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Prevention of Human Poliovirus-Induced Paralysis and Death in Mice by the Novel Antiviral Agent Arildone

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Arildone, a novel antiviral agent which blocks virion uncoating, was assessed for its ability to prevent paralysis and death in mice infected intracerebrally with a lethal dose of human poliovirus type-2 (strain MEF). Intraperitoneal administration of arildone suspended in gum tragacanth prevented paralysis and death in a dose-dependent manner (minimal inhibitory dose = 32 mg/kg, twice daily) and protected animals from virus challenges in excess of 20 50% lethal doses. Oral medication with arildone solubilized in corn oil was similarly effective in preventing poliovirus-induced paralysis and death. Arildone was therapeutically effective even when intraperitoneal medication was delayed for 48 h postinfection. Analysis of the virus titers in the central nervous system tissues of animals infected with 200 50% lethal doses demonstrated that arildone reduced titers in the brain and spine by approximately 3 and 4 $\log_{10}$ PFU per g of tissue, respectively, implying that direct inhibition of virus replication was responsible for host survival. Arildone is the first antiviral agent capable of preventing poliovirus-induced death in mice. The efficient inhibition of poliovirus replication described here demonstrates the potential usefulness of uncoating blockers in the systemic treatment of viral diseases.

Arildone [4-6-(2-chloro-4-methoxy phenoxy) hexyl]-3,5 heptanedione (Sterling Drug, Inc., New York) has been shown to selectively inhibit poliovirus replication in vitro by preventing virion uncoating (1, 6). The drug interacts directly with poliovirus capsids, making the virions more resistant to alkaline or thermal degradation (1). Results of investigations of arildone-resistant mutants suggest that viral capsid polypeptide VP1 is the site of virion-drug interaction (H. J. Eggers, Interferon Sci. Mem., March 1981).

Previous studies have shown arildone to be effective in inhibiting plaque production by Semliki Forest virus, vesicular stomatitis virus, coxackievirus A9, murine cytomegalovirus, herpes simplex virus types I and II, and poliovirus (4, 5). Of the susceptible viruses examined thus far in vitro, poliovirus appears to be the most sensitive to arildone. For this reason, poliovirus-infected mice were chosen as a model system for assessing the therapeutic efficacy of arildone. Mice infected intracerebrally (i.c.) with this virus develop flaccid limb paralysis and other clinical and pathological reactions characteristic of the disease in humans (2, 3). In this report, we describe the effectiveness of arildone in the prevention and treatment of poliovirus-induced symptoms. The results show that the blockage of virion uncoating was a valid indicator of the efficacy of arildone in vivo.

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MATERIALS AND METHODS

Virus and cells. The MEF strain of poliovirus type 2, originally obtained from the University of Pittsburgh Medical School, was plaque purified, propagated and titrated on HeLa Ohio cells (Flow Laboratories, Inc., Rockville, Md.), and stored as a cell-free virus stock at −70°C. The identity of plaque-purified virus was confirmed by serum neutralization test, using standardized poliovirus type 2 antiserum (American type Culture Collection, Rockville, Md.). HeLa Ohio cells were grown in monolayer culture in medium 199 (Flow) supplemented with 5% Bobby Calf serum (GIBCO Laboratories, Grand Island, N.Y.) and antibiotics (50 U of penicillin and 50 μg of streptomycin per ml).

Virus assays. Virus titrations were determined by plaque assay on HeLa Ohio cells (4) by using 0.5% SeaKem agarose overlays (Marine Colloids, Rockland, Maine). The antiviral activity of arildone was assessed with the drug present at various concentrations in the adsorption and overlay medium, followed by quantitation of visible plaques on the third day postinfection. To quantitate virus levels in the central nervous system of mice infected i.c. with 200 50% lethal doses (LD_{50}), four animals from each group (placebo nonparalyzed, placebo paralyzed, and arildone medicated) were sacrificed on various days postinfection; the brains (cerebrum and diencephalon) and spinal cords (including the brain stem and midbrain)

were removed, weighed, and homogenized with a Brinkmann Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.). Tissue homogenates were clarified by centrifugation, and serial 10-fold dilutions were made. Diluted virus was allowed to adsorb to the HeLa cell monolayers for 1 h; the homogenates were aspirated and the cultures were treated as described above.

Animal infection and drug dosing. Male and female ICR mice (Buckberg Farms) weighing 18 to 20 g were infected in the left cerebral hemisphere with 2 to 200 LD_{50} of virus (1 LD_{50} = $10^{3.0}$PFU) in a 0.03-ml volume. Arildone suspended in 1% gum tragacanth plus 2% Tween 80 (GT) at various doses was used for intraperitoneal (i.p.) injections. Animals were dosed with the required concentration 4 h before infection, 2 h postinfection, and twice daily (9 h apart) for 4 to 13 days. In one experiment, medications were initiated 48 h postinfection and given twice daily for 14 days. Arildone was solubilized in corn oil and administered intragastrically via a gavage needle. Medication (0.2 ml) were administered 4 h before infection, 2 h postinfection, and then twice daily for 13 days. Placebo-medicated animals were treated with the appropriate vehicle. Infected arildone-medicated, placebo-medicated, or nonmedicated mice were observed daily for a 21-day period, and the deaths were recorded to determine efficacy.

RESULTS

In vitro and in vivo anti-poliovirus activity of arildone. The anti-poliovirus-2 activity of arildone was demonstrated in a plaque reduction assay. The concentration of arildone necessary to inhibit plaque production by 50% was determined to be between 0.07 and 0.14 μM (Table 1). Previous studies have shown that at all concentrations tested, arildone has no effect on the cellular uptake of radioactively labeled precursors or the synthesis of protein, RNA, or DNA (6).

TABLE 1.

Arildone concn (μM)	PFU[a]	% Plaque reduction
0	65.0	
0.02	54.0	17
0.04	45.0	31
0.07	36.3	44
0.14	20.3	69
0.27	8.3	83
0.54	1.0	98
1.08	0.0	100

[a] Average number of plaques in three monolayers.

The systemic effects of arildone were studied in mice infected intracerebrally with various doses of poliovirus. When animals infected with 2 LD_{50} were medicated i.p. with arildone suspended in GT, dose dependent prevention of poliovirus-induced death was observed (Table 2, experiment 1). At 20 days postinfection, 26, 42, 78, 94, and 100% survivors were observed in groups medicated with arildone at 0, 32, 63, 125, and 250 mg/kg twice daily, respectively. In two experiments, animals medicated with the highest dose were maintained for >100 days without evidence of symptoms.

Onset of paralysis was more rapid, and the percentage of survivors 20 days postinfection was reduced to 3%, when animals were infected with 20 LD_{50} and given a placebo (Table 2, experiment 2). Despite the higher challenge dose of virus, 100% of the animals medicated with arildone survived the 20-day observation period without symptoms.

In the aforementioned studies, i.p. medications were administered for 14 days. The medication period could be reduced to 5 days with only a minimal increase in mortality (Table 2, experiment 3).

The ability of arildone to prevent paralysis and death in animals with a well-established infection was examined in animals infected 48 h before initiation of treatment. Under these conditions, 87% of the drug-treated animals survived the 20-day period postinfection, compared with only 30% of the recipients of placebo (Table 2, experiment 4).

Arildone suspended in GT or solubilized in corn oil was administered orally to mice infected with 2 LD_{50}. The oil formulation was significantly more effective than the GT suspension in preventing poliovirus-induced death (Table 3, experiments 5 and 6). At the completion of the observation period, only 59% of the group medicated with the GT suspension were alive compared with 93% in the group medicated with the oil formulation.

Effect of arildone on the replication of poliovirus in the central nervous system of mice. Animals infected i.c. with 200 LD_{50} were medicated i.p. with arildone (250 mg/kg) suspended in GT or placebo-GT 4 h before infection and 12 h postinfection and twice daily for only 3 days. Under these conditions, 90% of the placebo and nonmedicated animals were paralyzed or had died by day 6 postinfection, compared with the arildone-medicated group, in which 100% remained symptom free (data not shown). Figure 1 shows the effect of i.p. administered arildone on poliovirus replication in the brain and spinal cord of infected animals. Virus titers in the brain increased rapidly in the first 48 h postinfection, reaching a mean titer of 8 $\log_{10}$ PFU per g of tissue on day 2 postinfection. After day 3 postinfection, the placebo-treated group consisted of three different populations: symptom-free, paralyzed, and dead animals. The placebo group was divided for assay purposes into paralyzed and nonparalyzed animals after day 3 (dead animals were not analyzed because of autolysis). The mean virus titer decrease to 6.2 $\log_{10}$ on day 5

TABLE 2.

Expt no. (size of inoculum)	Arildone treatment regimen			No. of expts × no. of mice challenged per expt	% Survivors[a]		
	Route of Administration/ vehicle	Treatment schedule	Individual dose (mg/kg)		Day after challenge		
					5	10	20
1 (2 LD_{50})	i.p./GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	5 × 10	98 ± 2.0	46 ± 5.1	26 ± 5.1
			32	5 × 10	100 ± 0	88 ± 3.8	42 ± 2.5
			63	5 × 10	95 ± 2.0	90 ± 3.2	78 ± 3.8
			125	5 × 10	100 ± 0	100 ± 0	94 ± 2.4
			250	5 × 10	100 ± 0	100 ± 0	100 ± 0
2 (20 LD_{50})	i.p./GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	3 × 10	97 ± 3.3	23 ± 12.0	3 ± 3.3
			250	3 × 10	100 ± 0	100 ± 0	100 ± 0
3 (2 LD_{50})	i.p./GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 5 days thereafter	0	3 × 10	100 ± 0	43 ± 3.3	20 ± 1.0
			250	3 × 10	100 ± 0	100 ± 0	93 ± 6.6
4 (2 LD_{50})	i.p./GT	Doses delivered 48 h postchallenge and twice daily for 14 days thereafter	0	3 × 10	97 ± 3.3	50 ± 5.8	30 ± 10.0
			250	3 × 10	100 ± 0	97 ± 3.3	87 ± 3.3
5 (2 LD_{50})	Oral/GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	4 × 10	92 ± 4.8	52 ± 4.8	25 ± 9.5
			250	4 × 10	100 ± 0	87 ± 6.3	59 ± 6.3
6 (2 LD_{50})	Oral/corn oil	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	3 × 10	90 ± 10.0	40 ± 5.8	30 ± 10.0
			250	3 × 10	100 ± 0	100 ± 0	93 ± 3.3

[a] Geometric mean ± standard error of the mean.

FIG. 1. Effect of arildone on virus replication in the brain and spinal cord of mice infected i.c. with 200 LD_{50}. Animals were medicated i.p. with placebo GT or arildone (○) at 250 mg/kg twice daily for 3 days. After day 4, the placebo group consisted of symptom-free (●), paralyzed (■), and dead animals. Four animals from each group on each day were sacrificed, and the virus titer was determined in the central nervous system tissue. The data are expressed as the mean ± standard deviation.

postinfection in the placebo nonparalyzed group was most likely a reflection of the selection for animals with the lowest virus titers. Paralyzed placebo-medicated mice on days 5 and 6 demonstrated consistent virus titers of between 6.7 and 6.9 $\log_{10}$ PFU per g of tissue. Maximal mean virus titers of 5.2 $\log_{10}$ in the brains of arildone-treated animals were observed on day 2 postinfection and dropped to 4.4 $\log_{10}$ on days 3 through 6, despite the cessation of medication after day 3.

Virus replication to a definable threshold in the spinal cords of polio-infected mice can be correlated with the onset of paralysis (3). In this model, the threshold is approximately 7 $\log_{10}$ PFU per g of spinal cord tissue (weight was determined for the spinal cord including vertebrae) (Fig. 1). Mean virus titers in the nonparalyzed placebo-medicated group, which increased from 4.5 $\log_{10}$ on day 1 postinfection to 6.5 $\log_{10}$ on day 3, never exceeded 7 $\log_{10}$. All eight of the paralyzed placebo-medicated animals analyzed on day 5 and 6, however, had extremely consistent virus titers of 7.4 ± 0.13

$\log_{10}$ PFU per g of tissue. Since none of the arildone-medicated animals became paralyzed, they were not expected to have virus titers of 7 $\log_{10}$ or higher. The mean virus titer of arildone-treated animals was consistently lower than the paralytic threshold, never exceeding 3.6 $\log_{10}$ (Fig. 1). The differences between the placebo-treated and arildone-treated animals increased to greater than 4 $\log_{10}$ on days 5 and 6, despite the cessation of arildone medications on day 3. The significance of this result will be discussed later.

DISCUSSION

The mode of action of arildone is unique among antiviral agents in that it acts by preventing the uncoating of virions (6). The results presented here demonstrate that agents with this mode of action reduce virus replication in vivo. In these studies, arildone exhibited both prophylactic and therapeutic activity in experimentally infected mice. When administered prophylactically, arildone effectively prevented poliovirus-induced death in a dose-dependent manner. This prophylactic activity extended to animals challenged with doses of virus as high as 200 LD_{50} (2×10^5 PFU). The virus-specific activity of arildone was also apparent from the low toxicity of the drug. The acute oral LD_{50} for arildone in mice is in excess of 8 g/kg (the highest level tested). Monkeys and rats medicated daily for 5 weeks with 1,000 mg/kg exhibited no untoward reactions (H. P. Drobeck, personal communication).

An important test of any anti-infective agent is its ability to reverse the symptoms evoked by the infectious agent or prevent appearance of symptoms once infection has been clearly established. Arildone administered 48 h after establishment of infection effectively prevented development of paralysis and death in mice. Poliovirus-induced paralysis in mice, however, is an irreversible process leading to death of placebo-treated mice within 24 h. Medication with arildone lengthened significantly the period between onset of paralysis and death (data not shown).

Arildone exists as a suspension in gum tragacanth and Tween 80 but can be dissolved in oils such as corn oil. The activity of the dissolved drug was significantly greater than that of the suspended agent, presumably reflecting increased oral absorption.

The replication of poliovirus in the central nervous system of mice inoculated i.c. with 200 LD_{50} was markedly reduced by arildone therapy. Previous studies have shown that mice infected i.c. with poliovirus under the conditions described here do not exhibit an immune response (2). Thus, it appears that the reduction in poliovirus titers in vivo and the survival of treated animals are direct results of inhibition of replication by arildone. The results shown in Fig. 1 also demonstrate the persistence of arildone activity after the cessation of therapy, implying a relatively long half-life for the compound in central nervous system tissue.

In summary, arildone represents a nontoxic, virus-specific agent with demonstrated therapeutic efficacy. The results demonstrate that prevention of the virion uncoating process is a novel and potentially viable approach to antiviral chemotherapy.

LITERATURE CITED

1. **Caliguiri, L. A., J. J. McSharry, and G. W. Lawrence.** 1980. Effect of arildone on modifications of poliovirus *in vitro*. Virology **105**:86–93.
2. **Jubelt, B., G. Gallez-Hawkins, O. Narayan, and R. T. Johnson.** 1980. Pathogenesis of human poliovirus infection in mice. I. Clinical and pathological studies. J. Neuropathol. Exp. Neurol. **39**:138–148.
3. **Jubelt, B., O. Narayan, and R. T. Johnson.** 1980. Pathogenesis of human poliovirus infection in mice. II. Age-dependency of paralysis. J. Neuropathol. Exp. Neurol. **39**:149–159.
4. **Kim, K. S., V. J. Sapienza, and R. I. Carp.** 1980. Antiviral activity of arildone on deoxyribonucleic acid and ribonucleic acid viruses. Antimicrob. Agents Chemother. **18**:276–280.
5. **Kuhrt, M. F., M. J. Fancher, V. Jasty, F. Pancic, and P. E. Came.** 1979. Preliminary studies of the mode of action of arildone, a novel antiviral agent. Antimicrob. Agents Chemother. **15**:813–819.
6. **McSharry, J. J., L. A. Caliguiri, and H. J. Eggers.** 1979. Inhibition of uncoating of poliovirus by arildone, a new antiviral drug. Virology **97**:307–315.