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Short Communication

Efficacy of ASP2151, a helicase–primase inhibitor, against thymidine kinase-deficient herpes simplex virus type 2 infection in vitro and in vivo

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ABSTRACT

ASP2151 was developed as a novel inhibitor of herpes simplex virus (HSV) and varicella-zoster virus helicase–primase. The anti-HSV activity of ASP2151 toward a clinical HSV isolate with acyclovir (ACV)-resistant/thymidine kinase (TK)-deficiency was characterized in vitro and in vivo using a plaque reduction assay and the ear pinna infection in mice. The IC_{50} ranged from 0.018 to 0.024 μg/ml, indicating the susceptibility of TK-deficient HSV-2 was similar to that of wild-type HSV-2 strains. Anti-HSV activity of ASP2151 in vivo was evaluated in mice infected with wild-type HSV-2 and TK-deficient HSV-2. ASP2151 significantly reduced the copy numbers of wild-type HSV-2 and TK-deficient HSV-2 at the inoculation ear pinna, while valacyclovir significantly reduced the copy number of wild type HSV-2 but not that of TK-deficient HSV-2 in the inoculated ear pinna. Thus, ASP 2151 showed therapeutic efficacy in mice infected with both wild-type and TK-deficient HSV-2. In conclusion, ASP2151 is a promising novel herpes helicase–primase inhibitor that indicates the feasibility of ASP2151 for clinical application for the treatment of HSV infections, including ACV-resistant/TK-deficient HSV infection.

Since the late 1970s, synthetic nucleoside analogues targeting viral DNA synthesis such as acyclovir (ACV), penciclovir, valacyclovir, and famciclovir have been developed and provided for the treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. However, the emergence of ACV-resistant HSV has been observed during prolonged ACV treatment (Balfour, 1983; Balfour et al., 1994; Burns et al., 1982; Chakrabarti et al., 2000; Chen et al., 2000; Crumpacker et al., 1982; Erlich et al., 1989; Gupta et al., 2000; Sacks et al., 1989; Shimada et al., 2007; Wade, 1993). Although recurrence of ACV-resistant HSV is not common, there are reports of current lesions caused by ACV-resistant virus in immunocompromised patients during ACV therapy (Morfin et al., 2000a,b; Safrin et al., 1991; Saijo et al., 2002; Sasadeusz and Sacks, 1996; Shimada et al., 2007). Thymidine kinase (TK)-deficient mutants resistant to ACV exhibit attenuation in the mouse model (Field and Darby, 1980), but some TK-deficient HSV cause progressive diseases in humans, and preserve pathogenicity in mice (Erlich et al., 1989; Kaplowitz et al., 1991; Sacks et al., 1989; Shimada et al., 2007; Swetter et al., 1998). The limitations of the current therapies highlight the need to develop novel anti-herpes drugs with potent antiviral activity based on alternative mechanisms of action.

HSV has variety of HSV-dependent enzymes, including protease and protein kinase, those are involved in the metabolism of nucleotides, DNA, and synthesis of DNA. Recently, there have been reports of newly synthesized selective inhibitors targeting these HSV-dependent enzymes. ASP2151 (amenamevir, Fig. 1) is an anti-herpes agent that targets the helicase–primase complex and reportedly has antiviral activity against wild-type HSV-1 and -2 and VZV in vitro (Chono et al., 2010). It is important to assess the activity of ASP2151 against ACV-resistant HSV for development of novel therapeutics. We have a paired clinical isolates with TK-positive and TK-deficient HSV-2 isolated from the genital lesion and the whitlow of the same patient, respectively (Shimada et al., 2007) and the paired clinical isolates which shows pathogenicity in mice were used for the efficacy comparison of ACV and ASP2151 to TK-deficient HSV-2 infection.

ASP2151 was synthesized by Astellas Pharma Inc. (Tokyo, Japan), and acyclovir was purchased from Sigma, St. Louis, MO. Both were dissolved in dimethylsulfoxide just before use.

To evaluate the antiviral efficacy of ASP2151, a plaque reduction assay was performed as described previously (Kurokawa et al., 1993; Miwa et al., 2005; Okuda et al., 2004; Yoshida et al., 2005). Briefly, Vero cells in 6 cm petri dishes were infected with 100 plaque-forming units of three HSV-2 strains, the wild-type HSV-2 (OOM strain), genital isolate (TK-positive), and whitlow isolate (TK-deficient), for 1 h. Then the cells were overlaid with 0.8%

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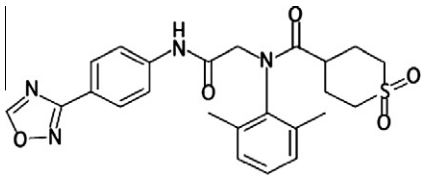

Fig. 1. Structure of ASP2151 (amenamevir).

nutrient methylcellulose medium containing various concentrations of the test compounds (ACV alone: 0, 0.25, 0.5, 1, or 2 μg/ml or ASP2151 alone: 0, 0.0015, 0.003, 0.006, 0.012, 0.024, or 0.048 μg/ml), and 3 days later, fixed with 5% neutral formalin and stained with methylene blue. The number of plaques was counted under a dissecting microscope. The 50% inhibitory concentration for plaque formation (IC_{50}) was defined as the concentration at which the plaque number decreased to half of that in cells cultured without the addition of antiviral drugs. The IC_{50} was determined by using the computer program Microplate Manager III (BioRad, Hercules, CA).

We also investigated the efficacy of ASP2151 on wild-type HSV-2 and TK-deficient HSV-2 infection in a mouse ear pinna infection model. Female BALB/c mice (7 weeks old) were purchased from Sankyo Labs Service Co. Ltd., Tokyo, Japan. All animal experimental procedures conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Experimentation Guidelines of the University of Toyama. The ear pinna was used to evaluate the temperature-sensitive pathogenicity of the TK-deficient whitlow isolate because this clinical isolate causes cutaneous lesions on the cooler ear pinna but not in the skin of the warmer midflank (Shimada et al., 2007). Both ear pinnas were scratched with a bundle of 27-gauge needles and left and right ear pinnas were infected by spreading 5 μl of viral solution containing 2.5×10^4 PFU at 37 °C of genital (TK-positive) and whitlow (TK-deficient) isolates, respectively. ASP2151 was dissolved in 25% (v/w) PEG400 and 25% (v/w) Cremophor EL solution before use. ASP2151 (3 mg/kg per dose), valacyclovir (30 mg/kg per dose), or the vehicle as a control were orally administered by gavage at 2 h before infection and twice daily for 8 days until the ear pinnas were harvested.

The amount of virus in the ear pinna was assessed by quantitative PCR (Aldea et al., 2002; Phromjai et al., 2007; Shimada et al., 2007). Briefly, all mice were euthanized 8 days after HSV-2 infection, and ear pinnas were collected and weighed. DNA was extracted from the ear pinnas using a nucleotide extraction kit (Qiagen, Tokyo, Japan), and the amount of HSV-2 DNA was monitored by real-time PCR based on SYBR green incorporation using 5′-GCTCGAGTGCGAAAA-AACGTTC-3′ and 5′-CGGGGCGCTCGGC TAAC-3′ as PCR primers for amplification of UL30 encoding the DNA polymerase gene. To normalize each of the DNA extracts, eight serial HSV-2 genomic DNA sequences with known copy numbers were used as a standard control. The copy numbers of the ear pinna HSV-2 DNA suspended in 20 μl of total reaction volume were quantified using SYBR Premix Ex Taq (Takara Bio Inc., Kyoto, Japan) using a Thermal Cycler Dice TP800 (Takara Bio) and Thermal Cycler Dice Real Time System Software and expressed as the number of copies of HSV-2 DNA per 1 mg of ear pinna.

Statistical analysis of the HSV-2 DNA copy numbers among treatment groups was accomplished using one-way analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) test. The change of body weight in the treatment groups was evaluated by one-way ANOVA followed by Fisher's PLSD as the post-hoc test. Statistical differences were considered significant at $P < 0.05$.

The susceptibilities of viruses to ACV and ASP2151 are shown in Table 1. The IC_{50} of ACV was similar in clinical isolates of HSV-2 (OOM) and the genital isolate, but the ACV-resistant/TK-deficient HSV-2 had about 50 times lower susceptibility to acyclovir than the other HSV-2 strains. On the other hand, the IC_{50} of ASP2151 was similar for all three HSV-2 strains including an ACV-resistant/TK-deficient HSV-2, and the IC_{50} to ASP2151 was about 30 times lower than that to ACV.

Table 1
Susceptibility to anti-herpetic drugs of OOM strain and paired clinical isolates of HSV-2 (μg/ml).

Strain	Acyclovir	ASP2151
OOM	0.730 ± 0.169	0.022 ± 0.002
Genital isolate (TK-positive)	0.666 ± 0.115	0.024 ± 0.003
Whitlow isolate (TK-deficient)	37.510 ± 5.018	0.018 ± 0.004

The antiviral activity (IC_{50}) of acyclovir and ASP2151 toward virus strains are expressed as the mean (μg/ml) ± S.E. of four independent plaque reduction assays. The whitlow isolate was significantly resistant to acyclovir ($P < 0.0005$) compared to OOM and a genital isolate by the Student's *t*-test.

Fig. 2 shows the changes in body weight of the three groups. The body weight loss was significantly less in the ASP2151 group than the vehicle group at 5–6 days after virus infection ($P < 0.05$). However, there was no significant difference between the ASP2151 and valacyclovir treatment groups.

The efficacy of ASP2151 and valacyclovir was evaluated by the reduction in the number of copies in the infected ear pinna. The amounts of virus in the ear pinna in mouse groups treated with ASP2151, valacyclovir, and vehicle are shown in Fig. 3. The numbers of virus copies of the TK-positive HSV-2 in the ASP2151 and valacyclovir treatment groups were significantly lower than those in the vehicle groups ($P = 0.019$ and $P = 0.005$, respectively), but no significant difference between ASP2151 and valacyclovir treatment groups was observed. On the other hand, the numbers of virus copies of TK-deficient HSV-2 in the ASP2151 treatment group was significantly lower than those of vehicle and valacyclovir treatment groups ($P = 0.001$ and $P = 0.009$, respectively). There was no significant difference between the vehicle and valacyclovir treatment groups. Thus, ASP2151 was effective against TK-deficient HSV-2, while valacyclovir was not.

ASP2151 showed potent anti-HSV activity not only against the wild-type HSV-2 but also against ACV-resistant/TK-deficient HSV-2 both in vitro and in vivo. In a guinea pig model of genital herpes using wild-type HSV-2, ASP2151 showed the superior to valacyclovir in both potency and efficacy (Katsumata et al.,

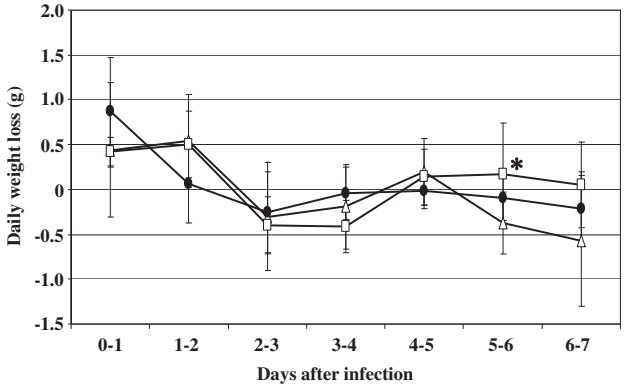

Fig. 2. Changes in body weight of mice in ASP2151, valacyclovir, and vehicle treatment groups. Open squares (ASP2151, $n = 9$) and closed circles (valacyclovir, $n = 10$) and open triangles (vehicle, $n = 10$) indicate the mean ± S.D. of weight loss of each group, respectively. The body weight was measured at the same time daily using a finely calibrated animal-weighing balance. $^*P < 0.05$ by comparison of ASP2151 group versus vehicle group at 5–6 days after infection by the one-way ANOVA followed by Fisher's PLSD.