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In vitro and in vivo antiviral activity of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU) and related compounds*

Haruhiko Machida and Shinji Sakata

Research Laboratories, Yamasa Shoyu Company Ltd., Choshi, 288 Japan

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Summary

BV-araU and related compounds such as CV-araU, IV-araU and BV-araUMP showed marked activity against herpes simplex virus type 1 (HSV-1) in human embryonic lung fibroblast cells. BV-araU, CV-araU and BV-araUMP were also effective in mice infected intracerebrally with HSV-1. Especially, when mice were infected with a low dose of virus, both intravenous and oral treatment with BV-araU proved capable of increasing the mean survival time and decreasing the final mortality of the infected mice. The in vivo anti-HSV-1 activity of BV-araU was comparable to that of BVDU. BV-araU exhibited little toxicity for mice.

BV-araU; BV-araUMP; BVDU; HSV-1 encephalitis

Introduction

The synthesis of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)-uracil (BV-araU) (Fig. 1) has been reported [2,13]. It was found to be a potent inhibitor of the replication of herpes simplex virus type 1 (HSV-1) in human embryonic lung fibroblast (HEL-F) cells [8,10] and primary rabbit kidney cells [5], without toxicity for the host cell. The anti-HSV-1 potency is critically dependent on the choice of the cell

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Abbreviations used: BV-araU, 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil; CV-araU, 1-β-D-arabinofuranosyl-*E*-5-(2-chlorovinyl)uracil; IV-araU, 1-β-D-arabinofuranosyl-*E*-5-(2-iodovinyl)uracil; BV-araUMP, 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil 5'-monophosphate; araT, 1-β-D-arabinofuranosylthymine; vinyl-araU, 1-β-D-arabinofuranosyl-5-vinyluracil; IDU, 5-iodo-2'-deoxyuridine; ACV, acyclovir [9-(2-hydroxyethoxymethyl)guanine]; BVDU *E*-5-(2-bromovinyl)-2'- deoxyuridine; FIAC, 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine; FMAU, 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-methyluracil.

system [3,12]: it is most active in human diploid fibroblasts and least effective in monkey kidney cells.

In the present paper, we further show (i) the in vitro activity of BV-araU and related compounds against HSV-1 and HSV-2 and (ii) the efficacy of BV-araU and BV-araU 5'-monophosphate (BV-araUMP) in the treatment of HSV-1 encephalitis in mice.

Materials and Methods

Cells and virus

HEL-F cells were used for all in vitro antiviral tests. As challenge viruses 3 strains of HSV-1 and 3 strains of HSV-2 were used. HSV-1 strain VR-3 and HSV-2 strain MS were kindly supplied by Dr. S. Yamazaki, National Institute of Health of Japan, and HSV-1 strains MP and CHR-3 and HSV-2 strains HG-52 and UW-268 were kindly provided by Dr. K. Hayashi, Institute of Medical Science, University of Tokyo.

In vitro antiviral activity

HEL-F cells grown in a multiwell plate (Linbro FB-12-TC) were infected with about 50 plaque-forming units of HSV-1 or HSV-2 per well. After 1 h adsorption, the inoculum was removed and the cultures were overlaid with maintenance medium containing 0.5% Noble agar (Difco), 50 μg/ml of DEAE-dextran and an appropriate amount of the test compound in serial half-$\log_{10}$ dilutions. After 2–4 days of incubation, plaques were counted microscopically without staining.

In vivo efficacy against encephalitis induced by HSV-1 in mice

Groups of 4-wk-old male random-bred albino Jcl:ICR Swiss mice, weighing 18–22 g, were infected intracerebrally (i.c.) with 3–30 LD_{50} of HSV-1 strain VR-3. The infected mice were treated with drugs twice daily starting at 4 h post-infection. The differences in mean survival times and mortality of control and drug-treated groups were evaluated by Student's *t*-test or Fisher's exact test (or χ square test with Yates' correction), respectively.

Acute toxicity for mice

Acute toxicity was determined using groups of 10 uninfected 6-wk-old male Jcl:ICR Swiss mice, as described previously [7].

Compounds

BV-araUMP was prepared from BV-araU by phosphorylation with phosphoryl-chloride [14]; BV-araUMP was obtained as free acid and neutralized to sodium salt when used. BVDU [*E*-5-(2-bromovinyl)-2'-deoxyuridine] was synthesized by the method of Bergstrom and Ogawa [1]. FIAC [1-(2-fluoro-2-deoxy-β-D-arabino-furanosyl)-5-iodocytosine] and FMAU [1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-methyluracil] were kindly supplied by Dr. J.J. Fox, Sloan-Kettering Institute, New York, and ACV [acyclovir; 9-(2-hydroxyethoxymethyl)guanine] was a gift of Dr. W. Wilson, Nippon Wellcome Co. Ltd., Osaka. The other 5-substituted araU derivatives were synthesized as described previously [1].

Results

In vitro antiviral activity

As shown previously, BV-araU exhibits marked activity against HSV-1 in a viral CPE inhibition assay [8,10]. Like BV-araU, BV-araUMP, IV-araU [1-β-D-arabinofuranosyl-*E*-5-(2-iodovinyl)uracil] and CV-araU [1-β-D-arabinofuranosyl-*E*-5-(2-chlorovinyl)uracil], also show marked anti-HSV-1 activity in this system. Against HSV-2, however, all BV-araU analogues show relatively low activity.

BV-araU and its congeners have now been evaluated for anti-HSV-1 and HSV-2 activity by the plaque reduction method. As demonstrated in Fig. 2, BV-araU was highly effective in inhibiting plaque formation of all 3 HSV-1 strains. In this respect, BV-araU was as effective as FIAC. However, BV-araU exhibited low activity against HSV-2 (Fig. 2). For BV-araU and various other thymidine analogues, 50% plaque reduction doses were calculated upon graphical plotting of plaque formation versus

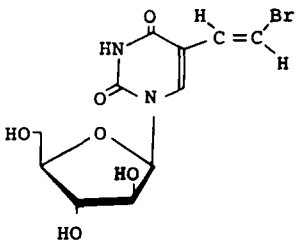

Fig. 1. Structural formula of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU).

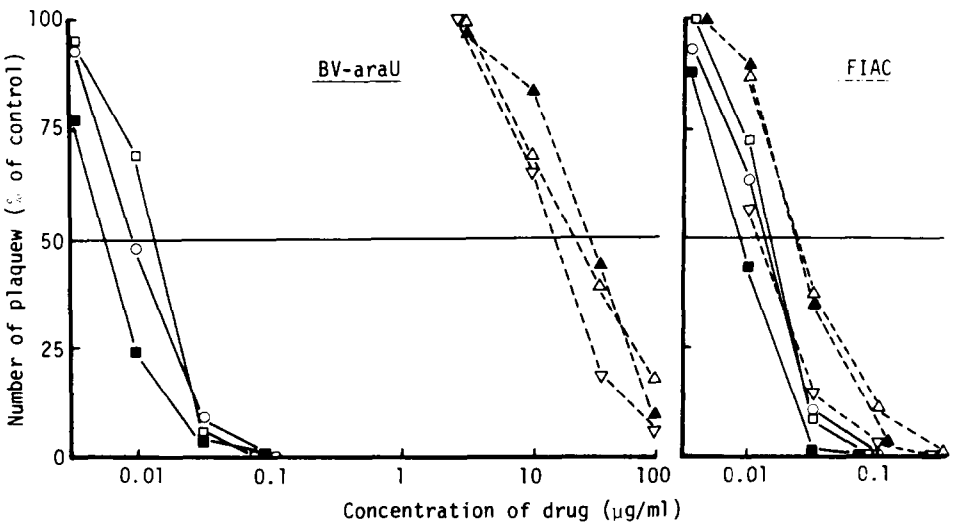

Fig. 2. Inhibition of plaque formation of HSV-1 strain VR-3 (○), CHR-3 (□), MP (■), HSV-2 strain MS (△), UW-268 (▽) and HG-52 (▲) by BV-araU and FIAC.

concentration of the test compound. The values thus obtained are presented in Table 1. These values represent averages of 2–4 separate experiments. The anti-HSV-1 activity of BV-araU and the BV-araU analogues was comparable to that of BVDU, FIAC and FMAU. However, FIAC and FMAU were highly active against HSV-2 whereas the halogenovinyl derivatives were not (Table 1).

Therapeutic efficacy on HSV-1 encephalitis in mice

As shown in Table 2, multiple oral doses of 50 mg/kg or 100 mg/kg of either BV-araU, CV-araU, araT or BVDU were effective in increasing the mean survival time of mice infected i.c. with 30 LD_{50} of HSV-1 in agreement with previous findings [9]. Intraperitoneal treatment with 50 mg/kg or 100 mg/kg of BV-araU and BV-araUMP and 100 mg/kg of CV-araU also increased the mean survival time of the infected mice (data not shown). However, neither of the drugs brought about a marked increase in the final survival rate of the infected mice, whether treated p.o. or i.p. This was most likely due to the relatively high dose of the virus inoculum (30 LD_{50}). When the virus inoculum was decreased (6 LD_{50}), p.o. treatment with 50 mg/kg or 200 mg/kg of BV-araU effected a statistically significant increase in the final mortality rate of the infected mice (Table 3). Treatment with 12.5 mg/kg or 25 mg/kg of BV-araU was effective in increasing the mean survival time but did not significantly decrease the final mortality rate. As seen in Table 4, p.o. treatment with BV-araU was almost as effective as BVDU and araT in increasing the mean survival time and in decreasing the mortality rate. Intravenous treatment with BV-araU was also effective in both decreasing mortality and increasing the mean survival time (Table 5). Likewise, i.v. treatment with BV-araUMP (50 mg/kg) and BVDU (200 mg/kg) significantly reduced the mortality rate. Thus, in terms of efficacy in the HSV-1 encephalitis model in mice, BV-araU seems to be comparable to BVDU and araT.

TABLE 1

Anti-HSV activity of thymidine analogues, as determined by the plaque reduction method

Compound	50% plaque reduction dose (μg/ml)					
	HSV-1			HSV-2		
	VR-3	CHR-3	MP	MS	UW-268	HG-52
BV-araU	0.010	0.014	0.006	7.3	11.7	26.3
CV-araU	0.018	0.017	0.017	19.5	8.4	26.6
IV-araU	0.014	0.016	0.005	4.3	4.2	18.0
BV-araUMP	0.013	0.011	0.005	15.0	15.1	26.9
AraT	0.12	0.19	0.29	0.22	0.52	0.66
Vinyl-araU	0.021	0.016	0.024	1.25	2.82	3.98
IDU	0.28	0.40	0.66	1.95	6.1	6.2
BVDU	0.017	0.014	0.011	10.8	15.0	5.8
ACV	0.041	0.049	0.020	0.11	0.051	0.03
FIAC	0.013	0.015	0.008	0.019	0.011	0.023
FMAU	0.005	0.006	0.003	0.011	0.004	0.007

TABLE 2

Effect of peroral (p.o.) treatment with BV-araU, CV-araU, araT and BVDU on HSV-1 encephalitis in mice infected with a high virus dose

Treatment		Survivors/total	Mean survival time (days ± S.E.)
Compound	Dose (mg/kg)		
PBS (control)		0/19	4.0 ± 0.22
BV-araU	50	2/10	7.1 ± 1.22 $P < 0.001$**
BV-araU	100	3/10 $P < 0.05$*	6.6 ± 0.42 $P < 0.001$
CV-araU	50	2/10	5.9 ± 0.48 $P < 0.001$
CV-araU	100	1/10	5.9 ± 0.26 $P < 0.001$
AraT	50	2/9	6.9 ± 0.46 $P < 0.001$
AraT	100	2/9	7.4 ± 0.72 $P < 0.001$
BVDU	50	1/10	5.8 ± 0.32 $P < 0.001$
BVDU	100	2/9	5.7 ± 0.18 $P < 0.001$

Mice were infected i.c. with 30 LD_{50} of HSV-1 VR-3 and treated p.o. with drugs twice daily for 4.5 days.
*Fisher's exact test.
**Student's *t*-test.

TABLE 3

Effect of peroral (p.o.) treatment with BV-araU on HSV-1 encephalitis in mice infected with low virus dose

Dose of BV-araU (mg/kg)	Survivors/total	Mean survival time (days ± S.E.)
0 (control)	1/20	5.1 ± 0.34
12.5	2/20	6.1 ± 0.45 $P < 0.05$**
25	3/20	6.4 ± 0.36 $P < 0.01$
50	7/20 $P < 0.05$*	6.8 ± 0.23 $P < 0.001$
200	13/20 $P < 0.01$	7.1 ± 0.46 $P < 0.01$

Mice were infected i.c. with 6 LD_{50} of HSV-1 VR-3 and treated p.o. with drugs twice daily for 4.5 days.
*χ^2 test with Yates' correction.
**Student's *t*-test.

Toxicity for mice

In preliminary toxicological studies BV-araU was found to exhibit little, if any, toxicity for mice. The LD_{50} value of BV-araU was about 3300 mg/kg upon a single i.p. administration. The LD_{50} values for subcutaneous and p.o. administration were > 5 g/kg and > 10 g/kg, respectively. BV-araUMP was somewhat more toxic than BV-araU upon i.p. administration (LD_{50} = 1750 mg/kg). Its LD_{50} value upon i.v. injection was 1360 mg/kg. In a separate set of experiments the toxicity of BV-araU was compared to that of BVDU. The LD_{50} values of BV-araU and BVDU, both injected i.p., were estimated to be 3154 mg/kg (2919–3417 mg/kg) and 1466 mg/kg (1327–1620 mg/kg), respectively. The difference in the LD_{50} values of BV-araU and BVDU was statistically significant at $P < 0.05$.

TABLE 4

Effect of peroral (p.o.) treatment with BV-araU, BVDU and araT on HSV-1 encephalitis in mice infected with low virus dose

Treatment		Survivors/total	Mean survival time (days ± S.E.)
Compound	Dose (mg/kg)		
PBS (control)		5/26	6.3 ± 0.42
BV-araU	50	12/20 $P < 0.025$*	7.4 ± 0.65 $P < 0.01$**
BV-araU	200	14/20 $P < 0.005$	9.7 ± 0.99 $P < 0.01$
BVDU	50	9/20	8.5 ± 0.37 $P < 0.01$
BVDU	200	16/20 $P < 0.001$	10.3 ± 2.59 $P < 0.01$
AraT	50	11/20 $P < 0.05$	8.1 ± 0.63 $P < 0.05$
AraT	200	12/20 $P < 0.025$	11.1 ± 1.00 $P < 0.001$

Mice were infected i.c. with 3 LD_{50} of HSV-1 VR-3 and treated p.o. with drugs twice daily for 4.5 days.
*χ^2 test with Yates' correction.
**Student's t-test.

TABLE 5

Effect of intravenous (i.v.) treatment with BV-araU, BV-araUMP and BVDU on HSV-1 encephalitis in mice

Treatment		Survivors/total	Mean survival time (days ± S.E.)
Compound	Dose (mg/kg)		
PBS (control)		1/32	5.7 ± 0.28
BV-araU	50	5/20 $P < 0.05$*	7.5 ± 0.38 $P < 0.01$**
BV-araU	200	10/20 $P < 0.001$	8.0 ± 0.47 $P < 0.001$
BV-araUMP	50	8/20 $P < 0.005$	7.0 ± 0.33 $P < 0.01$
BV-araUMP	200	4/20	8.5 ± 0.43 $P < 0.001$
BVDU	50	3/20	6.9 ± 0.43 $P < 0.01$
BVDU	200	13/20 $P < 0.001$	13.3 ± 1.38 $P < 0.001$

Mice were infected i.c. with 6 LD_{50} of HSV-1 VR-3 and treated i.v. with drugs twice daily for 6.5 days.
*χ^2 test with Yates' correction.
**Student's t-test.

Discussion

BV-araU, CV-araU, IV-araU and BV-araUMP are highly effective in inhibiting plaque formation of HSV-1. They are slightly more active than vinyl-araU against HSV-1. The anti-HSV-2 activity of CV-araU and IV-araU was as low as that of BV-araU. BV-araUMP also exhibited a poor activity against HSV-2. BVDU is also notorious for its much lower activity against HSV-2 than HSV-1. The differential susceptibility of HSV-1 and HSV-2 to inhibition by BVDU, BV-araU and the other halogenovinyl derivatives may be related to deficient phosphorylation of the 5'-monophosphates to 5'-diphosphates in HSV-2 infected cells [4].

BV-araU and BV-araUMP were found to be effective in both increasing the mean survival time and decreasing the mortality of mice infected i.c. with HSV-1. In the treatment of herpetic encephalitis, BV-araU was as effective as BVDU. The latter compound has been reported to be highly efficacious against experimental encephalitis caused by HSV-1 infection [6,11]. However, the in vivo efficacy of these halogenovinyl derivatives was lower than expected from their in vitro activity. The in vitro anti-HSV-1 activity of these compounds was much higher than that of araT, whereas their in vivo efficacy was similar. We are now searching the optimal treatment schedule for the therapeutic use of BV-araU and BV-araUMP.

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