paired sera. In the clinical laboratories, indirect immunofluorescent antibody assay is more commonly used rather than neutralization assays, as the latter is more cumbersome and requires handling live viruses. It is also possible to diagnose SARS by detection of viral antigen in the serum using monoclonal and polyclonal antibodies against the N protein. The sensitivity of assays is inversely proportional to the time after onset of illness and serum antibody titre, and the sensitivity is much lower when tested on non-serum clinical specimens.

Strategies of therapy and therapeutic options

Traditionally, there were no effective antiviral agents for coronaviruses and initial efforts focused on the use of currently available drugs, either conventional antiviral agents or non-antivirals with inhibitory effects on SARS-CoV. When SARS-CoV was better characterized in terms of virology and pathogenesis, attempts were made to target specific pathways or viral molecules using novel compounds. Another approach to therapy was the use of agents that augment the immune system or provide specific antibodies using passive immunization (Table 1). Summaries of the agents that had been tested or undergone clinical trials were recently published.[3–8] It has to be noted that none of the potential antiviral agents has undergone randomized controlled clinical trials to assess their efficacies.

The pulmonary pathology of SARS consists of prominent inflammation with diffuse alveolar damage, presence of multinucleated pneumocytes, squamous metaplasia of the epithelium and sometimes bronchiolitis obliterans organizing pneumonia-like lesions. Viral particles are commonly seen within the pneumocytes. Haemophagocytosis is sometimes seen in the lungs.[9] In the absence of solid information based on controlled trials, the most important aspect of the management of SARS is ventilatory support, together with the use of antibacterial agents necessary to control secondary bacterial infections. The use of non-invasive ventilation as initial ventilatory support in SARS patients was associated with reduced intubation need and mortality in a cohort of patients in Hong Kong. There were no systematic surveys on the prevalence of bacterial co-infection at presentation or the incidence of nosocomial superinfection. In some series, patients developed nosocomial bacteraemia, catheter-related sepsis or nosocomial pneumonia due to *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae* or *Escherichia coli*.[10] Effective antiviral therapy to control viral replication and hence tissue damage and inflammation are highly desirable. The role of antiviral therapy is supported by the fact that viral load is positively correlated with the development of organ dysfunction and death.[11] The key organs of replication of SARS-CoV are the lungs (in pneumocytes) and alimentary tract (in enterocytes). Another issue to be considered

Table 1. Strategies for antiviral treatment of SARS-CoV infection

	Currently available drugs for human use	Investigational agents
Viral targets		
viral entry and fusion	chloroquine	convalescent plasma[a], monoclonal antibodies, peptides representing different regions of ACE2, luteolin, other small molecules, peptides targeting S protein
viral replication	chloroquine	calpain inhibitors
viral protease	protease inhibitors (lopinavir/ritonavir[a] and nelfinavir)	quercetin
viral RNA synthesis and gene expression	ribavirin[a] and indomethacin	siRNA
Immunomodulation	interferon alfacon-1[a]	interferon-α and interferon-β
Unknown or other mechanisms	nitric oxide[a], niclosamide and reserpine	glycyrrhizin, baicalin, valinomycin, nitric oxide donors (e.g. *S*-nitroso-*N*-acetylpenicillamine)

[a]Have been used in human subjects for the treatment of infection.

in the antiviral therapy of SARS is the time of initiation of treatment. Viral load in the respiratory tract increases in the first 10–15 days after the onset of disease and then decreases thereafter, coinciding with seroconversion of the patients.[10,12] Therefore, even if antivirals were started ~5 days after the onset of symptoms, such treatment is still potentially useful in halting the progression of infection, provided that the agents are potent enough to inhibit viral replication. Although immunomodulatory agents—primarily corticosteroids—were widely used during the SARS epidemic to avoid excessive tissue damage due to cytokine dysregulation, the benefit of this has not been conclusively demonstrated.[8] In some studies, the use of corticosteroids was associated with an increase in the plasma viral load.[13] Other complications include opportunistic infections such as aspergillosis and late sequelae, including avascular osteonecrosis, which occurred in 9.9% of the patients in a cohort in Hong Kong.[14,15]

To initiate an infection, the first step is the entry of viral particles into susceptible host cells. SARS-CoV enters host cells through the binding of the spike (S) protein to angiotensin-converting enzyme 2 (ACE2) and CD209L. Neutralizing antibodies have been shown to be protective in animal studies. Specific neutralizing antibodies are unlikely to be present in the general population at the onset of a new epidemic. Neutralizing antibodies can be elicited by an effective vaccine, and this is an active area of research. Alternatively, passive immunization can be achieved by using convalescent plasma from SARS patients. This has been used in a small number of SARS patients in Hong Kong and Taiwan in a non-randomized fashion during the initial outbreak with some clinical benefits, including a decrease of plasma viral load from $\sim 10^5$ copies/mL to undetectable levels 24 h after plasma transfusion.[16–18] There is a theoretical possibility that plasma containing neutralizing antibodies may be harvested from individuals immunized with the SARS-CoV vaccines. However, whether such plasma from such individuals will contain therapeutic levels of antibodies is unknown. Subsequently, various human monoclonal antibodies to the S protein have been shown to be able to neutralize the virus and therefore may potentially be useful for therapeutic or prophylactic purposes. Another approach to inhibiting viral entry and fusion is the use of synthetic peptides and small molecules that block the interaction between the S protein and ACE2, examples of which include peptides representing different regions of ACE2, recombinant proteins targeting the heptad repeats of S protein, quercetin and luteolin.[19–21]

The other major target of antiviral therapy is the protease of coronaviruses. The main protease $3CL^{pro}$ of SARS-CoV is essential for viral replication. Lopinavir/ritonavir (Kaletra) was the earliest combination used for the treatment of SARS patients. The main antiviral activity resides in the lopinavir component. In a series of SARS patients treated with lopinavir/ritonavir plus ribavirin as the initial regimen in Hong Kong, the treatment group had a lower incidence of adverse outcomes than the control group receiving only ribavirin in terms of overall death rate, intubation rate and rate of use and mean dose of corticosteroids.[22] In another Hong Kong series of SARS patients, the use of lopinavir/ritonavir plus ribavirin was associated with a lower incidence of acute respiratory distress syndrome, nosocomial infection, use of corticosteroids and death, lower viral loads in nasopharyngeal aspirates and higher peripheral lymphocyte counts when compared with historical controls who had received ribavirin alone.[23] More recently, nelfinavir has also been found to have antiviral activities against SARS-CoV in Vero E6 cells, although it did not reduce the viral load in the lungs of infected mice.[24,25] The beneficial effects of protease inhibitors may be due to mechanisms in addition to the direct inhibition of viral replication. *In vitro*, the $3CL^{pro}$ and other proteins of SARS-CoV apparently contributed to the development of apoptosis of host cells.[26] The protease inhibitors have been shown to be potent modulators of apoptosis in HIV infection; this immunomodulatory action is increasingly recognized as an important benefit of protease inhibitors in the treatment of HIV infection.[27] Whether the protease inhibitors demonstrate similar anti-apoptotic effects in SARS-CoV infection remains to be confirmed. Therefore, consideration of the use of protease inhibitors (in a clinical trial setting) for the treatment of SARS should be given priority in the future.

The purine nucleoside analogue ribavirin, a broad-spectrum antiviral agent, has been tested and used for the treatment of SARS since the early phases of the outbreak. There is no standardized regimen of ribavirin in this setting. Adult dosages of 8 mg/kg iv every 8 h for 14 days, 8 mg/kg iv every 8 h for 5 days followed by 1200 mg orally every 8 h for a total of 10–14 days, 2 g iv loading followed by 1 g iv every 6 h for 4 days and then 500 mg iv every 8 h for 3 days have been used, or 2.4 g orally for one dose followed by 1.2 g orally every 8 h (400 mg iv every 8 h for those who cannot tolerate oral medications) for 12 days.[10,28–30] Its antiviral activities can be attributed to the inhibition of guanosine triphosphate synthesis and viral RNA polymerase activities. In addition, release of pro-inflammatory cytokines is also reduced by ribavirin. The *in vitro* activities of ribavirin on SARS-CoV are highly variable, depending on the type of cells used for antiviral assays. When tested on Vero cells, ribavirin demonstrated little-to-no activity on SARS-CoV, presumably due to the lack of phosphorylation of ribavirin in this cell line. However, ribavirin at clinically achievable concentrations possessed significant inhibitory activities when tested in other cell lines such as Caco-2.[31] Animal studies with ribavirin have not been encouraging.[25] The clinical benefits of ribavirin alone from the case series are likewise uncertain. Anaemia is the main adverse reaction associated with the use of ribavirin. In a Canadian cohort of 110 patients, 61% of those who received ribavirin had evidence of dose-related haemolytic anaemia, 46% had hypomagnesaemia, 58% developed hypocalcaemia and 29% had both hypocalcaemia and hypomagnesaemia.[32] Although ribavirin alone is unlikely to possess substantial antiviral activities at clinically used dosages, it may be considered for use in combination with other agents, especially type I interferons, which have synergistic activities with ribavirin when tested *in vitro*.[31,33] Therefore, the use of ribavirin in this infection should be undertaken in clinical settings as part of a combination therapy.

Interferons are some of the more promising agents for the control of SARS-CoV infection. A large number of interferons belonging to the three classes (α, β and γ) have been tested for their antiviral activities against SARS-CoV *in vitro* and in animal models. Interferon-α and -β have consistently been shown to be active *in vitro*, with interferon-β appearing to be the most active of the three classes of interferons. In an uncontrolled clinical study, the use of corticosteroids with interferon alfacon-1 (a synthetic interferon-α), appeared to result in improvements in oxygenation and more rapid resolution of chest radiograph abnormalities.[34]

Certain unconventional agents have been investigated for their antiviral activities against SARS-CoV. Chloroquine is one of the better studied compounds. It possesses *in vitro* antiviral activities

against SARS-CoV and HCoV-229E, and the anti-inflammatory properties of chloroquine have been postulated to be beneficial for the treatment of SARS.[35] The mechanism of action of chloroquine is unknown, but may involve alterations of ACE2 glycosylation and endosomal pH. The antiparasitic agent, niclosamide,[36] extracts from herbal medicines (e.g. glycyrrhizin and baicalin);[37] and non-steroidal anti-inflammatory agents such as indomethacin have all been reported to inhibit SARS-CoV *in vitro*.[38] Experience from clinical or animal studies for these agents is lacking.

Development of new therapeutic agents for human use is a lengthy process, and this is unlikely to be of immediate benefit in the wake of new epidemics due to novel pathogens. The appropriate use of existing agents is the only viable option under such circumstances. Based on available information, the most suitable candidates for clinical trials in the event of another outbreak of SARS appear to be protease inhibitors (lopinavir and nelfinavir), interferons (interferon alfacon-1, interferon α-n1, interferon α-n3 and interferon β-1b) and convalescent plasma (which is stocked by some institutions such as the Hong Kong Red Cross Blood Transfusion Service). These have been used with some success in uncontrolled clinical trials, and their efficacy has been supported by laboratory data. Combination therapy is another possibility that has not been fully explored; chloroquine and ribavirin are possible agents to consider in this regard, and a protease inhibitor combined with ribavirin is another option for clinical studies.

Prevention of SARS-CoV infection

In addition to the obvious use in the treatment of SARS-CoV infections, effective antivirals could potentially be important for prophylaxis in exposed individuals, such as laboratory and healthcare workers, as well as for preventing outbreaks in institutions. Although no studies have addressed the safety and effectiveness of antiviral prophylaxis, this should be considered in situations in which healthcare workers are exposed during aerosol-generating procedures or when there is exposure to a high viral load (as in laboratory accidents). These are the circumstances where the risk of transmission is the highest during epidemic and post-epidemic periods. Intranasal interferons have been used with some success in the prevention of experimental coronavirus and rhinovirus upper respiratory tract infections, and this mode of chemoprophylaxis warrants further studies for SARS-CoV.[39,40] The use of convalescent plasma is another option for prophylaxis, although this has not been tested clinically. Intranasal or systemic interferon and convalescent plasma for prophylaxis should be considered in the face of a new outbreak because both these agents are relatively safe. The major caution in the use of antibodies for immunoprophylaxis is the possible enhancement of viral infectivity by the antibodies, a situation that has occurred in feline coronaviruses.[41]

Active immunization against SARS-CoV has been attempted by a number of approaches, including the use of inactivated whole viruses, recombinant protein fragments and subunits, DNA vaccines and viral vectors carrying the target proteins.[3] Neutralizing antibodies against the S1 is the main correlate with immunity. Protective efficacy of the candidate vaccines is mainly studied in animals so far, and only few vaccines have entered Phase 1 human trials.[42]

SARS-CoV is not unduly resistant to chemical disinfectants (such as alcohols, sodium hypochlorite and povidone–iodine) and heat (such as 56°C for 60 min or 60°C for 30 min), but can survive in faecal and respiratory specimens for over 7 days at room temperature. The use of common disinfectants in the hospital or laboratory is adequate. However, the fact that 30% of the SARS cases occurred in healthcare workers means that infection control within the hospital is a crucial component of management. In addition, the last cases of SARS occurred in 2004 and involved three incidents (in Singapore, Taiwan and Beijing) of laboratory-acquired cases in the post-outbreak period. These incidents were not related to failures in disinfection, but to accidents and possible breaches in biosafety practice. In general, contact and droplet precautions are the quintessential infection control measures, whereas airborne precautions are necessary in aerosol-generating procedures such as bronchoscopy.

The importance of personal hygiene could also be important in the community setting. Intrafamilial transmission of SARS during the epidemic had occurred, and household members' secondary attack rate ranged from 6.2% to 10.2% in Toronto, Singapore and Hong Kong, respectively.[43–45] In two of these cohorts, prolonged contact with the index patient was a risk factor for transmission and, interestingly, the Toronto series also showed that hand hygiene practices may also affect the risk of transmission. Early recognition of the index case (to shorten the duration of exposure) and maintenance of good personal hygiene are prudent measures to minimize familial spread of the infection.

Antivirals against other non-SARS-CoV coronaviruses

Studies of antiviral therapy against coronaviruses other than SARS-CoV have been scarce. There have not been clinical trials on therapy of infections caused by HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU1. Limited *in vitro* data suggested that intravenous immunoglobulins, heptad repeat 2 peptide, siRNA and some other chemicals may have inhibitory activities on HCoV-NL63, and saikosaponins (a group of oleanane derivatives from certain medicinal plants) are inhibitory to HCoV-229E.[46,47]

Transparency declarations

None to declare.

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