Activity of Arildone with or without Interferon against Acute Hemorrhagic Conjunctivitis Viruses in Cell Culture

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Arildone (WIN 38020; 4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3,5-heptanedione) inhibited the infectivity of acute hemorrhagic conjunctivitis viruses in tissue culture. Arildone did not inhibit interferon (IFN) production or IFN activity. Treatment of cultures with combinations of arildone and IFN resulted in an additive inhibition of acute hemorrhagic conjunctivitis virus production.

Arildone is a broad-spectrum antiviral agent that has been shown to selectively inhibit replication of picornaviruses (1). The mechanism of action of arildone is consistent with inhibition of the uncoating process (1, 8). Arildone has been shown to prevent paralysis in mice infected with poliovirus (7). Our *in vitro* studies were designed to investigate the possible use of arildone in the treatment of acute hemorrhagic conjunctivitis (AHC) caused by enterovirus type 70 (EV70) and coxsackievirus type A24 (CA24) (2, 10, 12). The approach was to determine whether arildone can inhibit replication of AHC viruses in cell culture, affect interferon (IFN) production and antiviral activity, and inhibit replication when used in combination with IFN.

The effect of arildone on EV70 and CA24 production *in vitro* was determined as follows. Human amnionic WISH cells (Flow Laboratories, Inc., McLean, Va.) were infected with EV70 (strain SEC146/71) or CA24 (strain SEC1/75) (9) at a low multiplicity of infection (10^{-4} PFU per cell) to simulate the early stages of natural infection. Fresh minimal essential medium with Earle salts supplemented with 2% fetal bovine serum, 100 U of penicillin per ml, and 100 μ g of gentamicin per ml (EMEM) and containing various concentrations of arildone (Sterling-Winthrop Research Institute, Rensselaer, N.Y.) was added 2 h postinfection. Arildone was dissolved in dimethyl sulfoxide and then diluted in EMEM. Culture fluids were harvested when 100% cell death was observed microscopically (at approximately 30 h) in virus controls. Virus yields were determined as previously described (4). Virus control media contained 0.01% (vol/vol) dimethyl sulfoxide and had no effect on AHC virus replication (data not shown). Arildone at 1 and 10 μ g/ml effected significant reductions of EV70 and CA24 (1 to 4 $\log_{10}$ U) (Table 1). CA24 was 10 to 100 times (1 to 2 $\log_{10}$ U) more susceptible to the antiviral effects of arildone (10 μ g/ml) than was EV70. The reduction in virus yield increased as the concentration of arildone increased. These results indicated that arildone can inhibit AHC virus spread in cell cultures at levels that are similar to those reported previously for other picornaviruses (1) and provided encouragement for evaluations of the effects of arildone treatment against natural infections with AHC viruses.

The effect of arildone on EV70 and CA24 production and on the action of IFN was investigated to determine whether

arildone treatment would adversely affect IFN production. Specifically, cells were first treated with arildone (10 μ g/ml) in EMEM for 8 h. The cells were then washed and challenged with EV70 (5 PFU per cell). Culture fluids were harvested after 24 h of incubation at 37°C and assayed for production of IFN (5). Treatment of cells with 1 and 10 μ g of arildone per ml did not significantly affect the levels of IFN produced in response to EV70 infections (0.5 $\log_{10}$ U reduction) (Table 1). IFN activity in culture fluids was identified as IFN- β by neutralization with antiserum to human IFN- β (10^3 U/ml; Barbara Dalton, Smith Kline & French Laboratories, Philadelphia, Pa.). In addition, the effect of arildone antiviral activity on IFN was determined by assaying human fibroblast IFN (10^5 U/ml; Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) in the presence of arildone (5). Titration of IFN in the presence of arildone (1.0 and 10 μ g/ml) did not significantly affect the antiviral action of IFN- β against EV70, CA24, and Sindbis virus (data not shown). Taken together, these results indicate that arildone treatment of cells does not affect the production and action of IFN. Our findings are consistent with the observation that arildone does not affect macromo-

TABLE 1. Effect of different concentrations of arildone on AHC Virus and on IFN production

Concn of arildone (μg/ml)	Reduction (log ₁₀ PFU/ml) of ^a :				Log ₁₀ reduction in EV70-induced IFN production ^b
	EV70		CA24		
	Expt 1	Expt 2	Expt 1	Expt 2	
0.01	0.08	0.03	0.16	NR ^c	ND ^d
0.10	0.26	0.37	NR	NR	ND
1.00	1.14	1.21	1.02	1.32	0.5
10.00	1.84	1.52	3.97	2.88	0.5

^a Culture fluids from three cultures were pooled, diluted, and assayed in triplicate for virus content. $\log_{10}$ PFU per milliliter reduction was calculated by subtracting the mean plaque count obtained in each test culture from the mean plaque count obtained in the virus control.

^b Culture fluids from three cultures were pooled and assayed for IFN. The $\log_{10}$ unit reduction in IFN was calculated by subtracting the $\log_{10}$ IFN titer produced in test cultures from the $\log_{10}$ IFN titer produced in virus control cultures. Reduction (0.5 $\log_{10}$) is not significant since the standard error of this IFN test is within this limit (5) and IFN levels were within 10^1 to $10^{1.5}$ U/ml.

^c NR, No reduction detected.

^d ND, Not done.

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FIG. 1. Inhibitory effect of IFN alone and in combination with arildone against CA24 and EV70. Each point represents the amount (mean) of virus in pooled culture fluids harvested from three cultures. Each sample was assayed in triplicate. (Ten micrograms of arildone per ml alone [no IFN] reduced the yield of virus by about $2 \log_{10}$ U.)

lecular synthesis or cellular transport systems (8). Thus, these results suggest that treatment of AHC patients with arildone should not affect the action of endogenously produced IFN (3, 6). It is suspected that arildone will reduce the levels of virus and then IFN- β production will decrease because IFN- β production is dependent upon virus infection in vivo (6).

We also investigated the activity of combinations of arildone and IFN- β against AHC viruses in vitro to determine whether potentiation would occur. Potentiation was expected since we have found that the combination of antibody and IFN inhibits AHC virus production synergistically (4). The methods used were similar to those reported for determining the combined effects of IFN and antibody (4), except that arildone was substituted for antibody. Briefly, human WISH cells were infected (10^{-4} PFU per cell) and then treated with different concentrations of arildone, IFN, or combinations of arildone and IFN. Virus yields from infected cultures incubated for 24 h were compared. The results indicate that the combined inhibitory effect of arildone and different concentrations of IFN against AHC viruses was additive (11) (Fig. 1). For example, arildone alone (10 μ g/ml) reduced CA24 and EV70 yields in preinfected cultures by approximately $2 \log_{10}$ (Fig. 1). IFN- β alone (500 U/ml) reduced CA24 and EV70 yields by 2.0 and 1.5 $\log_{10}$ U, respectively. The combination of arildone (10 μ g/ml) and IFN- β (500 U/ml) reduced CA24 and EV70 yields

by 3.5 to 4.0 $\log_{10}$ U, respectively. Similarly, the reduction in AHC virus yields with combinations of 1 μ g of arildone per ml (about 1.0 $\log_{10}$ U) and IFN- β (see Table 1) was additive (data not shown). These findings suggest that efficacy may not be increased by treatment of AHC with combinations of arildone and IFN since increasing the dose of either arildone or IFN alone should have the same inhibitory effect as treatment with the combination of arildone and IFN. However, if arildone is shown to be toxic for the eye or if resistant viruses arise, then combinations of arildone and IFN may be more beneficial. Animal studies are needed to establish parameters for the treatment and prevention of AHC.

The reason that the inhibitory effect of combinations of arildone and IFN was additive and not synergistic, as with antibody and IFN (4), is not known. The difference is probably related to the mechanism(s) of the inhibitory action of arildone and polyclonal antibody. Further studies are being done to identify this mechanism(s).

In summary, these in vitro studies indicate that arildone can inhibit AHC viruses without affecting nonspecific host defense factors (e.g., IFN production and action). In addition, the inhibitory effects of combination of arildone and IFN were shown to be additive. Additional studies are needed to determine the efficacy of arildone against AHC in vivo.

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