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Review

Treatment of picornavirus infections

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Abstract

The picornaviruses are a diverse group of viral pathogens that together comprise the most common causes of infections of humans in the developed world. Within the picornavirus family are three well-known groups of human pathogens-the enteroviruses (including polioviruses, coxsackieviruses, and echoviruses), the rhinoviruses, and the hepatoviruses (including hepatitis A). Recently, the parechoviruses (formerly, echoviruses 22 and 23) have been classified as a fourth genus of human picornaviruses. This article will focus on the enteroviruses and rhinoviruses agents, for which substantial effort has been expended and recent successes reported towards the development of safe and effective antiviral therapy. © 2002 Published by Elsevier Science B.V.

Keywords: Picornavirus; Therapy; Prophylaxis; Rhinovirus; Enterovirus

1. Virology

The human enteroviruses (EVs) comprise more than 60 and the rhinoviruses (RVs) more than 100 distinct serotypes within the family Picornaviridae ('pico' meaning small, 'rna' for ribonucleic acid) (Miller, 1997). The EVs are also frequently classified by their sub-genus names: polioviruses, coxsackieviruses A and B, echoviruses and the newer numbered EVs. Picornaviruses are small (27–30 nm diameter; 1.34 g/ml buoyant density), consisting of a simple viral capsid and a single strand of positive (message) sense RNA. EVs are acid and ether-stable and grow optimally at core body temperature (36–37 °C); RVs are acid-labile and grow best at the temperature in the nasal passages (33 °C). The picornaviral capsid contains four proteins, VP1–VP4, arranged in sixty repeating protomeric units of an icosahedron (Rueckert, 1990). These sixty repeating protomeric units are arranged in twelve pentamers (VP2) and twenty hexamers (VP1 and VP3). Variations within the capsid proteins VP1–VP3 are responsible for the antigenic diversity among the viruses. VP4 is not present on the viral surface; rather it is in close association with the RNA core functioning as an anchor to the viral capsid. Destabilization of VP4 results in viral uncoating. The atomic structure of numerous EVs and RVs

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have been resolved by X-ray crystallographic studies and reveal a deep cleft or canyon in the center of each protomeric unit into which the specific cellular receptor for the viruses fits when virus encounters a susceptible host cell (Hogle et al., 1985; Racaniello, 1995). This canyon is important in several of the antiviral strategies discussed below.

The RNA genome of the EVs and RVs is approximately 7.4 kb in length and serves as a template for both viral protein translation and RNA replication, the latter accomplished via a double-stranded replicative intermediate form of RNA (Johnson and Sarnow, 1995). A single reading frame begins several hundred nucleotides from the 5′ end and terminates several dozen nucleotides from the 3′ end, just upstream from a poly-A tail. The untranslated sequences at both the 5′ and 3′ ends are involved in viral regulatory activities such as replication and translation. A single polyprotein is translated from the open reading frame. Post-translational modification is accomplished by virus-encoded proteases and results in the generation of the four capsid proteins, as well as, the enzymes necessary for replication and translation (Hellen and Wimmer, 1995). Within hours of infecting a host cell, all host cell function is aborted and the cell becomes a factory for viral replication. Cell death is by lysis, with release of progeny virus into the immediate environment of the cell and the resumption of the viral infection cycle with attachment of new virus particles to neighboring cells.

2. Clinical significance

2.1. Rhinoviruses

The RVs are responsible for more than half of all cases of the ‘common cold’ (Makela et al., 1998), which in turn is responsible for 25 million days of missed work, 23 million days of missed school, and 27 million physician visits each year in the US alone (Turner, 1998). The common cold is also the most frequent cause of inappropriate antibiotic use in the US (Gonzales et al., 1998; Nyquist et al., 1998). The median duration of RV cold is 11 days, but up to one-quarter last ≥ 2 weeks (Arruda et al., 1997). In a recent study of more severe viral respiratory tract infections, the median duration of illness as defined by complete resolution of both respiratory and systemic symptoms was 14 days (Hayden et al., 1999).

Infections with the RVs are associated with a number of upper and lower respiratory tract complications in both adults and children (Table 1). Viral respiratory tract infections, particularly due to the RVs, are the most important predisposing factor to acute otitis media (Pitkaranta et al., 1998). Most cases of acute sinusitis are thought to result from bacterial disease secondary to a preceding viral upper respiratory tract infection. Sinus abnormalities are frequently detectable during uncomplicated colds, as well (Gwaltney et al., 1994). RV infections are major factors in the induction of acute exacerbations of asthma in adults (Nicholson and Kent, 1993) and in children (Johnston et al., 1995). RV infections are also associated with lower respiratory tract syndromes in other specific patient populations. In children with cystic fibrosis, picornaviruses (RVs or EVs) were detected in about one-fifth of exacerbations and colds were associated with deterioration in pulmonary function testing (Collinson et al., 1996). Among adults aged 60–90 years residing in

Table 1
Clinical illnesses caused by picornavirus infections

Rhinoviruses
Upper respiratory tract illness
Otitis media
Sinusitis
Exacerbations of asthma, cystic fibrosis, chronic bronchitis
Lower respiratory tract illness—infants, elderly, immunocompromised
Enteroviruses
Non-specific febrile illnesses
Upper respiratory tract illness
Otitis media
Hemorrhagic conjunctivitis, herpangina, hand-foot-mouth syndrome
Pleurodynia
Myocarditis
Aseptic meningitis
Encephalitis
Neonatal sepsis-like syndrome

the community, RV infection was associated with lower respiratory tract symptoms in 65%; 40% consulted their doctor, and 76% of these received antibiotics (Nicholson et al., 1996). The overall burden of RVs in elderly people may approach that of influenza (Nicholson et al., 1996). Up to 40% of exacerbations in patients with chronic bronchitis may be associated with RV infections (Gwaltney, 1989). In infants aged less than 12 months, RV infections have been associated with hospitalization for lower respiratory tract illness, particularly bronchiolitis (Schmidt and Fink, 1991), deterioration in those with bronchopulmonary dysplasia (Chidekel et al., 1994) and fatal pneumonia in myelosuppressed patients (Ghosh et al., 1999).

2.2. Enteroviruses

The polioviruses, the prototypic EVs, were recognized by their paralytic potential as early as the 14th century, BC in Egyptian art. Summer epidemics, of paralytic poliomyelitis ravaged the United States through the 1950s. Since the introduction of vaccines in the late 1950s and early 1960s, much of the developed world is now virtually free of poliovirus disease. In many developing countries, eradication programs have made dramatic progress (CDCP, 1996, 1997).

With the control of poliovirus infections in much of the world, attention has turned to the nonpolio EVs. It is estimated that between 10 and 15 million people in the US annually develop symptomatic nonpolio EV infections (Table 1). Most of these patients have non-specific febrile syndromes often with constitutional and/or respiratory symptoms, with or without rashes (Strikas et al., 1986). Most affected patients are young infants in whom differentiation of mild, nonpolio EV illness from more alarming causes of fever and rash is extremely difficult. One prospective study found that 13% of babies born in the summer months were infected with EVs during the first month of life; 21% of the infected babies were hospitalized for suspected bacterial sepsis and received antibiotics or antiherpes therapy (Jenista et al., 1984). Most EV-associated respiratory illnesses are indistinguishable from those due to other respiratory viruses, including the RVs. Several EV respiratory syndromes have distinctive characteristics, including hemorrhagic conjunctivitis, herpangina, hand-foot-mouth syndrome and pleurodynia (Cherry, 1998). Recent pandemics of EV hemorrhagic conjunctivitis (Wadia et al., 1983) and hand-foot-mouth syndrome (Ho et al., 1999) have been associated with concurrent severe neurologic and/or pulmonary complications.

The EVs are among the most commonly identified etiologies of myocarditis, causing between 25 and 35% of cases for which a cause is found (Martino et al., 1995). Neonates and young infants (≤ 6 months of age) are particularly susceptible to EV myocarditis (see below), but most cases occur in young adults between the age of 20 and 39 years.

Meningitis is a frequent manifestation of EV infection, which is in turn the most common cause of meningitis in the US. The severity of EV meningitis varies with host age and immune status (Rotbart, 1997). The duration of illness due to EV meningitis is typically longer than 1 week, but many patients, particularly adults, may have symptoms that persist for 2 or more weeks (Rotbart et al., 1998); the youngest children have the shortest disease duration. Encephalitis due to the EVs is well documented but uncommon (Whitley et al., 1989; Modlin et al., 1991). Unlike aseptic meningitis, which may have prolonged morbidity but from which recovery is generally the rule, encephalitis due to the EV may have more profound acute disease and long-term sequelae.

Neonatal infection with the EVs poses the greatest risk for severe disease when illness develops in the first days of life; this pattern suggests possible transplacental acquisition (Abzug et al., 1993). Disseminated intravascular coagulation and other findings of 'sepsis' result in an illness that may be indistinguishable from that due to overwhelming bacterial infection. Mortality is typically due to hepatic failure or myocarditis.

EVs have been implicated in several chronic illnesses (Dalakas, 1995; Rewers and Atkinson, 1995) including juvenile onset diabetes mellitus, chronic fatigue syndrome, dermatomyositis and polymyositis, congenital hydrocephalus and amyotropic lateral sclerosis. Evidence for these associ-

ations has been largely from serologic or from nucleic acid hybridization studies; definitive proof is lacking and confirmatory studies remain to be done (Dalakas, 1995; Muir et al., 1996). Persistent EV infections occur in agammaglobulinemic patients; manifestations almost always include meningoencephalitis (McKinney et al., 1987; Webster et al., 1993). Half of all patients with persistent EV meningoencephalitis have concomitant dermatomyositis or polymyositis. These observations confirm the important role of antibody in EV clearance, an unusual phenomenon because many other viruses are contained largely by cell-mediated immunity. A syndrome of late-onset muscular atrophy and pain has been reported in individuals who suffered paralytic poliomyelitis 20–40 years previously (Dalakas et al., 1984); evidence for persistent or latent infection in these individuals has been conflicting.

3. Stepping stones on the path towards antiviral therapy for the picornaviruses

Important scientific accomplishments over the past five decades have paved the way for development and testing of antiviral therapy for the EVs and RVs. The first successful propagation of a virus in continuous cell culture lines was achieved with poliovirus by Enders and colleagues (Enders et al., 1949). This Nobel Prize-winning advance facilitated the development of poliovirus vaccines, allowed for the identification of the other picornavirus serotypes, and established the gold standard diagnostic test for many viruses. The determination of the complete genomic sequence for many of the RVs and EVs provided us with genetic structure-function information with which antiviral strategies can be devised (Hellen and Wimmer, 1995). The three-dimensional capsid structure for the EVs and RVs at atomic resolution identified the 'canyon' on each protomeric face of the virus into which the host cell receptor fits, and beneath which a 'pore' opens into a hydrophobic pocket; capsid-inhibiting compounds act in that pocket (Hogle et al., 1985; Racaniello, 1995). Specific host cell receptors have now been identified for numerous EV serotypes (Racaniello, 1995; Bergelson et al., 1994, 1997), providing yet another potential target in antiviral strategies. Finally, rapid and sensitive molecular diagnostic methods for the EVs have been developed (Rotbart et al., 1994), providing an important prerequisite for clinical trials.

4. Model systems for studying antiviral therapy of picornaviruses

The picornaviruses are readily propagated in tissue culture using cells of human or monkey derivation. Buffalo green monkey kidney (BGM), human rhabdomyosarcoma (RD), human embryonic lung, and primary cynomolgous monkey kidney cells are the preferred lines used to isolate the EVs (Dagan and Menegus, 1986). The group B coxsackieviruses grow best in BGM cells whereas echoviruses grow best in RD cells. The RVs grow optimally in human embryonic lung fibroblast (HELF) lines, such as, WI-38 and MRC-5. Human embryonic kidney cells and several HeLa cell clones also support the growth of RVs (Landry, 1999). Ideal growth conditions for the EVs are at core body temperature (37 °C), whereas, RVs grow optimally at temperatures closer to that of the nasopharynx (33 °C). Antiviral assays demonstrating the inhibitory effects of an anti-picornaviral compound generally involve the demonstration of protection against virus-induced cytopathic effect in susceptible cell lines (Buontempo et al., 1997; Woods et al., 1989). Plaque reduction, virus yield and cytopathic effect reduction assays in 96-well format are traditionally used to assess antiviral activity. Novel approaches to detecting capsid inhibitor compounds (see below) involving ICAM-1 (Last-Barney et al., 1993) and thermal stabilization (Rombaut et al., 1991) have also been employed.

Murine models of human EV infections have been the most commonly studied animal system. Suckling mice can be readily infected with the Barty strain of echovirus 9 (McKinlay et al., 1986; Bultman et al., 1983) and coxsackievirus A9 (Woods et al., 1989; Melnick and Godman, 1951). Infected animals develop flaccid limb paralysis as a result of infection and intragastric administra-

Table 2
Therapeutic strategies and candidate compounds for treatment of picornaviral infections

Target	Compound class
Cell susceptibility	Interferons
Viral attachment and binding to host cells	Antibodies, soluble ICAM
Viral uncoating/capsid function	Capsid-function inhibitors
Viral replication	Enviroxime-like compounds
Viral protein synthesis	3C protease inhibitors

tion of a capsid-inhibitor agent is capable of preventing the development of paralysis (Woods et al., 1989; McKinlay et al., 1986). Adult mice can be infected intracranially with poliovirus and protected from development of paralysis by oral administration of capsid-inhibitor agents (Buontempo et al., 1997; McKinlay and Steinberg, 1986; Jubeit et al., 1989). Similarly, coxsackievirus B3 can infect adult mice resulting in myocarditis with involvement of multiple organ systems (Klingel et al., 1996); again, a capsid function inhibitor, has been shown to be orally effective in markedly reducing viral titers in all organs tested and preventing death of the mice in this latter model system (Pevear et al., 1999). Human RVs have only been reported to successfully infect primates (Dick, 1968; Pinto and Haff, 1969) and no practical animal model has been developed for the RVs.

5. Approaches to treatment and clinical trials

There are several steps in the replication cycle of the picornaviruses that are potential targets in antiviral therapy. Cell susceptibility, viral attachment, viral uncoating, viral RNA replication, and viral protein synthesis have all been studied as potential strategic targets of anti-picornaviral compounds (Table 2). The following sections briefly review these targets, the mechanisms of action of anti-picornavirus compounds directed at these targets and the clinical trials performed to date.

6. Interferon

lnterferons are potent, selective mediators of cellular changes which induce a number of antiviral, anti-proliferative, and immunological effects, all of which collectively affect host cell susceptibility to picornavirus infection (Capobianchi et al., 1991; Geniteau-Legendre et al., 1987; Kandolf et al., 1985; Kishimoto et al., 1988; Langford et al., 1985, 1988; Lopez-Guerrero et al., 1990; Okada et al., 1992; Sasaki et al., 1986). The cellular antiviral effects of interferons are mediated through specific receptor-signal transduction pathways. In conjunction with double stranded RNA, interferons induce the expression of proteins, some of which mediate an antiviral activity. The best described pathways are: (1) 2′,5′-adenylate synthetase; (2) double stranded RNA dependent protein kinase; and (3) the Mx proteins. Through transfection/expression systems, an isoform of the 2′,5′-adenylate synthetase system has been linked to the inhibition of replication of picornaviruses (Chebath and Benech, 1987). Clinically, children with acute EV meningitis have significant elevations in endogenous interferon levels in the CSF (Chonmaitree and Baron, 1991; Ichimura et al., 1985), which may be important in recovery from the infection. Although alpha interferon itself is a very potent inhibitor of picornavirus infection, additive or synergistic protective effects are seen when used in conjunction with capsid-inhibiting compounds (Langford et al., 1985), nucleoside analogs (Okada et al., 1992), or gamma interferon (Fleischmann et al., 1984). Interferons may also work in conjunction with humoral antibodies and macrophages to eliminate picornavirus infections (Geniteau-Legendre et al., 1987).

Intranasal interferon has been shown to be effective as prophylaxis for RV colds in several studies (Hayden et al., 1986; Hayden and Gwaltney, 1983; Merigan et al., 1973; Samo et al., 1983; Greenberg et al., 1982). Additional studies demonstrated significant efficacy against naturally acquired RV infections and against contact spread of RVs within family groups after experimental induction of a natural cold (Hayden et al., 1986; Douglas et al., 1986). Side effects of interferon included nasal irritation and stuffiness, and mu-

cosal ulceration (Hayden et al., 1986; Samo et al., 1983). Administered therapeutically, 1 day after experimental RV infection, intranasal interferon had no effect on development of infection or symptoms, but did result in moderate reductions of virus shedding and cold symptoms (Hayden and Gwaltney, 1984). Additional studies with low-dose intranasal interferon also demonstrated a lack of efficacy in post-exposure prophylaxis of RV infections in families (Monto et al., 1989). Despite in vitro efficacy noted above, interferons have not been clinically evaluated in EV infections.

7. Immunoglobulins

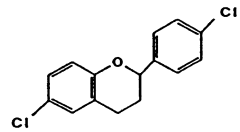
The host clears EV infections primarily via humoral immunity. As noted above, patients who lack antibody because of congenital or acquired immunodeficiencies are uniquely susceptible to infections with the EVs (McKinney et al., 1987). Similarly, normal neonates are at high risk for severe EV disease because of a relative deficiency of EV antibodies (Abzug et al., 1993; Modlin et al., 1981). Antibodies act by binding to EVs and preventing attachment and binding to host cells, which correlates with 'neutralization' of EVs observed in cell cultures treated with antibody.

Antibodies, in the form of Immune serum globulin, have been used prophylactically and therapeutically against the EVs in two clinical settings: the neonate and the immunocompromised host. As noted above, neonates may develop an overwhelming sepsis syndrome from transplacental/peripartum acquisition of EV infection. The high mortality rate of this disease, coupled with the known association of severe EV disease with absolute or relative antibody-deficiency states, has prompted numerous investigators to administer antibody preparations to neonates with EV sepsis. Anecdotal reports of clinical success with maternal serum or plasma (Jantausch et al., 1995) or commercial immunoglobulin preparations (Black, 1983; Johnston and Overall, 1989) against a variety of EV serotypes causing neonatal sepsis have been reported; other reports describe progressive disease and death despite such therapy (Wong et al., 1989). A blinded, randomized controlled study was too small to demonstrate the clinical benefits but did show a reduction in viral titer in babies receiving intravenous immunoglobulin preparations that were subsequently shown to contain high antibody titers to the infecting serotype (Abzug et al., 1993). Individuals with congenital or acquired antibody deficiencies are also at risk of severe EV infections (see above). Prior to the availability of intravenous immunoglobulin preparations, mixed results were reported with intramuscular and/or intrathecal administration of immunoglobulin preparations. As with neonatal sepsis, some antibody-deficient patients appeared to benefit by supplemental immunoglobulin, others progressed and died despite therapy (McKinney et al., 1987). Since known antibody-deficient patients have begun receiving maintenance supplementation with intravenous immunoglobulin, the incidence of chronic, progressive EV meningoencephalitis has fallen (demonstrating the prophylactic benefit of these preparations) and the clinical profile of patients developing such infections has been modified (Webster et al., 1993). Therapeutic efficacy in established EV meningoencephalitis in antibody-deficient patients has only been anecdotally studied.

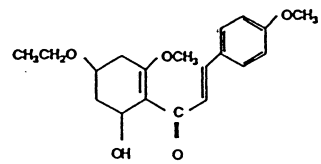
8. Capsid-inhibiting compounds

Capsid-inhibiting compounds block viral uncoating and/or viral attachment to host cell receptors. As noted above, the resolved three-dimensional structure of the EVs reveal a 'canyon' formed by the junctions of VP1 and VP3. Beneath the canyon lies a 'pore' that leads to a hydrophobic pocket into which a variety of diverse hydrophobic compounds can bind (Table 2, Fig. 1). Although the compounds integrate into a virus capsid via a number of noncovalent, hydrophobic-type interactions, the affinity is high with constants ranging from 2.0×10^{-8} to 2.9×10^{-7} M (Fox et al., 1991). Several factors appear to correlate with the abilities of a compound to function within the hydrophobic pocket and to manifest antiviral activity including, the number

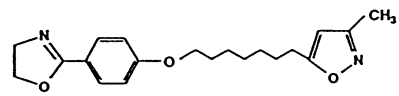
4',6'-dichloroflavan



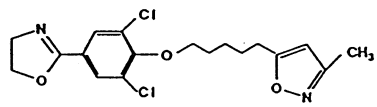
Ro09-0410 (chalcone)



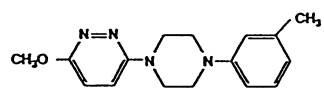
WIN 51711



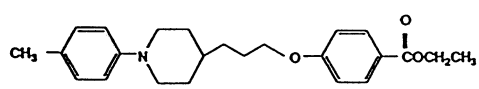
WIN 54954



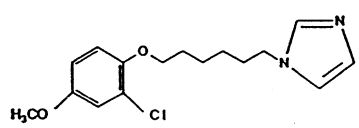
R61837



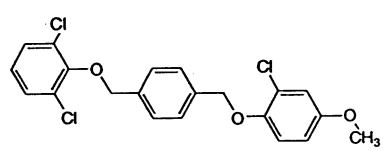
R77975 (pirodavir)



SCH 38057



SCH 48973



VP 63843 (pleconaril)

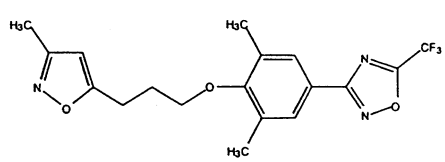


Fig. 1. Chemical structures of representative capsid function-inhibitor compounds with anti-picornaviral activity.

of molecules of compound active in each virion; the location of the compound activity within the hydrophobic pocket; the length and space-filling properties of the molecule; and most importantly, the extent of hydrophobic interactions with amino acids in the pocket. With regards to those factors, enhanced potency appears to correlate with increased number of molecules of compound per pocket (Fox et al., 1991; Zhang et al., 1991), proximity of compound to the opening of the pocket (Zhang et al., 1991), increased hydrophobic energy resulting from filling a greater proportion of the pocket by the compound (Fox et al., 1991; Zhang et al., 1991, 1992) and absence of bulky amino acid substitutions (mutations) within the pocket structure (Heinz et al., 1989; Heinz, 1990; Pevear et al., 1989).

Several potential mechanisms of action of picornavirus inhibition by compounds that affect the function of the virus capsid have been hypothesized. Filling the hydrophobic pocket results in increased stability of the virus, making the virus more resistant to uncoating. The increased stability of the virus-compound complex is evidenced by the resistance to thermal inactivation (Rombaut et al., 1985). This property can be used as a rapid screen in order to identify molecules with binding avidity; the majority, but not all, compounds with potent antiviral activity also result in thermal stability. It is also possible that a degree of capsid flexibility may be required for uncoating, and activity of these compounds within the hydrophobic pocket may reduce this necessary flexibility, inducing a more rigid structure. Alternatively, changes in the conformation of the canyon floor as a result of drug activity within the underlying pocket may affect the attachment of the virus to the host cell receptor (Langford et al., 1988). It has been shown, however, that such perturbations in the canyon floor do not absolutely correlate with antiviral potency (Zhang et al., 1991, 1992). The capsid-inhibiting compounds vary in their spectrum of activity, perhaps as a result of factors such as pocket fit discussed above. Certain compounds demonstrate both anti-EV and anti-RV activity (Otto et al., 1985; Pevear, 1999), others are more selective to one picornavirus genus or the other (Buontempo et al., 1997; Cox et al., 1996).

Trials of the 'R' series of capsid-binding compounds developed by Jannsen Pharmaceuticals have been limited to intranasal administration to patients with RV colds (Al-Nakib et al., 1989; Barrow et al., 2001; Hayden et al., 1992). Pirodavir (R77975) and R61837 were efficaceous in experimentally-induced RV colds when these drugs were administered intranasally before or after infection, but prior to onset of symptoms (Barrow et al., 2001; Hayden et al., 1992); pirodavir required six times daily dosing, with efficacy loss at three daily doses (Hayden et al., 1992). Another series of capsid-binding compounds, the phenoxyl imidazoles, are broad spectrum inhibitors of the EVs, and demonstrate therapeutic oral efficacy in animal models (Buontempo et al., 1997; Cox et al., 1996). This series has limited potency against the RVs and further development of candidate drugs has been discontinued.

The 'WIN' series of compounds developed by Sterling–Winthrop Pharmaceuticals has been clinically evaluated in both RV and EV infections. The first compound of this group to advance to clinical trials was disoxaril (WIN 51711; Fig. 1). Disoxaril was moderately active against RVs in vitro and very active against EVs both in vitro and in vivo (McKinlay et al., 1986; McKinlay and Steinberg, 1986; Otto et al., 1985). The appearance of asymptomatic crystalluria in healthy volunteers prevented further clinical study. Shortening of the aliphatic chain from $n = 7$ to 5 and adding chloro-groups to the phenyl ring (Fig. 1) resulted in WIN 54954 which had broad, potent anti-RV and anti-EV activity in vitro and in vivo (Woods et al., 1989), including oral therapeutic efficacy in mice. Clinical efficacy was assessed in two RV (rhinovirus 23 and rhinovirus 39) challenge trials (Turner et al., 1993) and one EV challenge trial (coxsackievirus A21) (Schiff et al., 1992). Despite administering the compound prior to infection and achieving serum concentrations above the in vitro minimal inhibitory concentrations, both RV trials failed to show efficacy of WIN 54954 (Tebbe et al., 1997a); very low concentrations of the drug were found in nasal wash samples, the site of the experimental infection. In contrast, WIN 54954 significantly reduced the number and severity of colds induced by coxsack-

ievirus A21, and also significantly reduced nasal mucous discharge, respiratory and systemic symptoms, and viral titers (Tebbe et al., 1997b). The overall symptomatic attack rate was reduced from 15/23 patients in the placebo group to 3/27 in the WIN 54954 treated groups ($P = 0.0001$). This study represents the first demonstration of oral efficacy of an anti-EV agent; the differences in results compared with those in the RVs studies using the same compound are enigmatic since the MIC for one of the RV serotypes was identical to that of the coxsackievirus A21 strain used. The fact that EV infections are systemic, usually with a viremic phase, may explain the enhanced EV efficacy of an orally-active compound which achieves good blood levels over the effect seen in RV infections, which are limited to the upper airway where drug distribution may have been insufficient. WIN 54954 was not further developed for clinical use because of adverse reactions of flushing and rash, possibly related to concomitant alcohol ingestion by study volunteers.

Pleconaril (3–13,5-dimethly-4-[[3-methyl-5-isoxazolyl)propyl]phenyl]-5-(trifluoromethyl)-1,2,4-oxadiazole) (Fig. 1) is the first of a new generation of metabolically stable capsid function-inhibitors; this compound has been more extensively evaluated in clinical trials than any other anti-picornaviral agent. Pleconaril has demonstrated broad spectrum and potent anti-EV and anti-RV activity and is highly orally-bioavailable (Pevear et al., 1999; Kearns et al., 1998, 1999; Abdel-Rahman and Kearns, 1999). In a mouse model of multi-organ system infection following intracranial inoculation of EVs, pleconaril, a capsid-inhibitor compound, has been shown to reduce viral titers in all affected organs and to prevent death of the animals (Pevear et al., 1999). High levels of pleconaril are achieved in the central nervous system and in the nasal epithelium (McKinlay, personal communication). Pharmacokinetic studies of pleconaril have been undertaken in adults, children, and neonates (Kearns et al., 1998, 1999; Abdel-Rahman and Kearns, 1999). In adults, the pharmacokinetics of pleconaril are best characterized as a one-compartment open model with first order absorption (Abdel-Rahman and Kearns, 1999), indicative of a compound that is readily absorbed into and distributed throughout the water compartment. Concentrations of pleconaril 12 h after a single oral dose remain 2.5-fold greater than required to inhibit 95% of EVs in vitro. Neonates and older children have similar PK profiles (Kearns et al., 1998, 1999). Oral bioavailability, in animals and humans, approaches 70%.

In pre-clinical trials, pleconaril was devoid of cardiovascular and central nervous system side effects and no significant differences from placebo have been noted in adverse events in any of the clinical trials to date (see below).

In a challenge study of coxsackievirus A21 respiratory infection, 33 volunteers were randomized to receive either 400 mg of pleconaril or matching placebo, orally, 14 h before inoculation with virus (Schiff et al., 1996). Beginning after inoculation, subjects received 200 mg capsules twice daily for 6 days. Pleconaril had a significant beneficial effect on symptom scores, global assessment, fever, and nasal mucous production, with 41% of placebo treated subjects experiencing moderate colds versus none in the pleconaril treated group. Peak viral titers which occurred on the peak day of symptoms were reduced by greater than 99% in the pleconaril group compared with the placebo group (Schiff et al., 1996).

In a placebo-controlled trial of pleconaril in 221 pediatric patients with EV meningitis, significant reductions in the total morbidity (composite measurement of all disease symptoms) and global assessment (caregiver's assessment of patient's illness) scores were documented for the overall study population treated with pleconaril (Sawyer et al., 1999). Headache duration was significantly reduced by pleconaril treatment in children older than 8 years. Responses were noted as early as 24 h after initiation of treatment. Viral shedding from the throat was also reduced in the pleconaril-treated group compared with placebo (Sawyer et al., 1999).

Pleconaril has also been studied in adult patients with EV meningitis (Shafran et al., 1999). In a double-blind, placebo-controlled trial, 198 patients aged 14–65 years received either 200 mg of pleconaril three times per day for 7 days, or placebo. Those receiving pleconaril had a 2-day

reduction in duration of headache and a 2-day faster resolution of all symptoms of meningitis. Pleconaril-treated patients also returned to work or school 2 days faster (Shafran et al., 1999).

Pleconaril has been used by compassionate release for approximately 400 patients with potentially life-threatening EV infections, 38 of whom have been followed long enough to assess therapeutic responses (Rotbart and Webster, 2001). Among 16 antibody-deficient patients with chronic EV meningoencephalitis, 12 showed some clinical improvement and three others stabilized concurrent with therapy. Six of eight of these patients cleared the virus and eight of nine had improvement in other laboratory parameters. Clinical responses were also seen in three of four patients with severe neonatal EV disease, three of four cases of myocarditis, three of three patients with chronic EV infection related to bone marrow transplant, two of three patients with vaccine-associated or wild-type poliomyelitis, and one (of one) patient with post-polio muscular atrophy syndrome (Rotbart and Webster, 2001).

In a double-blind, placebo-controlled study of 1024 adults with viral respiratory infection during the fall rhinovirus season, patients receiving pleconaril recovered from all cold symptoms and returned to overall wellness (measured via a global assessment score) 3.5 days sooner than patients receiving placebo (Hayden et al., 1999). Individual symptoms (including nasal congestion, rhinorrhea, and pharyngitis) each resolved 1–2 days sooner in the pleconaril-treated patients.

In two follow-up double-blind, randomized, placebo-controlled studies of rhinoviral respiratory infection in adults involving more than 2000 patients in 200 centers across the US and Canada, benefit of pleconaril was again demonstrated (Hayden et al., 2001). Pooled data from the two studies showed statistically significant differences in the median number of days to illness alleviation (approximately 1 day reduction with reduction in severity of symptoms seen within the first 24 h), as well as statistically significant reductions in total symptom scores, days of cold medication use, nights of sleep disturbance, and nasal tissue use (Hayden et al., 2001).

In all clinical studies till date (Schiff et al., 1996; Sawyer et al., 1999; Shafran et al., 1999; Hayden et al., 1999, 2001; Rotbart and Webster, 2001), a very favorable safety profile has been observed with pleconaril. There have been no differences in serious adverse events between treatment and placebo groups; a slight increase in the incidence of nausea has been seen in pleconaril treated patients in certain studies and with certain dosing regimens (Rotbart and Liu, 2001). The favorable safety profile and paucity of adverse events are probably the result of the unique site of action of the compound on the viral capsid.

9. Enviroxime-related compounds

Enviroxime [2-amino-l-(isopropyisulfonyl)-6-benzimidazole phenyl ketone oxime is a prototype compound for a series of molecules with broad anti-EV and anti-RV activity developed by Lilly Pharmaceuticals (DeLong et al., 1978a,b; Wikel et al., 1980). The mechanism of action of these compounds has been suggested to be the inhibition of RNA replication via targeting the 3A protein coding region of the viruses (Heinz and Vance, 1995). The drugs apparently prevent formation of the RNA replicative intermediate, a complex dependent on both viral proteins 3A and 3AB (Heinz and Vance, 1995), and hence, the formation of new plus-strand RNA molecules. Vinyl acetylene benzimidazoles derivatives of enviroxime provide improved bioavailability of the compounds; flouridation of these latter structures further enhances blood levels in animal models (Tebbe et al., 1997a,b). This class of compounds can be added to tissue culture systems several hours after viral inoculation without loss of antiviral activity, again reflecting their action at a later stage of the viral life cycle (i.e. RNA replication).

Enviroxime resulted in modest clinical and virologic benefit in some studies (Phillpotts et al., 1981, 1983) and no benefit in others (Hayden and Gwaltney, 1982; Miller et al., 1985). Problems with poor pharmacokinetics and undesirable toxicology and side effects resulted in discontinuance of that program. Newer derivatives of enviroxime

(see above) promise better bioavailability and tolerance, but have not been clinically evaluated.

10. 3-C protease inhibitors

A series of compounds are under development that targets the 3-C protease of picornaviruses resulting in inhibition of viral protein synthesis, via blocking viral specific protein processing (Patick et al., 1997). Published results are limited to those with tripeptide aldehydes derived from the sequence of a natural 3-C cleavage site, Leu–Phe–Gln. Anti-enzyme activity is potent ($K_i = 6$ nM) with high therapeutic indices in vitro. Like the RNA inhibitors discussed above, time of addition with the protease inhibitors is several hours without loss of antiviral activity. These compounds appear to have both anti-RV activity and anti-EV activity, and appear to have an antiviral effect even if initiated (in the animal model) several hours after infection. Clinical trials in RV upper respiratory infections for the lead compound, AG7088 have been discontinued as of this time in an attempt to improve characteristics of the formulation.

11. Drug resistance

Enterovirus mutants that are resistant to the antiviral effects of the capsid inhibitors have been extensively studied; resistance to other anti-picornaviral compounds has not been thoroughly studied because of the lack of progress of these other compounds in the clinical arena. Mutants have only been isolated in the laboratory and express varied levels of resistance (Heinz et al., 1989). Mutants showing resistance to low concentrations of capsid-inhibitors generally are the result of mutations in the hydrophobic pocket of amino acids with bulkier side chains. It is thought that these side chains sterically block the activity of the earlier capsid inhibitors. Mutants with resistance at only high drug concentrations retain some sensitivity to the capsid inhibitor compounds. These mutations map to regions near the canyon floor that are disrupted upon drug activity. Thus far, the mutants studied have shown cross-resistance to early agents of different structural classes (Ninomiya et al., 1990).

In a study of the early capsid inhibitor WIN 54954 in human subjects infected with coxsackievirus A21, no resistant virus was detected (McKinlay, unpublished data). In a study conducted with laboratory-isolated drug resistant mutants of RVs, infectivity of the virus was reduced significantly (Yasin et al., 1990). This result is consistent with laboratory observations with drug-resistant coxsackievirus B3 which showed a marked reduction in virulence in mice compared with wild type virus (Pevear et al., 1999). Further, pleconaril resistant mutants of human rhinovirus type 1A shared severely attenuated growth characteristics in cell culture (D. Pevear, personal communication). Although recovery of resistant strains is not uncommon under laboratory conditions, resistance has not been found associated with clinical failure in patients treated till date.

12. Summary

Infections with the picornaviruses cause significant morbidity and even mortality. Progress toward the development of anti-picornaviral therapy has been substantial. In vitro efficacy of several classes of antiviral agents against the enteroviruses (including those targeting cell susceptibility, virus attachment, virus uncoating, viral RNA replication and protein production) has led to clinical evaluation of the most promising compounds. Only immunoglobulin is currently available for use in treatment of picornavirus infections, and the indications for such therapy are very limited. Of those in active development, the capsid function-inhibitor compounds have received the most clinical study and the leading drug, pleconaril, has now been shown to be efficacious in naturally-occurring enterovirus and rhinovirus infections. Application for approval of pleconaril in the treatment of respiratory infections due to the picornaviruses is currently pending before the FDA. Ultimately, the variety of different approaches available to treat these infections may make combination therapy even more

effective than treatment with any single compound.

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