Oral Efficacy of WIN 51711 in Mice Infected with Human Poliovirus

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WIN 51711, a new broad-spectrum anti-picornavirus agent, prevented the development of paralysis and subsequent death in mice infected intracerebrally with a lethal dose of human poliovirus type 2 (MEF strain). The prophylactic efficacy of intragastrically administered WIN 51711 was dose dependent over the 3.9- to 62.5-mg/kg (twice daily) dose range, with a minimal significantly effective dose of <15.6 mg/kg per dose (twice daily) (P < 0.008). An oral four times a day dosage regimen initiated 48 h postinfection with WIN 51711 doses as low as 12.5 mg/kg was effective in significantly reducing poliovirus-induced paralysis and death compared with a placebo. Viral titers in the brains and spines of mice infected intracerebrally with 200 50% lethal doses of poliovirus were reduced by 3 to 5 log_{10} PFU/g in the WIN 51711-medicated group compared with placebo-mediated animals. The potent in vitro and in vivo anti-picornavirus activity of WIN 51711 makes it a potentially useful drug for the treatment of enterovirus infections in humans.

Although researchers in the field of antiviral chemotherapy have been encouraged by the discovery of drugs for the treatment of herpesvirus and influenza virus infections, therapy of disease caused by picornaviruses remains elusive. Arildone, an aryl diketone compound synthesized at the Sterling-Winthrop Research Institute, has been shown to possess in vitro and in vivo activity against poliovirus (1, 4, 5, 6). Synthesis of analogs of the arildone molecule produced new chemical entities with broad-spectrum in vitro antipicornavirus activity. One of the most active of these in terms of level of potency and spectrum was WIN 51711 (see Fig. 1). Based on its in vitro potency (7), WIN 51711 was tested for systemic efficacy in the human poliovirus model in mice. In this model, intracerebral inoculation of mice with poliovirus results in flaccid limb paralysis and subsequent death (2, 3), a clinical picture similar to that observed in severe poliovirus infections in humans. The data presented here show that WIN 51711 is orally effective in preventing poliovirus-induced paralysis and death in mice.

MATERIALS AND METHODS

Mice. Albino, female ICR mice weighing 18 to 21 g were used in all experiments. Mice were purchased from Buckberg Farms, Thomkinds Cove, N.Y.

Virus. Poliovirus type 2, MEF strain, was originally obtained from the University of Pittsburgh and had undergone many tissue culture passages in our laboratory. Virus identity was confirmed by standardized poliovirus antisera obtained from the American Type Culture Collection. For the tests reported here, the virus was grown at a low multiplicity of infection in HeLa (Ohio) tissue culture cells and stored at −70°C in heat-sealed ampoules.

Infection of mice. For the first phase of the experimental work, mice were infected with 2 to 20 50% lethal doses (LD_{50}; 500 PFU) of virus via the intracerebral route. Three hundredths of a milliliter was injected into the left cerebral hemisphere through a 5/8-in. (1.6-cm), 26-gauge needle. In later experiments measuring infectivity titers in tissues in which a fulminating infection was desired, 200 LD_{50} was used as the infectious dose and was given as described above.

Antiviral and placebo treatments. WIN 51711 (Fig. 1) is practically insoluble in water at neutral pH (maximum solubility, 0.5 µg/ml [pH 7.0]). In the studies reported here, both the free base (therapy studies) and methanesulfonate salt of WIN 51711 (prophylaxis and virus titer study) were used. These two forms of WIN 51711 can be used interchangeably, since they are equally efficacious and produce similar levels in serum when administered orally by gavage in 1% gum tragacanth (data not shown). A 1% gum tragacanth suspension was used as a placebo in all studies.

Experimental design. (i) Prophylactic efficacy. In the prophylactic tests, medication (in divided doses) with WIN 51711 was administered to mice by oral gavage (0.2 ml per dose) at levels ranging from 7.8 to 125 mg/kg per day. Dosing was initiated 2 h prior to infection, then continued on a twice daily (b.i.d.) basis for a total of 10 days. The viral inoculum was 2 LD_{50}, which had been shown previously to result in 70 to 100% mortality at 20 days postinfection. The mice were observed b.i.d. at the time of medication and also during the postmedication period for a total of 20 days. Appropriate placebo-mediated animals were included. (ii) Therapeutic efficacy. In experiments designed to determine whether therapy with WIN 51711 is effective when delayed until extensive virus replication has occurred, mice were infected with 20 LD_{50} of virus and medicated orally with WIN 51711 at doses ranging from 12.5 to 100 mg/kg beginning 48 h postinfection and continued four times daily (every 6 h) for 8 days.

Infectivity titers in brain and spinal cord. The effect of the WIN compound on virus proliferation in the central nervous system was determined in animals infected intracerebrally with 200 LD_{50} of virus. WIN 51711 was administered orally at 100 mg/kg starting 2 h prior to infection and continued b.i.d. for 4 days postinfection. Placebo-mediated mice were given 1% gum tragacanth alone, and all animals were observed over a 7-day postinfection period for evidence of flaccid paralysis or death. The brains and spinal columns were removed from four WIN 51711-medicated and four placebo-mediated animals on each of the 4 days following infection. Paralysis was defined as apparahal in the placebo group on day 3 postinfection. No paralysis was seen in the WIN 51711-mediated group throughout the 5-day period.

The animals were sacrificed by cervical dislocation, and the brain (cerebrum and diencephalon) and spinal cord (spinal cord, brain stem, and midbrain) were removed from
each animal and weighed. The weighed tissues were homogenized in a Brinkmann Instruments, Inc., Polytron homogenizer and centrifuged at 2,000 × g for 15 min. The supernatant material was stored in Nunc screw-capped ampoules at −70°C in a mechanical freezer. The virus contents of the supernatants were determined on 1-day-old confluent HeLa (Ohio) cell sheets in a standard plaque assay with six-well Costar plastic plates (7).

RESULTS

Prophylactic effect on survival. Dose-dependent oral prophylactic efficacy of WIN 51711 demonstrated in mice infected with a lethal dose of poliovirus is shown in Fig. 2. At the end of the test period (20 days postinfection), only 24% of the placebo-medicated mice were alive, compared with 45, 69, and 89% of the animals medicated with 3.9, 5.6, and 62.5 mg/kg b.i.d., respectively (n = 30 per dose group). Statistical analyses of the data by Mantel-Cox and Breslow analyses of variance showed that medication with WIN 51711 at 15.6 mg/kg resulted in a significant reduction in mortality (P < 0.008) compared with the placebo-medicated group. The minimal effective dose under these conditions, therefore, was greater than 3.9 and less than 15.6 mg/kg b.i.d.

Therapeutic effect on survival. Dose-dependent therapeutic efficacy of WIN 51711 in mice infected with 20 LD50 is shown in Fig. 3. Oral dosing was initiated 48 h postinfection and was continued every 6 h for 8 days. Twenty mice per dose group were used in three separate experiments, and the results are shown as the mean survival on each day (n = 60 per dose group). On day 10 postinfection, only 5% of the placebo-medicated animals were alive and symptom free, compared with 43, 62, 68, and 84% of mice medicated with WIN 51711 at doses of 12.5, 25, 50, and 100 mg/kg, respectively. Statistical analysis of the data showed that medication with WIN 51711 at 12.5 mg/kg resulted in significant protection (P < 0.001).

Infectivity titers in brain and spinal cord. Infectivity titers in the brain and spine were quantitated in mice infected intracerebrally with 200 LD50. When mice were infected with this high inoculum, the first deaths appeared in the placebo group on day 3 postinfection, and all the mice in this group were dead by the postinfection day 7. In contrast, 90% of mice medicated with WIN 51711 at 100 mg/kg b.i.d. remained alive and asymptomatic through day 7 postinfection.

The mean PFU per g of tissue found in brain and spinal cord on days 1 through 4 postinfection are graphically illustrated in Fig. 4. WIN 51711 reduced the virus titer in both tissues by >3.5 log/g of tissue on observation day 4 compared with paralyzed mice.

When brain titers were analyzed by a general linear model procedure (Duncan's Multiple Range Test for variables), a highly significant difference was seen between the medicated and placebo-medicated groups of mice (P < 0.0001). No significant difference was noted between the paralyzed and nonparalyzed placebo groups.

The greatest effect of WIN 51711 medication was on poliovirus titers in the spinal cord. Viral titers in WIN 51711-medicated animals were reduced by >5 log PFU/g of tissue compared with paralyzed placebo animals on day 4 postinfection.

DISCUSSION

Picornaviruses are the etiologic agents for the majority of diagnosable viral upper respiratory infections, as well as potentially serious central nervous system infections in humans. Previous studies have demonstrated the in vitro potency and spectrum of WIN 51711 against a number of rhino- and enterovirus serotypes (7). The data presented here demonstrate the oral prophylactic and therapeutic potency of WIN 51711, a new anti-picornavirus agent, in a model of human enterovirus disease in mice. Other studies (B. A. Steinberg, A. A. Visosky, J. A. Frank, Jr., and M. A. McKlinay, 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 433, 1984) have shown that WIN 51711 is

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FIG. 1. WIN 51711: 5-{7-[4-(4,5-dihydro-2-oxazolyl)phenoxyl]heptyl}-3-methylisoxazole.
active in infant mice infected with a lethal strain of echovirus type 9.

Poliovirus infection of mice results in a fulminating infection leading to paralysis and subsequent death. This disease process differs from that seen in humans in that the virus, once inoculated intracerebrally, does not elicit the production of neutralizing antibodies (2), and second, once paralysis has begun in the mouse, its progress is inexorable, leading to death within 24 h. When the symptoms of disease are evident (i.e., flaccid limb paralysis), virus titer in the spinal cord has reached a maximum, and irreversible neurologic damage has occurred. Therapy must be initiated, therefore, prior to the appearance of symptoms to be effective. The studies reported here show that WIN 51711 was effective in preventing death due to poliovirus when therapy was initiated 48 h after infection, at which time multiple rounds of viral replication and dissemination within the central nervous system has occurred.

Viral titers in the central nervous system were markedly reduced in mice medicated with WIN 51711 compared with the placebo group. Further, viral titers in spinal cord tissue of WIN 51711-medicated mice never approached the titer level previously shown to correlate with the onset of paralysis and subsequent death (7 log/g of spinal tissue). In the absence of detectable immune response in the mouse to intracerebral inoculation of poliovirus, the data suggest that mice infected with poliovirus via the intracerebral route were protected by the direct inhibitory effect of WIN 51711 on viral replication.

Mode of action studies have shown that WIN 51711 interacts directly with virion capsid proteins to prevent uncoating of the viral nucleic acid (M. P. Fox, M. J. Otto, W. S. Shave, and M. A. McKinlay, Abstr. 24th ICAAC, abstr. no. 431, 1984). The compound has no effect on mammalian cell growth at levels that interfere with virus replication. Consistent with the virus-specific mechanism if action, the acute toxicity of WIN 51711 in mice and rats was low, with an LD₅₀ exceeding 8,000 mg/kg, and no significant toxicity was observed in mice, rats, or monkeys orally administered WIN 51711 for 1 month at doses of 100 mg/kg. In addition, WIN 51711 was not shown to be mutagenic in the mammalian cell forward mutation assay and the Ames test, both with and without metabolic activation (R. Fabian, personal communication). The virus-specific mode of action, minimal toxicity, and demonstrated in vitro and systemic potency of WIN 51711 make it a candidate for clinical evaluation as a broad-spectrum anti-picornavirus agent.

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LITERATURE CITED