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# Evaluation of anti-herpesvirus activity of (1′*S*,2′*R*)-9-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl]-guanine (A-5021) in mice

Satoshi Iwayama [a,*], Yuko Ohmura [a], Katsuya Suzuki [a], Nobukazu Ono [a], Harumi Nakazawa [a], Miho Aoki [a], Itsuya Tanabe [a], Takaaki Sekiyama [a], Takashi Tsuji [a], Masahiko Okunishi [a], Koichi Yamanishi [b], Yukihiro Nishiyama [c]

[a] *Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan*
[b] *Department of Microbiology, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c] *Laboratory of Virology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-0065, Japan*

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**Abstract**

The anti-herpesvirus activity of (1′*S*,2′*R*)-9-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl]guanine (A-5021) was evaluated in murine cells and in several murine models of herpes simplex virus (HSV) infection. Against HSV type 1 (HSV-1), A-5021 was 15–30- and 30–60-fold more active, and against HSV type 2 (HSV-2), it was 2- and 8-fold more active than acyclovir and penciclovir in Balb/3T3 cells, respectively. When antiviral compounds were administered orally (once daily) to mice infected intraperitoneally with HSV-1 (Tomioka), A-5021 was more active than acyclovir or famciclovir in spite of its relatively low oral bioavailability. A-5021 was as active as penciclovir when the antiviral compounds were given intravenously (three times daily) to mice infected intraperitoneally with HSV-2 (186). In mice with a cutaneous HSV-1 (KOS) infection, three times daily oral therapy with A-5021 at 25 mg/kg per day produced more significant reduction in severity of skin lesions than equivalent treatment with acyclovir or famciclovir. In mice infected intracerebrally with HSV-1 (Tomioka), complete survival was observed in the group treated intravenously with A-5021 at 25 mg/kg per day (three times daily), while more than 50% of mice died in the groups treated intravenously with acyclovir of up to 100 mg/kg per day (three times daily). Moreover, A-5021 was more effective than acyclovir in clearing infectious virus from the brain. These findings demonstrate that A-5021 has potent anti-HSV activity in several murine models. 

*Keywords:* Animal model study; Anti-herpes compound A-5021; Herpes simplex virus (HSV-1, HSV-2)

* Corresponding author. Tel.: +81-44-2105844; fax +81-44-2105873.

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## 1. Introduction

Against the diseases caused by herpes simplex virus (HSV) and varicella-zoster virus (VZV), acyclovir (ACV) (O'Brien and Campoli-Richards, 1989) is widely used as a systemic (intravenous and oral) or topical treatment. Recently, famciclovir (FCV) (Vere Hodge et al., 1989b; Pue and Benet, 1993) and valaciclovir (Beauchamp and Krenitsky, 1993; Soul-Lawton et al., 1995), the oral prodrugs of penciclovir (PCV) (Bacon and Schinazi, 1993; Boyd et al., 1993; Sutton and Kern, 1993; Vere Hodge and Cheng, 1993) and ACV, respectively, have also been approved. These prodrugs improve the patient's burden by reducing dosing frequency in comparison with that for oral ACV.

Sekiyama et al. (1998) reported the synthesis of a series of novel nucleoside analogs. Among them, (1′*S*,2′*R*)-9-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl]guanine (A-5021) has been shown to possess a potent and selective anti-herpetic activity (Iwayama et al., 1998a; Ono et al., 1998). In plaque reduction assays, A-5021 is more active than ACV or PCV against HSV type 1 (HSV-1), HSV type 2 (HSV-2), VZV, and human cytomegalovirus, and shows considerably more prolonged antiviral activity than ACV when infected cells are treated for a short time (Iwayama et al., 1998a).

In the present study, to investigate the therapeutic potential of A-5021 for use in the systemic treatment of herpesvirus infection, the antiviral activity of this compound was studied in several murine models of HSV infection using clinically relevant administration routes (intravenous and oral routes). Part of this study has been presented in preliminary form (Iwayama et al., 1997).

## 2. Materials and methods

### 2.1. Compounds

A-5021 (Sekiyama et al., 1998), ACV (Shiragami et al., 1995), FCV, and PCV (Geen et al., 1990) were prepared by Ajinomoto (Kawasaki, Japan). The compounds were dissolved in saline (pH, approximately 11) or suspended in 1% carboxymethyl cellulose for intravenous or oral treatment, respectively. For antiviral therapies, compounds were given once daily or three times daily (every 6 h between 08:00 and 22:00 h) for 5 consecutive days. When it was practically difficult to administer compounds three times on the first day of administration, the remaining administration(s) was/were done on the sixth day from the starting day of the administration in accordance with the dosing interval to preserve the total number of doses (15 times) and the actual dosing period (5 days).

### 2.2. Cell cultures, viruses, and virus assays

Balb/3T3 clone A31 cells (Dainippon Pharmaceutical, Osaka, Japan) were grown in Dulbecco's modified Eagle's medium supplemented with 10% inactivated fetal bovine serum (FBS), and used to determine in vitro potencies of the antiviral compounds by a plaque reduction assay as described previously (Iwayama et al., 1998a). Vero C1008 cells (Dainippon) were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% inactivated FBS, and used to determine virus titers in tissue samples. The viruses used were HSV-1 (Tomioka), HSV-1 (KOS), and HSV-2 (186). Virus stocks were prepared in Vero C1008 cells.

### 2.3. Mice

CDF1 male mice (Charles River Japan, Kanagawa, Japan) were purchased at 4 weeks of age, and were used for pharmacokinetic studies, and for intraperitoneal and intracerebral infection studies, about 1 week later. BALB/c AnNCrj male mice (Charles River Japan) were purchased at 5–6 weeks of age and used for cutaneous infection study about 1 week later.

### 2.4. Pharmacokinetics in mice

Antiviral compounds were administered as a single intravenous or oral dose at 30 mg/kg. After drug administration, three mice at each time point

were bled by cardiac puncture into tubes containing heparin under ether anesthesia at 1, 5, 10, 30, 60, 120, 240 and 480 min after intravenous dosing, and at 5, 15, 30, 60, 120, 240 and 480 min after oral dosing.

A-5021 levels were analyzed by the pre-column derivatization method (Yonekura et al., 1993) with modifications. After addition of 9-[(*Z*)-4-hydroxy-2-buten-1-yl]guanine to the plasma samples as an internal standard (I.S.), the mixture was applied to a $C_{18}$ reversed-phase cartridge (Bond Elut) (Varian, Harbor City, CA) equilibrated with distilled water. After washing with distilled water, A-5021 and I.S. were eluted with methanol, and the eluate was dried in vacuo. The residue was dissolved with 200 μl of distilled water, and 60 μl of the solution was mixed with 30 μl of 100 mM phenylglyoxal (PGO) (Aldrich, Milwaukee, WI) in 2-methoxyethanol. The mixture was incubated at 42°C for 10 min to derivatize A-5021 and I.S., and the resulting PGO derivatives were analyzed by a high-performance liquid chromatography (HPLC) system (Hitachi, Tokyo, Japan). A total of 30 μl of the mixture was injected into the HPLC system. After elution from a guard column (Inertsil ODS-2; 10 × 4.0 mm I.D.) (GL Sciences, Tokyo, Japan) with 12% acetonitrile in 20 mM phosphate buffer (pH 6.5) at a flow rate of 1 ml/min, the PGO derivatives were introduced to an L-column ODS (250 × 4.6 mm I.D.) (Chemicals Inspection & Testing Institute, Tokyo, Japan) utilizing column switching, and then eluted with 20% acetonitrile in 20 mM phosphate buffer (pH 6.5) at a flow rate of 1 ml/min. Peaks were detected by fluorescence (excitation at 350 nm, emission at 510 nm). The limit of quantification of A-5021 in plasma was 5 ng/ml.

For quantification of ACV, plasma from each mouse was treated with an equal volume of 20% trichloroacetic acid. After centrifugation to remove precipitated proteins, the supernatants were analyzed by means of the HPLC system (Hitachi) using a YMC-Pack ODS AM-302 column (150 × 4.6 mm I.D.) (YMC, Wilmington, NC) with a linear gradient of acetonitrile from 0 to 8% in 0.1% trifluoroacetic acid over 20 min at a flow rate of 1 ml/min. The eluate was monitored by absorbance at 254 nm. The limit of quantification of ACV in plasma was approximately 70 ng/ml.

The area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was obtained from the mean plasma concentration–time profile extrapolated to infinity using the linear trapezoidal method.

### 2.5. Intraperitoneal infection

Mice were inoculated intraperitoneally with either HSV-1 (Tomioka) at $2.4 \times 10^5$ PFU per mouse or HSV-2 (186) at $2.8 \times 10^4$ PFU per mouse in 0.2 ml of phosphate-buffered saline (approximately 2500 or 100 times the 50% lethal dose, respectively). Against HSV-1, antiviral compounds were given orally once daily, and against HSV-2, they were given intravenously three times daily, starting approximately 3 h after infection. Survival was assessed daily until 21 days after infection, and the effective dose which conferred 50% survival ($ED_{50}$) on day 21 after infection was calculated by probit analysis.

### 2.6. Cutaneous infection

Inoculation of mice was performed according to the method of Simmons and Nash (1984) with modifications. In brief, each right flank was clipped and depilated with depilation cream, and the mice were anesthetized by intraperitoneal injection of 2,2,2-tribromoethanol (Aldrich, Milwaukee, WI). A 20-μl drop containing $3–4 \times 10^6$ PFU of HSV-1 (KOS) was placed on the skin of the posterior flank. A total of 20 scarifications were made through the drop with a 25-gauge needle. The virus solution was removed 2 min after the scarification. Antiviral compounds were given orally or intravenously three times daily starting at 1 or 4 days (delayed therapy) after infection. Lesions were assessed using the following criteria: (0) no sign of infection; (1) swelling and erythema at the inoculation site; (2) many small vesicles or a few large vesicles around the inoculation site; (3) zosteriform vesicles extending downward from the inoculation site to the midline of the abdomen; (4) ulceration at the inoculation site or zosteriform crust in a small region; (5) zosteriform ulceration or zosteriform crust with ulceration in a small region; (6) zosteriform ulcer-

ation or zosteriform crust with ulceration in a mid-sized region; and (7) severe zosteriform ulceration in a large region. The severity of skin disease was quantified by determining the mean area under the lesion score–day curve (AUC) calculated by averaging the AUCs of each mouse in each group.

### 2.7. Intracerebral infection

Mice were infected intracerebrally with 20 μl of virus suspension containing 100 PFU of HSV-1 (Tomioka) (16 times the 50% lethal dose) in phosphate-buffered saline. A-5021 or ACV was given intravenously three times daily starting 3–4 h after infection. Survival was assessed daily until 21 days after infection. In parallel with the survival test, viral replication in the brains of mice administered A-5021 or ACV at 25 mg/kg per day was investigated. The infection and treatment regimes were the same as for the survival test. The experiment was terminated when 50% or more of mice died during the survival test in the corresponding group. Whole brains of mice were removed on days 1, 2, 3, 4 and 5 after infection, and homogenized in cold EMEM supplemented with 10% inactivated FBS with glass homogenizers. After three freezing–thawing cycles, debris was removed by low-speed centrifugation, and the supernatant was tested for plaque production in Vero cells. The detection limit of the assay was $3.3 \times 10^2$ PFU/g of tissue. When no plaque was observed, the titer was taken as half of the detection limit, $1.7 \times 10^2$ PFU/g of tissue. The brains of ten surviving mice treated with A-5021 at 25 mg/kg per day in the survival study were also used on day 37 after infection.

### 2.8. Statistical analysis

Statistical evaluation of differences in the number of mice surviving by day 21 after infection between the control group and the treatment groups was performed using the two-tailed Fisher's exact test. To preserve the overall type I error rate of 0.05, the significance level of each test was adjusted by Bonferroni's inequality. The adjusted significance levels were 0.005 ( = 0.05/10), 0.0042 ( = 0.05/12), and 0.0063 ( = 0.05/8) in studies with intraperitoneal HSV-1, intraperitoneal HSV-2, and intracerebral HSV-1 infections, respectively.

For comparison of AUCs in the cutaneous infection studies and of virus titers in the brains in the encephalitis study, the data were normalized by logarithmic transformation, and comparisons of multiple groups were performed by analysis of variance. If a statistically significant difference was detected, ad hoc pairwise multiple comparisons were made with the Tukey HSD test for multiple comparisons. A level of $P < 0.05$ was considered significant. Calculations were made with the software program SAS 6.10 (SAS Institute).

## 3. Results

### 3.1. Antiviral activity in murine cells

Before starting in vivo evaluation in mice, the antiviral activity of A-5021 was determined in murine cells by a plaque reduction assay. Against HSV-1, A-5021 was 15–30- and 30–60-fold more active, and against HSV-2, 2- and 8-fold more active than ACV and PCV, respectively (Table 1).

### 3.2. Pharmacokinetics in mice

In order to compare the pharmacokinetics of the compounds in mice, mean plasma concentration–time profiles and the area under the plasma concentration–time curves ($AUC_{0-\infty}$) after intravenous and oral administration were evaluated (Fig. 1, Table 2). At 30 mg/kg, the $AUC_{0-\infty}$ for

Table 1
Anti-herpesvirus activity of A-5021, ACV and PCV in Balb/3T3 cells

| Virus | Strain | $EC_{50}$ (ng/ml)[a] | | |
|---|---|---|---|---|
| | | A-5021 | ACV | PCV |
| HSV-1 | Tomioka | 1.2 | 32 | 67 |
| | KOS | 1.3 | 20 | 37 |
| HSV-2 | 186 | 15 | 35 | 120 |

[a] Measured by a plaque reduction assay. All values listed are the average results of three to four experiments.

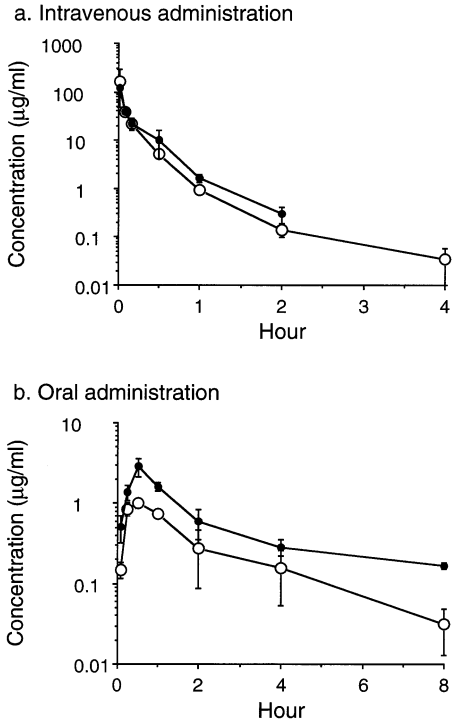


Fig. 1. Mean plasma concentration–time profiles for A-5021 and ACV following single intravenous (a) or oral (b) administration. (○) A-5021; (●) ACV. Error bars indicate standard deviations. The concentrations for ACV at 4 and 8 h and for A-5021 at 8 h after intravenous administration were below the detection limit of each compound.

A-5021 following intravenous administration was almost equal to that for intravenously administered ACV. When administered orally, however, the $AUC_{0-\infty}$ for A-5021 was smaller than that for ACV, resulting in a lower bioavailability of A-5021 than ACV.

Table 2
Bioavailability of A-5021 and ACV following a single-dose administration to mice at 30 mg/kg[a]

| Parameter (units) | A-5021 | ACV |
|---|---|---|
| $AUC_{0-\infty}$ i.v.[b] (μg h/ml) | 19.6 | 19.7 |
| $AUC_{0-\infty}$ oral (μg h/ml) | 2.1 | 5.2 |
| Oral bioavailability (%) | 10.7 | 26.4 |

[a] Derived from the mean plasma concentration–time profile extrapolated to infinity. Plasma samples were collected from three mice per point.

[b] i.v., intravenous.

### 3.3. Activity against intraperitoneal HSV-1 infection

Mice were infected intraperitoneally with HSV-1 (Tomioka), and A-5021, ACV or FCV, was administered orally once daily (Table 3). This treatment regimen may not be optimal for ACV and FCV; however, it was chosen to investigate the potential of A-5021. In this experiment, all vehicle-treated mice died within 12 days after infection, while A-5021 administration resulted in a significant reduction in mortality at 3 mg/kg per day (90% survival, $P < 0.005$). ACV and FCV showed significant activity at 40 and 12.5 mg/kg per day (80 and 70% survival, $P < 0.005$), respectively.

### 3.4. Activity against intraperitoneal HSV-2 infection

Against intraperitoneal HSV-2 (186) infection, A-5021, ACV or PCV, was administered intravenously three times daily (Table 4). In this experiment, mice in the vehicle-treated group began to die from 7 days after infection, and by day 9, all mice had died. A-5021 achieved a significant reduction in mortality at 350 mg/kg per day (80% survival, $P < 0.0042$), and PCV showed a significant effect at 175 and 350 mg/kg per day (70% survival, $P < 0.0042$). ACV did not show any significant effect up to 175 mg/kg per day. When ACV was administered at 350 mg/kg per day, 50% of mice died within 2 days after infection (during the treatment period), and the other surviving mice showed marked body weight loss during the treatment period (data not shown). The reason why these mice died remains to be investigated.

### 3.5. Activity against cutaneous HSV-1 infection

HSV-1 (KOS) was used for the cutaneous infection model. When this virus strain was used, most mice survived without therapy; however, skin le-

Table 3
Effect of oral treatment with A-5021, ACV or FCV on survival of mice infected intraperitoneally with HSV-1 (Tomioka)

| Antiviral compound[a] | Dose (mg/kg per day) | No. of survivors[b]/total no. of mice | $ED_{50}$ (mg/kg per day) |
|---|---|---|---|
| Vehicle | 0 | 0/10 | |
| A-5021 | 3 | 9/10[c] | 0.85 |
| | 1.5 | 6/10 | |
| | 0.75 | 5/10 | |
| ACV | 40 | 8/10[c] | 23 |
| | 20 | 4/10 | |
| | 10 | 1/10 | |
| FCV | 25 | 7/10[c] | 12 |
| | 12.5 | 7/10[c] | |
| | 6.25 | 2/10 | |
| | 3.13 | 0/10 | |

[a] Antiviral compounds were given orally once daily for 5 consecutive days starting approximately 3 h after infection.
[b] Measured at 21 days after infection.
[c] Significantly different ($P<0.005$) from vehicle-treated control. The $P$ value was adjusted for multiple comparisons using Bonferroni's inequality.

sions developed at the infection site and then spread laterally, resulting in zosteriform symptoms. Thereafter, the symptoms gradually improved until the mice were finally cured (data not shown). Therefore, this model is suitable for comparing the effects of antiviral therapies on herpetic skin lesions.

First, the effect of A-5021, ACV or FCV treatment was studied in this model with therapy starting at 1 day after infection (Fig. 2). Antiviral compounds were administered orally three times daily at 25 mg/kg per day. All compounds demonstrated significant therapeutic effects as measured by the area under the lesion score–day curve (AUC) ($P < 0.05$). Furthermore, A-5021 produced a more significant reduction in the severity of disease than ACV or FCV ($P < 0.05$). When A-5021, ACV or PCV was administered intravenously at 10 mg/kg per day using the same dosing schedule as in the above experiment, similar results were obtained: namely, A-5021 was significantly more active than ACV or PCV (mean AUC $\pm$ SE: $2.6 \pm 0.7$ vs. $11.5 \pm 2.1$ and $32.5 \pm 10.7$, respectively; $P < 0.05$).

Second, the effect of delayed therapy was examined. To achieve a therapeutic effect in this trial, a higher dose of antiviral compound was needed than when therapy was started 1 day after infection. A-5021, ACV or PCV, was administered intravenously at 100 mg/kg per day three times daily starting 4 days after infection (Fig. 3). Although no significant difference was observed between the AUCs by analysis of variance ($P = 0.0664$), the treatment with A-5021, ACV or PCV, resulted in a trend of reduced severity of the mean lesion score in comparison with the treatment with vehicle, and A-5021 was the most potent among the three.

### 3.6. Activity against HSV-1 encephalitis

Mice were infected intracerebrally with HSV-1 (Tomioka). A-5021 or ACV was given intravenously three times daily (Figs. 4 and 5). In this experiment, all mice in the vehicle-treated group died within 8 days. A-5021 achieved a complete survival at 25 mg/kg per day ($P < 0.0063$), while ACV administration resulted in no significant survival even at 100 mg/kg per day (Fig. 4). In parallel with the survival test, viral replication in the brains of mice administered A-5021 or ACV at 25 mg/kg per day was investigated (Fig. 5). In the vehicle-treated mice, virus titers in the brain reached a peak level at 2 days after infection, and were maintained at this level. A-5021 rapidly reduced virus replication relative to the control on day 1, maintained the titer at an almost 10-fold lower level than in the control, and finally resulted

Table 4
Effect of intravenous treatment with A-5021, ACV or PCV on survival of mice infected intraperitoneally with HSV-2 (186)

| Antiviral compound[a] | Dose (mg/kg per day) | No. of survivors[b]/total no. of mice | $ED_{50}$ (mg/kg per day) |
|---|---|---|---|
| Vehicle | 0 | 0/10 | |
| A-5021 | 350 | 8/10[c] | 124 |
| | 175 | 6/10 | |
| | 87.5 | 4/10 | |
| | 43.75 | 2/10 | |
| ACV | 350 | 4/10 | ND[d] |
| | 175 | 3/10 | |
| | 87.5 | 3/10 | |
| | 43.75 | 3/10 | |
| PCV | 350 | 7/10[c] | 137 |
| | 175 | 7/10[c] | |
| | 87.5 | 5/10 | |
| | 43.75 | 0/10 | |

[a] Antiviral compounds were given intravenously three times daily for 5 consecutive days starting approximately 3 h after infection.
[b] Measured at 21 days after infection.
[c] Significantly different ($P<0.0042$) from vehicle-treated control. The $P$ value was adjusted for multiple comparisons using Bonferroni's inequality.
[d] ND, not determined.

in complete disappearance of the virus from the brain. The virus titer in the brains of A-5021-treated mice on days 1, 2 and 3 after infection was significantly lower than that in the brains of the control mice ($P < 0.05$), while ACV showed a tendency to reduce virus titer, but not to a statistically significant degree. None of the surviving mice in the treatment groups showed any unusual behavior.

## 4. Discussion

The results of our experiments indicate that A-5021 was highly effective in the treatment of various HSV-infected murine models, including those of intraperitoneal, cutaneous, and encephalitic HSV infection. In mice infected intracerebrally with HSV-1, the mean virus titer in the brains of vehicle-treated mice increased rapidly, reached a peak level (approximately $10^5$ PFU/g of brain tissue) at 2 days after infection, and was thereafter maintained at this level (Fig. 5), resulting in the death of all mice (Fig. 4). Intravenous treatment with ACV at 25 mg/kg per day delayed the increase of mean virus titer; however, the mean virus titer finally reached a level close to the peak level observed in the vehicle-treated mice (Fig. 5), and more than 50% of the mice died (Fig. 4). It seems likely that this peak level of virus titer

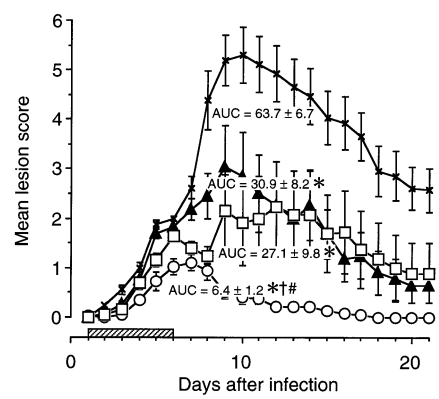


Fig. 2. Effect of oral A-5021, ACV, or FCV administration on the development of skin lesions in mice cutaneously infected with HSV-1 (KOS). Groups of ten to 13 mice were treated with antiviral compounds three times daily at 25 mg/kg per day. AUC is presented as mean ± standard error. Striped bar, antiviral therapy; (×) vehicle; (○) A-5021; (▲) ACV; (□) FCV; $*P < 0.05$ in a comparison between vehicle- and drug-treated groups; $\dagger P < 0.05$ in a comparison between A-5021- and ACV-treated groups; $\# P < 0.05$ in a comparison between A-5021- and FCV-treated groups. Error bars indicate standard errors.

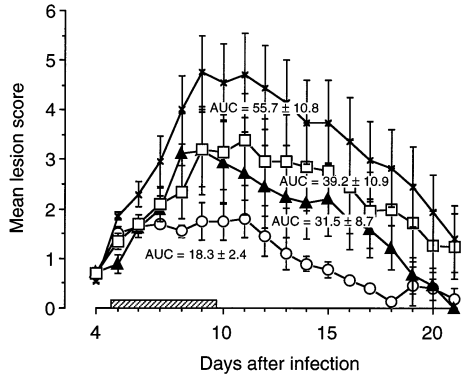


Fig. 3. Effect of delayed therapy with intravenous A-5021, ACV or PCV administration on the development of skin lesions in mice cutaneously infected with HSV-1 (KOS). Groups of nine to ten mice were treated with antiviral compounds three times daily at 100 mg/kg per day. AUC is presented as mean ± standard error. Striped bar, antiviral therapy; (×) vehicle; (○) A-5021; (▲) ACV; (□) PCV. Error bars indicate standard errors.

represents the limit above which the mice could not survive and, therefore, may be the most important factor determining the survival of mice. When the mice were treated with A-5021, the mean virus titer was maintained at a level almost 10-fold lower than the peak level observed in vehicle-treated mice, and this probably allowed the mice to survive.

When the therapy was started 4 days after infection in mice cutaneously infected with HSV-1, higher doses of antiviral compounds were required to show efficacy than when the therapy was started 1 day after infection. Under this condition, A-5021 showed a trend of superior therapeutic activity over ACV or PCV (Fig. 3). These findings may indicate that viral replication from 4 days after infection was still important for developing skin lesions, although the viral replication in the first 4 days after infection would seem to have been the greatest determinant of the lesion development thereafter. Furthermore, the results may suggest that a more potent in vitro antiviral activity will result in a greater improvement in clinical response even if the start of the therapy is delayed.

The findings on the pharmacokinetics of A-5021 suggest that the superior therapeutic activity of A-5021 in the HSV-1-infected mice was not due to the improvement of the plasma pharmacokinetic properties, but rather a reflection, at least in part, of its potent in vitro antiviral activity. It has been reported that in mice infected intranasally with HSV-1 or HSV-2, single daily subcutaneous doses of PCV are more effective than equivalent treatment with ACV (Boyd et al., 1988), and this may be explained by the fact that PCV shows more persistent antiviral activity than ACV in cell culture due to the stability of the nucleoside triphosphate in infected cells (Vere Hodge and Perkins, 1989a; Earnshaw et al., 1992; Bacon et al., 1996). It has also been demonstrated that A-5021 shows persistent antiviral activity in infected cells in addition to its potent anti-her-

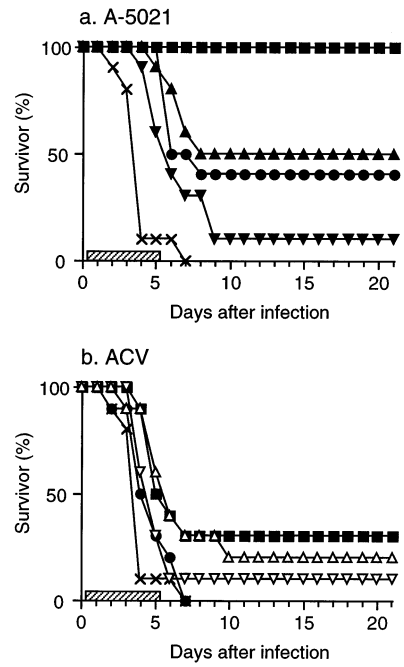


Fig. 4. Effect of intravenous A-5021 or ACV administration on the survival of mice infected intracerebrally with HSV-1 (Tomioka). Groups of ten mice were treated with A-5021 (a) or ACV (b) three times daily at the following doses: 0 (vehicle) (×); 100 (△); 50 (▽); 25 (■); 12.5 (●); 6.3 (▲) and 3.1 mg/kg per day (▼). The striped bar indicates the period of antiviral therapy. The reduction in the mortality rate achieved by A-5021 at 25 mg/kg per day by day 21 after infection was significant ($P < 0.0063$) compared with that in the vehicle-treated control. The $P$ value was adjusted for multiple comparisons by Bonferroni's inequality.

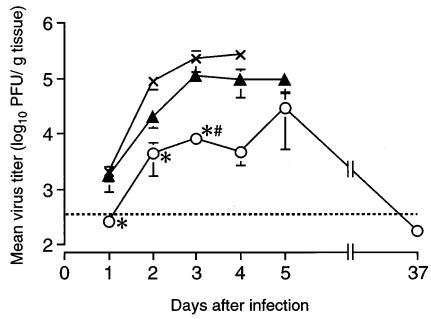


Fig. 5. Effect of intravenous A-5021 or ACV administration on virus replication in the brains of mice infected intracerebrally with HSV-1 (Tomioka). Mice were treated with A-5021 or ACV at 25 mg/kg per day. The treatment schedule was the same as that in Fig. 4. Virus titers in the brains were determined by a plaque assay in Vero cells. Virus titration was terminated when 50% or more of mice died during the survival test in the corresponding group (Fig. 4). All values represent the mean titers of four mice except those for A-5021 on day 37 (ten mice), ACV on days 4 (three mice) and 5 (two mice), and vehicle on day 4 (three mice). Error bars indicate standard errors. ( × ) vehicle; (○) A-5021; (▲) ACV; $*P < 0.05$ in a comparison between vehicle- and drug-treated groups; $\#\ P < 0.05$ in a comparison between A-5021- and ACV-treated groups; (----) limit of detection.

pesvirus activity (Iwayama et al., 1998a). This property of A-5021 may also contribute to its potent anti-herpesvirus activity in various murine models.

The $EC_{50}$ values in murine cells (Balb/3T3; Table 1) were approximately 10-fold lower than those reported in human cells (MRC-5; Iwayama et al., 1998a) for all of the antiviral compounds tested against both HSV-1 and -2. Sutton and Boyd (1993) reported that the $EC_{50}$ values against HSV-1 (SC16) for ACV and PCV in Balb/3T3 cells are 0.02 and 0.08 μg/ml, respectively. These values are comparable to our results in this study. Braitman et al. (1991) also reported that the anti-HSV-1 and -2 activity of SQ33054, ganciclovir and ACV were higher in murine cells than in human cells, and suggested that this may be a reflection of the differences in cellular metabolism in different cells.

The oral bioavailability of A-5021 in mice was lower than that of ACV when administered at 30 mg/kg (Table 2). Rats have been considered a good model for human oral bioavailability of ACV (Beauchamp and Krenitsky, 1993), and in our preliminary study on rats, A-5021 showed a limited oral bioavailability of around 16% (Iwayama et al., 1998b). To enhance the oral bioavailability of A-5021, we are now screening several series of oral prodrugs of A-5021 (Iwayama et al., 1998b).

In most of the experiments in the present study, in order to investigate the strength of the activity of A-5021, we used equal doses of A-5021 and the standard compounds ACV, PCV and FCV. Many studies have described the high level of efficacy of these standard compounds (O'Brien and Campoli-Richards, 1989; Sutton and Kern, 1993). The compounds could also be expected to show satisfactory efficacy if administered at sufficiently higher doses in our models. However, it should be noted that A-5021 may have an advantage in clinical cases in which even maximum dosages of clinically available compounds have limited efficacy (e.g. herpes encephalitis). In our murine models, differential activity of A-5021 and ACV against HSV-1 was greater than against HSV-2. This finding seems to correspond with the activities of these compounds in murine cells (Table 1). We have previously reported that, in human cells, A-5021 is 17- and 2-fold more potent than ACV against clinical isolates of HSV-1 and -2, respectively (Iwayama et al., 1998a). Because there was a good correlation in the relative antiviral activities of those compounds between murine and human cells, and A-5021 also has a potent anti-VZV activity in human cells (Iwayama et al., 1998a), A-5021 may have good efficacy against herpesvirus infections in humans.

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