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Mini review

Treatment of human enterovirus infections

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1. Clinical importance

The enteroviruses (EVs) are among the most common and most important viral pathogens of humans (Cherry, 1987; Melnick, 1990). The paralytic potential of the polioviruses, the prototypic EVs, was recognized as early as the 14th century B.C. 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 (CDC, 1996, 1997a,b).

Control of poliovirus infections in much of the world has turned attention to the non-polio EVs, which include the coxsackieviruses, echoviruses, and newer numbered EVs (Table 1). In the US alone, the EVs are estimated to cause 5–10 million symptomatic infections annually (Strikas et al., 1986). In temperate climates, these infections occur during the summer and fall months; young

Table 1
The enterovirus serotypes

Subgroup	Serotypes
Poliovirus	1–3
Coxsackieviruses A	1–22, 24[a]
Coxsackieviruses B	1–6
Echovirus	1–9, 11–27, 29–31[a]
Numbered enteroviruses	68–71

[a] Coxsackievirus A23, echoviruses 10 and 28, and enterovirus 72 have been reclassified (34); echovirus 22/23 has been tentatively reclassified.

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Table 2
The most common and/or important clinical syndromes proven to be caused by the enteroviruses

Organ system	Disease
Neurologic	Aseptic meningitis Encephalitis Poliomyelitis Chronic meningoencephalitis (antibody-deficient patients)
Respiratory	Common cold Stomatitis/herpangina/hand-foot-mouth syndrome Pharyngitis, tonsillitis, rhinitis Pleurodynia (Bornholm disease)
Cardiovascular	Myocarditis Pericarditis
Miscellaneous	Febrile, exanthematous illness Neonatal sepsis

children are most commonly affected, as both the incidence and severity of EV infections vary inversely with the patient's age. In addition to the actual disease that they cause, EV infections cause clinical concern because they mimic illnesses due to other pathogens. Distinguishing EV infections from those due to common bacteria and other viruses is often difficult on clinical grounds alone (Cherry, 1987). Hence, unnecessary treatment for other infections is frequently instituted during EV infections.

The EVs are responsible for a wide array of clinical diseases affecting many organ systems (Table 2). Despite the name 'entero' viruses, enteric disease is not a prominent manifestation, although diarrhea and vomiting may be significant manifestations of certain outbreaks of 'summer flu' due to the EVs. It is important to note that no disease is uniquely associated with any specific EV serotype and that no serotype is uniquely associated with any one disease (Cherry, 1987; Melnick, 1990). This is true even of paralytic poliomyelitis, which has been associated with numerous non-polio EV serotypes (Grist and Bell, 1984; Melnick, 1990). For that reason, in most circumstances it is sufficient to speak of diseases that 'EVs cause', to diagnose 'an EV' in the laboratory, and to treat 'an EV' infection without necessarily specifying or identifying the particular serotype. Certain clinical syndromes are indeed more likely to be caused by one or a few serotypes (see below), but significant overlap exists among the serotypes and the diseases they cause.

The most common symptomatic manifestation of EV infection is a non-specific febrile illness, with or without a rash (Dagan, 1996). This so-called 'viral syndrome' is one of the most numerically important causes of fever among children, responsible for several million cases each summer (Strikas et al., 1986). Often the illness is accompanied by upper respiratory symptoms and is referred to as 'summer flu' or the 'summer cold' and may be indistinguishable from the same illness caused by influenza viruses or by rhinoviruses, their fellow picornaviruses (see below), typically in the fall and winter months (Cherry, 1987; Rotbart, unpublished data). By far the most vexing clinical EV syndrome that the physician encounters is aseptic meningitis (Rotbart, 1995). Aseptic meningitis is the most common infection of the central nervous system and EVs are the leading cause of aseptic meningitis in the United States, causing 75 000–200 000 cases each year (McKinlay, unpublished data; Rotbart, 1995). In young infants with the disease, clinical criteria to distinguish EV meningitis from that due to bacteria and herpes simplex virus are unreliable. As a result, thousands of children are hospitalized and treated with unnecessary antibiotics and antiherpes medications annually because of the fear that a case of meningitis is not due to an EV (Chonmaitree et al., 1988). EVs are also the most commonly identified cause of aseptic meningitis in adults, in whom the disease also usually results in hospitalization and full recovery takes on average more than 2 weeks (Rotbart et al., 1996). Additional acute clinical EV syndromes of significance include encephalitis, poliomyelitis (particularly due to the polioviruses), myocarditis (particularly due to the coxsackievirus B group), hemorrhagic conjunctivitis (particularly due to serotypes coxsackievirus A24 and enterovirus type 70), hand foot-mouth syndrome, Bornholm disease (pleurodynia), and overwhelming neonatal sepsis (particularly due to the echoviruses and coxsackieviruses type B). The last syndrome is thought to be due to

perinatal transmission, either transplacentally or during birth, from mother to infant.

In addition to the well-recognized acute EV diseases, 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 associations 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.

2. Characteristics of the viruses

The EVs comprise 66 distinct serotypes (Miller, 1997) within the family Picornaviridiae ('pico' meaning small, 'rna' for ribonucleic acid). The traditional taxonomic subgroups of EVs (Table 1) are based on the patterns of replication of the individual serotypes in various host cells and tissues. Like other picornaviruses, EVs 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). The capsid contains four proteins, VP1–VP4, arranged in sixty repeating protomeric units of an icosahedron (Rueckert, 1990). Variations within capsid proteins VP1–VP3 are responsible for the antigenic diversity among the EVs; neutralization sites are most densely clustered on VP1 (Rueckert, 1990). 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 two poliovirus serotypes, types 1 and 3, have been resolved by computerized crystallographic studies, and reveal a deep cleft or canyon in the center of each protomeric unit into which the specific cellular receptor for the EVs fits when virus encounters a susceptible host cell (Hogle et al., 1985). This canyon is important in several of the antiviral strategies discussed below.

The encapsidated RNA of the human EVs 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 at approximately nucleotide 740 from the 5′ end and terminates at approximately nucleotide 7370, leaving 740 bases at the 5′ end and 70 bases at the 3′ end (just upstream from a poly A tail) untranslated; these untranslated sequences are felt to be 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 two virus-coded proteases, and results in generation of the four capsid proteins as well as the enzymes necessary for replication and translation (Haller and Semier, 1995).

3. Milestones in the development of enteroviral therapy

Numerous scientific accomplishments over the past five decades have paved the way for development and testing of antiviral therapy for the EVs. The first successful propagation of a virus in continuous cell culture lines was achieved with poliovirus by Enders et al. (1949). This Nobel Prize-winning advance facilitated the development of poliovirus vaccines, allowed for the identification of the other EV serotypes, and established

the gold standard diagnostic test for many viruses. Cell culture remains the foundation of susceptibility testing for antiviral drugs. The determination of the complete genomic sequence for the polioviruses was followed by sequence determination for many of the non-polio EVs (Hellen and Wimmer, 1995), providing us with genetic structure-function information with which antiviral strategies can be devised. The three-dimensional resolution of the capsid structure for polioviruses, like that of the rhinoviruses, 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 drug-binding pocket; capsid-binding compounds (see Section 4.3) act at that site (Hogle et al., 1985). Specific host cell receptors have now been identified for numerous EV serotypes (Rotbart and Kirkegaard, 1992; Racaniello, 1995), 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, 1997), providing an important prerequisite for clinical trials.

4. Therapeutic strategies

As with other viral pathogens, there are several steps in the replication cycle of the EVs which are potential targets in antiviral therapy. Cell susceptibility, viral attachment and binding, viral uncoating, viral RNA replication, and viral protein synthesis have all been studied as targets of anti-EV compounds (Table 3). The following sections briefly review these targets and the mechanisms of action of relevant agents.

4.1. Interferon

Interferons 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 EV infection (Kandolf et al., 1985; Langford et al., 1985; Sasaki et al., 1986; Geniteau-Legendre et al., 1987; Kishimoto et al., 1988; Langford et al., 1988; Lopez-Guerrero et al., 1990; Capobianchi et al., 1991; Okada et al., 1992). The cellular antiviral effects of interferons are mediated through specific receptor-signal transduction pathways. In conjunction with double stranded RNA (dsRNA), interferons induce the expression of proteins, some of which mediate an antiviral activity. The best described pathways are: (i) 2′,5′-adenylate synthetase; (ii) dsRNA dependent protein kinase; and (iii) 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 (Ichimura et al., 1985; Chonmaitree and Baron, 1991), which may be important in recovery from the infection. Although alpha interferon itself is a very potent inhibitor of EV infection, additive or synergistic protective effects are seen when used in conjuction with capsid-binding compounds (Langford et al., 1985), nucleoside analogs (Okada et al., 1992), or gamma interferon (Pitkaranta et al., 1991). Interferons may also work in conjuction with humoral antibodies and macrophages to eliminate EV infections (Geniteau-Legendre et al., 1987).

Table 3
Therapeutic strategies and candidate compounds for treatment of enteroviral infections

Target	Compound class
Cell susceptibility	Interferons
Viral attachment and binding to host cells	Antibodies
	Capsid-binders[a]
Viral uncoating	Capsid-binders[a]
Viral replication	Enviroxime-like compounds
Viral protein synthesis	3C protease inhibitors

[a] Capsid-binding compounds include: rhodamine (Eggers et al., 1970); flavonoids (Ishitsuka et al., 1982); chalcones (Ninomiya et al., 1985); aralkylamino-pyridines (Kenny et al., 1987); oxazolinyl isoxazoles (Otto et al., 1985); pyridazinamines (Hayden et al., 1992); phenoxyl imidazoles (Rozhon et al., 1993; Buontempo et al., 1997)

4.2. Antibodies

The primary mechanism of clearance of EVs by the host is via humoral immunity. 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 (Modlin et al., 1981; Abzug, 1995). 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.

4.3. Capsid-binding compounds

Capsid-binding compounds inhibit viral replication by blocking viral uncoating and/or blocking viral attachment to host cell receptors. As noted above, the resolved three-dimensional structure of the EVs reveals a 'canyon' formed by the junctions of VP1 and VP3. Beneath the canyon lies a 'pore' which leads to a hydrophobic pocket into which a variety of diverse hydrophobic compounds can bind (Table 3; Fig. 1). Although the compounds bind to virus via a number of non-covalent, hydrophobic-type interactions, the binding affinity is high with affinity 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 bind within the hydrophobic pocket and to manifest antiviral activity, including: the number of molecules of compound bound to each virion; the location of the compound binding within the drug-binding 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 regard to those factors, enhanced potency appears to correlate with increased number of molecules of compound bound per virion (Fox et al., 1991; Zhang et al., 1991), proximity of compound binding to the opening of the pocket (Zhang et al., 1991), increased hydrophobic binding 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 substitions (mutations) within the pocket structure (Heinz et al., 1989; Pevear et al., 1989; Heinz, 1990).

Several hypotheses have been proposed for the mechanism of EV inhibition by the capsid-binding molecules. Filling the pocket results in increased stability of the virus, making the virus more resistant to uncoating. The increased stability induced by capsid binding 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 binding 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 binding within the underlying pocket may affect the attachment of the virus to the host cell receptor (Pevear et al., 1989). 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-binding 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-rhinoviral activity (Otto et al., 1985; McKinlay, unpublished data), others are more selective to one picornavirus genus or the other (Cox et al., 1996; Buontempo et al., 1997).

4.4. Enviroxime-related compounds

Enviroxime [2-amino-1-(isopropylsulfonyl)-6-benzimidazole phenyl ketone oxime] is a prototype compound for a series of molecules with broad anti-EV and anti-rhinoviral 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

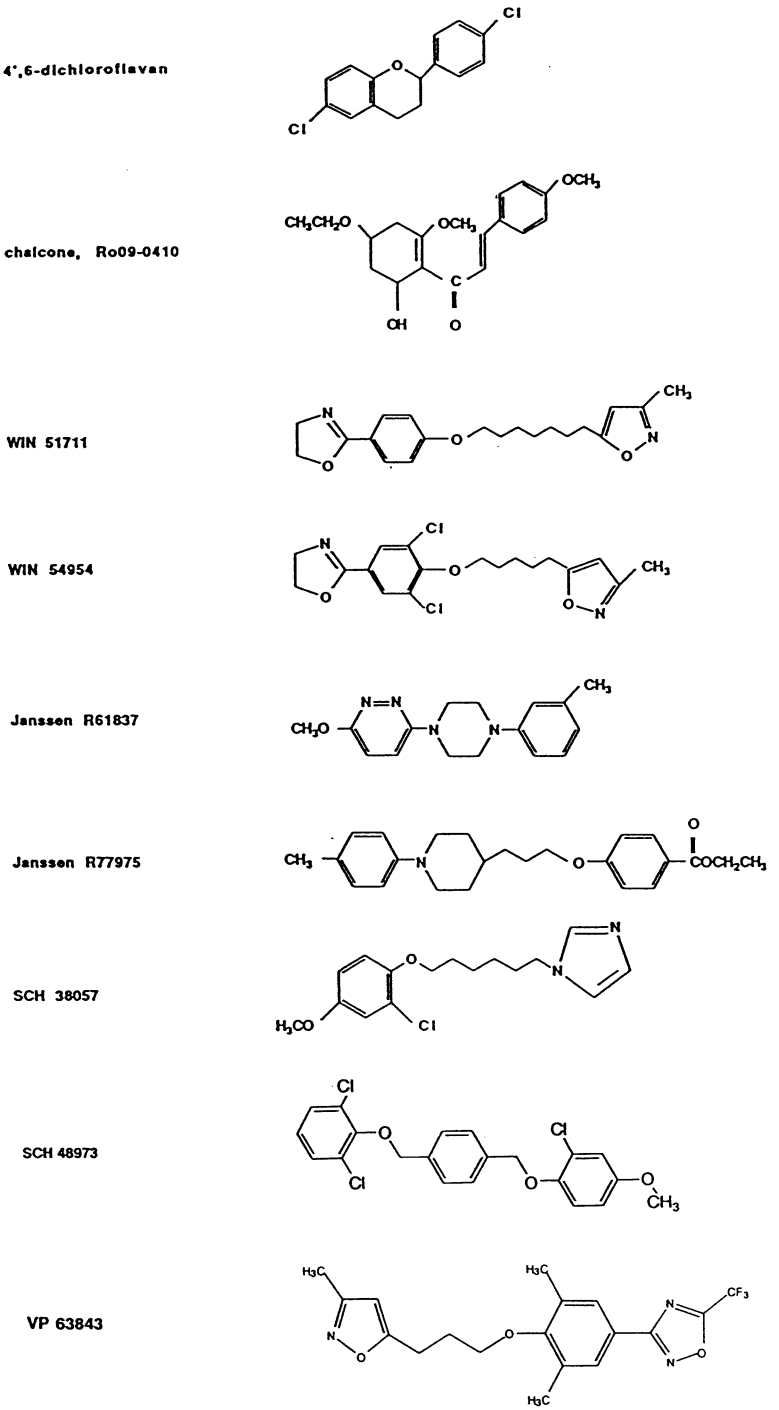

Fig. 1. Chemical structures of representative capsid-binding compounds with anti-picornaviral activity

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).

4.5. 3C protease inhibitors

A series of compounds under development by Agouron Pharmaceuticals targets the 3C 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 3C 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 antirhinoviral activity as well as anti-EV activity.

5. In vitro and in vivo models

The EVs are readily propagated in tissue culture (Melnick and Rennick, 1980). 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. Antiviral assays demonstrating the inhibitory effects of an anti-EV compound generally involve the demonstration of protection of virus-induced cytopathic effect in susceptible cell lines (Woods et al., 1989; Buontempo et al., 1997). 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 binding inhibitors (see below) involving ICAM-1 (Last-Barney et al., 1993) and thermal stabilization (Rombaut et al., 1991) have also been employed.

The mouse is the most common species used in models of human enterovirus infections. Suckling mice can be readily infected with the Barty strain of echovirus 9 (Bultman et al., 1983; McKinlay et al., 1986) and coxsackievirus A9 (Melnick and Godman, 1951; Woods et al., 1989). Infected animals develop flaccid limb paralysis as a result of infection and intragastric administration of a capsid-binding agent is capable of preventing the development of paralysis (McKinlay et al., 1986; Woods et al., 1989). Adult mice can be infected intracranially with poliovirus and protected from development of paralysis by oral administration of capsid binding agents (McKinlay and Steinberg, 1986; Jubelt et al., 1989; Buontempo et al., 1997). Coxsackievirus B3 can also infect adult mice resulting in myocarditis with involvement of multiple organ systems (Klingel et al., 1996). Pleconaril, a capsid binding agent (see Section 7), 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 (Jim Groarke, personnal communication).

6. Pharmacokinetics

In vivo models have also been used to study the pharmacokinetics of candidate anti-EV compounds. From the studies conducted to date, capsid binding compounds are efficacious when plasma levels are above the in vitro inhibitory levels. Analysis of plasma levels of a capsid binder in suckling mice showed that plasma levels exceed the in vitro inhibitory concentration for a portion of the dosing interval (Woods et al., 1989). In a clinical study (Schiff et al., 1996), plasma levels of pleconaril, a capsid binder, exceeded the in vitro inhibitory concentration of coxsackievirus A21 by 2–5-fold for the duration of the dosing interval (McKinlay, unpublished data). In this study, pleconaril was shown to be partitioned into lipophilic tissues with concentrations in brain and spinal cord

up to six times that observed in plasma. Concentrations in nasal epithelium are up to eleven times higher than plasma (McKinlay, unpublished data), correlating with clinical effects described below.

7. Clinical trials

Because of the potentially large market for common cold treatments, many of the anti-picornaviral compounds which have reached clinical trials have done so in the context of volunteer challenge studies or naturally-occurring infections with the rhinoviruses. The results of these studies are relevant to anti-EV therapy because of the close relationship between the two genera of picornaviruses as well as the propensity of EVs to cause a summer upper respiratory tract illness ('summer flu', 'summer cold'). Bioavailability and safety information will also be relevant in anticipated use of these compounds in more serious EV infections. Unfortunately, although numerous studies have been conducted, only a few classes of compounds have demonstrated clinically significant efficacy.

The clinical efficacy of intranasal interferon as prophylaxis for rhinovirus colds has been demonstrated in several studies (Merigan et al., 1973; Greenberg et al., 1982; Hayden and Gwaltney, 1983; Samo et al., 1983; Hayden et al., 1986; Hayden and Gwaltney, 1984). Additional studies demonstrated significant efficacy against naturally acquired rhinovirus infections and against contact spread of rhinoviruses within family groups after experimental induction of a natural cold (Douglas et al., 1986; Hayden et al., 1986). Side-effects of interferon included nasal irritation and stuffiness, and mucosal ulceration (Samo et al., 1983; Hayden et al., 1986). Administered therapeutically 1 day after experimental rhinovirus 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 postexposure prophylaxis of rhinovirus infections in families (Monto et al., 1989). Despite in vitro efficacy noted above, interferons have not been clinically evaluated in EV infections.

Immune serum globulin has 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; Valduss et al., 1993) 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 clinical benefit but did show a reduction in viral titer in babies receiving intravenous immunoglobulin preparations which were subsequently shown to contain high antibody titers to the infecting serotype (Abzug et al., 1995). Individuals with congenital or acquired antibody deficiencies are also at risk for severe EV infections (see Section 4.2). 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 maintanence 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.

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 Section 4.4) promise better bioavailability and tolerance, but have not been clinically evaluated. Similarly, the 3C protease inhibitor compounds have not been tested clinically in either EV or rhinovirus infections.

The most extensive clinical experience in treating EV and rhinovirus infections has been with the capsid-binding compounds (Fig. 1), to which the remainder of this section is devoted. Trials of the 'R' series of compounds from Janssen Pharmaceuticals have been limited to intranasal administration to patients with rhinovirus colds (Al-Nakib et al., 1989; Barrow et al., 1990; Hayden et al., 1992). Pirodavir (R77975) and R61837 were efficaceous in experimentally-induced rhinovirus colds when these drugs were administered intranasally before or after infection, but prior to onset of symptoms (Barrow et al., 1990; Hayden et al., 1992); pirodavir required six times daily dosing, with efficacy loss at three daily doses (Hayden et al., 1992).

A series of capsid-binding compounds developed by Schering-Plough are broad spectrum inhibitors of the EVs, and demonstrate therapeutic oral efficacy in animal models (Cox et al., 1996; Buontempo et al., 1997). This series has limited potency against the rhinoviruses and candidate drugs have not yet advanced to clinical trials.

The 'WIN' series of compounds, initially developed at Sterling-Winthrop Pharmaceuticals, have been clinically evaluated in both rhinovirus and EV infections. The first compound of this group to advance to clinical trials was disoxaril (WIN 51711; Fig. 1), an oxazoline analog and stable ester mimic of an earlier metabolically unstable compound (WIN 41137). Disoxaril was moderately active against rhinoviruses in vitro and very active against EVs both in vitro and in vivo (Otto et al., 1985; McKinlay and Steinberg, 1986; McKinlay et al., 1986). The appearance of asymptomatic crystalluria in healthy volunteers prevented further clinical study. Shortening of the aliphatic chain from $n=7$ to $n=5$ and adding chloro-groups to the phenyl ring (Fig. 1) resulted in WIN 54954 which had broad, potent anti-rhinovirus 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 rhinovirus (rhinovirus 23 and rhinovirus 39) challenge trials (Turner et al., 1983; Turner and Hayden, 1992) 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 rhinovirus trials failed to show efficacy of WIN 54954 (Turner et al., 1983; Turner and Hayden, 1992); 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 coxsackievirus A21, and also significantly reduced nasal mucous discharge, respiratory and systemic symptoms, and viral titers. The overall symptomatic attack rate was reduced from 15/23 patients in the placebo group to 3/27 in the WIN 54954 treated groups ($P=0.0001$) (Schiff et al., 1992). This study represents the first demonstration of oral efficacy of an anti-EV agent; the differences in results compared with those in the rhinovirus studies using the same compound are enigmatic since the MIC for one of the rhinovirus 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 rhinovirus infections, which are limited to the upper airway where drug biodistribution 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 alchohol ingestion by study volunteers.

The latest compound in the 'WIN' series is being developed by ViroPharma, Incorporated, and has been named pleconaril (VP 63843). Pleconaril (3-{3,5-dimethly-4-[[3-methyl-5-isoxa-

zolyl)propyl]phenyl]-5-(trifluoromethyl)-1,2,4-oxadiazole) has demonstrated broad spectrum and potent anti-EV and anti-rhinovirus activity and, like WIN 54954, is highly orally-bioavailable (Pevear et al., 1996). In pre-clinical trials, pleconaril was devoid of cardiovascular and central nervous system side-effects and no differences from placebo have been noted in adverse events in any of the clinical trials to date (Jon Rogers, personal communication). In a phase IIA challenge study of coxsackievirus A21 similar to that described above for WIN 54954, 33 seronegative (for coxsackievirus A21) 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 statistically significant effect on symptom scores, global assessment, fever, and nasal mucous production. Peak viral titers which occurred on the peak day of symptoms were reduced by greater than 99% in the pleconaril group compared to the placebo group (Schiff et al., 1996). There were no differences in adverse effects between study and control groups. The safety profile of pleconaril is attributed to the metabolic stability and viral specific nature of the mechanism of action (Fromtling and Castaner, 1997).

In a recently completed phase 2 study, therapeutic efficacy of pleconaril was evaluated in a double-blinded, placebo-controlled study of 39 patients with naturally-occurring EV meningitis. Preliminary results indicate a statistically significant shortening of disease duration (58% reduction from 9.5 days to 4.0 days; $P = 0.0008$) including reduction in severe incapacitating headache. Also reaching statistical significance were time to complete absence of headache (64% reduction from 18.3 days to 6.5 days; $P = 0.0008$), duration of analgesic use (54% reduction from 11.5 days to 5.3 days; $P = 0.026$), and total analgesic use (48% reduction; $P = 0.043$) (McKinlay, unpublished data). Additional studies of pleconaril in other EV diseases, including hand-foot-mouth syndrome, summer flu, and neonatal sepsis, are planned or already underway. The drug has been made available for compassionate use in antibody-deficient patients with chronic EV meningoencephalitis, patients with EV myocarditis, and neonates with EV sepsis.

8. Drug resistance

Enterovirus mutants which are resistant to the antiviral effects of the capsid binding agents have been extensively studied. Mutants can be isolated in the laboratory which express high and low levels of resistance (Heinz et al., 1989). High resistance mutants generally are the result of mutations in the drug binding pocket of amino acids with bulkier side chains. It is thought that these side chains sterically block the binding of the capsid binders. Low resistance mutants retain some sensitivity to the capsid binding compounds. These mutations map to regions near the canyon floor that are disrupted upon drug binding. Thus far, the mutants studied have shown cross resistance to capsid binding agents of different structural classes (Ninomiya et al., 1990).

Drug resistant EVs have not been reported from clinical studies conducted to date. In a study of the capsid binding inhibitor WIN 54954 in human subjects infected with coxsackievirus A21, no resistant virus was detected (McKinlay, unpublished data). In a clinical study conducted with laboratory-isolated drug resistant mutants of the closely related rhinoviruses, 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 (Groarke et al., 1995).

9. Summary and conclusions

Infections with the human EVs cause significant morbidity and even mortality. Progress toward the development of anti-EV therapy has been substantial. In vitro efficacy of several classes of antiviral agents against the EVs (including those targeting cell susceptibility, virus attachment and

binding, virus uncoating, viral RNA replication and protein production) has led to clinical evaluation of the most promising compounds. Of those in active development, the capsid-binding compounds have received the most clinical study and the leading compound, pleconaril, has now been shown to be efficacious in naturally-occurring, serious EV infection (aseptic meningitis). Widespread use of these compounds in less serious EV infections will depend upon the continued demonstration of safety and tolerability.

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