ELSEVIER

Antiviral Research 27 (1995) 355–365

Antiviral Research

Inhibition of the enteroviruses that cause acute hemorrhagic conjunctivitis (AHC) by benzimidazoles; Enviroxime (LY 122772) and Enviradone (LY 127123)

M.P. Langford *, W.A. Ball, J.P. Ganley

Department of Ophthalmology, Louisiana State University Medical Center, 1501 Kings Hwy / P.O.Box 33932, Shreveport, LA 71130-3932, USA

Received 24 October 1994; accepted 15 February 1995

Abstract

Enviradone (EvirD, (*E*)-1-[(1-methylethyl) sulfonyl]-6-(1-phenyl-1-propenyl)-1*H*-benzimidazole-2-amine) and Enviroxime (EvirX, 2-amino-1-(isopropyl-sulfonyl)-6-benzimidazole phenyl ketone oxime) inhibited enterovirus 70 (EV70) and coxsackievirus A24 variant (CA24v) infection of conjunctival and laryngeal cells. On average, the continuous presence of 1–3 μg of EvirD or EvirX/ml in cell cultures acutely infected with EV70 or CA24v inhibited virus production (> 2 $\log_{10}$ reduction) and 100% of the viral cytopathogenic effect (CPE). The 50% CPE inhibitory dose (ID_{50}) for EvirD and EvirX against 11 EV70 and 15 CA24v isolates ranged from 0.01 to 0.3 μg and 0.01–0.65 μg/ml, respectively. The mean ID_{50} for EvirD and EvirX against the 26 AHC viruses was 0.17 ± 0.12 μg and 0.13 ± 0.14 μg/ml, respectively. Pretreatment for 15 min with 3 μg EvirX/ml or for 1–2 h with 3 μg EvirD/ml protected conjunctival cells against viral CPE. The cells were resistant to infection for 1–2 h at 33 and 37°C after removal of EvirD and EvirX. The addition of 10 μg EvirD/ml up to 6 h or 10 μg EvirX/ml 1–2 h after low multiplicity infection inhibited viral CPE. Ten-fold less EvirD inhibited EV70 when added to glioma cells 2 h before infection than when added 2 h after infection. Our results indicate that EvirX and EvirD inhibit AHC viruses in vitro at concentrations that are not cytotoxic and suggest that EvirX or EvirD may be prove useful against AHC.

Keywords: Conjunctivitis, Enterovirus, Coxsackievirus, Eye disease, Antiviral agent

* Corresponding author. Fax: (1318) 675 6000.

SSDI 0166-3542(95)00019-4

1. Introduction

Epidemics of AHC are caused by enterovirus type 70 (EV70) or a variant of coxsackievirus type A24 (CA24v). The humoral immune responses to AHC viruses are transient, weak or undetectable (Kono et al., 1977; Christopher et al., 1982; Langford et al., 1985) and the postepidemic decline in seropositive individuals is thought to be associated with the recurrence of AHC epidemics (Bern et al., 1992). AHC epidemics and pandemics have occurred around the world during the past 25 years (Heirholzer and Hatch, 1985; Wright et al., 1992) with a periodicity similar to influenza (Yin-Murphy, 1984).

Cytolytic EV70 or CA24v infection of conjunctival cells causes the acute onset (< 12 h) of AHC (Wright et al., 1992). AHC is usually benign with total resolution of symptoms within 14 days. However, CA24v can infect the cells of the upper respiratory tract and cause a sore throat. EV70 is neurotropic and approximately 1 : 10,000 AHC patients, usually males 20–40 years of age, have mild to permanent weakness in the muscles of the face and/or extremities (Wadia, 1989).

The rapidness and ease of spread of AHC within the family and community, bilateral infection, and neurologic disease create unique economic and psychological problems during epidemics (Wolken, 1974; Kono, 1975). The growing concern over the evolution of more sight-threatening and neurovirulent AHC viruses and the absence of a vaccine for the prevention of AHC indicate the need for an effective antiviral treatment.

Benzimidazole compounds have been shown to inhibit picornavirus replication, although their mechanism of action is unknown (DeLong and Reed, 1980; Eggers, 1985). We present our investigation of the antiviral activities of 2 benzimidazole compounds, Enviroxime (EvirX) and Enviradone (EvirD), against several isolates of EV70 and CA24v. The results of our in vitro studies indicate that these compounds inhibit EV70 and CA24v replication and suggest they may be useful in the treatment of AHC.

2. Materials and methods

2.1. Tissue culture

Chang's human conjunctival cells (HCC), human HEp-2 cells (epidermoid carcinoma of the larnyx) and human glioma cells (Hs 683) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were maintained in F-10 medium supplemented with 10% newborn bovine calf serum (BCS) (GIBCO Laboratories, Grand Island, NY) and antibiotics. The cells were maintained and microtiter plate (MTP) cell cultures were prepared for assays and experiments in 96-Well Tissue Culture Clusters (Costar, Charlotte, NC) using standard medium and tissue culture techniques (Langford et al., 1986).

2.2. Viruses

Nine EV70 isolates and 15 CA24v isolates were provided by Drs. M. Yin-Murphy (Singapore University, Singapore), R. Kono (National Institutes of Health, Tokyo,

Table 1
ID_{50} of EvirD and EvirX against 11 EV70 and 15 CA24v

Virus	Origin	Ref.	m.o.i.	ID_{50} EvirD		ID_{50} EvirX	
				Expt. 1	Expt. 2	Expt. 1	Expt. 2
EV70							
J670/71	Hokkaido	Kono, 1975	5	0.15	0.20	0.10	0.07
J648/71	Mie, Japan	Mitsui et al., 1972	2	0.08	0.20	0.08	0.05
J648/71R	?	Langford et al., 1986	0.1	ND	0.01	ND	0.01
SEC146/71	Singapore	Yin-Murphy, 1984	3	0.10	0.15	0.10	0.03
SEC148/71	Singapore	Yin-Murphy, 1984	5	0.08	0.20	0.20	0.07
R20/71	Morocco	Nejmi et al., 1974	2	0.08	0.10	0.30	0.02
HK/71	Hong Kong	Chang et al., 1977	0.2	0.01	0.20	0.01	0.01
AE/72	Thailand	Thongcharoen et al., 1978	1	0.10	0.10	0.08	0.03
FSN-72	Thailand	Thongcharoen et al., 1978	11	ND	0.10	ND	0.10
FS-24/74	Thailand	Thongcharoen et al., 1978	8	0.10	0.30	0.08	0.10
SDDR/92	Dom. Rep.	Orillac and Langford, 1993	0.1	0.10	0.20	0.07	0.03
Mean				0.09	0.16	0.11	0.04
S.D.				±0.04	±0.08	±0.08	±0.03
CA24v							
SEC24/70	Singapore	Yin-Murphy, 1984	3	0.08	0.01	0.10	0.01
EH44/70	Singapore	Yin-Murphy, 1984	0.01	0.08	ND	0.30	ND
HK1/71	Hong Kong	Chang et al., 1977	0.05	0.20	ND	0.65	ND
BE65/75	Singapore	Yin-Murphy, 1984	0.01	0.10	ND	0.30	ND
75308/75	Sri Lanka	Higgins and Chapman, 1977	0.05	0.06	ND	0.10	ND
V3309/758	Vellore	Christopher et al., 1977	5	ND	0.30	ND	0.10
3751/75	Brunei	Bahrin et al., 1976	0.02	ND	0.20	ND	0.10
BD/75	Pakistan	WHO, 1986	0.10	ND	0.30	ND	0.10
E 2/78	Singapore	Yin-Murphy, 1984	0.01	0.30	0.30	0.30	0.10
SEC17/85	Singapore	Yin-Murphy, 1989	0.01	0.03	0.01	0.30	0.10
JAPAN 85	Japan	Miyamura et al., 1988	0.04	0.60	0.30	0.10	0.03
V3454/85	Vellore	Yin-Murphy, 1984	1	ND	0.30	ND	0.10
Tia 86	Taiwan	Chen et al., 1986	0.3	0.03	0.01	0.65	0.10
VW2156/86	Belgium	Yin-Murhhy, 1989	2	0.08	0.30	0.20	0.10
VI1992/87	Singapore	Yin-Murphy, 1989	0.4	0.30	0.30	0.10	0.10
Mean				0.17	0.20	0.28	0.08
S.D.				±0.17	±0.14	±0.20	±0.03
Mean E70/CA24				0.13	0.18	0.21	0.07
S.D.				±0.13	±0.11	±0.18	±0.04
Mean (AHC viruses)				0.17		0.13	
S.D.				±0.12		±0.14	

Japan) or C. Jayavasu (Ministry of Public Health, Bangkok, Thailand) (Table 1). J648/71R was adapted to infect rabbit eyes (Langford et al., 1986) and SDDR/92 was isolated from an AHC patient in the Dominican Republic in 1992 (Orillac and Langford, 1993). Poliovirus type 1 strains Sabin (PV-1S) and Mahoney (PV-1M) were obtained from the ATCC. Virus stocks were prepared in HEp-2 cells as previously described (Stanton et al., 1977) and stored at −100°C. EV70 titers were 5- to 10-fold lower in

HCC and human glioma cells than in HEp-2 cells. Notably, CA24v titers were 5-fold lower in HCC than in HEp-2 and CA24v replication was not detected in glioma cells.

2.3. Compounds

EvirD (LY 127123, (*E*)-1-[(1-methylethyl) sulfonyl]-6-(1-phenyl-1-propenyl)-1*H*-benzimidazole-2-amine), EvirX (LY 122772, 2-amino-1-(isopropyl-sulfonyl)-6-benzimidazole phenyl ketone oxime) and a placebo compound (LY 253963) were provided by Lilly Research Laboratories (Indianapolis, IN). The compounds were dissolved in dimethyl sulfoxide (1.0 mg/ml) and stored at 4°C until diluted in culture media.

2.4. Inhibition of virus production

Dilutions of EvirD and EvirX (0.5 $\log_{10}$ dilution series) in Eagle's minimum essential medium with Earle's salts (GIBCO) supplemented with 2% BCS and antibiotics (EMEM) (Langford and Kaiwar, 1990) were pipetted (100 μl/culture) onto triplicate MTP cultures of HCC or human glioma cells at various times prior to, at the time of, or at various times after EV70 or CA24v infection. The cultures were incubated at 37°C for 24 h, frozen, and virus yields in triplicate sham and treated cultures were estimated by virus plaque assay of culture fluids (Stanton et al., 1977). The mean $\log_{10}$ plaque forming units (PFU)/ml was determined for each concentration of EvirD and EvirX at each time point (Reid, 1968).

2.5. Inhibition of viral CPE

As above, 100 μl of each 0.5 $\log_{10}$ dilution of EvirD, EvirX or placebo in EMEM was pipetted onto triplicate MTP cultures of HCC or HEp-2 at various times prior to, at the time of, or at various times after EV70 or CA24v infection. The cultures were incubated at 33 or 37°C until 100% cell death was observed microscopically in the virus control. The cultures were fixed and stained with 1% crystal violet (w/v) in 20% methanol. Viral CPE was quantified spectrophotometrically (Dynatech, Guernsey, Channel Island, Great Britain) (Langford and Kaiwar, 1990). The mean optical density at 590 nm (OD_{590}) of crystal violet-stained cell monolayers was calculated and used to determine the CPE ID_{50}. The ID_{50} is the concentration of EvirD and EvirX that inhibited EV70 and CA24v CPE by 50% (i.e., the concentration that increased the OD_{590} in the virus infected culture to 50% of that in the cell control). The ID_{50} was calculated by dividing the mean OD_{590} from triplicate drug treated cultures by the mean OD_{590} from respective replicate untreated control cultures $\times 100$. The level of CPE inhibition at various times after removal of the EvirX and EvirD was determined as above except HCC were incubated in EMEM containing EvirX or EvirD, fresh EMEM pipetted onto each culture and then challenged with EV70 or CA24v at 1, 2 and 4 h after removal of the compounds.

2.6. Statistical analysis

Significance differences between means of virus groups were estimated by Student's *t*-test. The ANOVA test was used to analyze differences between treatments and linear

regression analysis was performed to assess drug kinetic differences. The computations were performed on an IBM PC XT equipped with Epistat statistical software.

3. Results

3.1. Inhibition of EV70 or CA24v production and CPE in HCC

EvirX and EvirD inhibited EV70 (J648/71 and SEC146/71) and CA24v (SEC24/70 and Tai 86) yields and CPE in HCC in a dose-dependent manner between 0.1 and 1.0 μg/ml (Fig. 1). The reduction in EV70 or CA24v yields in infected cultures treated with EvirD or EvirX was associated with an inhibition of viral CPE. For example, acutely infected cells incubated in medium containing 0.1 μg EvirD or EvirX/ml produced 0.1–1.5 $\log_{10}$ less virus than the virus controls. Acutely infected cells treated with 1 μg EvirX or EvirD/ml produced 2–3 $\log_{10}$ less EV70 or CA24v than the virus controls. Concomitantly, the OD_{590} of the infected cultures treated with 0.1 μg of EvirX/ml was 0.5–1.0 OD_{590} units lower than the OD_{590} of cultures treated with 1–3 μg of EvirX/ml and the OD_{590} of the cell control (not graphed). Notably, acutely infected cells continuously incubated in medium containing 1–3 μg EvirX or EvirD/ml inhibited virus production (> 2 $\log_{10}$ reduction) and inhibited viral CPE by 100%. Cytotoxicity was observed microscopically in control HCC cultures and in virus infected HCC incubated for 24 h in medium containing 10 μg EvirD/ml (note that cytotoxicity was associated with a decrease in the OD_{590} in cultures treated with 10 μg EvirD). Based upon the results of this and similar experiments in HCC and HEp-2 cells (not

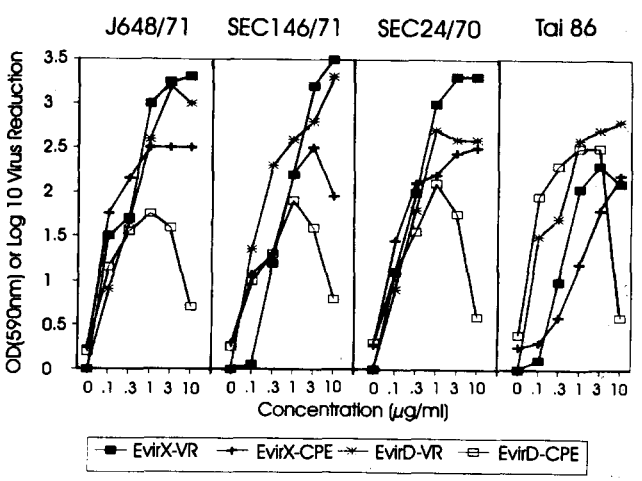

Fig. 1. Dose–response curve of the antiviral activities of EvirX and EvirD against EV70 (J648/71 and SEC146/71) and CA24v (SEC24/70 and Tai 86). Acutely infected HCC were incubated with various concentrations of the compounds and the mean $\log_{10}$ PFU virus reduction (VR) and CPE (OD_{590} of crystal violet stained cultures) determined. The S.D. were ≤ 0.5 $\log_{10}$ of the VR means and $\leq 20\%$ of the OD_{590} means. The results are from 3 independent experiments.

shown), the 50% drug cytotoxic doses (i.e., the concentrations that reduced the OD_{590} in treated cultures to 50% of that in the untreated cell control) for EvirD, EvirX, and placebo (LY 253963) were estimated to be 10 μg, 60–100 μg and 20 μg/ml, respectively.

3.2. ID_{50} of EvirX and EvirD against several epidemic isolates of EV70 and CA24v

The ID_{50} of EvirD and EvirX was determined for 11 EV70 and 15 CA24v in laryngeal carcinoma HEp-2 cells (Table 1). The ID_{50} for EvirD and EvirX against 11 EV70 ranged from 0.01 to 0.30 μg/ml with EvirD ID_{50} means of 0.09 ± 0.04 and 0.16 ± 0.08 μg/ml and EvirX ID50 means of 0.11 ± 0.08 and 0.04 ± 0.03 μg EvirX/ml in two experiments. The ID_{50} for EvirD and EvirX against 15 CA24v ranged from 0.01 to 0.65 μg/ml with EvirD ID_{50} means of 0.17 ± 0.17 and 0.2 ± 0.14 μg/ml and EvirX ID_{50} means of 0.28 ± 0.2 and 0.08 ± 0.03 μg/ml in two experiments. In comparison, the ID_{50} of PV-1M and PV-1S were 0.2–0.6 μg EvirD/ml and 0.1–0.3 μg EvirX/ml (not shown). While individual isolates varied in their sensitivities to EvirD and EvirX, no statistical differences ($P > 0.4$) were detected between mean ID_{50}s of EV70 and CA24v isolates or experiments. The ID_{50} for EvirD and EvirX against the 26 AHC virus isolates were 0.17 ± 0.12 and 0.13 ± 0.14 μg/ml, respectively. Notably, the multiplicity of infection (m.o.i., ranging from 0.01 to 11 PFU/cell), country of origin, and year of isolation did not affect the ID_{50} of EvirD or EvirX.

3.3. Effect of pretreatment on prevention of EV70 and CA24v infection

No viral CPE was observed microscopically and control level OD_{590} were detected in EV70 and CA24v infected HCC cultures incubated in EMEM containing 3 μg EvirD or EvirX/ml for 2 and 4 h before infection (Fig. 2). Unexpectedly, the intracellular levels of EvirX reached after 15 min of preincubation were sufficient to protect cells against the CPE of EV70 and CA24v infections. In contrast, inhibition of EV70 and CA24v CPE by EvirD required 1–2 h of pretreatment.

3.4. Effect of EvirD and EvirX treatment applied after EV70 or CA24v infection

The addition of medium containing 3 or 10 μg EvirD or EvirX/ml to HCC cultures 1 h after infection with EV70 (J648/71) or CA24v (SEC24/70) (m.o.i.s of 1 and 3 PFU/cell, respectively) inhibited viral CPE (Fig. 3). Interestingly, 3 and 10 μg EvirD/ml were more effective at inhibiting the CPE of EV70 and CA24v in HCC than 3 and 10 μg EvirX/ml when added up to 2 h after infection. Notably, EV70 replication was inhibited by the addition of 10 μg EvirD/ml up to 6 h postinfection.

3.5. Decay of the antiviral activities of EvirX and EvirD

EV70 (J648/71, 1 PFU/cell) and CA24v (SEC24/70, 3 PFU/cell) CPE was inhibited by intracellular EvirX or EvirD up to 4 h postinfection at 33 and 37°C (Fig. 4). However, maximal inhibition of EV70 and CA24v CPE was detected in HCC cultures

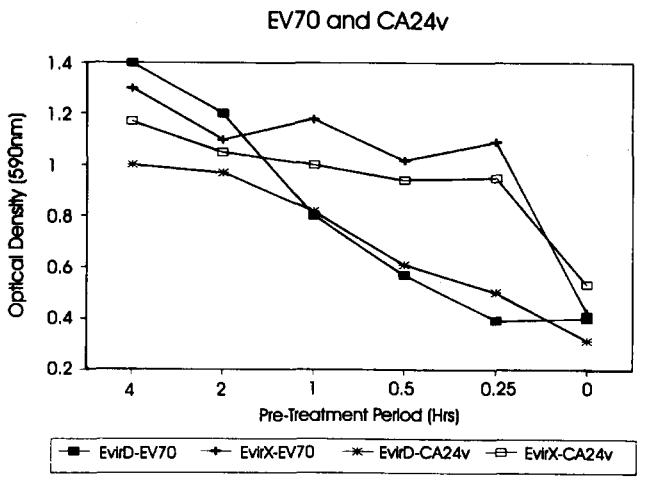

Fig. 2. Effect of pretreatment time on inhibition of viral CPE. Triplicate HCC cultures were incubated in medium containing EvirD or EvirX (3 μg/ml) for various times prior to removal and infection with EV70 (J648/71) or CA24v (SEC24/70). The cultures were fixed and strained with crystal violet when 100% cell death was observed in the virus control. The mean OD_{590} from 3 experiments are presented. S.D. of the means were ≤ 0.11 OD_{590} unit.

infected 1 h after the removal of media containing 10 μg of EvirD or EvirX/ml. The antiviral activities of EvirD and EvirX against EV70 and CA24v infection declined through 4 h. The antiviral activity of EvirX in cells cultured at 33°C declined more

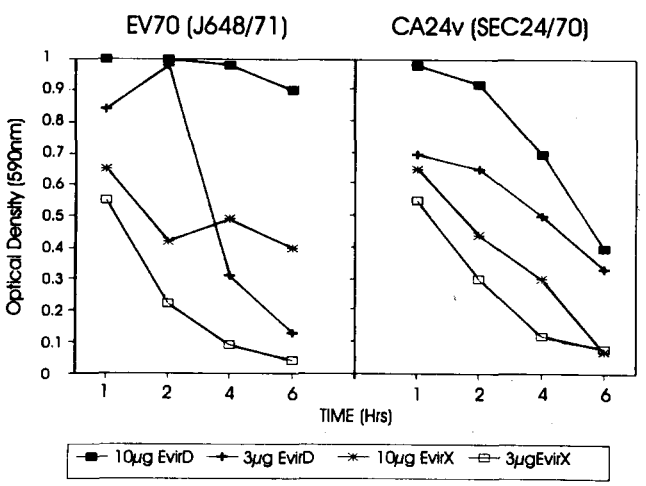

Fig. 3. Effect of EvirX and EvirD on inhibition of CPE in infected HCC. Medium containing 3 or 10 μg EvirD or EvirX/ml was added to EV70 or CA24v infected HCC cultures at various times following 1 h of incubation to allow virus adsorption. After 20 h (EV70) or 36 h (CA24v) incubation, the cells were stained and the mean OD_{590} from replicate cultures treated at each time point was determined ($n = 3$). The means from one experiment run in triplicate are presented (S.D. of the OD_{590} means ≤ 0.17).

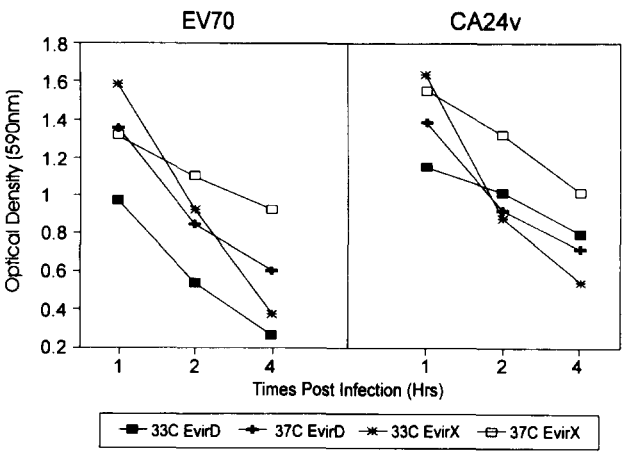

Fig 4. Decay of the antiviral activities of EvirD and EvirX with time. HCC were incubated in medium containing EvirD or EvirX for 2 h at 33 and 37°C and then infected with EV70 or CA24v at various times after the removal of the medium containing the EvirD or EvirX. The means and S.D. of the OD_{590} for the 33 and 37°C cell controls were 1.14 ± 0.08 and 1.4 ± 0.1, respectively. Mean OD_{590} from one experiment run in triplicate are plotted (S.D. ≤ 0.16 OD_{590} units).

rapidly than the antiviral activity in EvirD-treated cells and EvirX-treated cells incubated at 37°C ($P = 0.01$).

3.6. *Effect of time-of-addition of EvirD and EvirX on EV70 yields in glioma cells*

Incubation of human glioma cells for 2 h prior to EV70 (J648/71) infection in medium containing 10 μg EvirD or EvirX/ml or incubation of EV70 infected glioma

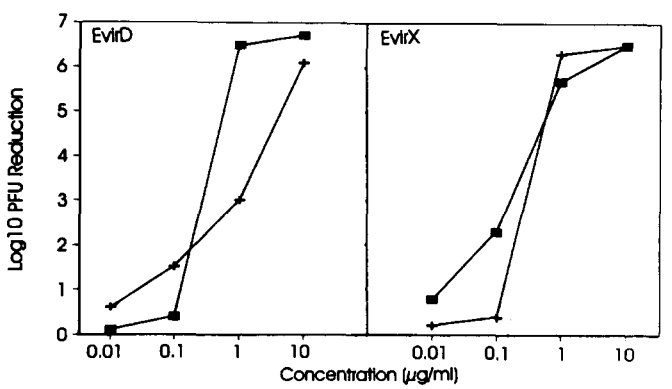

Fig. 5. Inhibition of EV70 production by different concentrations of EvirD and EvirX in human glioma cells. Glioma cells were incubated with medium containing various concentrations of EvirD or EvirX for 2 h prior to EV70 (J648/71) infection or for 2 h, 1 h after EV70 infection. The mean virus titers (PFU/ml) in triplicate treated and control cultures were determined by plaque assays ($n = 3$). The mean virus reductions in two experiments were calculated and plotted. S.D. were < 0.6 $\log_{10}$ PFU/ml.

cells in medium containing 10 μg EvirD or EvirX/ml beginning 2 h postinfection inhibited virus production by $\geqslant 10^6$ PFU/ml reduction (Fig. 5). Notably, approximately 10-fold less EvirD than EvirX (i.e. 1 μg versus 10 μg/ml) inhibited virus production by 10^6 PFU when added before infection rather than after infection ($P < 0.0001$). (Cytotoxicity was not observed in glioma cells under these experimental conditions.)

4. Discussion

We have shown that incubation of conjunctival (HCC), laryngeal (HEp-2) and glioma cells infected with EV70 or CA24v in medium containing 1–10 μg EvirD or EvirX/ml reduces viral production and inhibits viral CPE. The mean ID_{50} of EvirD and EvirX against EV70 and CA24v, isolated during epidemics spanning a 22-year period, were similar (0.17 and 0.13 μg/ml, respectively). The antiviral activities of EvirD and EvirX were demonstrable at 33 and 37°C. In addition, EvirX and EvirD protect cells against high multiplicity EV70 and CA24v infection. The maximum antiviral activity of EvirX was detected after 15 min of incubation while the antiviral activity of EvirD was detected after 1–2 h of incubation. The antiviral activities of EvirX and EvirD were dose-dependent and detectable up to 1–2 h after the removal of extracellular EvirX and EvirD. These results suggest that EvirD and EvirX may inhibit AHC virus infection in vivo.

Benzimidazoles have been shown to inhibit picornavirus replication by an unknown mechanism (Tamm and Eggers, 1963) at levels that are 10-fold less than their cytotoxic levels (Bucknall, 1967). Our results are consistent with these observations, in that, EvirD and EvirX exhibited maximal antiviral activity against EV70 and CA24v at concentrations of 1–3 μg/ml/24 h. However, cytotoxicity was detected in HCC and HEp-2 cells incubated in medium containing $\geqslant 10$ μg EvirD or > 30 μg EvirD/ml/24 h. Thus, our results indicate that the therapeutic indices for EvirD and EvirX are $\geqslant 10$ and suggest that a therapeutic effect may be achieved in the eye without drug-induced complications.

Taken together, our results indicate that AHC viruses are inhibited by EvirX and EvirD and suggest that EvirX or EvirD may prove useful in the treatment of AHC. Also, the results suggest that EvirX may prove to be more efficacious than EvirD since the antiviral activity of EvirX is detected earlier than EvirD and EvirX appears to be less toxic. Although side effects and poor antiviral activity have been reported in human clinical trials with EvirX against rhinovirus infection (Hayden and Gwaltney, 1982; Phillpotts et al., 1983), it may be possible to apply therapeutic levels of EvirX to the conjunctiva without ocular toxicity. Thus, careful animal model testing is planned to assess cytotoxicity and the therapeutic effects of these compounds against AHC viruses.

Acknowledgements

This study was supported in part by a Summer Student Research Award (W.A.B.) from the Office of the Dean, Louisiana State University Medical Center, Shreveport,

LA. We thank Christopher Duggan for excellent technical support and Eli Lilly Research Laboratories for generously providing the compounds. The authors gratefully acknowledge the contributions and encouragement of Dr. Marguerite Yin-Murphy and Dr. Reisaku Kono. The authors have no proprietary interests in these compounds.

References

Bahrin, M.A., Joshi, N.D. and Yin-Murphy, M. (1976) A coxsackievirus type A24 epidemic conjunctivitis in Brunei. Southeast Asian J. Trop. Med. Public Health 7, 355–358.

Bern, C., Pallansch, M.A., Gary, H.E., Alexander, J.P., Torok, T.J., Glass, R.I. and Anderson, L.J. (1992) Acute hemorrhagic conjunctivitis due to enterovirus 70 in American Samoa: serum-neutralizing antibodies and sex-specific protection. Am. J. Epidemiol. 136, 1502–1506.

Bucknall, R.A. (1967) The effects of substituted benzimidazoles on the growth of viruses and the nucleic acid metabolism of host cells. J. Gen. Virol. 1, 89–94.

Chang, W.K., Liu, K., Foo, T.C., Lam, M.W. and Chan, C.F. (1977) Acute hemorrhagic conjunctivitis in Hong Kong, 1971–75. Southeast Asian J. Trop. Med. Public Health 8, 1–6.

Chen, C.-W., Lin, K.-H. and Ko, L.S. (1986) Isolation of CA24v from acute hemorrhagic in Kao-hsiung, Taiwan. Mthly. Rept. Infect. Agents 7, 42.

Christopher, S., John, T.J., Charles, V. and Ray, S. (1977) Coxsackievirus A 24 variant EH24/70 and enterovirus type 70 in an epidemic of acute haemorrhagic conjunctivitis: a preliminary report. Indian J. Med. Res. 65, 593–595.

Christopher, S., Theogaraj, S., Godbole, S. and John, T.J. (1982) An epidemic of acute hemorrhagic conjunctivitis due to coxsackievirus A24. J. Infect. Dis. 146, 16–19.

DeLong, D.C. and Reed, S.E. (1980) Inhibition of rhinovirus replication in organ culture by a potential antiviral drug. J. Infect. Dis. 141, 87–91.

Eggers, H.J. (1985) Antiviral agents against picornaviruses. Antiviral Res. Suppl. 1, 57–65.

Hayden, F.G. and Gwaltney, J.M. (1982) Prophylactic activity of intranasal enviroxime against experimentally induced rhinovirus type 39 infection. Antimicrob. Agents Chemother. 21, 892–897.

Heirholzer, J.C. and Hatch, M. (1985) Acute hemorrhagic conjunctivitis. In: R.W. Darrell (Ed.), Viral Diseases of the Eye, Lea and Febiger, Philadelphia, pp. 165–196.

Higgins, P.G. and Chapman, T.E. (1977) Coxsackievirus A24 and acute hemorrhagic conjunctivitis in Sri Lanka. Lancet 1, 361.

Kono, R. (1975) Apollo 11 disease or acute hemorrhagic conjunctivitis: a pandemic of a new enterovirus infection of the eyes. Am. J. Epidemiol. 101, 383–390.

Kono, R., Miyamura, E., Tajiri, E., Sasagawa, A., Phuapradit, P., Roongwithu, N., Vejjajiva, A., Jayavasu, C., Thongcharoen, P., Wasi, C., Rodprassert, P. (1977) Virological and serological studies of neurological complications of acute hemorrhagic conjunctivitis in Thailand. J. Infect. Dis. 135, 706–713.

Langford, M.P., Barber, J.C., Sklar, V.E.F., Clark, S.W., Patriarca, P.A., Onarato, I.M., Yin-Murphy, M. and Stanton, G.J. (1985) Virus-specific early appearing neutralizing activity and interferon in tears of patients with acute hemorrhagic conjunctivitis. Curr. Eye Res. 4, 233–239.

Langford, M.P., Yin-Murphy, M., Barber, J.C., Heard, H.K. and Stanton, G.J. (1986) Conjunctivitis in rabbits caused by enterovirus type 70 (EV70). Invest. Ophthalmol. Vis. Sci. 27, 915–920.

Langford, M.P. and Kaiwar, R. (1990) An assay for the detection of interferon dependent and interferon independent antibody activities. J. Virol. Meth. 30, 151–160.

Mitsui, Y., Kajima, M., Matsumara, K. and Shioda, H. (1972) Hemorrhagic conjunctivitis: a new type of epidemic viral keratoconjunctivitis. Jpn. J. Ophthalmol. 16, 33–40.

Miyamura, K., Yamashita, K., Takeda, N., Ogino, T., Utagawa, E., Yamazaki, S., Fukumura, K., Uehara, T. and Shinjo, N. (1988) The first epidemic of acute hemorrhagic conjunctivitis due to coxsackievirus A24 variant in Okinawa, Japan, in 1985–1986. Jpn. J. Med. Sci. Biol. 41, 159–174.

Nejmi, S., Gaudin, O.G., Chomel, J.J., Baaj, A.J., Sohier, R. and Bosshard, S. (1974) Isolation of a virus responsible for an outbreak of an acute conjunctivitis in Morrocco. J. Hyg. 72, 181–183.

Orillac, R. and Langford, M.P. (1993) Acute hemorrhagic conjunctivitis epidemic in the Dominican Republic. Eye 7, 479–480.

Phillpotts, R., Wallace, J., Tyrrell, D. and Tagart, V. (1983) Therapeutic activity of enviroxime against rhinovirus infection in volunteers. Antimicrob. Agents Chemother. 23, 671–675.

Reid, D.W.B. (1968) Statistical methods in virology. In: Rhodes, A.J. and Van Rooyen, C.E. (Eds.), Textbook of Virology for Students and Practitioners of Medicine and Other Health Sciences, Williams and Wilkins, Baltimore, pp. 228–231.

Stanton, G.J., Langford, M.P. and Baron, S. (1977) Effect of interferon, elevated temperature, and cell type on replication of acute hemorrhagic conjunctivitis viruses. Infect. Immun. 18, 370–376.

Tamm, I. and Eggers, H.J. (1963) Specific inhibition of replication of animal viruses. Science 142, 124–126.

Thongcharoen, P., Wasi, C., Pimolpan, V. and Panpatana, P. (1978) Etiological study on acute hemorrhagic conjunctivitis in Thailand, 1971–1975. J. Med. Assoc. Thailand 61, 195–201.

Wadia, N.H. (1989) Neurological manifestations of enterovirus 70: a 15-year review from India. In: Y. Uchida, K. Ishii, K. Miyamura and S. Yamazaki (Eds.), Acute Hemorrhagic Conjunctivitis; Etiology, Epidemiology and Clinical Manifestations, Karger, New York, pp. 251–266.

Wolken, S.H. (1974) Acute hemorrhagic conjunctivitis. Surv. Ophthalmol. 19, 71–84.

WHO (1986) Acute hemorrhagic conjunctivitis. Wkly. Epidemiol. Rec. 61, 254.

Wright, P.W., Strauss, G.H. and Langford, M.P. (1992) Acute hemorrhagic conjunctivitis. Am. Family Phys. 45, 173–178.

Yin-Murphy, M. (1984) Acute hemorrhagic conjunctivitis. Prog. Med. Virol. 29, 23–44.

Yin-Murphy, M. (1989) Epidemics of acute hemorrhagic conjunctivitis by coxsackievirus A24 variant. In: Y. Uchida, K. Ishii, K. Miyamura and S. Yamazaki (Eds.), Acute Hemorrhagic Conjunctivitis; Etiology, Epidemiology and Clinical Manifestations, Karger, New York, pp. 57–65.