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Efficacy of oral treatment with BV-araU against cutaneous infection with herpes simplex type 1 in shaved mice

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Summary

The effect of oral BV-araU was tested in cutaneous model infections of shaved Balb/c mice with herpes simplex virus type 1 (HSV-1). Progression of cutaneous symptoms associated with cutaneous infection with HSV-1 F strain was inhibited by BV-araU at doses of 20 and 50 mg/kg twice daily, beginning one day post-infection, resulting in significant increase in the survival rate. Onset of disease was suppressed in most animals receiving 100 mg of BV-araU per kg. BV-araU (20 mg/kg or more) also significantly increased the survival rate of mice infected with HSV-1 WT-51 strain. The efficacy of BV-araU was not affected by gender or age (6–9 weeks) of the mice. BV-araU was effective even when the treatment was started 2.5 days post-infection. The efficacy of BV-araU against F strain infection was comparable to that of acyclovir, but acyclovir showed therapeutic effects at lower doses compared with BV-araU against WT-51 strain infection. Against infection of cyclophosphamide-treated immunosuppressed mice with HSV-1 KOS(S) strain, BV-araU decreased the morbidity rate and severity of symptoms at doses of 200 and 100 mg/kg, respectively, and all mice given 50 mg of BV-araU or more per kg survived, suggesting oral efficacy can be achieved against HSV-1 infections in immunosuppressed individuals.

BV-araU; HSV-1; Mouse model; Skin infection; Immunocompromised

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Introduction

1-β-D-Arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU) exhibits potent antiviral activities against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) in cell culture (Machida et al., 1981; Machida, 1986). Its clinical efficacy for the treatment of zoster in immunocompetent patients has been demonstrated in Japan (Niimura et al., 1990). Marked effectiveness of BV-araU was also demonstrated against intracerebral (i.c.) and intraperitoneal (i.p.) infections with HSV-1 in mice (Machida and Sakata, 1984; Machida and Takezawa, 1990). The efficacy of BV-araU against i.c. infections was much more marked than that of acyclovir, although acyclovir seemed more efficacious than BV-araU against i.p. infections (Machida et al., 1990). Among various animal models of HSV-1 infection, the cutaneous infection model in mice, as well as the encephalitis model (i.c. infection), is considered to closely simulate HSV-1 infections in humans. Local treatment with 10% BV-araU in DMSO is effective in inhibiting the onset of cutaneous symptoms by HSV-1 infection in hairless mice (De Clercq, 1984). However, no study was reported on the effect of systemic administration of BV-araU in this system. We now report on the marked therapeutic effects of oral BV-araU on HSV-1 cutaneous infections in immunologically normal and immunosuppressed shaved Balb/c mice.

Materials and Methods

Animals

Male and female Balb/c mice (6–8 weeks of age) had been bred in this laboratory starting from the colony obtained from Clea Japan Inc. or were purchased from Clea Japan Inc. The backs of the mice were shaved with a razor 1 or 2 days before virus inoculation. To induce immunosuppression, mice were treated i.p. with 200 mg of cyclophosphamide (Aldrich Chemical Co., Inc.) per kg body weight one day before virus inoculation according to the method of Ikeda et al. (1988).

Virus

Three strains of HSV-1 were used; strain WT-51, a recent clinical isolate from a patient with herpes keratitis, was donated by Dr. Kurimura, Research Institute for Microbial Diseases of Osaka University, F strain was obtained from Dr. Hayashi of Kobe Environment and Health Research Institute, and KOS(S) strain, one of the sub-strains of the KOS strain, was kindly supplied by Dr. Y. Ozaki, Shiga Medical College. Strain KOS(S) was avirulent for normal mice, and lethal infection was not caused in them by i.p. inoculation (Ijichi et al., 1990). All three strains used were quite susceptible to BV-araU and

TABLE 1

Susceptibilities of HSV-1 strains to BV-araU and acyclovir determined in mouse 3T3 cells and HEL cells

Cells	Drug	50% plaque reduction dose[a] (μg/ml) for:		
		WT-51	F	KOS(S)
Balb/3T3	BV-araU	0.135	0.125	0.137
	Acyclovir	0.076	0.079	0.064
HEL	BV-araU	0.030	0.036	0.082
	Acyclovir	0.097	0.138	0.118

[a]ED_{50} values were determined by the plaque reduction method.

acyclovir when tested in mouse cells (Balb/3T3 cells) by a plaque reduction method (Table 1). There was little difference in susceptibilities of these strains to each drug in human embryonic lung fibroblast (HEL) cells, too. Generally, these HSV-1 strains were slightly less susceptible to BV-araU than acyclovir in mouse cells, while they were more susceptible to BV-araU in HEL cells.

Viral inoculation

The shaved backs of Balb/c mice were abraded with a needle, and 15 μl of a viral suspension containing HSV-1 WT-51 strain (1.5×10^4 plaque forming units, pfu), F strain (1.5×10^5 pfu for normal mice and 5×10^3 pfu for immunosuppressed mice) or KOS(S) strain (3.3×10^5 pfu) was inoculated onto the backs according to the method of Nagafuchi et al. (1979). All WT-51 and F strain infected mice showed severe zosteriform skin lesions, and most of them died between 6–9 days post-infection. For KOS(S) strain-infected mice the skin lesions spontaneously resolved 2 to 3 weeks after inoculation, while immunosuppressed mice all died due to the KOS(S) strain infection.

Drug treatment and evaluation of efficacy

Drugs were dissolved in physiological saline or suspended in physiological saline containing 0.5% carboxymethylcellulose (Nacalai Tesque, Inc.). In general, the drugs were orally administered in a volume of 1.0 ml/100 g body weight twice a day for 7 days starting one day after infection. The control mice received physiological saline. Mice were observed for skin lesions and death twice a day. The degree of the symptoms was scored according to the following criteria:

Score 1: just perceptible small vesicles, erythema, or crust in local region.

Score 2: many small vesicles or large vesicles in local region, or zosteriform crust in small region.

Score 3: erosion, ulceration, crust with ulceration in local region, zosteriform erythema and vesicles or erosion in small region, or zosteriform crust in middle region.

Score 4: zosteriform erosion with ulceration or crust with ulceration in small region, zosteriform erosion in middle region, or zosteriform crust.
Score 5: zosteriform ulceration or crust with ulceration in middle region, or zosteriform erosion or crust with ulceration.
Score 6: zosteriform ulceration with erosion or ulceration with crust.
Score 7: severe zosteriform ulceration.
Score 8: severe zosteriform ulceration with systemic symptoms.

Differences in the mean lesion score at each time point between control and drug-treated groups and in the mean survival rate were statistically evaluated by the Mann-Whitney U-test and chi-square test with Yates' correction, respectively.

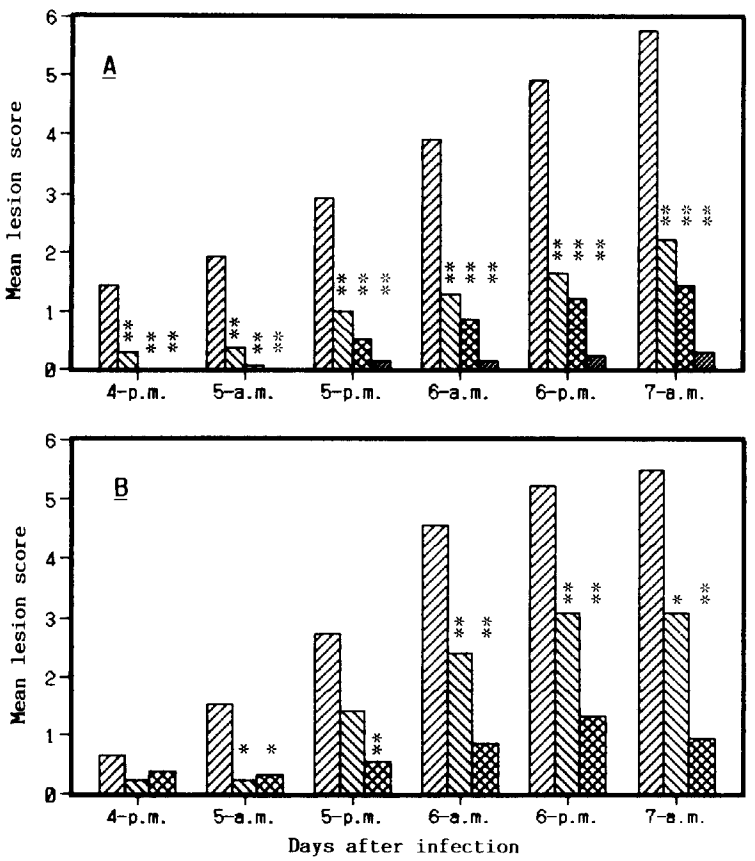

Fig. 1. Effect of oral treatment with BV-araU on cutaneous infections with HSV-1 F and WT-51 strains in shaved Balb/c mice. Seven-week-old shaved male Balb/c mice, in groups of 14 animals, were infected with HSV-1 F strain (A) or eight-week-old female mice were infected with WT-51 strain (B). Infected mice were treated orally with BV-araU twice daily for 7 days beginning one day after inoculation. ▨ control, ▧ 20 mg/kg, ▩ 50 mg/kg and ▩ 100 mg/kg. $^{*}P < 0.05$, $^{**}P < 0.01$ by Mann-Whitney U-test.

Results

Effects of BV-araU on cutaneous infections with HSV-1 F and WT-51 strains in normal mice

Fig. 1 illustrates changes in mean lesion score of cutaneous symptoms in shaved Balb/c mice infected with HSV-1 F or WT-51 strain. BV-araU at a dose of 20 mg/kg or higher twice a day markedly inhibited progression of cutaneous symptoms, resulting in a significant increase in the survival rate. Zosteriform symptoms occurred in no mice given 100 mg of BV-araU per kg body weight. BV-araU also significantly suppressed symptoms and increased the survival rate at doses of 20 and 50 mg/kg in animals infected with the HSV-1 WT-51 strain (Fig. 1B). However, slightly more marked inhibition of the progression of cutaneous symptoms and earlier complete cure were seen in F strain infection than in WT-51 strain infection.

Effects of sex and age of mice and drug treatment schedule

Whether or not the sex and age (factors related to the host) affect therapeutic efficacy of BV-araU was determined in experiments conducted in the same way. Efficacy of BV-araU found in female mice infected with either HSV-1 F or WT-51 strain was comparable to that in males (data not shown). BV-araU also showed marked efficacy regardless of age of infection of mice ranging from 6 to 9 weeks (Fig. 2).

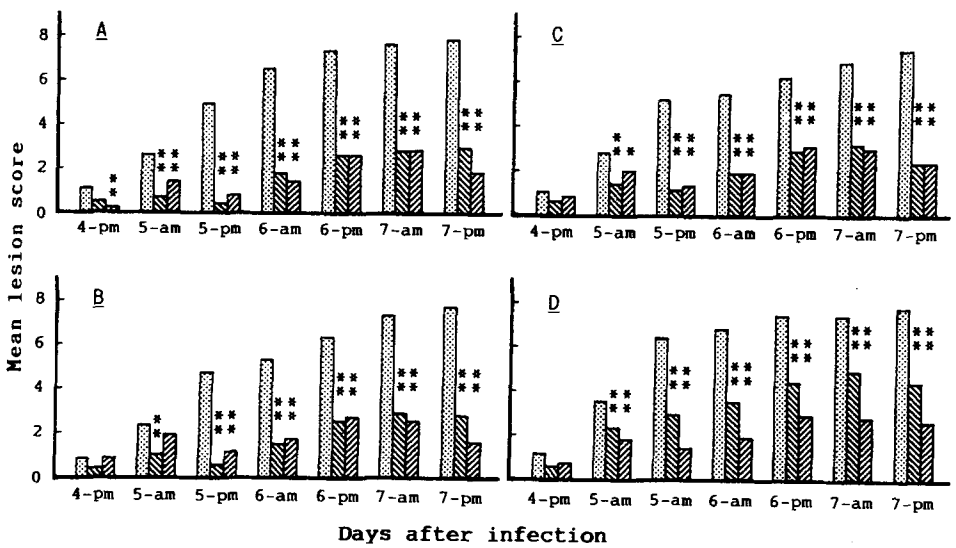

Fig. 2. Effect of oral treatment with BV-araU on cutaneous infection with HSV-1 WT-51 strain in shaved Balb/c mice of different ages. Shaved female Balb/c mice of 6- (A), 7- (B), 8- (C), and 9-weeks (D) of age, in groups of 9 or 10 animals, were infected with HSV-1 WT-51 strain and treated with BV-araU twice daily for 7 days beginning one day after inoculation. ▒ control, ▨ 50 mg/kg and ▨ 100 mg/kg. $^{*}P < 0.05$, $^{**}P < 0.01$ by Mann-Whitney U-test.

BV-araU at a dose of 100 mg/kg significantly suppressed the progression of symptoms and increased the survival rate even when the treatment was initiated 2.5 days after infection with WT-51 strain. However, it was not effective when treatment was initiated 4.5 days post-infection (Fig. 3A). The efficacy of 4-day treatment was comparable to that of 6- or 8-day treatment when the treatment was initiated one day after infection. At a dose of 20 mg/kg, BV-araU was effective in inhibiting progression of symptoms when the treatment started either 2 or 3 days after infection with F strain (Fig. 3B), but failed significantly to increase the survival rate of infected mice.

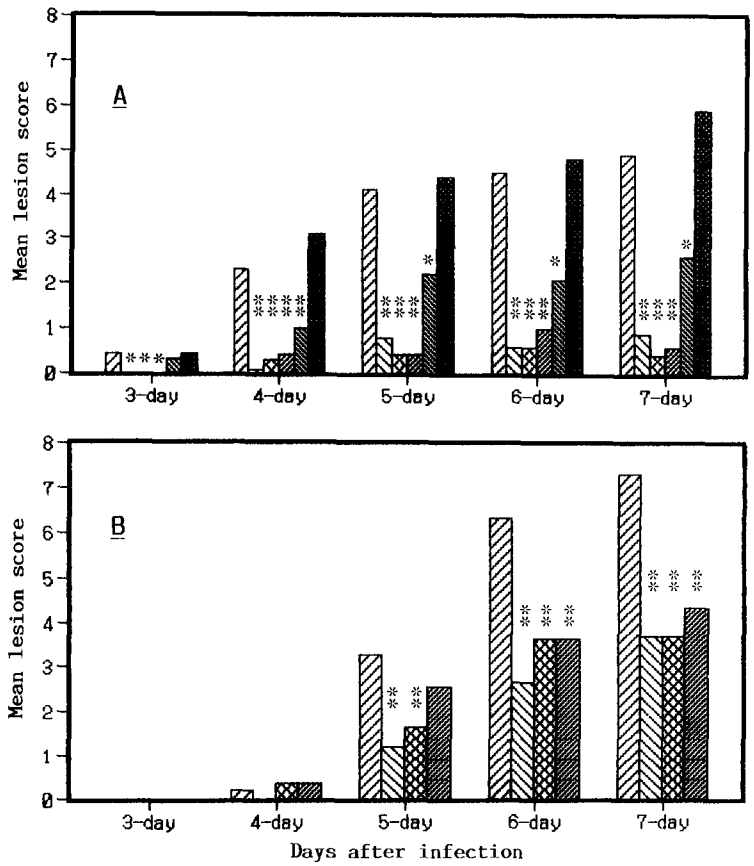

Fig. 3. Influence of treatment schedule on the efficacy of oral BV-araU against cutaneous infection with HSV-1 in shaved Balb/c mice. (A) Seven-week-old shaved female Balb/c mice, in groups of 16 animals, were infected with HSV-1 WT-51 strain. (B) Eight-week-old shaved male Balb/c mice, in groups of 14 animals, were infected with HSV-1 F strain. Infected mice were treated with BV-araU (100 mg/kg in A, and 20 mg/kg in B) twice daily for various periods; (A) control, day 1–8; day 1–6; day 1–4; day 2.5–10; and day 4.5–10; (B) control; day 1–7; day 2–8; and day 3–9. $^{*}P < 0.05$, $^{**}P < 0.01$ by Mann-Whitney U- test.

TABLE 2

Effects of oral treatments with BV-araU and acyclovir on cutaneous infection with HSV-1 in shaved Balb/c mice

Virus strain	Treatment		No. of survivors per total	No. of mice of which the maximum symptom score is:				Mean survival time (days ± SE)
	Drug	Dose (mg/kg)		<1	<2	<3	>4	
F	Saline (control)		1/14	0	0	1	13	6.88 ± 0.32
	BV-araU	5	1/15	0	0	2	13	7.00 ± 0.33
	BV-araU	10	3/12	0	0	4	8	7.06 ± 0.52
	BV-araU	20	7/14[a]	4	2	3	5[a]	8.07 ± 0.52
	Acyclovir	5	2/14	0	1	3	10	7.29 ± 0.35
	Acyclovir	10	7/14[a]	1	1	1	11	7.93 ± 0.51
	Acyclovir	20	12/13[b]	5[c]	1	2	5[a]	7.5
WT-51	Saline (control)		0/13	0	0	0	13	6.77 ± 0.24
	BV-araU	20	2/14	1	0	0	13	7.25 ± 0.25
	BV-araU	50	6/13[c]	2	0	3	8[c]	10.07 ± 0.89[d]
	BV-araU	100	14/14[b]	8[c]	3	2	1[b]	
	Acyclovir	20	13/14[b]	4	0	2	8[c]	7
	Acyclovir	50	14/14[b]	10[b]	1	2	1[b]	
	Acyclovir	100	14/14[b]	12[b]	0	1	1[b]	

Shaved 7-week-old Balb/c mice, in groups of 12 to 15 animals, were infected with HSV-1 F strain or WT-51 strain, and treated with BV-araU or acyclovir twice daily for 7 days beginning one day post-infection.

[a] $P < 0.01$ by χ^2-analysis with Yates' correction.

[b] $P < 0.001$ by χ^2-analysis with Yates' correction.

[c] $P < 0.05$ by χ^2-analysis with Yates' correction.

[d] $P < 0.01$ by Student's t-test.

Comparison of efficacies of BV-araU and acyclovir

BV-araU and acyclovir were equally effective in inhibiting the progression of symptoms due to infection with HSV-1 F strain (Table 2). Both drugs significantly suppressed the progression of symptoms at a dose of 10 mg/kg. However, the minimum dose of BV-araU required for significant increase in the survival rate was 20 mg/kg, while that of acyclovir was 10 mg/kg. In animals infected with WT-51 strain, both BV-araU at a dose of 50 mg/kg and acyclovir at a dose of 20 mg/kg significantly inhibited the progression of cutaneous symptoms and increased the survival rate. All mice given BV-araU (100 mg/kg) or acyclovir (50 and 100 mg/kg) survived.

Efficacies for cutaneous infection in immunosuppressed mice

Efficacies of oral BV-araU against cutaneous infection with HSV-1 KOS(S) strain in immunosuppressed mice and normal mice were examined. Skin lesions developed were spontaneously resolved in most normal mice within three weeks after infection (Fig. 4), and no mice died. In contrast, immunosuppressed mice

died after showing severe symptoms. BV-araU at a dose of 200 mg/kg strongly inhibited the progression of symptoms in all normal mice and completely suppressed onset of lesions in about a half of the infected mice. The treatment of immunosuppressed mice showed both delay of onset in all mice and inhibition of the progression of symptoms in most mice. The lesions were completely resolved in most mice within two weeks after infection. Even at lower doses (50 and 100 mg/kg), BV-araU was effective in inhibiting progression of symptoms in the KOS(S) strain infected immunosuppressed mice (data not shown), and mice receiving 50 mg of BV-araU per kg of body weight or more all survived. The survival rate of mice given 20 mg of BV-araU per kg body weight was 81%, while that of control mice was 12.5% ($P < 0.001$ by χ^2-analysis with Yates' correction).

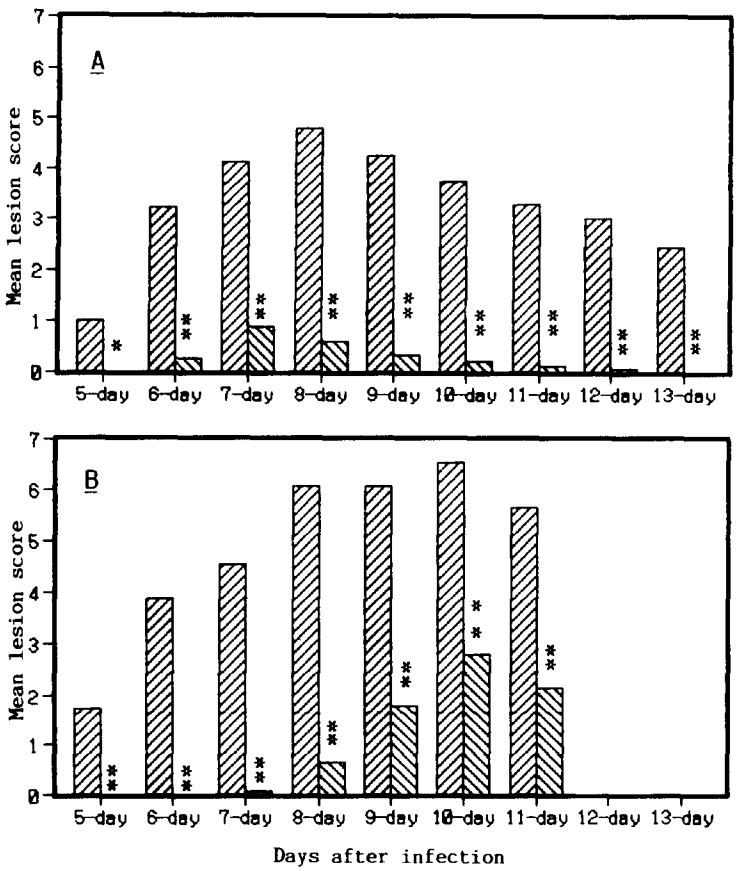

Fig. 4. Effect of oral treatment with BV-araU on cutaneous infection with HSV-1 KOS(S) strain in shaved Balb/c mice. Eight-week-old shaved male normal (A) or immunosuppressed (B) Balb/c mice, in groups of 15 animals, were infected with HSV-1 KOS(S) strain and treated with physiological saline (▨) or 200 mg of BV-araU per kg body weight (▧) twice daily for 7 days beginning one day after inoculation. Since most of cyclophosphamide-treated control mice died 12 days post-infection, the statistical analysis was not available for cyclophosphamide-treated groups on that day and later. * $P < 0.05$, ** $P < 0.01$ by Mann-Whitney U-test.

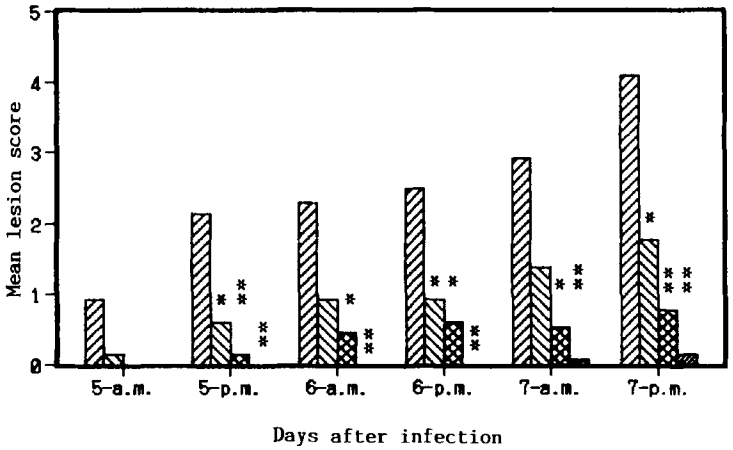

Fig. 5. Effect of oral treatment with BV-araU on cutaneous infection with HSV-1 F strain in shaved immunosuppressed Balb/c mice. Groups of 13 or 14 8-week-old, cyclophosphamide-treated shaved female Balb/c mice were infected cutaneously with 5×10^3 pfu of HSV-1 F strain and treated orally with BV-araU at various doses twice daily for 7 days beginning one day after inoculation. ▨ control, ▩ 20 mg/kg, ▩ 50 mg/kg, and ▩ 100 mg/kg. $^{*}P < 0.05$, $^{**}P < 0.01$ by Mann-Whitney U-test.

Against infection of immunosuppressed mice with F strain, BV-araU also markedly inhibited the progression of skin symptoms (Fig. 5). The survival rates of the F strain-infected immunosuppressed mice in control and BV-araU-treated groups at doses of 20 mg/kg, 50 mg/kg, and 100 mg/kg were 7%, 46%, 38%, and 86% ($P < 0.001$), respectively.

Discussion

HSV-1 mouse skin model infection presents cutaneous symptoms in animals. To evaluate the potential clinical efficacy of BV-araU in humans, it is useful to examine the efficacy of oral administration of BV-araU in this model, although the primary target of BV-araU therapy is herpes zoster of which the causative virus is different. As presented in this study, oral BV-araU showed therapeutic efficacy vis-a-vis cutaneous infection models in shaved Balb/c mice, inhibiting progression of cutaneous symptoms and increasing the survival rate at doses of 20 mg/kg or higher twice daily. BV-araU exhibited greater therapeutic efficacy against i.c. and i.p. infections with a moderately virulent virus strain of HSV-1 (WT-51 strain) than against infections with a strongly pathogenic strain (VR-3 strain; Machida and Takezawa, 1990). Therapeutic efficacy of BV-araU against cutaneous F strain infection observed in the present study seems to be a little more marked than that against WT-51 strain infection. The slight difference between therapeutic efficacies is likely to be related to the degree of virulence of these strains, because the virus inoculum size needed to induce cutaneous infection was about 10^4 pfu for WT-51 strain and about 10^5 pfu for F strain,

suggesting that WT-51 strain is slightly more virulent in this cutaneous infection system.

BV-araU showed significant therapeutic efficacy even when the treatment was initiated 2.5 days after infection, indicating that it may not be necessary to start the treatment immediately after infection. However, BV-araU at a dose of 20 mg/kg twice a day failed to increase significantly the survival rate when the treatment started 2 days post-infection or later, suggesting that a relatively large dose is needed when start of the treatment is delayed after infection.

Since herpes virus infections often occur in immunocompromised patients, antiherpesviral agents should have potency for not only immunocompetent individuals but also immunocompromised patients. Oral BV-araU showed marked therapeutic efficacies for cutaneous infections in both immunologically normal and immunosuppressed mice. There was no sex and age (6–8 weeks) differences in the efficacy. BV-araU showed efficacy comparable to acyclovir against HSV-1 F strain infection in this model. Although the primary clinical indication of BV-araU should be herpes zoster because of its stronger antiviral activity against VZV (Machida, 1986), the present findings, coupled with its potent anti-VZV activity, suggest that BV-araU is promising as an oral antiviral agent effective for herpes virus infections.

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