Anti-Herpes Simplex Virus Activity of Moronic Acid Purified from *Rhus javanica* In Vitro and In Vivo

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ABSTRACT

*Rhus javanica*, a medicinal herb, has been shown to exhibit oral therapeutic anti-herpes simplex virus (HSV) activity in mice. We purified two major anti-HSV compounds, moronic acid and betulonic acid, from the herbal extract by extraction with ethyl acetate at pH 10 followed by chromatographic separations and examined their anti-HSV activity in vitro and in vivo. Moronic acid was quantitatively a major anti-HSV compound in the ethyl acetate-soluble fraction. The effective concentrations for 50% plaque reduction of moronic acid and betulonic acid for wild-type HSV type 1 (HSV-1) were 3.9 and 2.6 µg/ml, respectively. The therapeutic index of moronic acid (10.3–16.3) was larger than that of betulonic acid (6.2). Susceptibility of acyclovir-resistant virus is a current problem (Pass et al., 1979; Norris et al., 1988; Erlich et al., 1989). The therapeutic index of moronic acid (10.3–16.3) was larger than that of betulonic acid (6.2). Susceptibility of acyclovir-phosphonoacetic acid-resistant HSV-1, thymidine kinase-deficient HSV-1, and wild-type HSV type 2 to moronic acid was similar to that of the wild-type HSV-1. When this compound was administered orally to mice infected cutaneously with HSV-1 three times daily, it significantly retarded the development of skin lesions and/or prolonged the mean survival times of infected mice without toxicity compared with the control. Moronic acid suppressed virus yields in the brain more efficiently than those in the skin. This was consistent with the prolongation of mean survival times. Thus, moronic acid was purified as a major anti-HSV compound from the herbal extract of *Rhus javanica*. Mode of the anti-HSV activity was different from that of ACV. Moronic acid showed oral therapeutic efficacy in HSV-infected mice and possessed novel anti-HSV activity that was consistent with that of the extract.

Herpetic infection is common in humans and causes several infectious diseases such as labial herpes, genital herpes, keratitis, and encephalitis. These clinical symptoms often become severe in immunosuppressed patients with acquired immunodeficiency syndrome and in organ transplant recipients (Pass et al., 1979; Norris et al., 1988; Erlich et al., 1989). The herpetic infection has been successfully treated with acyclovir (ACV) (Meyers et al., 1982; Fiddian et al., 1984; Dunkle et al., 1991; Whitley et al., 1991). However, the appearance of ACV-resistant virus is a current problem (Pass et al., 1979; Sibrack et al., 1982; Norris et al., 1988; Erlich et al., 1989; Oliver et al., 1989; Birch et al., 1990; Nugier et al., 1992; Reusser et al., 1996). Thus, the development of new anti-herpes simplex virus (HSV) agents is needed.

We previously selected 12 herbal extracts with oral therapeuatic anti-HSV type 1 (HSV-1) activity in a cutaneous infection model in mice from 142 herbal extracts (Kurokawa et al., 1993b). Four of the 12 herbs augmented oral therapeutic efficacy of ACV in mice (Kurokawa et al., 1995a) and showed potent anti-HSV-1 activity against infection with ACV/phosphonoacetic acid (PAA)-resistant (AP) HSV-1 and wild-type HSV type 2 (HSV-2) strains in vitro and in vivo (Kurokawa et al., 1995a, b). These four herbal extracts also exhibited prophylactic efficacy against recurrent HSV-1 disease in mice (Kurokawa et al., 1997). Prophylactic treatment with *Rhus javanica* L. among the four alleviated spontaneous and UV-induced recurrent HSV-2 genital disease in guinea pigs (Nakano et al., 1998). Its oral administration has been already used clinically for the treatment of chronic disease such as gastric and duodenal ulcer, umpsa, and so on in tradiational therapy based on the information accumulated historically (Kurokawa et al., 1993b). Furthermore, the extract of *R. javanica* was not mutagenic in a mutation assay using *Salmonella typhimurium* and *Escherichia coli* (unpublished data). Thus, *R. javanica* would be a possible candidate for anti-HSV medicines and may supplement the anti-HSV activity of ACV. Recently, we purified an anti-HSV compound, eugenin, from *Geum japonicum* Thunb. (whole plant) and

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**ABBREVIATIONS:** ACV, acyclovir; AP*, acyclovir/phosphonoacetic acid-resistant; CC₅₀, 50% cytotoxic concentration; EC₅₀, effective concentrations for 50% plaque reduction; EtOAc, ethyl acetate; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; PAA, phosphonoacetic acid; R. javanica, *Rhus javanica* L.; TK*, thymidine kinase-deficient.
Syzygium aromaticum (L.) Merr. et Perry (flower bud) among the four herbal extracts (Kurokawa et al., 1998) and shown that the anti-HSV activity of eugenin is different from those of anti-HSV nucleoside analogs. In a series of our studies, we isolated two anti-HSV compounds, moronic acid and betulonic acid, from the extract of R. javanica in this study. The moronic acid was identified as a major anti-HSV compound from R. javanica. This compound exhibited novel anti-HSV activity that was different from that of ACV.

Materials and Methods

Viruses and Cells. HSV strains used were the wild-type type 7401H HSV-1 (Kurokawa et al., 1993b), thymidine kinase-deficient (TK−) HSV-1 (B2006) (Dudds and Kit, 1964), and wild-type HSV-1 (Ito-1262) (Kurokawa et al., 1995a, b). These virus stocks were prepared from infected-Vero cells as reported previously (Kurokawa et al., 1993b, 1998). Vero cells were grown and maintained in Eagle’s minimum essential medium supplemented with 5% and 2% calf serum, respectively.

Plaque Reduction and Cytotoxicity Assays. Fractions obtained in each step for separations were examined for their anti-HSV-1 activity in the plaque-reduction assay. Duplicate cultures of Vero cells in 60-mm plastic dishes were infected with 100 plaque-forming units of wild-type HSV-1 for 1 h. The cells were overlaid with 5 ml of nutrient methylcellulose (0.8%) medium containing various concentrations of samples and then cultured at 37°C for 2 to 3 days. The cells were fixed and stained, and the numbers of plaques were counted as described previously (Kurokawa et al., 1995a). The effective concentrations for 50% plaque reduction (EC50) were determined from a curve relating the plaque number to the concentration of samples (Kurokawa et al., 1995a).

Cytotoxicity of herbal extracts was evaluated by the extent of omission of uninfected cells from the surface of stained dishes in the plaque-reduction assay as described previously (Kurokawa et al., 1993b). Cytotoxicity of purified compounds was examined by measuring their effects on the incorporation of [methyl-3H]thymidine (3.11 TBq/mmol; Amersham, Buckinghamshire, UK) into DNA of Vero cells as described previously (Kurokawa et al., 1993b). Cytotoxicity of purified compounds was also examined by measuring their effects on the growth of Vero cells (Kurokawa et al., 1995a, 1998). Briefly, Vero cells were seeded at a concentration of 5 × 103 cells/well onto 24-well plates and grown at 37°C for 2 days. The culture medium was replaced by fresh medium containing compounds at various concentrations, and the cells were further grown for 2 days. The cells in triplicate wells for each concentration were treated with trypsin, and the number of viable cells was determined by trypan blue exclusion test. The 50% cytotoxic concentration (CC50) was determined graphically as described previously (Kurokawa et al., 1995a).

Preparation of Herbal Extracts. Anti-HSV compounds were purified from the hot-water extract of R. javanica (Tochimoto Tenkaido, Osaka, Japan) using extraction with ethyl acetate (EtOAc) and chromatographic separations guided by anti-HSV-1 activity. The dried materials (500 g) were boiled in water (5.2 liters) at 20 mg/ml. The suspension was boiled for 10 min and centrifuged at 3000 rpm for 15 min, and then a small aliquot of its supernatant (hot-water extract) was used for the plaque-reduction assay.

TABLE 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Eluent</th>
<th>Weighta</th>
<th>EC50b</th>
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<td>246</td>
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<tr>
<td>1-4</td>
<td>40% CHCl3/hexane</td>
<td>787</td>
<td>3.8</td>
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<tr>
<td>1-5</td>
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<td>57</td>
<td>&gt;20</td>
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<td>2</td>
<td>20% EtoAc/benzene</td>
<td>110</td>
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<td>3</td>
<td>20% EtoAc/benzene</td>
<td>80</td>
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<td>20% EtoAc/benzene</td>
<td>70</td>
<td>&gt;10</td>
</tr>
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<td>5</td>
<td>20% EtoAc/benzene</td>
<td>50</td>
<td>&gt;20</td>
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<td>30% MeOH/CHCl3</td>
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</tr>
<tr>
<td>7</td>
<td>MeOH</td>
<td>40</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

a Amount of each fraction recovered from EtOAc-soluble fraction (4.85 g).
b Fractions with anti-HSV-1 activity selected for further fractionations.

We previously examined the antiviral activity of serum obtained from guinea pigs administered with herbal extracts (Kurokawa et al., 1996), and the active fractions of sera showing antiviral activity were rapidly and effectively separated based on their chemical properties, including stability in acidic and alkaline solutions (Kurokawa et al., 1993a). Thus, we applied this procedure to the fractionation of hot-water extract of R. javanica. The hot-water extract was divided into three fractions, each containing 5 ml, and then 1 N NaOH solution or hydrochloric acid was added to adjust the pH of the divided extracts to 4, 7, and 10. Each group was extracted three times with an equal volume of EtOAc, and the EtOAc-soluble fractions were dried in vacuo. The residue was dissolved in dimethyl sulfoxide at 20 mg/ml and examined for anti-HSV-1 activity in the plaque-reduction assay. Because the EtOAc-soluble fraction of pH 10 exhibited the strongest anti-HSV-1 activity among the three groups, the remaining hot-water extract was extracted with EtOAc (6 liters × 3) at pH 10 by a countercurrent extraction method. The EtOAc-soluble extracts were collected and dried in vacuo. The residue was 8.73 g and 1.8% of the hot-water extract.

Fractionation of EtOAc-Soluble Fraction. EtOAc-soluble fraction (4.85 g) was chromatographed on Wak gel C-200 (2.5 × 18 cm; Wako Pure Chemical Industry Co., Osaka, Japan) to obtain seven fractions (fractions 1–7 in Table 1). The first fraction (fraction 1, 3.81 g) showed the strongest anti-HSV-1 activity among all fractions separated was chromatographed on silica gel 60 (2.5 × 24 cm; Merck) eluting with the solvent of increasing polarity to obtain 10 fractions (fractions 1-1 to 1-10 in Table 1). Each fraction was dried in vacuo, dissolved in dimethyl sulfoxide at 20 mg/ml, and examined for anti-HSV-1 activity by the plaque-reduction assay. As shown in Table 1, fractions 1–4 and 1–7 exhibited stronger anti-HSV-1 activity than the other six fractions. Fraction 1–4 (787 mg) and fraction 1–7 (440 mg) were further separated into six fractions (fractions 1–4-1 to 1–4-6) and seven fractions (fractions 1–7–1 to 1–7–7), respectively, by Wak gel C-200 chromatography for the repurification of anti-HSV-1 compounds. Fraction 1–5 (658 mg) and fraction 1–6 (73 mg) were recrystallized for purification. Each fraction separated or recrystallized was analyzed by a preparative thin-layer chromatography [precoated Merck Kieselgel 60 F254 plate (0.25 and 0.5 mm), Tokyo, Japan]. Compound 1 of 292, 385, 73, and 58 mg (total, 808 mg) was prepared in quantity from fraction 1–4–5, fraction 1–5–1, fraction 1–6, and fraction 1–7–3, respectively. However, the amount of compound 2 (140 mg) purified from fraction 1–4–4 was smaller than that of compound 1.

Preparation of EtOAc-soluble fraction at pH 10

Fractions 1 to 7 were separated from EtOAc-soluble fraction at pH 10 by Wako gel C-200 chromatography. Fraction 1 was separated to fractions 1–1 to 1–10 by silica gel 60 chromatography. Each fraction was examined for its anti-HSV-1 activity in plaque reduction assay.
pound 1, and this compound was quantitatively minor. Chemical structures for these compounds were determined by their melting points (mp), Yanagimoto micro melting point apparatus; Yanagimoto Co., Kyoto, Japan), IR spectra (Hitachi 260–10 spectrometer in KBr disc; Hitachi, Japan), UV spectra (Shimadzu UV-2200 UV-VIS spectrophotometer in CHCl₃; Kyoto, Japan), ¹H and ¹³C NMR spectra (JEOL JNM-GX 400 NMR spectrometer; Akishima, Japan), and optical rotation data (JASCO DIP-360 digital polarimeter in CHCl₃; Nagoya, Japan).

**Animals.** Female BALB/c mice (6-week-old, 17–19 g) were purchased from Sankyo Labo Service Co., Ltd. (Tokyo, Japan). The mice were housed five per cage in a temperature-controlled room, with food and water ad libitum and under a 12-h light/dark diurnal cycle (light at 7:00 AM). The temperature in the room was kept at 24 ± 2°C. The mice were acclimated for at least 3 to 4 days before starting any experimental procedure. The animal experimentation guidelines of Toyama Medical and Pharmaceutical University were followed in animal studies.

**Mouse HSV-1 Infection.** BALB/c mice were cutaneously infected with wild-type HSV-1 (1 × 10⁶ plaque forming units/mouse) after scarification of the shaved right midflank with a 27-gauge needle as described previously (Kurokawa et al., 1993b, 1995a). Moronic acid (0.2, 1, 5, or 10 mg/kg) was orally administered once at 8 h before and three times daily for 7 successive days after viral inoculation. Based on body surface area, these doses of moronic acid correspond to the conventional doses of hot-water extract of *R. javanica* used for humans (Kurokawa et al., 1993b, 1995a). The development of skin lesions and death was observed three times daily, and the severity of the lesions was scored as described previously (Kurokawa et al., 1993b, 1995a): 0, no lesion; 2, vesicles in local region; 4, erosion and/or ulceration in local region; 6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and 12, death. The infected mice were fed and observed for at least a month to determine their mortality rates.

**Determination of Virus Yields in Skin and Brain.** Virus yields in the skin and brain were determined in infected mice. Mice were cutaneously infected with wild-type HSV-1 and moronic acid was orally administered at doses of 1, 5, or 10 mg/kg following the same schedule as described above. Their brain and skin [whole lesions that include the area (5 × 5 mm) encompassing the inoculation site] were removed under ether anesthesia on days 2, 3, 4, and 6 after infection and homogenized in 2 ml of PBS as described previously (Kurokawa et al., 1995a). The homogenate was centrifuged at 3000 rpm for 15 min, and the virus yield in the supernatant was determined by the plaque assay on Vero cells (Kurokawa et al., 1993b).

**Toxicity Assay in Mice.** Moronic acid was examined for its toxicity in uninfected mice. Five mice in each group were administered with moronic acid (1, 5, or 10 mg/kg) for 7 days following the same schedule used in infected mice. The uninfected mice were weighed on 1 to 7, 15, 21, and/or 36 days after initial administration on day 0. The mortality rates of mice was calculated on day 30.

**Statistical Analysis.** Student’s *t* test was used to evaluate the significance of differences in mean survival times and mean times at which skin lesions were initially scored as 2 or 6 after infection. Significance of differences in virus yields in organs, and mean weights of mice in groups were also evaluated by the Student’s *t* test. The repeated measure ANOVA with Dunn’s procedure as a multiple comparison procedure was used to analyze the interaction between moronic acid and water in mean skin lesions for 3 to 9 or 10 days after infection. Statistical differences in the mortality were evaluated using Fisher’s exact test. A P < 0.05 value was defined as statistically significant.

**Results**

**Identification of Moronic Acid and Betulonic Acid.** Moronic acid and betulonic acid were isolated from the hot-water extract of *R. javanica*, and their chemical structures were identified by physical and spectral analyses. The hot-water extract was first extracted with EtOAc at pH 4, 7, or 10, and the EC₅₀ values of these EtOAc-soluble fractions were 27.5, 16.0, or 7.4 ± 2.0 µg/ml, respectively. The EtOAc-soluble fraction at pH 10 showed the strongest anti-HSV-1 activity among the three extracts, although it showed a moderate cytotoxicity. This EtOAc-soluble fraction was separated by Wako gel C-200 and silica gel 60 chromatography as shown in Table 1. Fractions 1-4 to 1-7 exhibited stronger anti-HSV-1 activity than others. Among these fractions, fractions 1-4 and 1-7 were further separated by Wako gel C-200 rechromatography and fractions 1-5 and 1-6 were recrystallized. Two compounds (compounds 1 and 2) were purified from the separated or recrystallized fractions with strong anti-HSV-1 activity. Chemical structures for compounds 1 and 2 were determined by comparing their physical and spectral data with those of the literatures (Majumder et al., 1979; Gonzalez et al., 1983; Ahsan et al., 1995). Compounds 1 and 2 were identified as moronic acid and betulonic acid, respectively, whose presence has not been previously reported in *R. javanica* (Fig. 1). In each step of fractionations, we selected fractions showing the lowest EC₅₀ value for the next fractionation. Moronic acid was quantitatively the main compound (16.7%) in the EtOAc-soluble fraction at pH 10. Thus, we purified moronic acid and betulonic acid as major anti-HSV-1 compounds that were quantitatively separable from the hot-water extract of *R. javanica*.

**Anti-HSV Activity of Moronic Acid and Betulonic Acid.** Anti-HSV activity and cytotoxicity of moronic acid and betulonic acid were examined against wild-type HSV-1 strain in the plaque-reduction assay. The EC₅₀ values of moronic acid and betulonic acid were 3.9 and 2.6 µg/ml, whereas their CC₅₀ values were 40 to 63.4 and 16.2 µg/ml, respectively (Table 2). Therapeutic index (CC₅₀/EC₅₀) of moronic acid (10.3–16.3) was more than 1.7- to 2.6-fold larger than that of betulonic acid (6.2). Moronic acid was less cytotoxic than betulonic acid.

Anti-HSV activity of moronic acid was examined against wild-type HSV-1, AP⁺ HSV-1, TK⁻ HSV-1, and wild-type HSV-2 strains as well as wild-type HSV-1 strain. As shown in Table 2, all four strains used were similarly susceptible to moronic acid. Thus, this compound exhibited a potent and novel antiviral activity against AP⁺ HSV-1, TK⁻ HSV-1, and wild-type HSV-2 strains as well as wild-type HSV-1 strain.

**Therapeutic Efficacy of Moronic Acid on a Murine HSV-1 Infection Model.** Therapeutic efficacy of moronic acid was examined in a cutaneous HSV-1 infection model in mice. We previously showed that oral administration of the hot-water extract (250 mg/kg) of *R. javanica*, which corre-

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**Fig. 1.** Structures of moronic acid (1) and betulonic acid (2) isolated from *R. javanica.*
sponded to the dose for human use in mice, exhibited significant therapeutic efficacy in this murine model (Kurokawa et al., 1995a, b). Because the moronic acid content of the hot-water extract was 0.3%, 0.75 mg of moronic acid would be contained in 250 mg of the hot-water extract. Thus, we used the range of 0.2 and 10 mg/kg of moronic acid as the doses for human use of the hot-water extract in mice. As shown in Table 3 and Fig. 2, moronic acid at all doses used (0.2, 1, 5, and 10 mg/kg) significantly delayed the development of skin lesions and/or prolonged mean survival times compared with the control ($p < .05$ or $p < .01$ by the Student’s $t$ test and/or the repeated measure ANOVA). Thus, moronic acid exhibited therapeutic efficacy at doses corresponding to human use of the hot-water extract of $R. javanica$ in mice.

Toxicity of moronic acid was examined in mice at the doses of 1, 5, and 10 mg/kg as used for HSV-1 infected mice (data not shown). At these doses, no lethal toxicity was observed in uninfected mice at least for 30 days after initial administration. There was no significant difference in the mean weights between treated and untreated mice on days 7, 21, or 36 ($p > .05$). Similar results were observed on days 1 to 6 and 15 tested (data not shown). Moronic acid was not significantly toxic at the doses used in mice.

**Effect of Moronic Acid on Virus Yields in the Skin and Brain of Infected Mice.** Anti-HSV-1 activity of moronic acid was evaluated in the skin and brain of HSV-1-infected mice. Table 4 shows the virus yields in the skin and brain removed from infected mice on various days after infection. Moronic acid had a tendency to reduce virus yields in the skin compared with the controls, although the reduction of virus yields was not statistically significant. In the brain, 1 and 10 mg/kg moronic acid reduced virus yield to 83.9% of the controls on day 4 after infection, and the virus yields for 1, 5, and 10 mg/kg were 80.7% to 85.6%, 72.2%, and 57.0%, respectively, of the controls on day 6 after infection. Moronic acid dose-dependently reduced virus yields in the brain on day 6 after infection. The percent virus yields in the brain of mice treated with moronic acid (1, 5, and 10 mg/kg) were 1.11- to 1.65-fold less than those in the skin. Thus moronic acid exhibited stronger anti-HSV-1 activity in the brain of infected mice than in the skin.

**Discussion**

We have been studying the antiviral activity of herbal extracts of medicinal herbs for their possible use in the management of viral infection in humans. The medicinal herbs can be easily obtained in China and Japan and have been safely and cautiously managed for the treatment of chronic diseases based on the information on adverse reactions accumulated historically in traditional therapy (Kurokawa et al.,...
In this study, we purified moronic acid and betulonic acid as anti-HSV compounds from hot-water extract of \textit{R. javanica} that exhibited prophylactic and therapeutic anti-HSV-1 activity in mice and guinea pigs (Kurokawa et al., 1993b, 1995a, 1995b, 1997; Nakano et al., 1998). Moronic acid was qualitatively and quantitatively a major anti-HSV compound in the extract. It exhibited significant therapeutic HSV-1 activity in a cutaneous infection model in mice. Thus, moronic acid may be mainly responsible for therapeutic activity of the hot-water extract, although betulonic acid may somewhat contribute to the therapeutic activity of hot-water extract.

Hot-water extract of \textit{R. javanica} showed anti-APr HSV-1, TK$^-$ HSV-1, and HSV-2 activity in vitro and in vivo (Kurokawa et al., 1993b, 1995a). Mode of its anti-HSV action was indicated to be different from those of ACV and PAA (Kurokawa et al., 1995a, b). In this study, moronic acid purified from \textit{R. javanica} was also shown to exhibit anti-HSV activity against APr HSV-1, TK$^-$ HSV-1, and wild-type HSV-2 strains as well as the wild-type HSV-1 strain (Table 2). Thus, the anti-HSV action of moronic acid was consistent with that of the hot-water extract, and the mode was different from that of ACV and PAA. Because combination of ACV with the hot-water extract of \textit{R. javanica} strongly potentiated the anti-HSV activity of ACV more than its additive anti-HSV activity in vitro (Kurokawa et al., 1995a), this strong com-

### TABLE 4

Effects of moronic acid on virus yields in skin and brain of wild-type HSV-1-infected mice

<table>
<thead>
<tr>
<th>Days after Infection</th>
<th>Treatment</th>
<th>Dose</th>
<th>Mean ± S.D. Virus Yield$^a$</th>
<th>Skin</th>
<th>Brain</th>
<th>Skin/Brain$^b$</th>
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<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td></td>
<td>log$\text{10}$ PFU/organ</td>
<td>N.D.</td>
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<td>N.D.</td>
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<tr>
<td>2</td>
<td>Control (water)</td>
<td>10</td>
<td>5.32 ± 0.77 (100)$^c$</td>
<td>N.D.$^d$</td>
<td>N.D.$^d$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moronic acid</td>
<td>1</td>
<td>6.14 ± 0.43 (93.5)</td>
<td>2.86 ± 0.15 (100)</td>
<td>1</td>
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<tr>
<td></td>
<td>Moronic acid</td>
<td>1</td>
<td>6.33 ± 0.28 (96.3)</td>
<td>&lt;2.40 ± 0.00 (83.9)</td>
<td>&gt;1.11</td>
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<td>6.37 ± 0.34 (100)</td>
<td>4.09 ± 0.31 (100)</td>
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<td>Moronic acid</td>
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<td>6.07 ± 0.19 (95.3)</td>
<td>3.50 ± 0.40 (85.6)</td>
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<td>6.00 ± 0.09 (94.2)</td>
<td>2.33 ± 0.33 (57.0)$^e$</td>
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</tr>
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<td>3</td>
<td>Control (water)</td>
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<td>5.56 ± 0.09 (100)</td>
<td>N.D.$^d$</td>
<td>N.D.$^d$</td>
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<tr>
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<td>5.46 ± 0.40 (98.2)</td>
<td>N.D.$^d$</td>
<td>N.D.$^d$</td>
<td></td>
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<tr>
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<td>Moronic acid</td>
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<td>5.09 ± 0.36 (91.5)</td>
<td>N.D.$^d$</td>
<td>N.D.$^d$</td>
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<tr>
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<td>1.16</td>
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<td>Moronic acid</td>
<td>1</td>
<td>5.58 ± 0.17 (109.0)</td>
<td>2.65 ± 0.30 (72.2)</td>
<td>1.51</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Virus yields represent geometric mean ± S.D. per organ of three mice in each group.

$^b$ Skin/brain represents ratio of percentage of virus yields of skin over brain for each treatment.

$^c$ Numbers in parentheses represent percentage of virus yields.

$^d$ Not detected.

$^e$ $p < .01$ versus control by Student’s $t$ test.

1993b). In this study, we purified moronic acid and betulonic acid as anti-HSV compounds from hot-water extract of \textit{R. javanica} that exhibited prophylactic and therapeutic anti-HSV-1 activity in mice and guinea pigs (Kurokawa et al., 1993b, 1995a, 1995b, 1997; Nakano et al., 1998). Moronic acid was qualitatively and quantitatively a major anti-HSV compound in the extract. It exhibited significant therapeutic HSV-1 activity in a cutaneous infection model in mice. Thus, moronic acid may be mainly responsible for therapeutic activity of the hot-water extract, although betulonic acid may somewhat contribute to the therapeutic activity of hot-water extract.
bined activity probably resulted from the difference in anti-HSV actions of ACV and moronic acid as a major anti-HSV compound in the extract.

We previously showed that the hot-water extract of *R. javanica* exhibited oral therapeutic efficacy at the dose (250 mg/kg) corresponding to human use in mice as effectively as the oral administration of ACV at 5 mg/kg three times per day in mice (Kurokawa et al., 1993b, 1995a). The doses (0.2–10 mg/kg) of moronic acid used for oral administration to mice corresponded to its content of the hot-water extract (250 mg/kg) for human use and exhibited therapeutic efficacy in HSV-1-infected mice (Table 3 and Fig. 2). Thus, moronic acid at these doses was as effective against HSV-1 infection as ACV at 15 mg/kg/day. Moronic acid at these doses was not toxic in mice. Hot-water extract of *R. javanica* did not cause major adverse reactions, such as a weight loss, although it was effective against HSV infection in mice (Kurokawa et al., 1995a). Even in mice treated with combination of the extract with ACV (Kurokawa et al., 1995a) and in guinea pigs treated with the oral administration of the extract for 2 to 3 months (Nakano et al., 1998), no toxicity was observed. Therefore, moronic acid and the extract were similarly effective against HSV infection without toxicity.

Hot-water extract of *R. javanica* has been previously shown to have stronger anti-HSV-1 activity in the brain than in the skin, although ACV was more effective in reducing virus yields in the skin than in the brain of infected mice (Kurokawa et al., 1995a). Moronic acid showed stronger anti-HSV-1 activity in the brain of HSV-1-infected mice than in the skin (Table 4) similar to the hot-water extract of *R. javanica*, indicating different anti-HSV activity in the skin and brain between ACV and moronic acid. This may result from differences in their distribution in the body after absorption or their affinity for the central nervous system. Moronic acid may be expected to be beneficial in preventing central nervous system complications. Previously, we showed that the hot-water extract of *R. javanica* augmented the therapeutic anti-HSV-1 efficacy of ACV in their combination in mice and exhibited the different mechanism of anti-HSV activity from that of ACV (Kurokawa et al., 1995a). This augmented efficacy may be due to the different mechanisms of anti-HSV activity of moronic acid and ACV and their different anti-HSV activities in skin and brain. Because the hot-water extract showed prophylactic efficacy against recurrent HSV-1 diseases in mice (Kurokawa et al., 1997) and recurrent HSV-2 genital diseases in guinea pigs (Nakano et al., 1998), moronic acid may be expected to be effective for prophylaxis of recurrent HSV diseases.

Moronic acid was purified as a major anti-HSV compound from the hot-water extract of *R. javanica* that showed oral therapeutic efficacy in infected mice. It was characterized to exhibit novel anti-HSV activity in vitro and in vivo that was consistent with that of the extract. Moronic acid was verified to be a candidate of new anti-HSV agents. This compound and betulinic acid purified in this study are triterpenes, and some of them, such as betulinic acid and derivatives, have been reported to exhibit anti-human immunodeficiency virus (HIV) activity as inhibitors of HIV-1 entry (Kashiwada et al., 1996, 1997; Hashimoto et al., 1997; Labrosse et al., 1998). However, the mode of antiviral action of moronic acid against HSV may be different from that of betulinic acid against HIV. Thus, the further study of moronic acid may allow us to clarify the mechanism of anti-HSV action in vitro and its toxicological and anti-HSV therapeutic effects at high doses in vivo for clinical application. These studies are now in under way.

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References


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