CHAPTER 10

Arildone: A β-Diketone

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A. Introduction

A new class of antiviral compounds, the aryl β-diketones, was recently discovered. A few members of this group of compounds have been tested for antiviral activity with promising results. One of these compounds, arildone, was extensively studied and shown to be active against a wide variety of DNA and RNA viruses in vitro. In addition, arildone was shown to be effective against herpesvirus infections in vivo. Arildone and its metabolites were well tolerated in animal toxicity studies and they were not mutagenic. This drug shows promise as an effective antiviral agent for use in the treatment of cutaneous herpetic infections in humans. Currently, arildone is undergoing clinical trials for herpesvirus infections in the United States, Asia, and Europe. This review summarizes the current state of knowledge concerning the antiviral activity and possible mode of action of arildone.

B. Chemical Structure and Synthesis

During the course of routine screening of compounds for antiviral activity at the Sterling-Winthrop Research Institute, it was observed that several acyclic β-diketones of the general structure 1 (Fig. 1) inhibited the replication of equine rhinovirus in vitro (Diana et al. 1977 a, b, 1978 a, b). This observation was unusual in that acyclic β-diketones had not previously been shown to have antiviral activity, and thus represented a new class of antiviral drugs. In an effort to establish a structure–activity relationship, a substantial number of homologs were synthesized. The methylenedioxy diketone, structure 2 (Fig. 1), was the original compound. In order to maximize its antiviral activity, this compound was modified. The approach for chemical modification was to examine five parameters with respect to in vitro antiviral activity: (1) the size of the diketone moiety; (2) the necessity of the ethyl side chain; (3) the necessity of the double bond in the alkyl bridge; (4) the length of the alkyl bridge; and (5) the effect of various substituents on the ring structure. Following an aggressive synthetic effort, structure 3 (Fig. 1) emerged as the most promising candidate in this series, exhibiting in vitro antiviral activity against both equine rhinovirus and herpes simplex virus.

The activity against herpes simplex virus was of particular interest and prompted the synthesis of a related series of compounds similar to structure 4 (Fig. 1). This latter series of compounds offered two advantages in that they were simple to prepare and more amenable to chemical modification. The general scheme for their

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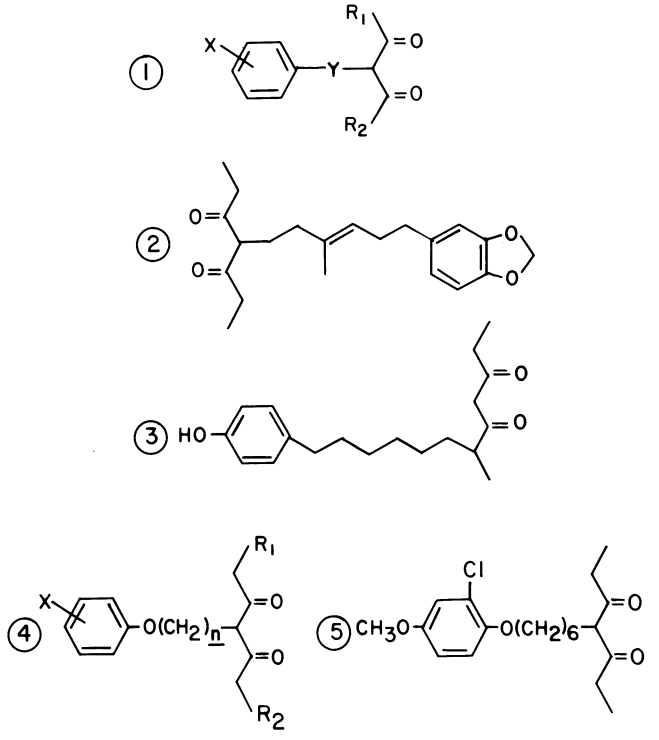

Fig. 1. Structure of arildone and some related compounds

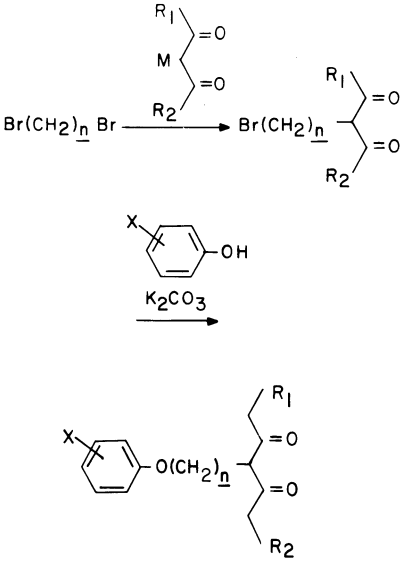

Fig. 2. Reactions involved in the synthesis of arildone and related arylalkyl diketones. R_1 and R_2 = H, CH_3 or C_2H_5; M = Li or Na; N = 3 to 10

synthesis is outlined in Fig. 2. Several homologs were prepared and tested for in vitro and in vivo antiviral activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2). The compound with the best in vitro and in vivo antiviral activity was structure 5 (Fig. 1). This compound, 4-[6-(2-chloro-4-methoxy)phenoxyl]hexyl-3,5-heptanedione, was given the name arildone. The compound was composed of a β-diketone separated from a substituted benzene ring by an alkyl chain of six carbons. Substituents of the benzene ring contributed to lipophilicity and substitution or addition of more hydrophilic substituents decreased the antiviral activity of the compound. An alkyl chain of six to eight carbon atoms had maximum activity, whereas shorter or longer alkyl chains decreased antiviral activity. Changes in the diketone portion of the molecule had less predictable effects on its antiviral activity. Arildone is a white solid in the form of irregular rod-shaped crystals. The compound is stable to light and heat, non-hydroscopic, and virtually insoluble in water (54 μ*M*).

C. Antiviral Effects

Arildone inhibited virus replication in vitro and in vivo. For in vitro testing, arildone was dissolved in dimethylsulfoxide (DMSO) followed by dilution in appropriate medium. For in vivo studies, arildone was usually prepared as a 4% or 8% solution in 90% DMSO, 10% polyethylene glycol 400, or in a cream base consisting of an oil in water emulsion.

I. In Vitro Studies

1. Inhibition of Cytopathic Effects in Cell Culture

The ability of arildone to inhibit viral multiplication, as determined by inhibition of cytopathic effects (CPE), was tested for two groups of DNA viruses and eight groups of RNA viruses (Diana et al. 1977a, b, 1978a, b). Virus was added to tube cultures of cell monolayers and allowed to adsorb at 37 °C for 60 min. Then medium containing varying concentrations of arildone was added and CPE determined microscopically. The results showed that the minimum inhibitory concentrations (MIC) ranged from 0.8 μ*M* for poliovirus to 16.2 μ*M* for HSV-1 and HSV-2 (Table 1). The maximum tolerated concentrations of arildone for the various cells ranged from 32.4 to 67.5 μ*M*. Higher concentrations caused intracellular granulation after 3 days of continuous exposure.

2. Plaque Reduction Tests

Because of the clinical importance of infections associated with herpesviruses and picornaviruses, further studies were conducted on the antiviral effect of arildone against members of these two groups of viruses.

a) Poliovirus 2

The effect of arildone on poliovirus plaque formation has been reported (McSharry et al. 1979; Kim et al. 1980). Virus was diluted in varying concentrations of aril-

done, adsorbed to monolayer cultures of HeLa cells at 37 °C for 60 min, the inoculum was aspirated and agar overlay medium containing appropriate concentrations of arildone was added. The results showed that the MIC of arildone for poliovirus 2 is less than 0.27 μ*M* (Table 1). This is considerably lower than that reported for the inhibition of CPE (0.8 μ*M*) and is due most likely to the fact that arildone was added at the same time as virus in the plaque reduction assay, whereas it was added after adsorption in the CPE assay.

b) Herpes Simplex Virus 1 and 2

Plaque reduction assays were performed on monolayer cultures of BSC_1 cells with various concentrations of arildone present both during the adsorption period and in the agar overlay medium (McSharry and Caliguiri 1979). The data showed that under these conditions the MIC of arildone for HSV-1 was less than 1.35 μ*M* (Table 1). Similar results (MIC of approximately 2.4 μ*M*) have been reported for HSV-1 and HSV-2 (Kim et al. 1980). Plaque reduction assays on 15 strains of HSV-1 and HSV-2 in which the arildone was added only in the agar overlay gave an MIC of less than 5.4 μ*M* (Pancic et al., unpublished work 1980). The MIC for some of the HSV isolates are presented in Table 1. The slight difference in the MIC (< 1.35 to < 5.4 μ*M*) could be due to strains of virus as well as to the time of addition of arildone.

c) Varicella Zoster Virus

The effect of arildone on the plaque forming ability of varicella zoster virus was tested in monolayer cultures of human melanoma cells (C. Grose, personal communication 1980). Various concentrations of arildone were added to the carboxymethylcellulose overlay; arildone was not present during the adsorption period. Arildone (2.7 μ*M*) reduced the number of plaques and, at higher concentrations, also reduced the size of the remaining plaques. The MIC is less than 2.7 μ*M* (Table 1). The results of these experiments show that arildone inhibited plaque formation by HSV-1, HSV-2, varicella zoster virus, and poliovirus 2 at concentrations that were well tolerated by the cell monolayer (Sects. F.I.2, F.I.3.a).

d) Other Viruses

Plaque reduction assays have been performed to determine the MIC of arildone for vaccinia virus, coronavirus, Sindbis virus, adenovirus, vesicular stomatitis virus (VSV), and influenza A_o/WSN (H_oN_1) virus. In these assays, arildone was present only after virus infection and did not prevent plaque formation by these viruses (Table 1). Kim et al. (1980) demonstrated a 50% plaque reduction of VSV by 13.5 μ*M* arildone using Liebowitz L-15 medium which contains galactose in place of glucose for the agar overlay, and Vero (monkey kidney) cells for the plaque assay. On the other hand, no reduction in pfu occurred in the presence of 27 μ*M* arildone when VSV was plaqued in MDBK (bovine kidney) cells in Dulbecco's medium containing 4,000 mg/l glucose in the overlay (J.J. McSharry, unpublished work 1980). This difference in susceptibility of VSV to arildone may be due to the use of different cells and overlay media in these experiments. The replication of VSV and HSV-1 in MDBK cells was poorly inhibited by arildone irrespective of

Table 1. Effect of arildone on virus infectivity

Virus	MIC (μ*M*)[a]	
	CPE	Plaque reduction
Poliovirus 2	0.8	< 0.27
Murine cytomegalovirus		10.8
Herpes simplex virus 1		
Sheely strain	16.2	< 1.35
Robinson strain		< 5.4
McKrae strain		< 5.4
Herpes simplex virus 2		
Curtis strain	16.2	< 5.4
75-1000 strain		< 5.4
Varicella zoster virus		< 2.7
Corona virus A59		>27
Vesicular stomatitis virus		
Indiana serotype	1.9	>27
Influenza A_0/WSN/(H_0N_1) virus		>27
Vaccinia virus	8.1	>13.5
Adeno virus		>27
Sindbis virus		>27

[a] Minimal inhibitory concentration expressed as μ*M* arildone required to reduce CPE or plaque formation by 50%

the media used, suggesting that MDBK cells are resistant to the antiviral effects of arildone (J. J. McSharry, unpublished work 1980). In addition, it was shown that media containing high concentrations of glucose (4,000–10,000 mg/l) reduced the antiviral activity of arildone against HSV-1 and HSV-2 (F. Pancic, unpublished work 1980). Currently, L-15 medium is routinely used for studies on the effect of arildone on HSV replication. Interestingly, there is no effect of glucose on the ability of arildone to inhibit poliovirus replication in HeLa or Vero cells (McSharry et al. 1979). Thus, the different results on effects of arildone against VSV were due to differences in cells and media. Table 1 presents a complete list of viruses tested by plaque reduction assays for arildone. Picornaviruses and herpesviruses were very sensitive to the drug, whereas other viruses were less sensitive. Concentrations of arildone in the range 13.5–27 μ*M* were toxic to some cultures and plaque assays could not be performed adequately at these higher concentrations.

3. Effect on Virus Yield

The effect of arildone on the yield of virus was determined for poliovirus 2, HSV-1 and HSV-2, VSV, murine cytomegalovirus virus (MCMV), Semliki Forest virus (SFV), and coxsackievirus A9 (Kim et al. 1980). Multiplicity of infection (m.o.i.) was between 0.1 and 1 pfu/cell and arildone was present from the time of adsorption throughout the entire virus growth cycle. Virus yield was determined by plaque assay 24 h after infection, except for MCMV which was assayed 72 h after

Table 2. Effect of arildone on virus replication

Virus	MIC (μM)[a]
Murine cytomegalovirus	<8.1
Semliki forest virus	<8.1
Vesicular stomatitis virus	2.7
Poliovirus 2	<2.7
Herpes simplex virus 1	2.7
Herpes simplex virus 2	<2.7
Coxsackievirus A9	<5.4

[a] Minimal inhibitory concentration expressed as μM arildone required to reduce the yield of virus by 50%

infection. The results (Table 2) showed: MIC for MCMV and SFV $< 8.1\ \mu M$; VSV and HSV-1 $2.7\ \mu M$; poliovirus 2 and HSV-2 $< 2.7\ \mu M$; and coxsackievirus $< 5.4\ \mu M$. Similar results have been presented for HSV-2 (Kuhrt et al. 1979). At m.o.i. 50, arildone ($5.4\ \mu M$) inhibited the yield of poliovirus by 3 log units (McSharry et al. 1979). At m.o.i. 1–10, $5.8\ \mu M$ arildone inhibited the yield of VSV by 50% (J.J. McSharry and L.A. Caliguiri, unpublished work 1979). The replication of Epstein–Barr virus lymphoblastoid cells was reduced by 50% in the presence of $\geqq 27\ \mu M$ arildone (Sumaya and Ench 1980). These results show that arildone inhibited the replication of a wide variety of DNA and RNA viruses in vitro at concentrations which did not inhibit cellular macromolecular synthesis (Sect. F.I.2). As in the plaque reduction tests, the yield of herpesviruses and picornaviruses were more sensitive to arildone than that of some other viruses.

II. In Vivo Studies Against Herpes Simplex Virus

The effect of arildone on HSV infection in the guinea pig has been extensively studied (Steinberg and Pancic 1976; F. Pancic, unpublished work 1980).

1. Arildone in DMSO

a) Effect on the Development of Lesions

In vivo studies have demonstrated that arildone is effective topically in a guinea pig skin infection produced by HSV-1 and HSV-2. Application of 8% arildone in cream formulation or 8% arildone in 90% DMSO five times daily starting 24 h postinfection suppressed the formation and progression of herpetic vesicles and significantly reduced virus titer in the lesion sites. Guinea pigs were infected intradermally with HSV-1 and 24 h later arildone was applied to the infected area. The effect of arildone on the development of herpetic vesicles was evaluated daily and the lesion was assigned a score based on severity (Steinberg and Pancic 1976; F. Pancic, unpublished work 1980). The effect of 4% and 8% arildone in DMSO on the development of herpetic vesicles was evident after 1 day therapy (Fig. 3). The growth of vesicles was arrested at the stage at which the therapy was initiated, and no new vesicles formed after that time. During the next 24 h therapy, drying, and

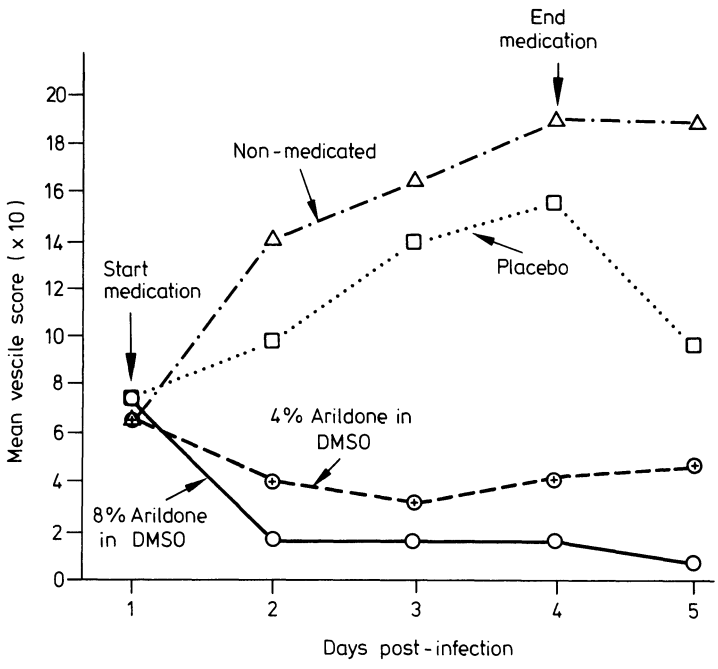

Fig. 3. The effect of 8% and 4% arildone in 90% DMSO on the development of herpetic vesicles in guinea pigs. Animals were infected intradermally with HSV-1. Medication was applied topically five times daily for 4 days. Scoring was as follows: 0 = no vesicles, no erythema; 0.5 = 1–4 small vesicles, barely raised; 1.0 = 1–4 raised vesicles, slight erythema; 1.5 = 1–4 large vesicles, pronounced erythema; 2.0 = 4–10 large vesicles, edematous tissue; 2.5 = > 10 large vesicles, partly coalescent, edematous tissue; 3.0 = coalescent vesicles, edematous tissue

crusting began in all arildone-treated sites, while in placebo DMSO-treated animals, drying of the skin was observed around existing vesicles, but not on the vesicles themselves. In those animals as well as in untreated ones, vesicles were moist and continued to increase in size. New vesicles were still in the process of forming in the placebo-treated animals. On the fourth and fifth days, the infection sites in the arildone-treated animals' skin was dry, smooth, slightly thickened, and showed no evidence of active infection. In placebo-treated animals, some crusting was observed at that time; however, each vesicle site was marked with a scab which, upon removal, revealed serous fluid under the crust.

Statistical analysis showed a significant difference between lesion scores of animals treated with 4% or 8% arildone and those of placebo-treated controls after 24 h treatment. The healing and drying of vesicles was observed after 24 h treatment in both groups treated with 4% and 8% arildone. The 8% arildone treated group showed a trend in the score that was significantly different from the placebo-treated group ($P=0.01$); the drug treatment results showed a decrease of score whereas the placebo score increased over time. Similar results were obtained with 4% arildone but to a lesser degree. Both drug concentrations showed overall mean scores that were significantly different from the placebo-treated group ($P=0.01$). There was no significant difference between 4% and 8% arildone in terms of

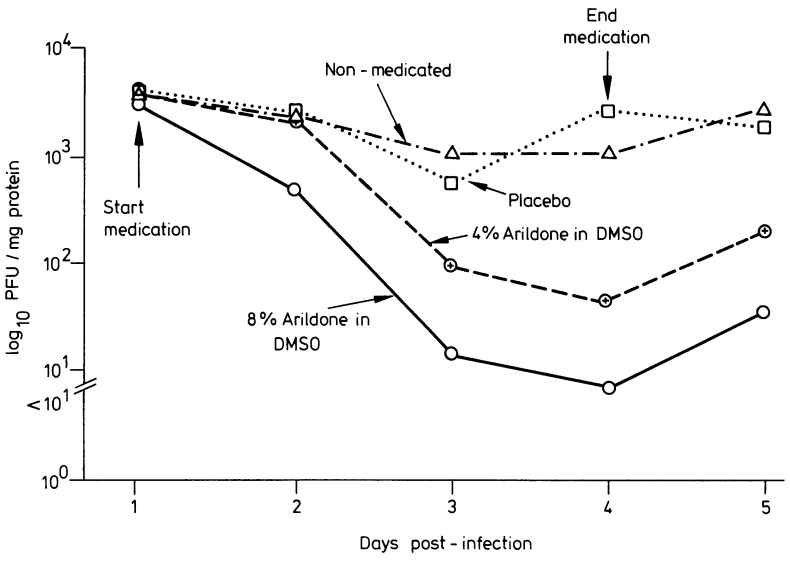

Fig. 4. The effect of 8% and 4% arildone in 90% DMSO on the virus content of herpetic lesions in guinea pigs. Animals were infected and medication applied as described in Fig. 3. The infected site was scraped into balanced salt solution with a sterile scalpel, virus was released by sonication, and large debris was removed by low speed centrifugation. The virus in the supernatant was quantitated by plaque formation and protein analysis

trends; however, the means were significantly different ($P=0.01$). There was a significant difference between the nonmedicated and placebo-treated groups in terms of trend over time ($P=0.01$), the latter having a flatter curve than the former. The overall means were comparable.

b) Effect on Virus Growth

For the purpose of determining virus titers in the lesion sites, guinea pigs were infected intradermally with HSV-1 and, from day 1 (24 h postinfection) through day 5, animals were killed daily. Virus was recovered from the skin by scraping the site with a sterile disposable scalpel and the amount of infectious virus was determined by plaque assay. Results showed that 4% and 8% arildone in 90% DMSO, applied to the skin of guinea pigs infected with HSV-1 starting 24 h postinfection, reduced the virus content in the lesions compared with virus content in placebo-treated and infected nonmedicated animals (Fig. 4); 4% and 8% arildone showed linear trends with time that were significantly different from placebo ($P=0.01$). Based on comparison between treatment means within a time period, the time–response curves diverged starting on day 3 (Fig. 4). There was no significant difference in trend between 4% and 8% arildone; however, within the time period comparison suggested a difference between the two starting on day 3 ($P=0.05$). There was no significant difference between the nonmedicated and placebo-treated groups, either in terms of trends with time or comparisons between treatment means within a time period.

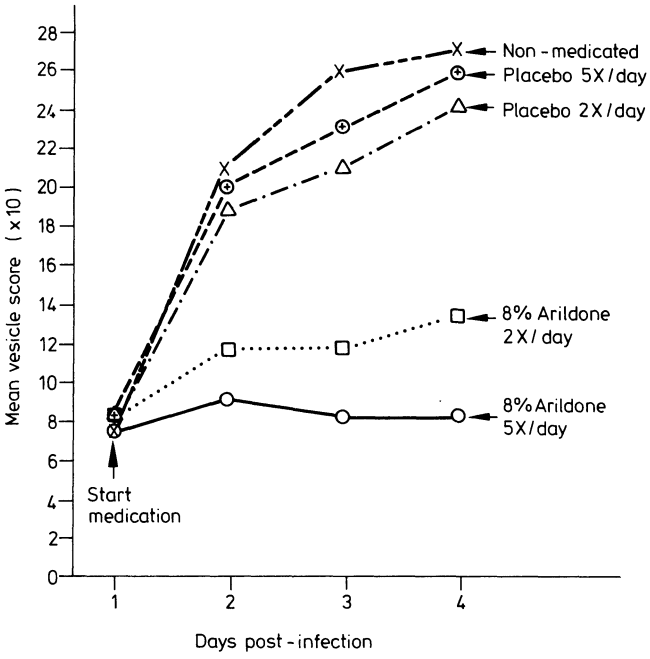

Fig. 5. The effect of 8% arildone in a cream base on the development of herpetic vesicles in guinea pigs. Animals were infected and medicated, and the results were scored as described in the legend for Fig. 3. Medication was applied either twice or five times daily for 4 days

2. Arildone in Cream

a) Effect on the Development of Lesions

The effect of the 8% arildone in cream preparation was also studied using the guinea pig skin infection model with HSV-1; 8% arildone in cream was effective in controlling the development of herpetic lesions in which the drug was applied either twice or five times daily, with the latter showing an overall greater effect (Fig. 5). The therapeutic effect was not as rapid and marked as that produced by arildone prepared in DMSO. The clinical process of the infection was arrested after 24 or 48 h therapy. The size of the herpetic vesicles remained constant and new ones failed to develop. The difference in the appearance of vesicles in arildone-treated and placebo-treated or untreated animals was even more evident on the third and fourth days of therapy when progressively larger and coalescing vesicles were observed in placebo-treated and untreated animals, while the vesicles in arildone-treated sites became smaller and crust formation began.

Statistical analysis of data showed a significant difference ($P=0.01$) between lesion scores of animals treated five times daily with arildone and those treated five times daily with placebo cream on days 2, 3, and 4 postinfection. The difference between lesion scores in animals treated twice daily with arildone compared with those treated twice daily with placebo was also statistically significant ($P=0.01$) on days 2, 3, and 4 postinfection. However, the scores were consistently lower for the group treated five times daily on all days compared with the group treated twice daily. There were no significant differences between the groups treated with placebo twice of five times daily and the nonmediated groups.

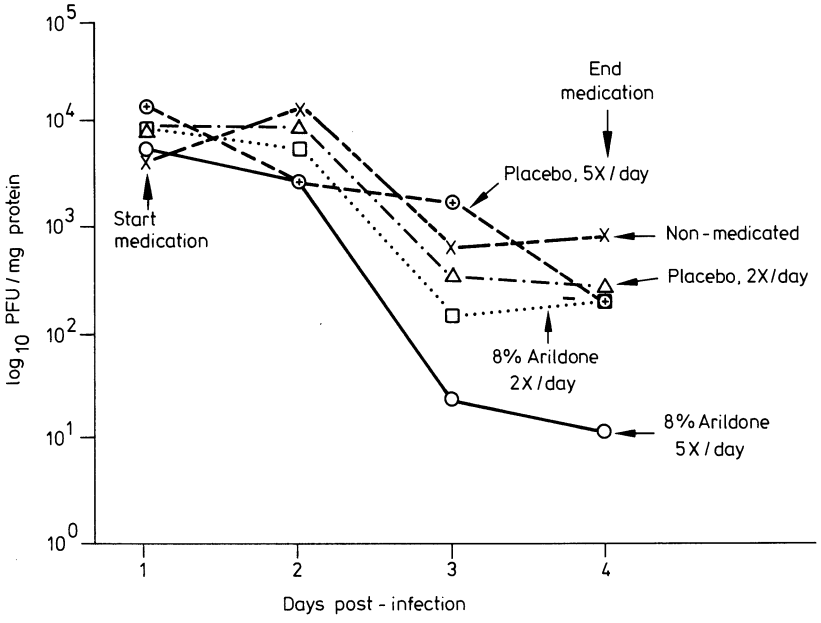

Fig. 6. The effect of 8% arildone in cream base on the virus content of herpetic lesions in guinea pigs. Animals were infected and virus was assayed as described in Fig. 4. Medication was applied to the infected area either twice or five times daily for 4 days

b) Effect on Virus Growth

Comparison of the effect of 8% arildone in cream on the virus growth in skin lesion after treatments twice or five times daily showed that virus titers were more significantly effected in animals treated five times daily with arildone, than in those treated only twice daily (Fig. 6). Arildone (8%) applied five times daily to the skin had a greater effect on the virus titer than when the compound was applied only twice daily. The statistically significant difference between virus titers in the skin scrapings of animals treated five times daily and corresponding placebo-treated animals was $P=0.01$ on days 3 and 4 postinfection. The virus titers in groups treated twice daily with arildone were not statistically different from the corresponding placebo-treated groups. There were no significant differences between either of the two placebo-treated groups (those treated twice or five times) daily and the nonmedicated animals. Similar results were obtained when other strains of HSV-1 and HSV-2 were used and 8% arildone applied topically either in DMSO or in a cream preparation. These results indicate that topical application of arildone may be useful in the treatment of cutaneous herpetic infection in humans.

D. Mode of Action

I. Herpes Simplex Virus

In a preliminary report, it was shown that 8.1 μM arildone prevented HSV-2 replication and the synthesis of HSV-2-specific DNA and proteins (Kuhrt et al.

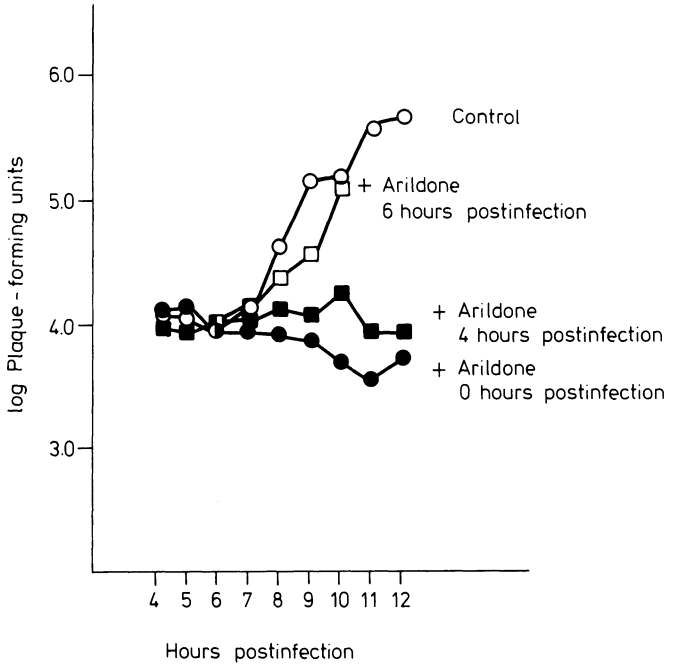

Fig. 7. Effect of adding arildone at various times after infection. BSC_1 cell monolayers were infected with HSV-2 at m.o.i. ~ 1. After the adsorption period, virus growth medium was added. At various times postinfection arildone (8.1 μ*M*) was added to the growth medium. The yield of virus 24 h postinfection was determined by plaque assay. (KUHRT et al. 1979)

1979). Addition of the drug during the first 6 h postinfection at low m.o.i. reduced the yield of virus, whereas addition of the drug after 6 h postinfection did not reduce virus yield (Fig. 7). These results showed that at low m.o.i. (1.0 pfu/cell), the sensitive step in HSV-2 replication was between 0 and 6 h postinfection. If arildone was removed from HSV-2-infected cells between 0 and 6 h postinfection, the yield of virus was not reduced, suggesting that the antiviral effect of arildone was reversible.

Arildone inhibited HSV replication by an unknown process, but it seemed to inhibit an early event since addition of the drug after 6 h postinfection did not change the course of the infection. One early event is the uncoating of HSV in the cytoplasm which occurs over a period of time and may not be complete until the virus enters the nucleus. The preliminary data on the arildone-sensitive stage in herpesvirus-infected cells are consistent with the idea that arildone prevents herpesvirus replication by inhibiting the uncoating of HSV in the cytoplasm of infected cells. Further studies with herpesviruses will be required to determine if arildone inhibits HSV uncoating. If arildone blocks uncoating during HSV infections, as it does in poliovirus infections (Sect. D.II), it will be very suggestive that the mechanism of action is the same for other viruses.

Table 3. Effect of arildone on early events in poliovirus replication[a]

Addition of compound (2.7 μ*M*)		PFU (% of control)
Adsorption at 0 °C	Incubation at 37 °C	
−	−	100
+	+	0
+	−	97
−	+	22

[a] HeLa cell monolayers were inoculated with poliovirus in the presence (+) or absence (−) of arildone (2.7 μ*M*) at 0 °C for 1 h. The inoculum was removed, the monolayers washed twice with ice-cold Eagle's MEM and the cells were incubated in the presence (+) or absence (−) of arildone at 37 °C for 1 h. The medium was aspirated and replaced with an agar overlay without arildone. Plaques were counted 48 h after incubation at 37 °C under an atmosphere of 5% CO_2. (McSharry et al. 1979)

Table 4. Effect of arildone on penetration of poliovirus[a]

Treatment	Infective centers per plate[b]	
	Untreated	Arildone (2.7 μ*M*)
End adsorption at 4 °C	4.6×10^4	5.1×10^4
Antibody added after incubation at 37 °C for:		
0 min	4.0×10^3	1.6×10^3
30 min	5.8×10^4	5.0×10^4
60 min	5.7×10^4	4.2×10^4

[a] Poliovirus was adsorbed by cells for 1 h at 4 °C in the presence or absence of 2.7 μ*M* arildone, and one set of cultures was assayed for infective centers without exposure to poliovirus-specific antibody. Other sets of cultures were exposed to poliovirus-specific antibody after additional incubation at 37 °C for various periods. (McSharry et al. 1979)

[b] Penetration was measured as infective centers no longer sensitive to poliovirus 2 antibody. Arildone was present in the treated cultures from the time of inoculation at 4 °C until the end of antibody treatment (30 min at 20 °C)

II. Poliovirus

The mode of action of arildone against poliovirus has recently been elucidated. Arildone inhibited poliovirus replication by preventing intracellular uncoating of the virion (McSharry et al. 1979). More recent studies suggested that arildone prevented intracellular uncoating of poliovirus by stabilizing the capsid proteins in such a way that these proteins cannot undergo the conformational changes required for uncoating and release of virion RNA (Caliguiri et al. 1980).

Table 5. Effects of arildone on photoinactivation of neutral red poliovirus[a]

Time (h)	Irradiation	Infectious centers	
		No arildone	Arildone (2.7 μ*M*)
0	−	2.8×10^4	1.6×10^4
	+	$<0.3 \times 10^0$	$<0.3 \times 10^0$
3	−	2.8×10^4	2.5×10^4
	+	2.0×10^4	1.3×10^1

[a] Virus grown in the presence of neutral red is photosensitive as long as the dye remains within the viral capsid. Neutral red poliovirus was adsorbed by GMK cell monolayers in the dark at 0 °C in the presence or absence of arildone (2.7 μ*M*). One set of cultures was irridated at the end of adsorption at 0 °C, or 3 h after transfer of cultures to 37 °C. The cells were detached from the monolayer, diluted and assayed for infective centers. (McSharry et al. 1979)

Previous experiments with plaque reduction assays suggested that arildone inhibited an early event in poliovirus replication, possibly adsorption, penetration, or uncoating. Arildone did not inhibit adsorption to or penetration of poliovirus into HeLa cells (McSharry et al. 1979). The data are presented in Table 3. When the drug was present only during incubation at 0 °C, poliovirus adsorbed to HeLa cells and formed plaques. When the drug was present during incubation at 0° and 37 °C or only during incubation at 37 °C, the number of plaques was reduced by 100% and 78%, respectively. The results of this experiment showed that arildone blocked an event which occured at 37 °C, after the adsorption period. The data (Table 4) showed that arildone did not prevent penetration of poliovirus into African green monkey kidney (GMK) cells after incubation at 37 °C for 30 or 60 min. In this experiment, poliovirus was adsorbed to GMK cells at 4 °C in the presence or absence of arildone. After the adsorption period, the plates were incubated at 37 °C and poliovirus-specific antibody was added at various times. Arildone did not prevent adsorption at 4 °C. Antibody did not reduce infectivity when added (immediately) after incubation at 37 °C, indicating that virus was still at the cell surface and susceptible to neutralization by antibody. After 30 and 60 min at 37 °C, antibody did not neutralize virus, indicating that the virus had penetrated the cell. The results of this experiment showed that arildone did not inhibit penetration of poliovirus into GMK cells. Arildone did prevent uncoating of poliovirus in GMK cells (McSharry et al. 1979). The data showed that, in the absence of arildone, virus was not sensitive to photoinactivation, indicating that it had undergone intracellular uncoating (Table 5).

The results of these experiments showed that arildone inhibited poliovirus replication by blocking uncoating of the virus after it had entered the cell and it was postulated that arildone inhibited the intracellular uncoating of poliovirus by a direct interaction of the drug with the viral capsid proteins. The isolation of drug-resistant mutants of poliovirus also suggested a direct interaction between arildone and the viral capsid proteins (L. A. Caliguiri, J. A. Laffin, M. Schrom, and

Table 6. Effect of arildone on thermal inactivation of poliovirus

Time (min)[a]	Poliovirus titer (pfu/ml)		
	Eagle's MEM	Eagle's MEM +0.01% DMSO	Eagle's MEM +2.7 μ*M* arildone +0.01% DMSO
0	3.4×10^9	4.3×10^9	4.0×10^9
2.5	2.5×10^9	2.3×10^9	4.5×10^9
5	2.2×10^8	2.4×10^8	2.3×10^9
10	2.3×10^7	6.1×10^7	2.5×10^9
20	1.3×10^6	3.4×10^6	2.2×10^9

[a] Time of incubation of poliovirus 2 at 47 °C. (Caliguiri et al. 1980)

Table 7. Effect of arildone on alkaline inactivation of poliovirus

Time (min)[a]	Poliovirus titer (pfu/ml)			
	Eagle's MEM	pH 10.5	0.01% DMSO pH 10.5	2.7 μ*M* arildone pH 10.5
0	9.2×10^7	7.3×10^7	9.1×10^7	8.5×10^7
15	3.0×10^7	2.7×10^7	3.0×10^7	6.4×10^7
30	8.4×10^6	1.4×10^7	1.1×10^7	8.1×10^7
60	8.1×10^6	5.1×10^4	1.6×10^4	5.4×10^7

[a] Time of incubation of poliovirus 2 at 40 °C at pH 10.5. (Caliguiri et al. 1980)

J. J. McSharry, unpublished work 1980; H. J. Eggers, personal communication 1979). To test this hypothesis, the effect of arildone on the thermal and alkaline degradation of poliovirus in vitro was studied (Caliguiri et al. 1980).

The results of these experiments showed that arildone prevented the loss of infectivity and the change in sedimentation due to heating poliovirus at 47 °C for various periods of time (Table 6, Fig. 8) and the loss of infectivity of poliovirus incubated at 40 °C at pH 10.5 (Table 7). The results presented in Fig. 9 show that arildone prevented the change in sedimentation profile of poliovirus incubated at 40 °C at pH 10.5 up to 20 min. However, after 60 min incubation under these conditions, arildone failed to protect poliovirus from changes in sedimentation rate. The fact that arildone protected poliovirus infectivity for 60 min incubation at 40 °C at pH 10.5, but did not completely stabilize the virus particle, suggests that heat and alkali inactivation of poliovirus occur via different mechanisms.

The results of these inactivation studies on the poliovirus showed that arildone stabilized poliovirus in the presence of heat and alkali. These results are in agreement with the previous studies, which showed that arildone stabilized the viral capsid in such a way that it can not uncoat after penetrating HeLa or GMK cells. Thus, the mode of action of arildone against poliovirus replication is to prevent uncoating by stabilizing the virion capsid proteins in such a manner as to prevent the conformational changes required for uncoating and release of viral RNA in the cytoplasm of host cells.

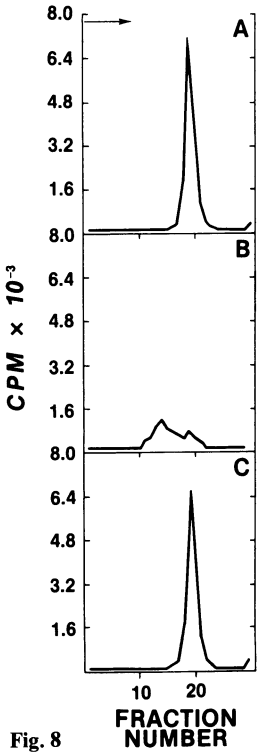

Fig. 8

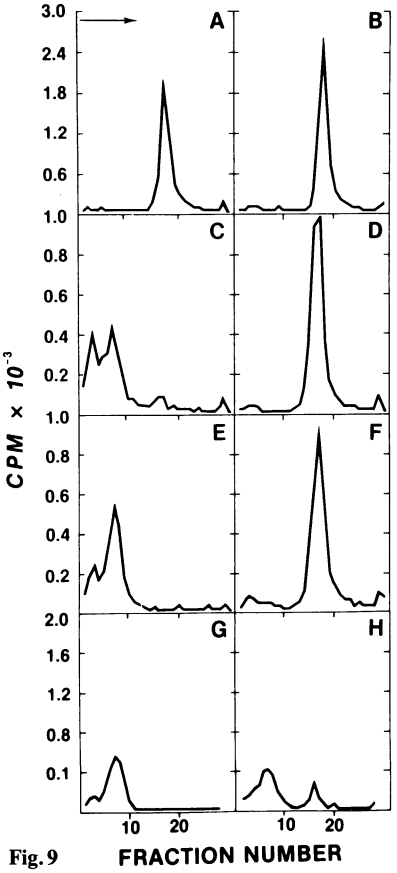

Fig. 9

Fig. 8 a–c. Sedimentation of poliovirus in sucrose gradients. Purified methionine ^{35}S labeled poliovirus 2 was sedimented in preformed linear 15%–30% sucrose reticulocyte standard buffer (w/w) gradients: **a** native, unheated poliovirus, **b** poliovirus incubated at 47 °C for 20 min in Eagle's MEM; **c** poliovirus incubated at 47 °C for 20 min in Eagle's MEM containing 2.7 μ*M* arildone and 0.01% DMSO. The arrow indicates the direction of sedimentation. (CALIGUIRI et al. 1980)

Fig. 9 a–h. Sedimentation of poliovirus in sucrose gradients after incubation at alkaline pH. Purified, methionine ^{35}S labeled poliovirus 2 was sedimented in preformed, linear 15%–30% sucrose reticulocyte standard buffer (w/w) gradients. Poliovirus incubated in glycine buffer, pH 10.5 at 40 °C for: **a** 5 min; **c** 10 min; **e** 20 min; and **g** 60 min. Poliovirus incubated in glycine buffer, pH 10.5 containing 2.7 μ*M* arildone at 40 °C for: **b** 5 min; **d** 10 min; **f** 20 min; and **h** 60 min. The *arrow* indicates the direction of sedimentation. (CALIGUIRI et al. 1980)

E. Metabolism

The following studies were performed at the Sterling-Winthrop Research Institute and the data, while not published, are on file. Because of space limitations, only a small portion of the studies are presented with accompanying data. Similar results were obtained from studies in other systems.

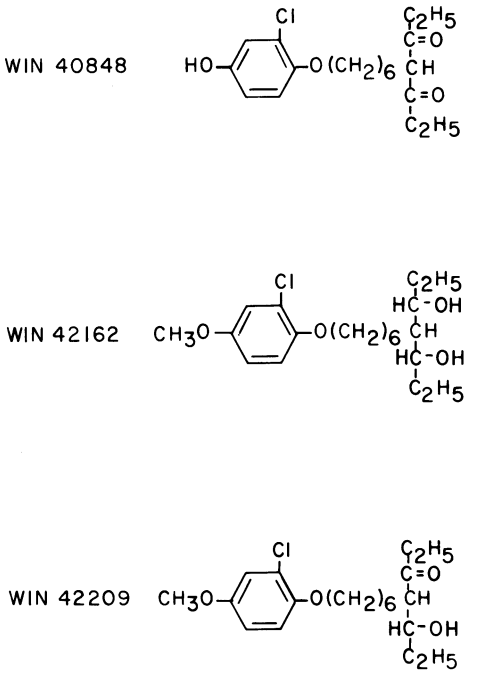

Fig. 10. Structures of some of the in vivo and in vitro metabolites of arildone

I. In Vitro Studies

1. Metabolism by CATR Cells

The metabolism of arildone ^3H was studied in normal human amnion (CATR) cells in tissue culture. CATR cells were incubated in medium containing 8.1 μM arildone ^3H for 24 h. After incubation, the media were pooled and sequentially extracted. The cells remaining affixed to the inside of the bottles were also extracted. Aliquots of each extract were spotted on thin layer chromatography (TLC) plates, along with arildone as a reference and the plates were developed. Large quantities of metabolites designated WIN 42162 and WIN 42209 were detected as well as a compound which matches the R_F of arildone. Their structures are shown in Fig. 10. In the extract of the medium, only metabolite WIN 42209 and the suspected arildone were present in significant amounts. In the extracts of the cells, metabolites WIN 42162, WIN 42209, and arildone were detected.

Mass spectral analysis of metabolites WIN 42162 and WIN 42209 identified these compounds as 4 [6-(2-chloro-4-methoxy)phenoxyl]hexyl 3,5-heptanediol and 4- [6-(2-chloro-4-methoxy)phenoxyl]hexyl -5-hydroxy-3-heptanone, respectively. Metabolite WIN 42162 was synthesized and chromatographed by TLC along with a CATR extract. A radioscan of the TLC plate showed metabolite WIN 42162 to have an identical R_F with the synthesized material. WIN 40848 (Sect. E.II.2) and WIN 42209 were active in vitro against HSV-1 and HSV-2. These two compounds inhibited virus-induced CPE at concentrations of 32.4 μM while WIN 42162 was inactive.

Table 8. Disposition of radioactivity following oral administration[a] of arildone ^{14}C

Specimen	Killing time					
	0.75 h			24 h		
	dpm/g[b]	(μg/g)	(% dose)	dpm/g[b]	(μg/g)	(% dose)
GI tract	1910	550	88.2	572	170	35.5
Liver	129	37	3.94	28.7	8.3	1.39
Adrenal glands	37.1	11	< 0.01	16.2	4.7	< 0.01
Spleen	35.7	10	0.05	14.0	4.1	0.03
Kidneys	29.7	8.7	0.16	18.0	5.3	0.12
Heart	16.7	4.7	0.03	8.67	2.3	0.02
Lungs	12.3	3.5	0.07	6.67	1.9	0.04
Carcass	9.53	2.8	4.41	26.0	7.5	33.4
Muscle	3.60	1.0		2.69	0.8	
Testes	3.33	1.0	0.02	18.0	5.2	0.09
Brain	3.33	1.0	0.02	0.57	0.6	< 0.01
Urine			1.83			25.6
Feces			0			3.17
Total recovery			98.7			99.4

[a] DMSO solution, 50 mg/kg; three animals
[b] dpm in thousands

II. In Vivo Studies

1. Disposition in Laboratory Animals

The adsorption and disposition of arildone ^{14}C were studied in rats after oral administration. Rats were given an oral dose of arildone ^{14}C in DMSO (50 mg/kg). The animals were killed at 0.75 or 24 h postmedication and disposition of radioactivity in various tissues, feces, and urine was determined. The results are presented in Table 8. Radioactivity in the gastrointestinal tract decreased from 88.2% of the dose at 0.75 h to 35.5% at 24 h. Liver radioactivity was equivalent to 37 and 8.3 μg/g of arildone at 0.75 and 24 h, respectively. Other tissues with high levels of arildone radioactivity at 24 h were kidney (5.3 μg/g), testes (5.2 μg/g), and carcass (7.5 μg/g). At 24 h, 25.6% of the dose was excreted in the urine, whereas only 3.2% appeared in the feces. The major repository for radioactivity at 24 h was the carcass which contained 33% of the dose.

The disposition of arildone after intravenous inoculation was also determined. Rats were inoculated intravenously with arildone ^{14}C (5 mg/kg) in DMSO. Blood samples were collected at various intervals after inoculation and radioactivity determined. The results are presented in Fig. 11. After intravenous administration of arildone ^{14}C, a biphasic decline in radioactivity was observed. The graphically determined half-lives were about 0.5 (α) and 5.5 h (β). The mean equivalent blood concentrations of arildone were 2.4 μg/ml at 3 min and 0.01 μg/ml at 24 h. The data, presented in Table 9, showed that, at 72 h postmedication, feces contained 57% of dose, urine 34.5%, gastrointestinal tract 2.7%, and remainder of animal

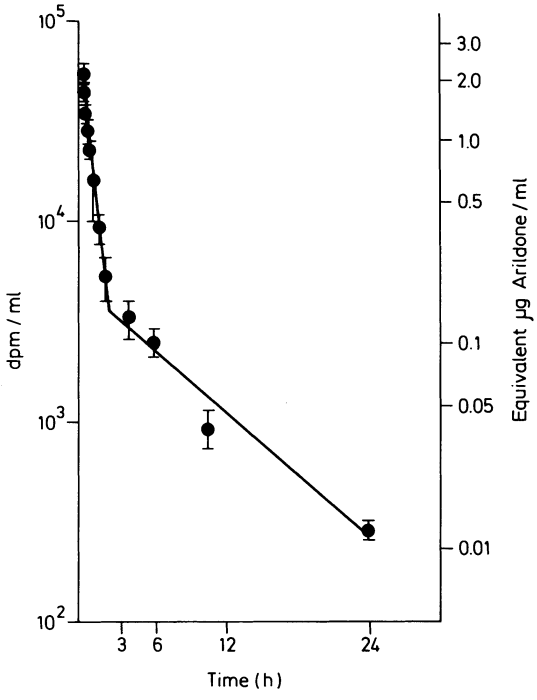

Fig. 11. Blood radioactivity after intravenous administration of arildone ^{14}C to rats. Arildone (5 mg/kg) in DMSO was injected into the external jugular vein of four rats. At various times postmedication, blood was withdrawn from the tail vein and radioactivity was determined

Table 9. Disposition of radioactivity following intravenous administration[a] of arildone ^{14}C

Specimen	% dose				
	Animal[b]				
	1	2	3	4	Mean ± standard error
Feces	51.0	69.4	49.2	58.5	57.0 ± 4.6
Urine	38.1	33.9	31.1	34.9	34.5 ± 1.4
GI tract	3.3	2.9	2.9	1.6	2.7 ± 0.4
Body[c]	1.5	1.7	2.4	1.4	1.8 ± 0.2
Total recovery	93.9	108	85.6	96.4	96.0 ± 4.6

[a] DMSO solution, 5 mg/kg
[b] Animals killed 72 h postmedication
[c] All tissues except GI tract

1.8%. Thus, after intravenous administration, arildone was rapidly removed from the blood and excreted in the feces and urine.

Similar studies of the disposition of radioactively labeled arildone administered to mice, rats, rabbits, and monkeys by various routes including intravenous inoculation in 70% ethanol, oral administration in gum tragacanth, or topical applica-

Table 10. Identification of fecal radioactivity following oral administration[a] of arildone ^{14}C

Fraction	% dose			
	Animal[b]			
	A	B	C	Mean ± standard error
Arildone	51.6	53.0	59.3	54.6 ± 2.4
WIN 40848	7.8	10.9	11.1	9.9 ± 1.1
Unidentified	10.0	15.4	11.6	12.3 ± 1.6
Nonextractable	27.8	4.2	4.3	12.1 ± 7.8
Total radioactivity	97.2	83.5	86.3	88.9 ± 4.0

[a] Gum tragacanth, 50 mg/kg
[b] Fecal samples, 0–24 h

tion in 8% cream to skin or vagina showed that arildone rapidly entered the various compartments of the body and was removed from the body quickly. Between 50% and 70% of the radioactivity was excreted in the feces and 20%–30% was excreted in the urine.

2. Metabolism in Laboratory Animals

Metabolic derivatives of arildone were isolated from the feces of rats fed arildone ^{14}C in gum tragacanth. Feces collected 10–24 h postmedication were extracted with hexane and then with ethyl acetate and the organic phases analyzed by thin layer chromatography. The data showed that approximately 55% of the radioactivity was recovered in the feces as arildone (Table 10). The desmethyl metabolite, WIN 40848 (Fig. 10), represented 10% of the dose. An unidentified polar component constituted 12% of the dose. The balance of the fecal radioactivity was not extractable with either hexane or ethyl acetate.

Mice, rats, and dogs were injected intravenously with arildone ^{14}C in DMSO. Blood was collected, the plasma separated and extracted with acetone and methanol. Urine from the dogs was assayed directly. The samples were analyzed for arildone and its various metabolites by high pressure liquid chromatography (HPLC). Arildone rapidly disappeared from the plasma of dog, mouse, and rat after intravenous administration of arildone ^{14}C. The rapid metabolism of arildone in the dogs was evidenced by the rapid decrease in the plasma concentrations of intact drug and the concomitant increase in the plasma concentration of two polar metabolites, chlorohydroquinone sulfate and 2-chloro-4-methoxyphenyl sulfate. Arildone had a graphically determined (from 5 to 60 min) half-life of approximately 20 min in the dog. The same pattern of metabolism was evident in the rat and mouse, although the mouse plasma contains an additional radioactive peak. All species showed evidence of WIN 40848 (Fig. 10); however the concentrations of this desmethyl metabolite were, in general, significantly lower than arildone. In the two dogs, the plasma concentrations of free chloromethoxyphenol were significantly lower than those of the conjugated chloromethoxyphenyl sulfate. However,

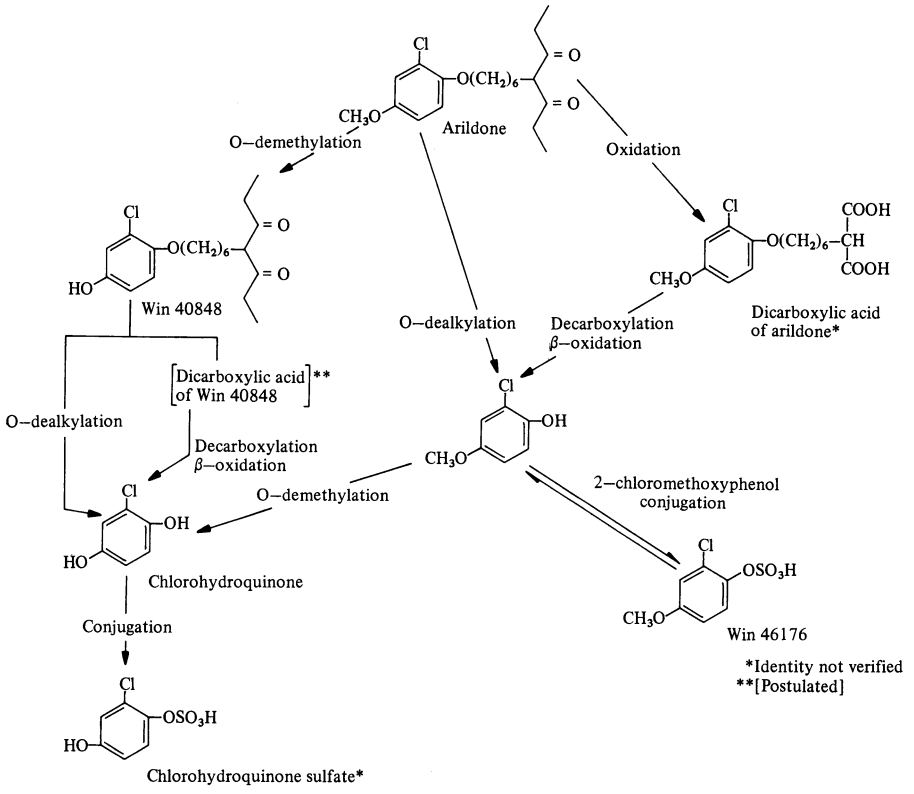

Fig. 12. Proposed pathway for arildone metabolism. * Identity not verified; ** postulated

in the rat and mouse, the concentrations of the free and conjugated chloromethoxyphenol were similar. The dog urine samples analyzed by HPLC had high concentrations of 2-chloro-4-methoxyphenyl sulfate (11.9%–12.2% of the dose), chloromethoxyphenol (4.4%–5.9% of the dose) and chlorohydroquinone sulfate (3.5%–8.1%) of the dose). Only trace amounts of arildone and WIN 40848 were present.

The proposed pathways for arildone metabolism are shown in Fig. 12. There are two possible metabolic routes that would yield phenolic end products. First, the entire side chain could be removed intact by O-dealkylation. Second, the branched chain could be oxidized to the corresponding dicarboxylic acid. This malonate type derivative could then undergo decarboxylation (as does malonic acid); the remaining chain could then be degraded by β-oxidation like a fatty acid. In either case, the free phenols are then conjugated and excreted.

F. Toxicology

Toxicologic studies necessary to assure the tolerance and safety of arildone in humans were performed at the Sterling-Winthrop Research Institute and are summarized here to give the reader an overview of the benign effects of arildone and some of its metabolites in various in vivo and in vitro model systems.

Table 11. Effect of arildone on HeLa cell macromolecular synthesis

Arildone (μ*M*)	Activity ($10^3 \times$ cpm)[a]					
	Leucine ^{3}H		Thymidine ^{3}H		Uridine ^{3}H	
	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble
0	2.0 (100)	7.2 (100)	6.2 (100)	40.3 (100)	48.0 (100)	119.3 (100)
2.7	2.1 (105)	7.2 (100)	4.9 (82)	32.3 (80)	54.0 (113)	101.4 (85)
27.0	2.1 (105)	6.8 (94)	5.2 (87)	26.5 (66)	33.0 (69)	70.9 (59)

[a] Average of four determinations; numbers in parentheses give the percentage of untreated control sample

I. In Vitro Studies

1. Mutagenic Evaluation of Arildone

Arildone was evaluated for mutagenic activity in the presence and absence of microsomal activation enzymes by the microbial assays developed by AMES et al. (1975) and at concentrations between 0.05 μg/plate and 2,000 μg/plate, arildone was not mutagenic. Arildone was not mutagenic in a mouse lymphoma assay, did not induce genetic damage in germ cells of rats fed up to 250 μg kg^{-1} day^{-1} for 5 days, nor did it cause any chromosomal abnormalities in bone marrow cells when rats were fed up to 250 μg kg^{-1} day^{-1}. The results of these tests showed that arildone was not mutagenic in vitro and did not cause chromosomal abnormalities or measurable genetic defects in vivo.

2. Effect of Arildone on Cellular Macromolecular Synthesis

A number of permanent cell lines were treated with various concentrations of arildone; 24 h after the addition of arildone to the cell monolayers, the cells were labeled with uridine ^{3}H, thymidine ^{3}H, or leucine ^{3}H to label RNA, DNA, or protein, respectively. At the end of the 30-min pulse, the medium was removed, the monolayers were washed thoroughly in cold PBS, extracted with 5% TCA, and TCA-insoluble material was solubilized with 0.1 *M* NaOH. TCA-soluble and TCA-insoluble radioactivity was measured by liquid scintillation counting in Biofluor (New England Nuclear Corporation). The results, presented in Tables 11 and 12 showed that 2.7 μ*M* arildone did not inhibit the transport of leucine ^{3}H or uridine ^{3}H into HeLa cells, whereas the transport of thymidine ^{3}H was inhibited by 18%. At the same concentration, the incorporation of leucine ^{3}H into protein was not affected, whereas the incorporation of thymidine ^{3}H into DNA was inhibited by approximately 20% and that of uridine ^{3}H was inhibited by 15%. At 1.4 μ*M*, arildone had little effect on the transport of precursors into acid-soluble

Table 12. Effect of arildone on BSC_1 cell macromolecular synthesis

Arildone (μM)	Activity ($10^3 \times$ cpm)[a]					
	Leucine ^3H		Thymidine ^3H		Uridine ^3H	
	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble
0	0.76 (100)	3.4 (100)	2.43 (100)	12.40 (100)	67.28 (100)	64.14 (100)
1.4	0.73 (96)	3.2 (94)	2.44 (100)	12.25 (99)	61.18 (91)	54.33 (85)
14.0	0.66 (87)	2.8 (82)	1.81 (74)	10.13 (82)	34.20 (51)	32.08 (50)

[a] Average of four determinations; numbers in parentheses give the percentage of untreated control sample

pools of BSC_1 cells (monkey kidney cell cultures) and only a slight effect on the incorporation of one of the precursors (uridine ^3H) into acid-insoluble material. Similar results have been obtained with MDBK, BHK21-F, CV1, and HKCC cells. The results of these experiments showed that arildone, at concentrations which were near or greater than the MIC of HSV and poliovirus (Sects. C.I.2a, C.I.2b), has only minimal inhibitory effects on cellular macromolecular synthesis.

3. Effect of Arildone and Its Metabolites on Cell Growth

a) Cell Culture Studies

The cytotoxicity and tolerance of arildone and its in vivo and in vitro metabolites WIN 42162, WIN 40848, and WIN 42209 (Sects. E.I, E.II) were studied in monkey kidney cell cultures (BSC_1). Cultivation of cells for 4 days in the presence of 5.4–21.6 μM arildone or its metabolites had no effect on the viability of BSC_1 cells. Higher concentrations of arildone or its metabolites inhibited growth. Cells treated with arildone (5.4–21.6 μM) could be passed in the presence of identical concentrations of the drug with no significant loss of viability. Arildone (32.4 μM) inhibited the viability of cells passed in its presence. Similar results were obtained after growth of primary rabbit kidney cells in arildone or its metabolic products. These results showed that arildone, at concentrations near or greater than the MIC for poliovirus and herpesviruses, did not inhibit cell growth.

b) Organ Culture Studies

Arildone was evaluated for cytotoxicity on ciliary cells of ferret and rhesus monkey trachea organ cultures. Cell viability was measured by observation of ciliary movement and vital dye staining. The maximum nontoxic concentration of arildone was 270–540 μM in ferret trachea organ culture and 540 μM in rhesus monkey trachea organ culture, indicating that arildone was 10–20 times less cytotoxic for tracheal organ cultures than for monolayer cultures of BSC_1 cells.

II. In Vivo Studies

Arildone was tested for acute oral toxicity in mice and rats. Studies were performed in young adult male mice and rats with suspensions of arildone in 1% gum tragacanth. The 7-day LD_{50} was > 8,000 mg/kg in both species. There were no deaths or overt symptoms at 2,000, 4,000, or 8,000 mg/kg. Arildone (8%) cream was studied in young adult male albino mice and rats. The arildone cream was administered by intubation. The 7-day LD_{50} was > 40 ml/kg in both species. There were no deaths in mice given 40 ml/kg and only two (of ten) deaths at that dose level in the rats.

Arildone (8%) cream was also studied topically on abraded and nonabraded sites on the backs of New Zealand White rabbits. No overt or adverse effects on the skin or the healing time, attributable to medication, were observed. No adverse effect on body weight occurred, and the results of hematologic studies and blood analyses were normal at the end of the 3-week treatment period. Gross and microscopic tissue evaluation of other organs revealed no drug-related changes in any of the medicated rabbits, and organ weights of liver, kidneys, and adrenal glands were normal. Plasma levels of arildone were determined on days 2, 9, and 22, about 2 h after the second daily application. There was no evidence of accumulation of the drug over the 3-week period.

In additional studies, arildone (8%) cream was studied for tolerance by intravaginal instillation in rabbits and dogs, three times a day over a 3-week period. No systemic or local adverse effects were observed when responses were compared with those seen in rabbits or dogs similarly medicated with the vehicle cream or in a control group (treated with water). No gross or microscopic tissue changes attributable to arildone medication were observed. Plasma levels of arildone determined on or about days 2, 8, and 21 were below detectable limits (25 ng/ml) in most samples. There was no evidence of drug accumulation over the 3-week period. Gross and microscopic examination of the external genitalia and vaginal tracts did not indicate any changes attributable to medication with arildone cream or its vehicle.

To assess the systemic toxicity, arildone was administered orally to rats and monkeys. Drug was administered to male and female albino rats in single daily doses of 40, 200, or 1,000 mg/kg for 1 month. The compound was very well tolerated at all dose levels. Growth, food consumption, and the results of hematologic studies and blood and urine analyses were normal. No gross or microscopic tissue changes were observed. Teratogenic potential of arildone was studied in rats and rabbits. Arildone was administered orally to pregnant albino rats in single daily doses of 200, 500, or 1,200 mg/kg from the 6th through the 15th day of gestation. No drug-induced gross skeletal or visceral teratogenic effects were observed at any dose level. There was no effect on litter size, number of resorptions, fetal weight, sex ratio, or pre- and postimplantation loss. Pregnancy rate, number of implantations, and number of corpora lutea were also normal.

The results of these toxicity studies showed that arildone and its metabolic derivatives are well tolerated in cellular and animal experimental models. From these results it has been suggested that arildone should be well tolerated in humans and hence a promising antiviral agent for the control of human viral diseases.

G. Summary and Perspectives

In vitro studies have shown that arildone inhibits cytopathic effect of a number of RNA and DNA viruses at concentrations ranging from 0.8 to 16.2 μ*M*. Plaque formation of 15 strains of HSV-1 and HSV-2 was inhibited in tissue culture with less than 5.4 μ*M* arildone. In addition, arildone at concentrations of 2.7 μ*M* inhibits the CPE of varicella zoster virus (C. Grose, personal communication). Other studies have shown that arildone is active against other herpesviruses such as murine cytomegalovirus (Kim et al. 1980) and Epstein–Barr virus (Sumaya and Ench 1980). Arildone was effective at drug concentrations which did not significantly inhibit cellular metabolism.

This potent activity against members of the herpesvirus group prompted animal studies and the therapeutic effect of arildone has been demonstrated in an experimental HSV-1 and HSV-2 skin infection in guinea pigs. Topical application of a solution of 4% or 8% arildone in 90% DMSO and 10% polyethylene glycol 400 as well as in an 8% arildone cream preparation suppressed development of vesicles when the treatment was initiated 24 h postinfection. Virus titers in the treated lesions were statistically lower in animals treated with both preparations. The 8% arildone DMSO preparation afforded the most rapid effect on the herpetic vesicles, aborting their development after only 24 h of five applications or less. Arildone in DMSO produced a greater reduction of virus replication in skin lesions than did the cream preparation. Both arildone preparations suppressed vesicles and reduced the virus titer of HSV-2 in the skin of guinea pigs; the DMSO formulation produced a significantly more rapid effect on vesicle suppression and a greater magnitude of virus reduction.

The most potent activity of arildone was against poliovirus; thus, the mode of action of the drug was studied first against this virus. Data indicate that arildone inhibits poliovirus replication by preventing intracellular uncoating of the virion (McSharry et al. 1979). Studies on purified poliovirus suggested that arildone prevents uncoating of the virion by stabilizing the protein–protein interactions of the capsid proteins (Caliguiri et al. 1980). Herpesvirus replication was inhibited by arildone by blocking an early step, one that occurs during the first 6 h after HSV infections, preventing virus replication. Although the mode of action of arildone against HSV is unknown, one possibility is that it blocks uncoating of the HSV nucleocapsid, either in the cytoplasm or in the nucleoplasm.

There are a number of analogs of arildone which possess antiviral activity in vivo and in vitro and are more soluble than arildone. WIN 41258-3, (4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3-diethyl-1-*H*-pyrazole methane sulfonate), and WIN 42202, an aryloxyalkyl phosphonate, are two compounds which are more soluble than arildone and have been shown to be effective against HSV-1 and HSV-2 infections of the mouse genital tract and guinea pig skin (Pancic et al. 1978, 1980a, b). Preliminary results indicated that these two compounds were no more toxic to cells than arildone (J.J. McSharry and L.A. Caliguiri, unpublished work 1980). In addition, these various modifications of the parent compound have led to changes in the antiviral spectrum of the drug. WIN 41258-3 does not inhibit poliovirus replication whereas it does inhibit HSV-1 and HSV-2 replication (L.A. Caliguiri, J.A. Laffin, and J.J. McSharry, unpublished work 1980). By corre-

lating the chemical change of the drug with the antiviral spectrum of these drugs it may be possible to elucidate the means for rational modification to enhance their clinical effectiveness.

Presently, a concerted effort is being made to determine the mode of action of arildone on HSV replication. The working hypothesis is that arildone inhibits HSV replication by blocking uncoating of the HSV nucleocapsid in a manner similar to that demonstrated for poliovirus. If arildone blocks HSV replication by preventing uncoating, then a single mechanism would be involved in the antiviral activity of the drug.

Arildone appears to be tolerated very well in experimental animals and may prove to be a useful drug in the treatment of cutaneous herpetic disease, especially herpes labialis, herpes genitalis, and varicella zoster in humans. The suppression of development of vesicles in the early phase, particularly with the DMSO preparation, may be a very significant achievement. The superior activity of arildone in a DMSO preparation is probably the result of deeper and more effective penetration of the drug, which may be of particular importance in varicella zoster patients. Currently, other vehicles and formulations are being studied which may provide a suitable delivery of arildone by the systemic route in herpetic and other viral infections. Clinical studies are under way to assess the effectiveness of arildone against herpesvirus infections in humans.

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