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In vitro and in vivo anti-herpes viral activities and biological properties of CV-araU

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Abstract

We compared the in vitro and in vivo antiviral effects against herpes simplex virus type 1 (HSV-1) and other biological properties of 1-β-D-arabinofuranosyl-5-[(*E*)-2-chlorovinyl]uracil (CV-araU) and 1-β-D-arabinofuranosyl-5-[(*E*)-2-bromovinyl]uracil (BV-araU, sorivudine). Both CV-araU and BV-araU exhibited antiviral activities against HSV-1 in the cell culture derived from mouse, though the activities were lower than those seen in human cells. For intraperitoneal and intracerebral infections in mice with HSV-1 strain WT-51, both compounds, administered twice daily, were effective in increase in the survival rate at doses of 15 mg/kg and 30 mg/kg, respectively. In pharmacokinetic analysis, both drugs were absorbed well in the rat gastrointestinal tract following oral administration. There was no difference between the metabolism of orally administered CV-araU and BV-araU in rats. High levels of the corresponding base were found in plasma after oral administration of CV-araU and BV-araU, but much lower base levels were seen after intravenous doses. Both drugs were resistant to degradation by rat liver enzymes.

Keywords: Herpes simplex virus type 1; Model infection; CV-araU; BV-araU; Sorivudine

1-β-D-Arabinofuranosyl-5-[(*E*)-2-bromovinyl]uracil (BV-araU, sorivudine), has demonstrated potent and selective antiviral activity against the herpes simplex virus type 1 (HSV-1) and the varicella-zoster virus (VZV) in cell culture (Machida et al., 1981; Machida and Sakata, 1984; Machida, 1986). BV-araU has also exhibited antiviral effects

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Table 1
Antiviral activities of CV-araU and BV-araU aginst HSV-1 strain WT-51 in HEL and Balb/3T3 cells

Compound		Concentration of drug (μg/ml) [a]	
		HEL	Balb/3T3
CV-araU	ED_{50}	0.016	1.67
	ED_{90}	0.059	> 32
BV-araU	ED_{50}	0.020	0.79
	ED_{90}	0.070	20.3

[a] Geometric mean of 2 separate experiments.

against several experimental HSV-1 infections in mice (Machida and Takezawa, 1990; Machida et al., 1990; Machida et al., 1992). Oral BV-araU has been used effectively in the treatment of patients with herpes zoster (Niimura, 1990; Hiraoka et al., 1991), and was approved for the treatment of zoster in Japan in 1993. A congener of BV-araU, 1-β-D-arabinofuranosyl-5-[(*E*)-2-chlorovinyl]uracil (CV-araU), is one of a series of 5-halogenovinyl-arabinofuranosyluracils synthesized in our laboratory (Sakata et al., 1980). CV-araU has been reported to exhibit potent and selective anti-HSV-1 and anti-VZV activities in human embryonic lung (HEL) cells, comparable to those of BV-araU (Machida et al., 1981; Machida et al., 1982), but has not been studied in great detail concerning in vivo efficacy. In the present study, we examined the in vitro and in vivo antiviral activities and other biological properties of CV-araU, in comparison to those of BV-araU.

In vitro antiviral activity of CV-araU against HSV-1 clinical isolate WT-51, which was also used in in vivo study, was compared with that of BV-araU. The method for the plaque reduction assay is described previously (Machida, 1990). Both CV-araU and BV-araU exhibited anti-HSV-1 activities in Balb/3T3 clone A31 cells derived from mouse. The mean 50% plaque reduction doses (ED_{50}) of CV-araU and BV-araU were 1.67 μg/ml and 0.79 μg/ml, respectively (Table 1). However, the ED_{50} values of CV-araU and BV-araU in Balb/3T3 cells were more than two orders of magnitude higher than those in HEL cells.

In vivo antiviral activities of drugs were evaluated in systemic infection and experimental encephalitis in mice. The HSV-1 clinical isolate WT-51 was used as a challenge in both model infections. The methods of inoculation, drug treatment, and evaluation of efficacies are described previously (Machida et al., 1990). In intraperitoneal (i.p.) infections, the CV-araU significantly increased the survival rate at a dose of 5 mg/kg, and BV-araU had a similar effect (Table 2, Exp. 1). When the experiment was repeated (Exp. 2), both drugs were efficacious at a dose of 15 mg/kg. In experimental encephalitis, the CV-araU significantly increased the survival rate at a dose of 30 or 100 mg/kg. BV-araU was able to increase the survival rate at a similar dose. Both drugs prolonged the survival time at a dose of 10 mg/kg.

Pharmacokinetic was studied in rats by the method as described previously (Ashida et al., 1993). Briefly, a dose of 100 μmol/kg of a drug was administered by gavage or intravenously (i.v.) to groups of five male, 8-week-old, SD rats. The concentrations of CV-araU, 5-[(*E*)-2-chlorovinyl]uracil (CVU), BV-araU, and 5-[(*E*)-2-bromovinyl]uracil

Table 2
Effect of CV-araU and BV-araU on infections with HSV-1 in mice

	Compound	Dose (mg/kg)	Survivors per total	Mean survival time (days ± S.E.)
i.p. infection				
Exp. 1	Control	–	3/20	7.7 ± 0.35
	CV-araU	5	10/20 $P < 0.05$ [a]	7.6 ± 0.42
		15	14/20 $P < 0.001$	10.3 ± 0.93 $P < 0.05$ [b]
		50	17/20 $P < 0.001$	10.0 ± 1.53
	BV-araU	5	13/20 $P < 0.001$	8.0 ± 0.51
		15	14/20 $P < 0.001$	9.3 ± 1.41
		50	18/20 $P < 0.001$	14.0 ± 3.00
Exp. 2	Control	–	3/20	7.8 ± 0.35
	CV-araU	5	4/20	7.6 ± 0.27
		15	15/20 $P < 0.001$	8.1 ± 0.75
		50	19/20 $P < 0.001$	6.5
	BV-araU	5	5/20	8.5 ± 0.51
		15	10/20 $P < 0.05$	7.8 ± 0.53
		50	18/20 $P < 0.001$	8.3 ± 0.25
i.c. infection				
Exp. 1	Control	–	2/19	4.8 ± 0.19
	CV-araU	10	1/19	7.4 ± 0.51 $P < 0.001$
		30	7/18	7.9 ± 0.84 $P < 0.001$
		100	17/19 $P < 0.001$	6.8 ± 1.25
	BV-araU	10	3/19	7.8 ± 0.43 $P < 0.001$
		30	13/19 $P < 0.001$	8.5 ± 0.91
		100	17/19 $P < 0.001$	8.6 ± 2.25
Exp. 2	Control	–	0/20	5.7 ± 0.62
	CV-araU	10	2/20	7.7 ± 0.55 $P < 0.01$
		30	12/20 $P < 0.001$	8.1 ± 0.54 $P < 0.01$
		100	18/20 $P < 0.001$	7.5 ± 0.71
	BV-araU	10	5/20	7.8 ± 0.47 $P < 0.01$
		30	16/20 $P < 0.001$	8.1 ± 0.66 $P < 0.05$
		100	16/20 $P < 0.001$	7.3 ± 0.15

[a] χ^2 analysis with Yates' correction.
[b] Mann-Whitney U-test.

(BVU) in plasma and urine were determined using high-pressure liquid chromatography (HPLC). Fig. 1 shows their blood concentrations after the i.v. or oral administration of CV-araU and BV-araU. Both CV-araU and BV-araU were absorbed well in the rat gastrointestinal tract. The blood levels of CVU following the i.v. administration of CV-araU was below detection limit by HPLC (1 μM) (Fig. 1A). In contrast, considerable amounts of CVU were found in the plasma after oral administration. Plasma levels of BVU following the i.v. administration remained low for 12 h (Fig. 1B). High levels of BVU were detected in the plasma after oral administration, even higher than those of CVU following oral administration of CV-araU. Fig. 2 shows the levels of CV-araU and BV-araU in rat urine following the i.v. or oral administration of the compounds. When

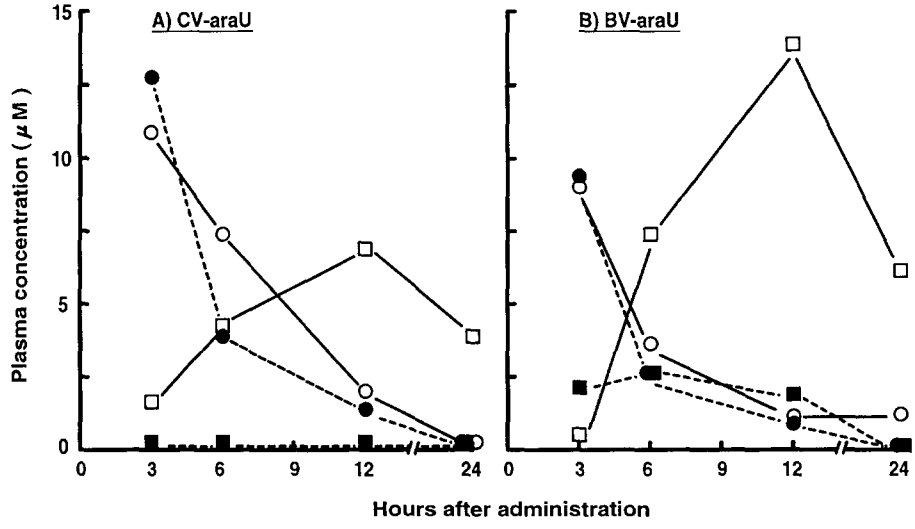

Fig. 1. Blood concentrations of CV-araU, CVU, BV-araU, and BVU in rats after administration of CV-araU and BV-araU. CV-araU (A) or BV-araU (B) was administered i.v. (broken lines) or orally (solid lines) at a dose of 100 μmol/kg. Blood concentrations of each unchanged nucleoside (circles) and deglycosylated base (squares) were determined by HPLC. Each point represents the mean of 5 rats.

drugs were administered i.v., over 70% of the dose was recovered from the urine collected for 24 h after administration. The levels of CV-araU and BV-araU recovered from the urine after oral administration were less than half of those present after i.v.

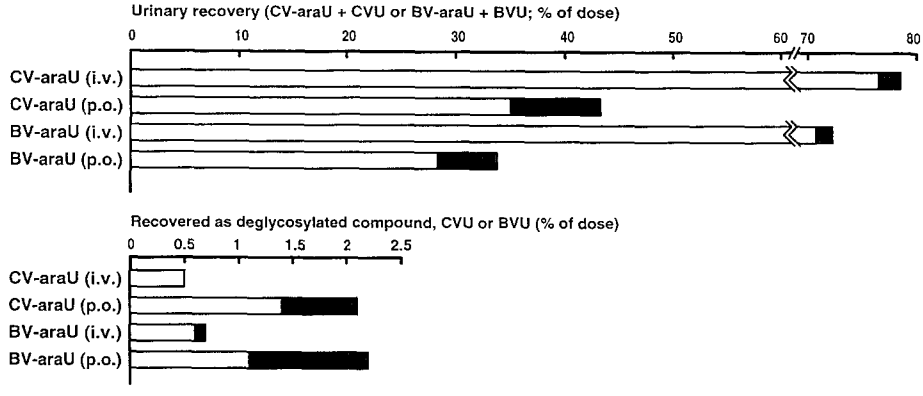

Fig. 2. Urinary recovery of CV-araU and BV-araU. Rats were given CV-araU or BV-araU orally (p.o.) or i.v., at a dose of 100 μmol/kg. Urine was collected for 0–12 h (open bars) and for 12–24 h (shaded bars) and analyzed by HPLC. Total urinary recovery, nucleoside plus base (upper), and recovery as deglycosylated compound, CVU or BVU (bottom) are expressed separately as a percentage of the dose. Each value represents the mean of 5 rats.

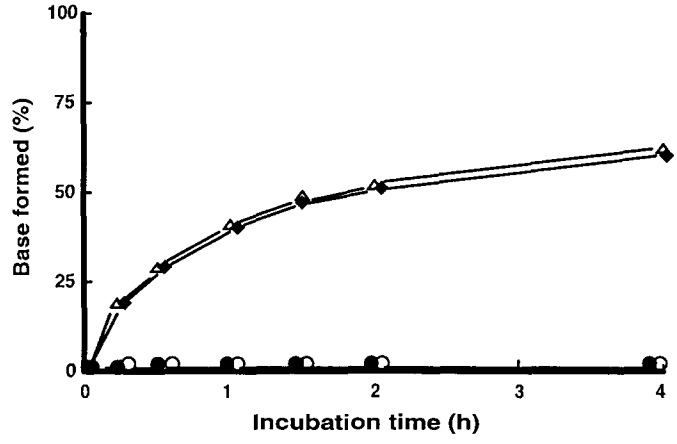

Fig. 3. Conversion of CV-araU and BV-araU to corresponding base by rat liver extract. CV-araU (○), BV-araU (●), BVDU (◆), and thymidine (△) were incubated with rat river extract at 37°C, and were analyzed by HPLC. Conversion to the base is presented as the molar percent of each compound in the reaction mixture. Each point represents the mean of two separate experiments.

administration. The amounts of CVU and BVU were approximately equal, regardless of administration method.

Deglycosylation of the drugs by rat river extract was studied by the method as described previously (Ashida et al., 1993). CV-araU and BV-araU containing an arabinose moiety were resistant to degradation by rat liver enzymes (Fig. 3). In contrast, a deoxyribonucleoside congener of BV-araU, *E*-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and thymidine were easily deglycosylated by rat liver extract.

The oral administration of CV-araU or BV-araU was effective against experimental HSV-1 infections in mice. A previous report indicates that BV-araU is readily absorbed in the gastrointestinal tract of rats (Nishimoto et al., 1990). Our study shows that CV-araU is also absorbed well in rats, and CV-araU exhibits anti-HSV-1 activity in the cells derived from mouse. These results support that the oral administration of CV-araU is effective against experimental infections in mice. In rats, CV-araU and BV-araU were metabolized in a similar manner; both were quickly broken down into the corresponding base following oral administration. In contrast, little or no BVU has been detected following the oral administration of BV-araU to germ-free rats (Ashida et al., 1993). Furthermore, only a minimal level of the base was seen after i.v. administration, and both CV-araU and BV-araU were resistant to degradation by rat liver enzymes. Thus, it appears that the degradation of orally administered CV-araU and BV-araU in rats is predominantly due to the action of enterobacteria.

In summary, our study shows that CV-araU, like BV-araU, exhibits marked antiviral activity in vivo and is well absorbed in the rat gastrointestinal tract. The substitution of bromine with chlorine at the halogenovinyl group appears to have little effect on in vivo antiviral activity and other biological properties.

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