

Antiviral chemotherapeutic agents against respiratory viruses: where are we now and what's in the pipeline?

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Purpose of review

The emergence of severe acute respiratory syndrome in late 2002 and the recent outbreaks of avian influenza in Asia are timely reminders of the ever present risks from respiratory viral diseases. Apart from influenza, there are no vaccines and very few antiviral chemotherapeutic agents available for the prevention and treatment of respiratory viral infections—the most common cause of human illness. If the current H5N1 avian influenza outbreak ever assumes the role of a pandemic, formidable technical difficulties relating to the properties of the agent, itself, will ensure that vaccines will only become available after a significant lead time and then only to a relatively small percentage of the population. The use of existing antivirals could be critical in limiting the initial spread of a pandemic, although their use in the control of epidemics caused by nonpandemic viruses has not been evaluated. It is against this background that a review of recent developments in respiratory antivirals has been undertaken.

Recent findings

The late 1990s were a period of unprecedented activity in the development of new and much superior antivirals for the treatment of influenza infections. However, during the past 2 to 3 years and largely for commercial reasons, there has been a decline in interest in their further development by major drug companies. This situation may soon change with the possible advent of new pandemic viruses, and moves are afoot in several countries to consider the stockpiling of antivirals. The neuraminidase inhibitors zanamivir and oseltamivir, and the M2 inhibitors amantadine and rimantadine, remain the only options for controlling respiratory disease caused by influenza viruses, although the latter two could not be used against very recent H5N1 strains. There are several other neuraminidase inhibitors in development. Compounds with activity against other respiratory viruses, notably rhinoviruses, are also in development, many based on a newer knowledge of viral protein structure and function (rational drug design).

Summary

The following is an overview of recent papers on the further development of neuraminidase inhibitors against influenza viruses and on recent development of newer antivirals against RSV and rhinoviruses. Where possible, comparisons are made with existing antivirals. For considerations of space, this review has been structured around stages in the replication cycle of significant respiratory viruses that have been traditionally used as targets for inhibition.

Keywords

antiviral agents, respiratory viruses, neuraminidase inhibitors

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Introduction

Viral respiratory infections are the most common cause of illness in humans, being responsible for millions of lost work hours and significant costs to the community from physician visits and hospitalizations [1]. The total annual costs of non-influenza-related viral respiratory infections for the United States, alone, have been estimated to be \$US 40 billion [2]. Of approximately 200 viral respiratory pathogens, the most important are influenza and respiratory syncytial viruses (RSVs). Other important human respiratory viruses include rhinoviruses, parainfluenza viruses, coronaviruses, coxsackieviruses, and adenoviruses [3]. Although rarely involved in clinically significant infections of the lower respiratory tract, common colds cause widespread illness and very high economic loss to the community. Rhinoviruses are responsible for about 50% of all common colds [4], with coronaviruses, parainfluenza viruses, coxsackieviruses, adenoviruses and, occasionally, influenza and RSV being responsible for most of the remainder. The human metapneumovirus [5] and a novel coronavirus, which has been recently shown to be the etiologic agent of severe acute respiratory syndrome [6–8], are examples of emerging respiratory viruses whose epidemiologic significance has yet to be determined.

In most countries, the only vaccines available for the prevention of viral respiratory infections are those used for the prevention of influenza. Vaccination is recommended for key risk groups, including the elderly and those with underlying chronic respiratory and other illnesses [9]. There are no vaccines available for the prevention of other clinically important respiratory viral dis-

eases, although several are in development. Alternative currently licensed therapies for the prevention and treatment of these infections, which have had limited application, include passive immunization for RSV using humanized antibodies [10], and antiviral drugs for influenza viruses and, in some situations, for RSV [11,12].

Current therapeutic options for respiratory virus infections

Antiviral drugs have a potentially critical role in the prevention and treatment of respiratory illness. Antiviral therapy has been used as a complementary strategy together with immunization in the prevention of influenza in the elderly [13]. It could be especially useful in the control of other respiratory viral infections, such as RSV, for which there are no vaccines. Major objections to the widespread use of antivirals are their cost, especially when used for prophylaxis, and the potential to allow selection of resistant strains that could be pathogenic but untreatable.

Ion channel blockers: amantadine and rimantadine

Amantadine (1-adamantanamine hydrochloride; Symmetrel) and its derivative rimantadine (α -methyl-1-adamantanemethylamine; Flumadine) are symmetric tricyclic amine compounds that inhibit strains of influenza A by blocking the M2 protein that functions as an ion channel [14,15]. The transmembrane domain of the M2 protein is highly conserved in all avian and human strains of influenza A, and both amantadine and rimantadine are effective at low doses against H1N1, H2N2, and H3N2 strains [16]. Both are ineffective against influenza B and C viruses, which do not use an M2 ion channel in the initial uncoating steps of replication.

Both drugs are approximately 70% effective in preventing influenza A when used prophylactically [17–19] and are effective in reducing the duration of viral shedding and the duration of symptoms when used as therapy in established infection [19–22]. Reductions of 1 to 2 days in the duration of symptoms have been demonstrated when treatment is commenced within 48 hours of the onset of illness [17,20]. However, there is no evidence that both amantadine and rimantadine can prevent complications associated with influenza infections [1•,13].

Some side effects are associated with the use of M2 ion channel inhibitors, which are reversible after cessation of treatment. These included gastrointestinal and central nervous system side effects, although these are lower for rimantadine than amantadine [17].

Resistance to both drugs develops quickly for all influenza A viruses. The current H5N1 virus that has occurred widely in Southeast Asia has been shown to have a resistant site in the M gene, so the M2 ion channel inhibitors could not be used to control this virus. Resistant strains spread quickly in institutions, such as nursing

homes, where the drugs are often used prophylactically in the face of an outbreak [23].

Neuraminidase inhibitors

Inhibition of the neuraminidase enzyme of both influenza A and B viruses prevents the spread of viral progeny in the respiratory tract by preventing the detachment of mature virions from the cell surface. The active site of the enzyme is highly conserved across all influenza A and B strains [24]. The determination of the three-dimensional structure of the influenza neuraminidase by X-ray crystallography [25] and subsequent computer modeling [26] has allowed the design and synthesis of specific inhibitors of the enzyme.

Zanamivir (Relenza)

Numerous randomized, placebo-controlled studies have shown that zanamivir is efficacious when used for the prophylaxis and treatment of naturally acquired influenza infections in healthy adults when used early following symptom onset. Trials in which zanamivir was used to treat naturally occurring influenza infections in adults have shown that the duration of symptoms was reduced by at least 1 day [27–29], and similar findings have been noted for children [30]. For high-risk patients with confirmed influenza infection, a meta-analysis of randomized, placebo-controlled trials showed a 2.5-day benefit in terms of symptom reduction and a 45% reduction in complications [31]. A randomized, double-blind, placebo-controlled trial assessing the efficacy of zanamivir showed that daily administration of 10 mg for 4 weeks was 67% effective in preventing illness and 87% effective in preventing febrile influenzal illness [32]. However, zanamivir has not been licensed for the prophylaxis of illness caused by influenza because of the limited amount of supporting data available at the time of its approval [33].

Zanamivir has poor oral bioavailability because of the presence of a positively charged guanidine group in its structure and is, therefore, administered topically by oral inhalation using a DiskHaler at a dosage of 10 mg, 12 hourly. Treatment must start as soon as possible after the onset of symptoms (within 48 hours) to be effective [28].

Adverse effects from zanamivir have been shown to be minimal. However, the administration of the powdered drug to the respiratory tract has been shown to exacerbate respiratory distress in patients with asthma or those with chronic obstructive pulmonary disease [34,35], which limits its use to individuals without compromised respiratory function.

Oseltamivir (Tamiflu)

Oseltamivir (RO 64-0796; GS 4104; Tamiflu) is an ethyl ester prodrug with high oral bioavailability that was developed by Gilead Sciences and F. Hoffman-La Roche [36]. It is converted to its active form oseltamivir carbox-

ylate (RO 64-0802; GS 4071) in the gut after ester hydrolysis [37]. The active form has been shown to be a very specific *in vitro* inhibitor of the neuraminidase enzyme of all influenza A and B strains when tested at low concentrations [38]. Oseltamivir carboxylate is also active against avian influenza viruses [39], including the H5N1 and H9N2 strains [40]. Oseltamivir has been shown to be effective in the treatment of naturally occurring influenza infections in adults [41,42]. The median time to illness resolution was reduced by approximately 30% (approximately 1.5 days) and illness severity by approximately 35% in patients receiving either 75 mg or 150 mg of drug b.i.d. for 5 days. Administration of the higher dose did not appear to affect the end-point or clinical benefit [41,42].

As with amantadine, rimantadine, and zanamivir, there are inconclusive data concerning the efficacy of oseltamivir in the prevention of influenza-related complications in high-risk patients [13]. However, recent meta-analysis of data from 10 placebo-controlled trials suggests that treatment with oseltamivir did reduce lower respiratory tract complications, antibiotic use, and rates of hospitalization in healthy and high-risk individuals [43]. There is also evidence that oseltamivir reduces the incidence of acute otitis media in children infected with influenza viruses [44].

Oseltamivir has been shown to be as efficacious as the other antiinfluenza drugs in preventing febrile, laboratory-confirmed influenza infections in healthy adults [45] and in preventing clinical influenza among household contacts of an infected patient [46]. It is well tolerated with few side effects, most of which can be avoided by ingestion with food.

Viral resistance to neuraminidase inhibitors

Mutants resistant to the neuraminidase inhibitors can be generated *in vitro* after several passages in the presence of drug [47–50] and have also been isolated from patients. Resistant virus was only recovered once from an immunocompromised child receiving zanamivir after an influenza B infection, 12 days after treatment was commenced [51]. Mutants resistant to oseltamivir have been isolated in 0.4% to 4.0% of patients, usually from those showing prolonged virus shedding [52•]. However, they do not lead to clinically resistant infection in the immunocompetent host, probably due to the ability of the immune system to eliminate them.

Mutants resistant to the neuraminidase inhibitors display a lower capacity to replicate in mice and ferrets, in comparison with nonresistant viruses [51,53] and, unlike the M2 ion channel inhibitors, appear unlikely to be easily transmissible. To date, there is no evidence of transmission between infected individuals [54].

Inhibitors of viral replication: ribavirin

Ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide; Virazole, Virazid, ICN Pharmaceuticals), an analogue of guanosine, has been a drug of interest for many years. It has *in vitro* activity against several unrelated RNA and DNA viruses, although its mechanism of action is unknown [55]. It has been suggested that ribavirin acts as a mutagen for RNA viruses, especially at higher concentrations [56•]. Ribavirin is only active *in vivo* against RNA viruses. In humans, ribavirin is effective against hepatitis C virus [57], RSV [58] and some arenaviruses, including Lassa fever virus [59]. It was first manufactured by ICN Pharmaceuticals in 1972 and is currently licensed for treatment of severe RSV disease in high-risk infants, Lassa fever virus infections, and for the treatment of hepatitis C in combination with interferon- α (Rebetron).

Ribavirin gained approval in the United States for the treatment of RSV infections in 1986, based on studies that demonstrated modest reductions in viral shedding and symptoms in previously healthy children [60] and in children at risk [61]. It is the only drug currently approved for the treatment of RSV infections, but its reported efficacy has been the subject of controversy. It is also used for the treatment of RSV in immunocompromised patients, such as peripheral stem cell transplants. In the years since its approval, there has been no consistent demonstration of a reduction in mortality rates, decreases in the need for mechanical ventilation, or length of administration [62,63].

Ribavirin is administered by means of a mask, tent, or mechanical ventilator for 12 to 18 hours daily for 3 to 7 days [64]. Oral administration has been associated with toxicity especially hemolytic anemia. Ribavirin is mutagenic for mammalian cells in culture and tetragenetic for several animals; procedures to protect health care workers and family from the aerosolized drug are necessary. This form of ribavirin used for treating RSV has few acute side effects but is unpleasant for the patient and has been associated with decreases in respiratory function. A more acceptable oral form of the drug has been investigated for the pre-emptive treatment of RSV infections in severely immunocompromised adults and children, such as patients undergoing bone marrow peripheral stem cell transplants [65,66]. Oral ribavirin in these patients was well tolerated, and symptoms caused by RSV infections were shown to resolve. Aerosolized ribavirin also has activity against influenza A, influenza B, and parainfluenza, but has no current role in the treatment of infections caused by these viruses [67].

Because its clinical benefits are limited and costs are high [68•], the American Academy of Pediatrics Committee on Infectious Diseases has, in recent years, revised its recommendation for the use of ribavirin in chil-

dren with serious RSV infections from *should be used to may be considered* [69].

There may be a role for ribavirin in the treatment of RSV infections in combination with passive immunization with humanized antibodies, such as RSVIG (Synergis). Some clinical benefit was demonstrated in a single study in which pediatric bone marrow transplant patients with RSV infections were treated with both [70].

What's in the pipeline?

The development of antiviral drugs requires a detailed knowledge of the replication of individual viruses and their intimate association with normal host cell activity. Early antivirals were associated with high host toxicity and low therapeutic indices, because highly selective drugs that blocked viral replication without adversely affecting the host cell were difficult to develop [71,72]. In the past, a series of compounds, many already in use for the treatment of other diseases, would be screened for antiviral activity. High-throughput screening allows thousands of compounds to be assessed for antiviral activity very quickly and, in a newer approach, compounds can now be synthesized according to the structure characteristics of a particular target protein, often based on its crystallographic structure (rational design).

Capsid-binding agents

The host cell receptor for rhinoviruses binds to a ligand within a depression or *canyon* on the surface of the viral capsid [73]. Several compounds that target this site have been developed. As a consequence of binding, the capsid loses its flexibility, which is essential for cellular uptake and uncoating [74]. One such compound is pleconaril (Picovir, ViroPharma) which, if given to patients with common colds within 24 hours, was shown to reduce time for alleviation of symptoms by 1 day when administered to infected patients at 400 mg b.i.d. for 5 days [52•].

Soluble intracellular adhesion molecule-1 for rhinoviruses

The intracellular adhesion molecule-1 acts as the cellular receptor for the major serotypes of the human rhinoviruses [73]. Recombinant soluble intracellular adhesion molecule-1 has been shown to competitively bind to the viral receptors of the rhinoviruses *in vitro* and to inhibit attachment and subsequent replication [74]. Clinical trials on infected volunteers have demonstrated that soluble intracellular adhesion molecule-1 caused reductions in disease severity and reductions in the levels of infectious virus [75].

Agents that inhibit cell-cell fusion

Several promising new anti-RSV compounds have been discovered through screening for antiviral activity. These compounds appear to block important protein-protein interactions or conformational changes within the F pro-

tein complex that are essential for replication [76]. Such compounds include peptides analogues of the F protein, or small molecules, such as the triphenol VP-14637 (ViroPharma), the disulphonated stilbenes CL-387626 [77], and RFI-641 (Wyeth-Ayerst) [78], and the benzimidazole derivative R-170591 (Janssen Pharmaceuticals) [76].

Agents that inhibit viral neuraminidase

Other compounds that specifically target the neuraminidase enzyme have been developed. These include A-315675 (Abbott Laboratories), a pyrrolidine-based compound shown to inhibit influenza A N1, N2, and N9 and B strain neuraminidases in enzyme assays and to inhibit influenza A and B virus replication in cell culture as effectively as zanamivir and oseltamivir carboxylate [79]. A-315675 was shown to be more active than oseltamivir carboxylate against both laboratory strains and clinical isolates of influenza B.

Other neuraminidase inhibitors, mostly analogues of zanamivir with differing side-chains, are also under development [80,81].

Agents that inhibit transcription or translation

Antisense therapeutics

Antisense oligonucleotides have been used to sterically block virus replication by binding to target viral RNA. Recently, this technology has been exploited to produce oligonucleotides specific for RSV genomic RNA which are complementary to repetitive gene-start sequences within the RSV genome and have been covalently linked with an oligoadenylate (2-5A) [82•]. The enzyme RNase L, an antiviral enzyme of the interferon system, is activated by the oligoadenylates and cleaves the target RSV genomic RNA. One such oligonucleotide, when administered to RSV-infected African green monkeys, caused a reduction in replication of RSV in the upper respiratory tract of up to four log₁₀ infectious doses [82•]. A theoretical advantage of this type of antiviral would be its potential to target conserved viral sequences. However, its widespread application may be limited by a lack of specificity, the degradation of the oligonucleotide by nucleases, and the low rate of uptake of oligonucleotides into cells [3].

Agents that inhibit viral protease enzymes

The translation of rhinovirus RNA yields a large polyprotein that is cleaved by a family of cysteine proteases, including the 3C protease. These proteases are highly conserved among human rhinovirus subtypes and lack homology with human proteases suggesting the possibility of a high therapeutic ratio. The process of posttranslational cleavage is critical for virus replication and is an obvious target for antiviral drugs.

Several inhibitors of the 3C protease have been investigated for use against human rhinovirus infections. The compound rupintrivir (AG7088, Agouron Pharmaceuticals) has been shown to have antiviral activity against the 48 human rhinovirus serotypes *in vitro*, with a mean 90% inhibitory concentration of 49 $\mu\text{g mL}^{-1}$ [83]. The drug has been recently shown to be well tolerated and safe when administered intranasally to healthy patients; therapeutic levels were shown to be present in the nose 9 hours after administration. It has recently been suggested that rhinovirus protease inhibitors may be modified to inhibit the severe acute respiratory syndrome coronavirus protease 3CLpro, because there appears to be a substantial degree of homology between the proteases of the two viruses [84].

Agents that inhibit posttranslational processing

A novel benzodithiin derivative, RD3-0028 (Rational Drug Design Laboratories), has been shown to be a specific inhibitor of RSV types A and B, and against clinical isolates in cell culture was shown to have activity superior to ribavirin [85]. When administered to RSV-infected mice by aerosol for 2 hours b.i.d., RD3-0028 reduced the pulmonary titer at doses significantly less than that of ribavirin [86]. Work performed by Sudo *et al.* [87] suggests that RD3-0028 inhibits RSV replication by interfering with the intracellular processing of the RSV F protein, or a step immediately after.

A new look at an old drug

The compound arbidol was created by the Center for Drug Chemistry in Moscow more than 20 years ago and is licensed for use as prophylaxis and treatment for influenza A and B infections in Russia. Arbidol has recently been shown to inhibit various human respiratory RNA viruses, including several strains of influenza A and B, RSV, parainfluenza type 3 (PIV3) and rhinovirus 14, as well as the avian coronavirus, infectious bronchitis virus, and Marek disease virus, an avian oncogenic herpesvirus. Its antiviral activity, expressed as 50% inhibitory concentrations in cell culture, for influenza A and B, RSV, PIV3, rhinovirus 14, poliovirus, IBV, and MDV, was 0.22 to 11.8 $\mu\text{g mL}^{-1}$ [88]. These findings confirm earlier clinical data from Russian workers that arbidol could be considered for use as a broad-spectrum antiviral.

Conclusion

Despite much activity during 40 years, there are no licensed vaccines available for the prevention of respiratory viral infections, other than influenza. For reasons relating to difficulties in the development of respiratory immunity, the properties of some of the viruses concerned and, in the case of RSV, the difficulties inherent in actively immunizing the neonatal child, this situation is likely to continue. Respiratory antivirals are therefore likely to have a critical role as a treatment option. Given the long lead times that would be likely before a vaccine

against pandemic influenza became available, they could be particularly useful in reducing the initial impact of infection. However, at issue is whether sufficient drugs could be stockpiled to meet this eventuality, and such matters are receiving the attention of public health authorities in many countries. Recent events relating to both pandemic influenza and severe acute respiratory syndrome are likely to provide new impetus in extending the range of available therapeutic agents.

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