
WIN 51711, a new systematically active broad-spectrum antipicornavirus agent
Picornaviruses are a major cause of viral-associated morbidity in humans, and can result in death in immunocompromised patients and neonates. The Picornaviridae family consists of two groups of viruses associated with human disease: the rhinoviruses and enteroviruses. The rhinovirus group consists of over 120 serotypes which are the causative agents of one-third to one-half of all upper respiratory illnesses, normally referred to as the 'common cold' (Couch, 1984). It is estimated that each individual, on average, experiences from two to five colds per year (Fox & Hall, 1980), resulting in approximately 250 million workdays of restricted activity per year in the US alone (Merigan, 1982).

The enterovirus group consists of at least 68 different viral serotypes, 63 of which are included in the following four subgroups: poliovirus (3 serotypes), Coxsackie A viruses (23 serotypes), Coxsackie B viruses (6 serotypes) and echorrhoviruses (31 serotypes). Five additional recently isolated serotypes have not been placed in any of four established subgroups due to changes in nomenclature.

Based on data from the Virus Watch Program, it is estimated that 5-15 million enteroviral infections occur each year in the US (Kogon et al., 1969), with only half of these infections resulting in symptomatic illness (Spigland et al., 1966). Clinical enteroviral syndromes range from mild upper respiratory disease with or without myalgia and fever, summer exanthems, herpangina and acute hemorrhagic conjunctivitis, to aseptic meningitis, myocardiitis/pericarditis, poliomyelitis, meningoencephalitis, infectious hepatitis and neonatal sepsis. The type of syndrome seen is largely dependent on the infecting serotype. The more serious syndromes such as poliomyelitis and aseptic meningitis are associated with only a few enterovirus serotypes, while mild upper respiratory disease and other less severe syndromes are associated with all serotypes.

At present, there is no specific treatment for picornavirus infections, although in excess of $1 billion is spent each year on palliative 'cold' remedies (Couch, 1984). In recent years, the clinical usefulness of a number of synthetic agents (enviroxime, dichloroflavan, Ro-09-0410) has been assessed in volunteers experimentally infected with rhinovirus (Hayden & Gwaltney, 1982; Phillpotts et al., 1983; Phillpotts et al., 1984). However, none of these compounds have demonstrated efficacy with the formulations and routes tested.

Recently, the synthesis of WIN 51711, a novel antiviral agent possessing activity in vitro against both enteroviruses and rhinoviruses, was reported (Diana et al., 1984):

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\begin{align*}
\text{CH}_3 & \quad \text{O} \quad \text{N} \\
\text{O} & \quad \text{(CH}_2\text{)}_2 \text{O} \\
\text{N} & \quad \text{H} \\
\end{align*}
\]

WIN 51711 has been shown to exert its antiviral effect through a direct interaction with picornaviral capsid protein(s) (Fox et al., 1984). Mechanism studies have demonstrated that the reversible drug-virus interaction 'stabilizes'
the virion conformation, thereby preventing thermally-induced conformational change in virion structure and loss of one of four viral capsid proteins, VP₆. The changes in virion structure which occur during thermal inactivation simulate the early events in the uncoating process. Similar effects have been noted for another synthetic antipicornavirus agent, Ro 09-0410 (Ninomya et al., 1984), which binds irreversibly to rhinovirus.

WIN 51711 was not virucidal and had no effect on the kinetics of ³H-uridine-labelled poliovirus-2 or rhinovirus-2 adsorption to HeLa (Ohio) cells (Fox et al., 1984). WIN 51711 had no detectable effect on penetration as measured by loss of sensitivity of infectious centers to antisera inactivation. More sensitive methods are being employed to investigate further early cell-virus interactions in the presence of WIN 51711. The antiviral effect of WIN 51711 has been shown to be mediated predominately through an inhibition of the virion uncoating process (Fox et al., 1984). Neutral red encapsidated poliovirus-2 or rhinovirus-2 rapidly become insensitive to photoinactivation during infection (Wilson & Cooper, 1963). In the presence of WIN 51711, however, virions remain intact and sensitive to inactivation. The following sequence of events theoretically takes place in cells infected in the presence of WIN 51711: (1) WIN 51711 interacts with virion capsid proteins (either before or after adsorption), stabilizing virion conformation, (2) virions adsorb to receptors on the cell surface without the loss of VP₆, (3) virions penetrate the plasma membrane at a normal rate by viropexis (Mandel, 1967) and, (4) intact stabilized virions remain in an endosome until degraded by cellular enzymes.

WIN 51711 is structurally related to arildone which has been shown to inhibit poliovirus replication in vitro and in vivo (Caliguiri, McSharry & Lawrence, 1980; McSharry, Caliguiri & Eggers, 1979; McKinlay et al., 1982). In contrast to arildone, WIN 51711 inhibits a broader spectrum of enteroviral and rhinoviral serotypes. In plaque and cytopathic effect reduction assays, WIN 51711 effectively inhibited all 33 rhinovirus serotypes tested at concentrations ranging from 0.02–6.2 mg/l, and also inhibited all echo-, Coxsackie A and B, and polio serotypes tested as well as EV-70 at concentrations ranging from 0.004–1.0 mg/l (Otto et al., 1985; Wilfert et al., 1984). Certain enterovirus and rhinovirus serotypes (Coxsackie B5, rhino-8 and rhino-41) appear to be only marginally sensitive to the antiviral effects of WIN 51711. Recently, strain-to-strain differences in drug sensitivity have also been observed for Coxsackie B3 and poliovirus-2. These differences in sensitivity can be attributed to the previously described mechanism of action of WIN 51711. The same variation in capsid protein amino acid sequence which underlies the antigenic differences between serotypes presumably alters the affinity of WIN 51711 for the virion, thereby preventing the stabilization of the nucleocapsid required for antiviral activity.

The systemic antiviral activity of WIN 51711 has been shown in two murine models of human enteroviral disease. Adult mice infected intracerebrally with human poliovirus type-2 (MEF strain) develop flaccid limb paralysis between 6 and 12 days after infection, leading to death within 24 h after the appearance of symptoms (Jubelt et al., 1980). Despite the severity of the central nervous system infection, WIN 51711 was prophylactically and therapeutically effective in preventing poliovirus-induced paralysis and death (Steinberg et al., 1984a, b). Daily oral doses of 8 mg/kg beginning 2 h prior to infection significantly reduced mortality. When therapy was delayed 48 h after infection, daily oral doses of 100 mg/kg (t.d) prevented death in 60% of the drug-treated group. The protective effect of WIN 51711 could be attributed to a marked 3–5 log pfu/g of tissue decrease in viral titres observed in spinal cords of drug-medicated mice infected intracerebrally with 200 LD₅₀.

The effect of WIN 51711 on a systemic enteroviral infection has been demonstrated in suckling mice infected with echovirus-9 (Barty strain) (Steinberg et al., 1984a). Subcutaneous inoculation of echovirus-9 into mice less than 24 h of age resulted in extensive polymyositis, progressive limb paralysis and eventual death (Bultmann et al., 1983). Single daily intraperitoneal doses of WIN 51711, beginning 2 h prior to infection, prevented the development of paralysis in a dose-dependent manner. The dose resulting in protection of 50% of the infected suckling mice was determined to be 7 mg/kg. When therapy was delayed until after extensive replication and tissue damage had occurred (48 h postinfection), approximately two-fold higher doses were required to obtain comparable results. Doses of 100 mg/kg of WIN 51711 completely inhibited virus replication. In this study, no virus was recovered from carcasses of drug-medicated animals, whereas titres in excess of 10⁶ pfu/animal were found in placebo-medicated animals.

WIN 51711 represents a significant new contribution to the rapidly developing field of antiviral research. As a specific inhibitor of the
picornaviral uncoating process, WIN 51711 has been shown to prevent the replication of a broad spectrum of clinically significant human picornaviruses in vitro as well as in vivo when administered orally to mice infected with lethal doses of polio- or echovirus. WIN 51711 was not mutagenic and was well-tolerated in acute or chronically treated rats, mice and monkeys. The potent efficacy of WIN 51711 in lethal animal models and the high degree of safety observed in toxicity testing make WIN 51711 a potential candidate for clinical evaluation against human picornaviral infections for which no therapy presently exists.

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References


