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Mini-review

Respiratory syncytial virus infections: Recent prospects for control

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Respiratory syncytial virus (RSV) infections remain a significant public health problem throughout the world, although recently developed and clinically approved anti-RSV antibodies administered prophylactically to at-risk populations appear to have significantly affected the disease development. Much effort has been expended to develop effective anti-RSV therapies, using both in vitro assay systems and mouse, cotton rat, and primate models, with several products now in various stages of clinical study. Several products are also being considered for the treatment of clinical symptoms of RSV. In this review, updates on the status of the approved anti-RSV antibodies, ribavirin, and recent results of studies with potential new anti-RSV compounds are summarized and discussed.

Keywords: Respiratory syncytial virus; Antivirals; Review

Contents

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1. Introduction

Respiratory syncytial virus (RSV) infections continue to be a serious public health problem throughout the world. The disease occurs during the winter months in temperate climates and, in the tropics, during the rainy season; evidence is accumulating, however, suggesting the RSV infection may be a year-round event in some areas (Halstead and Jenkins, 1998). The infection is considered the most important cause of lower respiratory tract infections worldwide in infants, particularly those less than 6 months of age, being responsible for high morbidity and mortality (Leung et al., 2005; Anon., 2005). RSV is also a significant cause of respiratory infection among the elderly (Thompson et al., 2003; Falsey and Walsh, 2006) and among bone marrow recipients (Ebbert and Limper, 2005). Vaccines are not available for protection against the RSV infection; indeed, a phenomenon known as "immunopotentiation" or "vaccine-enhanced disease" has been seen in the study of some potential RSV vaccines (Fulginiti et al., 1969; Kapikian et al., 1969; Kim et al., 1969).

The purpose of this review is to consider recent developments in antiviral agents which may have potential for treatment of this important virus infection, as well as to review information about those materials approved for prophylaxis or therapy that are presently in use.

2. In vivo test methodology

Two animal models have routinely been used to evaluate potential RSV inhibitors, these being the mouse and the cotton rat (*Sigmodon hispidus*). Rarely, non-human primates (African green monkeys and chimpanzees) have also been used; the paucity and high cost of primates have discouraged their widespread use, although some laboratories have turned to primate studies for late phases in RSV drug development (see Table 1). The guinea pig can develop an RSV-induced bronchiolitis and manifestations of asthma (Robinson et al., 1996; Bramley et al., 1999, 2003), but has rarely been used for antiviral studies. The mouse and cotton rat models offer similar parameters for evaluation of potential RSV inhibitors: moderate virus titers in tissues of the respiratory tract, and pulmonary histopathology. An effective anti-RSV dose has been defined as a 100-fold reduction in virus load in the lungs (Maggon and Barik, 2004); this is an inhibition generally greater than seen using ribavirin in mice or cotton rats (Table 1). In view of the questionable utility of ribavirin in the clinic (discussed in Section 4.6.1), this $2\log_{10}$ inhibition standard would appear appropriate, although an even greater titer inhibition would be desirable. Consideration also should be given to histopathological findings, although quantitation of such findings is difficult. Another concern in the interpretation of animal model results is the extrapolation to human outcomes of timing of treatment initiation relative to virus exposure in the animal model. In the laboratory animal, treatment is usually begun within 24 h of virus exposure; however, in the human infant, therapy usually would not begin until perhaps 3 days after manifestations of the RSV disease. Satisfactory comparisons of the results of animal testing to human studies have not yet been completed.

3. Prophylaxis

3.1. Anti-RSV antibodies

Until a safe and effective antiviral can be developed for treatment of RSV infections, prevention of the infection by use of anti-RSV antibodies appears to be the most acceptable approach. Two antibodies are currently approved for treatment of RSV disease: RSV-IGIV (RespiGam®), which is RSV immune globulin, and palivizumab (Synagis®), which is a chimeric humanized IgG monoclonal antibody, both produced by MedImmune Inc.

The RSV-IGIV is a preparation of polyclonal-concentrated RSV neutralizing antibody obtained from the sera of adult humans. An infusion of 750 mg/kg administered monthly to prematurely born infants has been reported to significantly decrease hospitalization and to reduce the number of hospital days with oxygen (PREVENT Study Group, 1997). However, the product is derived from blood, and consequently, has the potential to transmit blood-borne pathogens; further, its viscosity, coupled with required high volumes for administration, may lead to fluid overload. The material is usually given in a 2–4 h intravenous infusion (Kamal-Bahl et al., 2002). RSV-IGIV must be administered under medical surveillance. With the introduction of palivizumab, use of the product has dramatically declined (Barton et al., 2001).

Palivizumab, a humanized monoclonal antibody directed to an epitope in the A antigenic site of the F-protein of RSV, was approved in 1998 for the prophylaxis of infants at high risk for RSV infection. The product is 50–100 times more potent than RSV-IGIV (Johnson et al., 1997), and is now being used worldwide with considerable success as shown by randomized, double-blind, placebo-controlled trials (Cardenas et al., 2005). Palivizumab is licensed for intramuscular injection of 15 mg/kg administered at monthly intervals throughout the RSV season. No resistance to palivizumab has yet been reported, and all strains of RSV appear to be neutralized by it. Although this product has an excellent record in preterm infants, with a reported 78% exhibiting protection against RSV, infants with bronchopulmonary dysplasia or congenital heart disease have had a significantly lower rate of protection (Cardenas et al., 2005). This lesser effect has been attributed to an insufficient serum concentration, which would be alleviated by using a higher monthly dose (Cardenas et al., 2005).

An improved version of palivizumab, designated as MEDI-524 (Numax™), is now in Phase III clinical evaluation. This antibody appears to bind 70-fold better to the RSV F-protein than palivizumab (Wu et al., 2005). It is more potent in neutralizing RSV in vitro, and in RSV-infected mice (Mejias et al., 2005). Palivizumab exerts its protection by preventing the spread of virus into the lower respiratory tract, thus lessening the clinical manifestations of bronchiolitis; MEDI-524 also inhibits nasal replication of RSV, which may lead to inhibition of upper respiratory tract infections and otitis media (Cardenas et al., 2005).

Table 1
An overview of animal studies with potential RSV-inhibitory compounds

Compound	Animal model	Dosages used	Treatment route	Treatment schedule	Max. tissue virus titer reduction (log_{10})	Development status	Reference
Ribavirin	Cotton rat	200 mg/kg	i.p.	tid × 4 beg + 1 h	1.1	Approved 1986; high-risk patients use 1996	Hruska et al. (1982)
	Cotton rat	2 mg/ml	s.d.a.	Continuous beg + 1 h	1.0	Approved 1986; high-risk patients use 1996	Hruska et al. (1982)
	Cotton rat	90 mg/kg/day	i.p.	+24, 48, 72	1.7	Approved 1986; high-risk patients use 1996	Wyde et al. (1990a)
	Cotton rat	60 mg/ml	s.d.a.	2 h bid × 3 beg + 24 h	1.2	Approved 1986; high-risk patients use 1996	Wyde et al. (1987)
	Mouse	90 mg/kg/day	i.p.	tid × 3 beg + 24 h	1.8	Approved 1986; high-risk patients use 1996	Sudo et al. (1999)
VP-14637	Cotton rat	126 μg/kg	s.d.a.	bid × 4 beg + 24 h	2.1	Phase I, discontinued	Wyde et al. (2005)
BMS-433771	Cotton rat	25–200 mg/kg	p.o.	−1 h	∼1.0	Preclinical	Cianci et al. (2004a)
	Mouse	5–50 mg/kg	p.o.	bid × 5 beg − 1 h	1.25	Preclinical	Cianci et al. (2004a,c)
RFI-641	Cotton rat	0.2–3.3 mg/kg	i.n.	−2 h	0.6–3.2	Phase I, discontinued	Huntley et al. (2002)
	Mouse	0.08–1.3 mg/kg	i.n.	−2 h	1.5	Phase I, discontinued	Huntley et al. (2002)
	African green monkey	6 mg/kg	i.n.	−2 h	3.4	Phase I, discontinued	Huntley et al. (2002)
	African green monkey	6 mg/kg	i.n.	qd × 8 beg + 24 h	1.6	Phase I, discontinued	Huntley et al. (2002)
JNJ-2408068	Cotton rat	5 mg/ml	s.d.a.	15 min 0 or +24	1.9–3.7	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	+48 or +72 h	0.0	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	−24 h	≥2.0	Preclinical	Wyde et al. (2003)
	Cotton rat	5 mg/ml	s.d.a.	−48 or −96 h	0.0	Preclinical	Wyde et al. (2003)
MBX-300	Cotton rat	100 mg/kg/day	i.p.	qd × 3 beg + 1 h	1.5	Preclinical	Douglas (2004)
SiRNA	Mouse	3.5 μg/kg	i.n.	−4 h, +24 h or +72 h	2.0–3.0	Preclinical	Bitko et al. (2005)
	Mouse	3.5 μg/kg	i.n.	+96 h	<0.5	Preclinical	Bitko et al. (2005)
RBI-034	Cotton rat	50mg/kg	i.n.	qd × 3 beg + 1 h	1.1	Preclinical	Cramer et al. (2005)
	Cotton rat	50 mg/ml	s.d.a.	qd × 3 beg + 1 h	1.6	Preclinical	Cramer et al. (2005)
	Mouse	10 mg/kg/day	i.n.	−6 h, + 24, 72, 120 h	1.3	Preclinical	Cramer et al. (2005)
	African green monkey	50 mg/ml	s.d.a.	−6 h, + 24, 72, 120 h	4.0	Preclinical	Leaman et al. (2002)
A-60444	Nr[a]	Nr	Nr	Nr	Nr	Phase II clinical trials	Kelsey et al. (2004); Wilson et al. (2004); Carter et al. (2006)
Compound D	Mouse	0.4–4.1 mg/kg/day	i.n.	+3, 6 h, tid × 3	0.6	Phase II clinical trials	Liuzzi et al. (2005)
VX-497	Nr	Nr	Nr	Nr	Nr	Phase II vs. HBV	Markland et al. (2000)
Mycophenolate mofetil	Mouse	100 mg/kg/day	p.o.	qd × 5 beg + 24 h	Nr	Clinical use as immunosuppressant for transplant rejection	Roberts et al. (2001)
EICAR	Cotton rat	100 mg/kg day	i.p.	bid × 3 beg + 24 h	1.4	Preclinical	Wyde et al. (2000)
Pyrazofurin	Cotton rat	3 mg/kg/day	i.p.	qd × 3 beg + 24 h	1.0	Preclinical	Wyde et al. (1989)
LY253963	Cotton rat	1 mg/kg/day	i.p.	bid × 3 beg + 24 h	1.3	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)
	Cotton rat	3 mg/kg/day	i.p.	qd × 3 beg + 24 h	1.2	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)
	Cotton rat	10 mg/kg/day	p.o.	qd × 3 beg + 24 h	0.0	Phase II vs. influenza, withdrawn	Wyde et al. (1990b)

[a] Not reported.

4. Potential therapeutics

4.1. Virus targets

RSV belongs to the genus *Pneumovirus* of the family Paramyxoviridae. It is an enveloped virus containing a single-stranded, non-segmented, minus sense RNA. The RNA is 15 kDa and encodes 11 proteins, eight of which comprise the virion. Of the eleven proteins, three are surface glycoproteins designated as F-, G-, and the hydrophobic SH-protein. F- and G-proteins protrude through the envelope of the virus and are responsible for attachment (G-protein) and fusion (F-protein) to host cells. The F-protein is cleaved from a precursor F0-protein by cellular enzymes to produce the disulfide-linked F1 and F2 subunits that are the virion F-protein. SH is a small integral membrane protein whose function is not clear. It seems to be phosphorylated by an MAPK p38-dependant tyrosine kinase to achieve its normal cellular distribution in infected cells (Rixon et al., 2005). The inner portion of the envelope interacts with the mature (M) protein, which directs the assembly of virions within the inner side of the host cell membrane from which the viral envelope is derived. Within the envelope, posterior to the M-protein, is the nucleocapsid, which is made up of the major nucleocapsid N-protein that binds to genomic RNA, a phosphoprotein (P), a transcription anti-terminator factor or transcriptase processivity factor (M2-1), and the large polymerase subunit (L), which is a RNA-dependant RNA transcriptase. In addition, there are two non-structural proteins referred to as NS1 and NS2 and a regulatory protein known as M2-2.

Inhibitors of RSV that have been recently evaluated for efficacy fall into five general modes of action groups: those that inhibit attachment/fusion, oligonucleotides that target viral RNA, those that target the N-protein, those that inhibit some function of the virus RNA-dependant RNA polymerase, and those that inhibit inosine monophosphate dehydrogenase (IMPDH), although the latter may or may not represent the actual mode of inhibiting RSV replication.

4.2. Attachment/fusion inhibitors

The F- and G-proteins are involved in virus attachment and fusion, although the F-protein alone is sufficient to promote attachment to cells, subsequently leading to a productive viral infection (Karron et al., 1997). G-protein may simply enhance attachment to a target cell, but F-protein probably binds to a specific receptor (Techaarpornkul et al., 2001). The host cell receptor appears to be a glycosaminoglycan containing heparin sulfate (Hallak et al., 2000), since addition of heparin blocks virus attachment in vitro (Krusat and Streckert, 1997). Thus, numerous compounds of various classes have been synthesized which target attachment or the fusion activity of RSV, with fusion inhibitors predominating. The following reviews those that have proceeded to clinical trials and also more recently developed fusion inhibitors not yet announced to be in clinical trials. See Table 1 for an overview of each material which has undergone in vivo study which has been discussed in this review.

4.2.1. VP-14637

VP-14637 (5,5′-bis[1-(((5-amino-1*H*-tetrazolyl)imino)-methyl)] 2,2′,4″-methylidynetrisphenol; Fig. 1) was one of the first fusion inhibitors to progress to Phase I clinical trials. It is a triphenolic compound which apparently binds into the small hydrophobic cavity in the inner core of the F-protein, either preventing early transient Z conformation changes in the fusion process or by preventing formation of six-helix fusion core as the heptad repeats interact (Douglas et al., 2005). VP-14637 is a broad-spectrum inhibitor of RSV strains, inhibiting the virus in vitro at concentrations of 2 nM or less (Douglas et al., 2005; Wyde et al., 2005). In cotton rats, treatment by small droplet aerosol for 60 min significantly reduced mean lung virus titers (Wyde et al., 2005). VP-14637 was in phase I trials prior to a decision not to develop it further, in part due to developmental costs.

4.2.2. BMS-433771

Benzotriazole benzimidazoles represent another class of inhibitors that prevent fusion of RSV to host cell membranes. In particular, BMS-433771 (1-cyclopropyl-1,3-dihydro-3-[[1-(3-hydroxypropyl)-1*H*-benzimidazol-2-yl]methyl]-2*H*-imidazo[4,5-c]pyridin-2-one; Fig. 1), an azabenzimidazolone derivative, targets the hydrophobic pocket within the trimer of hairpins of the F-1 protein, a class I fusion protein (Cianci et al., 2004b). It apparently interferes with the normal association of the N- and C-terminal heptad repeats found within the binding pocket that occur as part of the fusion process (Cianci et al., 2005). BMS-433771 is active against multiple RSV strains, with a 50% inhibitory concentration (IC_{50}) of 20 nM (Cianci et al., 2004b). In a T cell-deficient BALB/c mouse model, the orally active compound (50 μg/kg) also significantly reduced virus titers in the lungs. The compound was well tolerated. The EC_{50} in the BALB/c mouse was determined to be 12 nM. The compound was somewhat less inhibitory to lung virus replication in cotton rats than in mice (Cianci et al., 2004a,c).

4.2.3. RFI-641

RFI-641 (4,4′-bis-4,6-bis-[3-(bis-carbamoylmethyl-sulfamoyl)-phenylamino]-[1,3,5] triazin-2-ylamino-biphenyl-2,2′-disulfonic acid; Fig. 1) from Wyeth–Ayerst (Pearl River, NY) is biphenyl triazine anionic compound that is an analog of CL-309623, a previously identified dendrimer-like stilbene inhibitor with anti-RSV activity (Gazumyan et al., 2000). Like the parent compound, RFI-641 inhibits RSV fusion mediated by the F-protein by directly interacting with that protein (Razinkov et al., 2002). The compound inhibited both A and B strains of RSV with EC_{50} values in the 20 nM range. It was also relatively non-toxic with selective indices ranging from 417 to 2500 (Douglas, 2004). The drug has been evaluated extensively in small animal models (Huntley et al., 2002) and in African green monkeys (Weiss et al., 2003). In the mouse, RFI-641 at 1.3 mg/kg delivered intranasally 2 h prior virus exposure reduced virus lung titers by 1.5 $\log_{10}$ plaque-forming units. In cotton rats using a similar prophylactic dosing regimen, doses up to 10 mg/kg reduced lung virus titers by >3 $\log_{10}$. In the primate, RFI-641 prophylactically dosed at 6 mg reduced

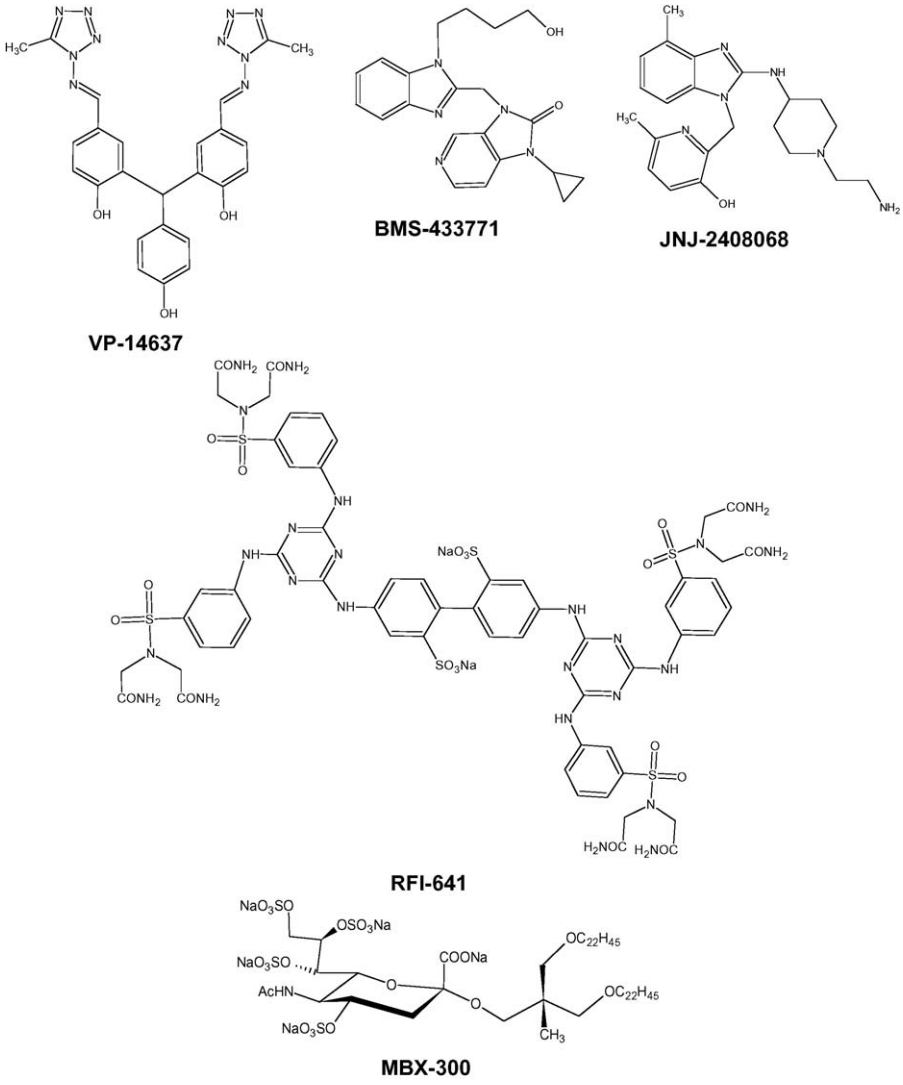


Fig. 1. Fusion inhibitors.

nasal virus titers by >3.4 $\log_{10}$ over a period of 10 days. Using intranasal administration, lung virus titers were only substantially reduced after a 2 h exposure to the compound (Weiss et al., 2003). When given 24 h after virus exposure and using daily doses thereafter, nasal virus titers were also significantly reduced (Huntley et al., 2002).

RFI-641 was in Phase II clinical trials in 2000–2001 for the secondary prevention and therapeutic treatment of RSV infections in adults (http://www.uchsc.edu/peds/research/ri/cto/resfund/index.htm).

4.2.4. JNJ-2408068

JNJ-2408068 (2-[[2-[[1-(2-aminoethyl)-4-piperidinyl]amino]-4-methyl-1*H*-benzimidazol-1-yl]-6-methyl-3-pyridinonol]; Fig. 1), is being developed by Johnson & Johnson (Raritan, NJ). It has very similar mode of inhibition to VP-14637 discussed earlier (Douglas et al., 2005). The compound is potent in vitro (EC_{50} = 0.16 nM), is not cytotoxic at concentrations >100 μM, and inhibits all members of the pneumovirus genera except for the murine pneumonia virus. In cotton rats, the compound was administered by aerosol for 15 min, either prior to or after virus exposure. Lung virus titers were reduced to below detectable limits (Wyde et al., 2003). It appeared well tolerated, but has limited oral bioavailability (Douglas, 2004).

4.2.5. MBX 300 (NMSO-3)

MBX 300 (Microbiotix, Worcester, MA) is [2,2-bis(docosyl-oxymethyl)propyl-5-acetoamido-3, 5-dideoxyl-4, 7, 8, 9-tetra-*O*-(sodium-oxysulfonyl) - D -glycero-D-galacto-2-nonulopyranosid]onate (Fig. 1). The compound apparently targets the attachment phase because its target is the G-protein. It is a specific inhibitor of RSV (Kimura et al., 2000) with EC_{50} values from 0.2 to 0.3 μM (Douglas, 2004). In cotton rats, when administered intraperitoneally at 100 mg/kg/day, lung virus titers were significantly reduced 3 days post-virus exposure (Douglas, 2004). According to Microbiotix, MBX 300 has undergone preliminary toxicology studies, including testing in Cynomolgus monkeys (http://www.microbiotix.com/pr030502.htm) and has specific and potent oral anti-RSV activity as well as an excellent safety profile. Microbiotix will be advancing MBX

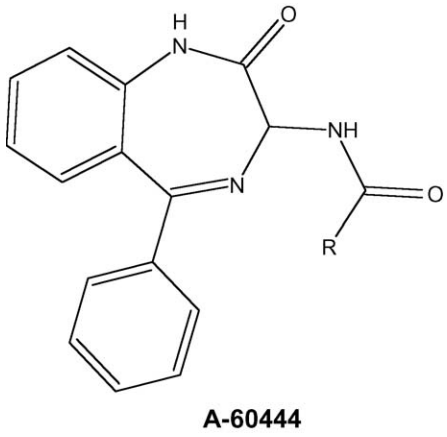
300 through preclinical development and expects to initiate clinical studies in the near future.

4.2.6. Small peptide fusion inhibitors

Recently, three peptides containing multiple copies of alternating HR1 and HR2 sequences of the terminal heptad repeats of the F-protein and denoted as 5-helix, HR121 and HR212 were designed to inhibit F-protein mediated fusion (Ni et al., 2005). The 5-helix, HR121 and HR212 proteins were functionally analogous to single HR1, HR1, and HR2 sequences of terminal ends of the F-protein, respectively. The three proteins were potent fusion inhibitors in vitro with IC_{50} values ranging from 3 to 8 μM as determined by a visual syncytial reduction assay. These peptides represent a targeted design approach for discovery of fusion inhibitors and could be lead compounds for the development of peptide RSV-peptide inhibitors.

4.3. Oligonucleotides that target viral RNA (antisense/siRNA)

Much has been reviewed regarding the theory, approaches used, and attempts to develop antisense oligonucleotides as therapies for RSV disease (Maggon and Barik, 2004; Cramer, 2005, Leaman, 2005). Strategies pursued have included antisense phosphorothioate oligodeoxyribonucleotides (ODN) (Jairath et al., 1997), short interfering double-stranded RNA molecules (siRNA) (Bitko et al., 2005), and 2–5 Å antisense chimeras (Barnard et al., 1999; Torrence, 1999). One phosphorothioate ODN targeted to repetitive intergenic sites with the RSV genome appeared significantly effective versus the virus (Jairath et al., 1997), but development never proceeded into the clinic, due, in part, to side effects (Siddiqui-Jain et al., 2002). Much study is underway with the siRNAs; a recent report by Bitko et al. (2005) showed that intranasal instillation of an in vitro-active siRNA into RSV-infected mice was significantly inhibitory to the infection. Treatment begun 4 h before the virus infection reduced the lung virus titers by 3 $\log_{10}$ and prevented pulmonary pathology from developing. When therapy began after virus exposure, the antiviral effect was progressively less, but continued to lower the virus titers. It should be noted that an siRNA being developed by Alnylam Pharmaceuticals, designated as ALN-RSV01, is claimed to be significantly inhibitory to RSV in vitro and in animal models, and the product, to be administered directly to the lungs, is now in Phase I clinical trials (Thomson CenterWatch Clinical Trials Listings Service; http://www.centerwatch.com/professional/cwpipeline/). Nothing has yet been published regarding the antiviral activity of ALN-RSV01. The use of 2–5 Å antisense strategy is also in developmental stages, but data have been recently published indicating this approach may also have promise. The 2–5 Å antisense chimera designed RBI034 has demonstrated potent anti-RSV efficacy in vitro (Xu et al., 2004) and aerosolized delivery reduced RSV infections in mice, cotton rats, and African green monkeys (Leaman et al., 2002; Cramer, 2005). Of potential significance is the observation that the combination therapy using RBI034 with ribavirin was more effective than either material used alone (Cramer et al., 2005). Some technical problems have slowed the progress of antisense antivirals; these include enhanced delivery to the target cells, a need to improve stability, a wider therapeutic window, and the challenge of large-scale synthesis (Maggon and Barik, 2004; Leaman, 2005).



A-60444

Fig. 2. N-protein inhibitor.

4.4. N-protein inhibitors

A-60444 (RSV-604) is a 1,4-benzodiazepine derivative with the general structural configuration as shown in Fig. 2 (Kelsey et al., 2004; Carter et al., 2006). In resistant mutant studies, the compound was found to be unique in that it apparently targets the N-protein of RSV (Wilson, 2004). The in vitro inhibitory activity is in the submicromolar range for both A and B RSV (Wilson et al., 2004). In Phase I clinical trials in the United Kingdom the compound was found safe and well tolerated without any serious adverse effects (Thomson CenterWatch Clinical Trials Listings Service; http://www.centerwatch.com/professional/cwpipeline).

Pharmacokinetic studies from this trial suggested that once daily dosing was feasible. The compound has now entered Phase II clinical trials (http://www.clinicaltrials.gov/ct/show/NCT00232635?order=1) to evaluate the antiviral effect of nasal/oral administration versus placebo in post-stem cell transplant patients with RSV infection and to assess the safety of the product. The pharmacokinetics of A-60444 in the presence of concomitant medications such as immunosuppressants and anti-fungal agents will also be studied. The open-label portion of the trial is now complete and the placebo-controlled trials are underway (Powell, CEO, Arrow Pharmaceuticals, personal communication). It is disappointing that detailed in vivo anti-RSV data are not yet available for this compound so that comparisons can be made to the other potential RSV inhibitors described in this review.

4.5. RNA-dependant RNA polymerase inhibitors

A number of imidazo[4,5-h]isoquinoline-7,9-dione inhibitors were synthesized that targeted the guanylylation of viral transcripts (5′ cap) of the RSV ribonucleoprotein (RNP) complex (Liuzzi et al., 2005). The most potent of these inhibitors was

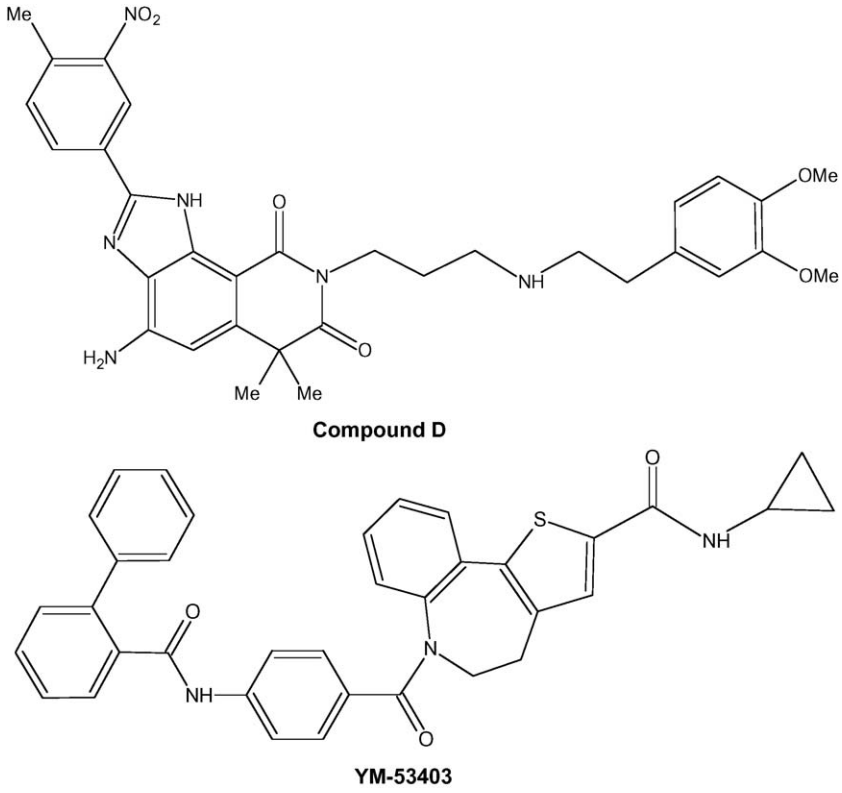


Fig. 3. RNA-dependent RNA polymerase inhibitors.

4-amino-8-(3-{[2-(3,4-dimethoxyphenyl)ethyl]amino}propyl)-6,6-dimethyl-2-(4-methyl-3-nitrophenyl)-1*H*-imidazo[4,5-h]-isoquinoline-7,9(6*H*,8*H*)-dione (Compound D; Fig. 3). These inhibitors may bind to a region in the L-protein with similarities to NDK motifs; NDK proteins play a role in maintaining the balance of intracellular nucleotide pools by exchanging gamma-phosphate groups from NTP to NDP. These compounds inhibited RSV replication in an ELISA-based assay with EC_{50} values ranging from 0.021 to 2.1 μM. Selectivity indices ranged from 30 to 400. In a mouse model, lung virus titers were reduced when the compounds were administered intranasally 3 and 6 h after virus exposure, then three times daily for 3 days at 0.4–4.1 mg/kg/day.

Recently, a novel benzazepine inhibitor of the L-protein was discovered from a large chemical library; it was designated as YM-53403 (6-{4-[(biphenyl-2-ylcarbonyl)amino]benzoyl}-*N*-cyclopropyl-5,6-dihydro-4*H*-thieno[3,2-*d*][1]benzazepine-2-carboxamide (Fig. 3) (Sudo et al., 2005). In a plaque reduction assay, the compound inhibited RSV replication at 0.2 μM. Mutant viruses with single point mutations in the L-protein (virus polymerase) were resistant to the antiviral effects of the compound and timing studies suggested that inhibition was maximal at around 8 h after virus exposure.

4.6. Inosine monophosphate dehydrogenase inhibitors

4.6.1. Ribavirin

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Virazole®) is the only antiviral drug currently approved for treatment of RSV infections. Ribavirin has inhibitory effects on a very broad spectrum of viruses, including RSV (Sidwell et al., 1972). The mechanism of viral inhibition by the drug is best described as multi-faceted and includes inhibition of IMPDH, inhibition of the 5′ cap formation of mRNA, and inhibition of viral polymerase by the phosphorylated forms of the compound, although the specific mechanism by which RSV is inhibited is not well documented (Sidwell, 1996). In early clinical studies, significant positive effects in RSV-infected infants were reported using ribavirin administered in a small-particle aerosol (Hall et al., 1983a,b; Taber et al., 1983; McIntosh et al., 1984; Barry et al., 1986); however, water was used as the placebo and has a bronchoconstricting effect by itself which may have affected the outcome of the studies. Subsequent trials, using saline as placebo, did not demonstrate the positive effects initially observed (Broughton and Greenough, 2004). Later studies have suggested that the aerosolized ribavirin may lessen post-bronchiolitic wheezing and reactive airway disease and reduce the viral load in the patient, but does not reduce the duration of hospitalization (Edell et al., 1999, 2002; Rodriguez et al., 1999; Guerguerian et al., 1999). Side effects (possible deterioration of respiratory function, anemia, teratogenicity) may also discourage use of this drug.

4.6.2. Other IMPDH inhibitors

The enzyme IMPDH catalyzes the conversion of inosine monophosphate to xanthosine monophosphate, which is an essential step in the de novo biosynthesis of guanine nucleotides

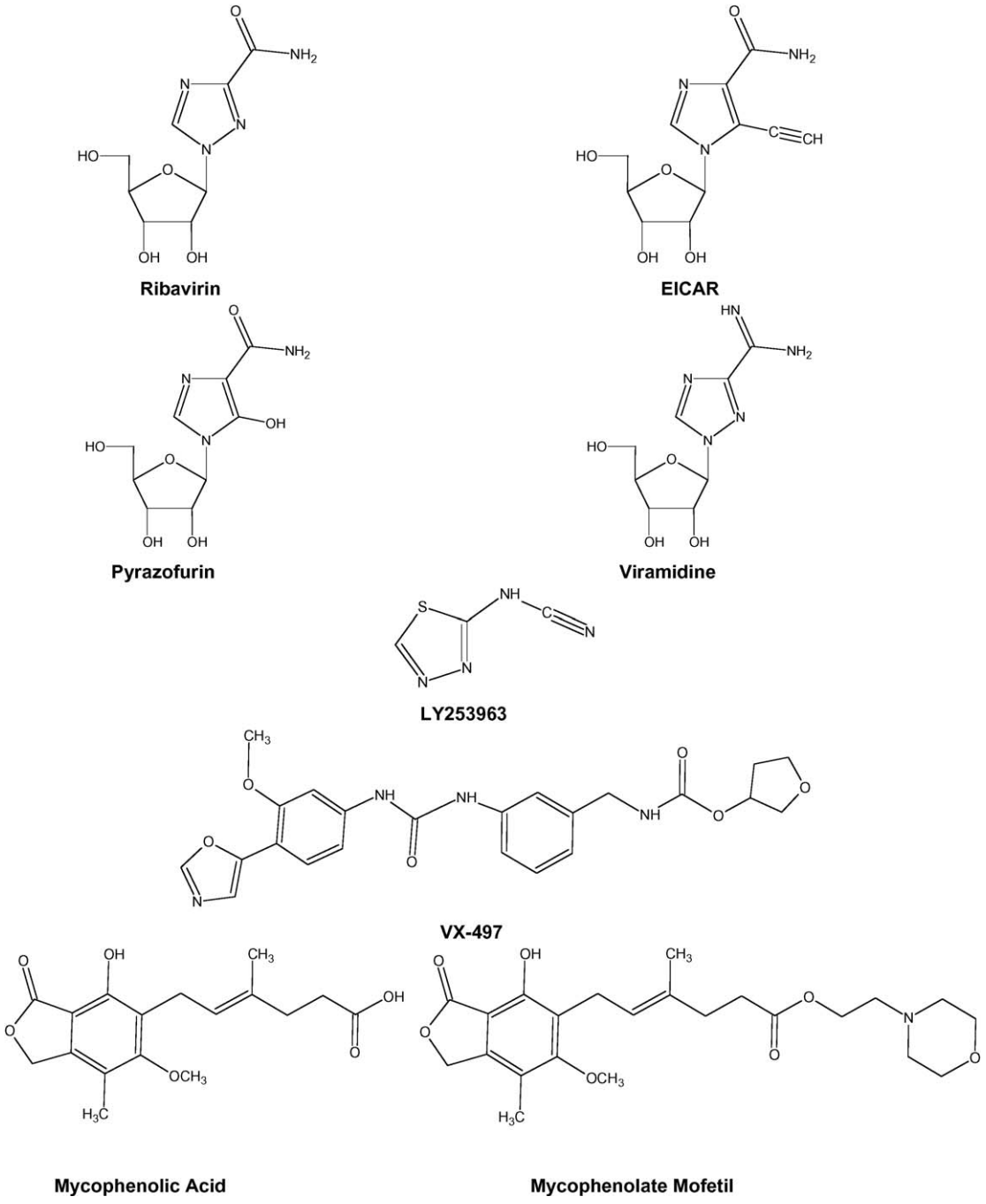


Fig. 4. IMPDH inhibitors.

leading to DNA and RNA synthesis. Inhibition of IMPDH thus reduces the amount of intracellular guanine nucleotides needed for RNA and DNA synthesis and consequently can result in significant antiviral effects, although such effects may also be associated with inhibition of cell replication. A number of compounds in addition to ribavirin, which are considered IMPDH inhibitors, have exhibited significant anti-RSV activity. These include VX-497, mycophenolic acid, mycophenolate mofetil, EICAR, pyrazomycin, viramidine, and LY253963 (Fig. 4).

VX-497 is a selective, highly potent, reversible, and uncompetitive inhibitor of IMPDH; it is structurally unrelated to other IMPDH inhibitors. The compound has a broad-spectrum antiviral effect, inhibiting RSV with a 50% inhibitory concentration (IC_{50}) of 1.1 μM and a 50% cytotoxic concentration (CC_{50}) of 10.2 μM (Markland et al., 2000). This activity was approximately 20-fold more potent than ribavirin, but the therapeutic index of VX-497 was less than that of ribavirin. The compound, in combination with interferon alpha, is currently being developed by Vertex Pharmaceuticals (Cambridge, MA) for the treatment of hepatitis C (McHutchison et al., 2005).

Mycophenolic acid has a broad-spectrum antiviral effect similar to that of ribavirin (Ando et al., 1968; Cline et al., 1969;

Planterose, 1969), although RSV was not included among the viruses initially studied. Roberts et al. (2001) have described the compound to be more potent than ribavirin both as an antiviral agent against RSV and as an inhibitor of IMPDH. In vitro studies we have done (unpublished data) indicate the IC_{50} of mycophenolic acid versus the A2 strain of RSV was 0.2–0.7 μg/ml compared to ribavirin's IC_{50} of 1–4 μg/ml, but the compound was also more cytotoxic, the CC_{50} being 10 μg/ml compared to a value of 80–120 μg/ml for ribavirin. Mycophenolic acid is a recognized immunosuppressant (Mitsui and Suzuki, 1969). Mycophenolate mofetil (CellCept), the prodrug of mycophenolic acid, is used as an immunosuppressive in the therapy of transplant rejection (Danovitch, 2005). A single report (Roberts et al., 2001) has indicated oral administration of the compound significantly inhibited RSV-induced pneumonia in mice, with efficacy still seen when treatment initiation was delayed to 5 days after virus exposure.

EICAR (5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide) was initially reported by De Clercq et al. (1991) to have significant in vitro activity versus RSV, the IC_{50} being 0.2 μg/ml. Cytotoxicity was not seen at 400 μg/ml, the highest concentration evaluated. Significant efficacy was also seen using i.p. treatment of RSV-infected cotton rats (Wyde et al., 2000).

Pyrazofurin is another IMPDH inhibitor with significant RSV inhibitory effects, the IC_{50} ranging from 0.02 to 1 μg/ml, depending upon the virus strain (Kawana et al., 1985, 1987). Efficacy was also seen versus RSV in the cotton rat model treated with this compound (Wyde et al., 1989).

Viramidine (ribamidine), the 3-carboxamidine analog of ribavirin being developed by Valeant Pharmaceuticals (Costa Mesa, CA), has an anti-RSV IC_{50} of 16 μg/ml, which is slightly higher than that of ribavirin, but is also less cytotoxic, with a CC_{50} of >1000 μg/ml (Gabrielsen et al., 1992). No animal studies with RSV have been reported; however, studies we have run (Sidwell et al., 2005) with viramidine in comparison to ribavirin versus influenza virus infections in mice suggest that both compounds have a similar therapeutic index, although viramidine is not taken up by red blood cells in the efficient manner that is seen with ribavirin and hence appears to have an enhanced safety profile (Lin et al., 2003).

LY253963, the sodium salt of 1,3,4-thiadiazole-2-ylcyanamide and the prodrug of an IMPDH inhibitor was reported by Wyde et al. (1990b) to have in vitro efficacy against RSV that was approximately equivalent to that of ribavirin; intraperitoneal treatment of RSV-infected cotton rats was also protective, but oral therapy was not effective in the same study. The compound, also known to be a significant inhibitor of influenza virus (Hayden et al., 1990), failed in a clinical trial against influenza (Hayden et al., 1994) and has been reported to have developmental toxicity effects (Herman and Chay, 1998). In these latter studies, it was designated as LY217896.

The pyrazole dicarboxamide analog of ribavirin, designated as GR92938X, has been reported to be an inhibitor of RSV in vitro without inhibiting other viruses such as influenza and parainfluenza; it may be pertinent to note that this compound did not appear to inhibit IMPDH (Woods et al., 1994).

5. Treating RSV bronchiolitis

Finally, a number of compounds have been or are now in clinical trials to treat bronchiolitis, the inflammatory disease cause by RSV infection, as well as the reactive airway disease developing after the bronchiolitis (Bisgaard, 2003). Clinical trials with anti-inflammatories have often failed to demonstrate significant effects. Systemically administered prednisolone (Bulow et al., 1999) and topically applied fluticasone (Wong et al., 2000), budesonide (Cade et al., 2000), and deoxyribonuclease I (Nasr et al., 2001) were not found useful. Dexamethazone given by intravenous injection (Buckingham et al., 2002) also had little effect, although when administered by inhalation was concluded to possibly reduce length of hospitalization (Bentur et al., 2005). Porcine surfactant administered intratracheally to RSV-infected infants was considered to have some beneficial effect as seen by improved gas exchange and lessened conventional mechanical ventilation (Luchetti et al., 2002). Most of these therapies target the hyper-inflammatory response and not the agent inducing this response. A fully effective treatment might need to be a combination of anti-inflammatory agents to alleviate the life-threatening symptoms and a potent antiviral agent to eliminate the source of the inflammatory response. It is notable that a combination of palivizumab and a glucocorticosteroid had a significant effect in lessening pulmonary histopathology and also markedly reduced lung virus titers over 3 $\log_{10}$ in RSV-infected cotton rats (Prince et al., 2000). Such a combination would be especially valuable in patients who are not immunocompetent or are immunologically immature.

6. Conclusions

At present, the most effective means for control of RSV infections is the use of anti-RSV antibodies (RSV-IGIV, palivizumab, MEDI-524) administered prophylactically to at-risk patients. The RSV-IGIV has essentially been replaced now by the other, more recent and improved antibodies. Ribavirin, approved for clinical RSV use, has lost favor as a therapy for RSV infections. Selected compounds which have shown great promise in vitro and in animal models and are now in some phase of clinical study, include the fusion inhibitors RFI-641 and MBX 300, one or more antisense siRNA's, and the N-protein inhibitor A-60444. Anti-inflammatories including dexamethazone and porcine surfactant have exhibited some promise in clinical trials to reduce symptomology associated with RSV disease; use of these materials in combination with a drug which has specific anti-RSV activity may be an effective approach for better control of this disease.

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