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Suppression of recurrent genital herpes simplex virus type 2 infection by *Rhus javanica* in guinea pigs

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Abstract

Rhus javanica has been shown to exhibit anti-herpes simplex virus (HSV) activity and potentiate the anti-HSV activity of acyclovir in vitro and in vivo. This extract was examined for its suppressive efficacy on recurrent genital infection in guinea pigs. Guinea pigs were primarily infected intravaginally with HSV type 2 (HSV-2). Prophylactic oral administration, at the dose corresponding to human use, of *R. javanica* significantly reduced the incidence, severity and/or frequency of spontaneous and severe skin lesions as compared with latently infected guinea pigs administered with water. This prophylactic efficacy was confirmed by the crossover administration, for more than 2 months, of *R. javanica* and water to the infected guinea pigs. Toxicity, such as weight loss, from *R. javanica* administration was not observed in the guinea pigs. When recurrent HSV-2 disease was induced by ultraviolet irradiation 3 months after primary infection, the prophylaxis with *R. javanica* was also significantly effective in reducing the severity of ultraviolet-induced skin lesions. Thus, prophylaxis of recurrent genital HSV-2 infection with *R. javanica* may preserve the efficacy of acyclovir by reducing both the use of acyclovir and the appearance of acyclovir-resistant viruses.

Keywords: *Rhus javanica*; Prophylaxis; Herpes simplex virus; Recurrent genital herpes; Herbal extract

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1. Introduction

Recurrent genital herpes causes frequent episodes of genital lesions for several days approximately once a month (Corey et al., 1982; Douglas et al., 1984; Straus et al., 1988). This recurrent infection is sometimes associated with severe clinical symptoms in immunosuppressed patients with acquired immunodeficiency syndrome and in organ transplant recipients (Rand et al., 1977; Pass et al., 1979; Norris et al., 1988; Erlich et al., 1989; Reusser et al., 1996). Acyclovir (ACV) has been used for the prevention and treatment of the recurrent infection (Corey et al., 1982; Douglas et al., 1984; Straus et al., 1988). However, the appearance of ACV-resistant HSV strains has become evident in immunosuppressed patients undergoing ACV therapy (Norris et al., 1988; Erlich et al., 1989; Reusser et al., 1996) making the use of ACV, especially its long-term use, an issue. Thus, new preventive agents of the recurrent disease which can undergo long term usage may be useful in reducing the morbidity accompanied with recurrent genital herpes.

We have previously shown that the hot-water extract of *Rhus javanica* L. exhibits prophylactic and therapeutic efficacy against herpes simplex virus (HSV) type 1 (HSV-1) infection in mice (Kurokawa et al., 1993, 1995a,b, 1997). This extract was also effective against ACV-resistant HSV-1 and HSV type 2 (HSV-2) infection in mice (Kurokawa et al., 1995b) and augmented therapeutic efficacy of ACV in mice infected with HSV-1 (Kurokawa et al., 1995a). *R. javanica*, a medicinal herb, is available in Japan and China and is commonly used as a hot-water extract. Based on traditional therapeutic information accumulated historically, oral administration of *R. javanica* has been already used clinically for the treatment of chronic diseases such as gastric and duodenal ulcer, empyema, etc. (Jiangxu New Medical College, 1978; Kurokawa et al., 1993). Further, the extract of *R. javanica* was not mutagenic in a mutation assay using *Salmonella typhimurium* and *Escherichia coli* (unpublished data). This extract may be tolerated throughout its long-term administration period. Thus, the drinking of this extract, in a daily tea or coffee, may be prophylactically used to reduce frequent recurrent genital episodes and improve the quality of life. Such prophylaxis with the extract may result in preservation of the efficacy of ACV for the future. In this study, we examined the prophylactic efficacy of *R. javanica* on recurrent HSV-2 disease by using a genital infection model in guinea pigs (Stanberry, 1989, 1990a,b). We showed that prophylactic treatment with *R. javanica* alleviated spontaneous and ultraviolet (UV)-induced recurrent genital HSV-2 disease in guinea pigs. This prophylactic efficacy was ascertained by the crossover administration of water and *R. javanica* to infected guinea pigs with spontaneous recurrence.

2. Materials and methods

2.1. Virus and medicinal herbs

HSV-2 (ITO 1262 strain) was isolated from a lesion of genital herpes and used as a wild type HSV-2 strain (Kurokawa et al., 1995b). Virus stocks were prepared from the HSV-2 infected-Vero cell cultures as described previously (Kurokawa et al., 1995a). *R. javanica* (gall) was authenticated and preserved with the voucher samples at the Museum of Materia Medica, the Analytical Research Center for Ethnomedicines, the Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, Japan.

2.2. Preparation of herbal extracts

Hot-water extracts were prepared from *R. javanica* and *Terminalia chebula* RETZUS (*T. chebula*) as described previously (Kurokawa et al., 1993). Briefly, the dried herb was boiled under reflux and the aqueous extract was filtered and lyophilized. The lyophilized extract was supplied in drinking water for the oral administration to guinea pigs as follows: the extract was suspended in distilled water at 2.5 mg/ml and boiled for 10 min. Anti-HSV activity of different lots of the extracts was qualified by plaque reduction assay (Kurokawa et al., 1993, 1995a).

Fig. 1. Representative spontaneous recurrent skin lesions in the external genitalia of guinea pigs more than 1.5 months after primary infection. (a–f) represent score 0 (no lesion), 3 (weak erythema with swelling), 5 (strong erythema with swelling), 6 (strong erythema with vesicles and/or crust), 7 (strong erythema with erosion and crust), and 9 (erosion or ulcer without crust), respectively, as described in the text.

2.3. Spontaneous recurrent genital herpes model

Female Hartley guinea pigs (200–230 g, Sankyo Labo Service, Tokyo) were infected intravaginally with HSV-2 ($0.5–3.0 \times 10^4$ plaque forming units/guinea pig) as primary infection as described elsewhere (Stanberry, 1989, 1990a,b). To avoid subjective observation, the genital skin lesions were assessed and scored simultaneously, no less than two persons, at 1.5 or 3–5 months after primary infection; assessment and scoring as follows: 0, no lesion; 1, swelling; 2, weak erythema (less than 50% of genital skin); 3, weak erythema with swelling; 4, strong erythema (more than 50% of genital skin); 5, strong erythema with swelling; 6, strong erythema with vesicles and/or crust; 7, strong erythema with erosion and crust; 8, strong erythema with erosion; 9, erosion or ulcer without crust (Fig. 1). The latently infected guinea pigs were divided into two groups of ten guinea pigs. Each group containing five or six guinea pigs with no or weak skin lesions (score 0–3), three or four guinea pigs with strong skin lesions (score 4–8) and one guinea pig with erosion or ulcer (score 9). Each group of ten guinea pigs was again divided into two, allowing for five guinea pigs per cage to be fed together. Due to consumption, the herbal extracts (2.3 mg/ml) were supplied daily in 500 ml of drinking water, per cage, ad libitum for 2 months. Therefore, the average dose of the extracts per guinea pig (average weight of 400 g) was estimated as 625 mg/kg per day. The estimated dose corresponded to the conventional dose of dried medicinal herbs used for humans based on body surface area. Water was supplied for the control group. The develop-

ment of skin lesions was observed daily and the severity of the lesions was assessed and scored as described above. The incidence of spontaneous recurrent disease was evaluated by determining the rate of guinea pigs with exacerbation of skin lesions at least once in each group. The severity of spontaneous recurrent disease was determined by the duration of severe skin lesions (score 6–9) in each guinea pig. The mean frequency was determined from the number of times that skin lesions were continuously exacerbated and then ameliorated in each guinea pig: this frequency correlates with the number of peaks counted in a line in Fig. 2.

For crossover experiments, latently infected guinea pigs administered with water for 2 months were successively administered with *R. javanica* for the next 2–3 months, whereas the guinea pigs administered with *R. javanica* for 2 months were administered with water or *T. chebula* followed by water for the next 2–3 months. Significant prophylactic efficacy of *T. chebula* at 625 mg/kg per day was not clear on recurrent HSV-2 disease in guinea pigs (data not shown) and thus was used, instead of water, as an inactive control for the herbal extracts in the first crossover experiment. The change of skin lesions was observed daily and the severity of the lesions was assessed as described above. The body weights of latently infected guinea pigs were periodically measured during the crossover experiments to evaluate the toxicity of the herbal extracts. At the end of crossover experiments, all guinea pigs used were examined for their anti-HSV-2 antibody to confirm the infection with HSV-2 by using the enzyme-linked immunosorbent assay as described previously (Nagasaka et al., 1995).

2.4. UV-induced recurrent genital herpes model

Guinea pigs were primarily infected with HSV-2 as described above. Then 3 months later, the infected guinea pigs (400–510 g), without genital skin lesions were selected. Their genital skin was exposed to UV produced by a transilluminator (UVP, USA) emitting 8 mW/cm^2 at 302 nm for 10 min under anesthesia with sodium pentobarbitone (Stanberry, 1989, 1990a,b). The output of UV light was determined by a UV radiometer (UVP, USA). *R. javanica* (625 mg/kg per day) was orally administered daily to guinea pigs as a drinking solution for 10 days starting 5 days before UV irradiation. The incidence of UV-induced genital skin disease was evaluated by determining the rate of guinea pigs with erythema (more than 50% of genital skin) with swelling and/or vesicles in each group for 10 days after UV-irradiation. The severity of induced recurrent disease was determined by the duration of erythema with swelling and/or vesicles.

2.5. Statistical analyses

The Fisher's exact test or χ^2 test was used to evaluate the significant differences between the incidence of UV-induced skin disease and spontaneous skin disease in the two groups. The Mann–Whitney's *U*-test was used to evaluate the significant differences between the severity of skin lesions and the mean frequency of recurrent skin disease per guinea pig in two groups. A *P*-value of less than 0.05 was statistically defined as significant.

Fig. 2. Prophylactic effects of *R. javanica* on the spontaneous recurrent HSV-2 infection. Guinea pigs were intravaginally infected with 213HSV-2. The latently infected guinea pigs were divided into two groups 3 months later as described in the text. Each group contained 10 guinea pigs ($n = 10$). *R. javanica* (625 mg/kg per day) was supplied within drinking water. Crossover experiments were performed and *T. chebula* was used as an inactive control as described in the text. In (a), guinea pigs were administered with *R. javanica* for 2 months (day 0 to day 60, vertical line) and then the administration was changed to water after the first 2 months. In (b), guinea pigs were administered with water for 2 months (day 0 to day 60, vertical line) and then the administration was changed to *T. chebula* (day 60 to day 90, broken line) and water after the first 2 months. The development of skin lesions was observed daily and the severity of the lesions was scored. Each line shows changes of skin score of one guinea pig in each group.

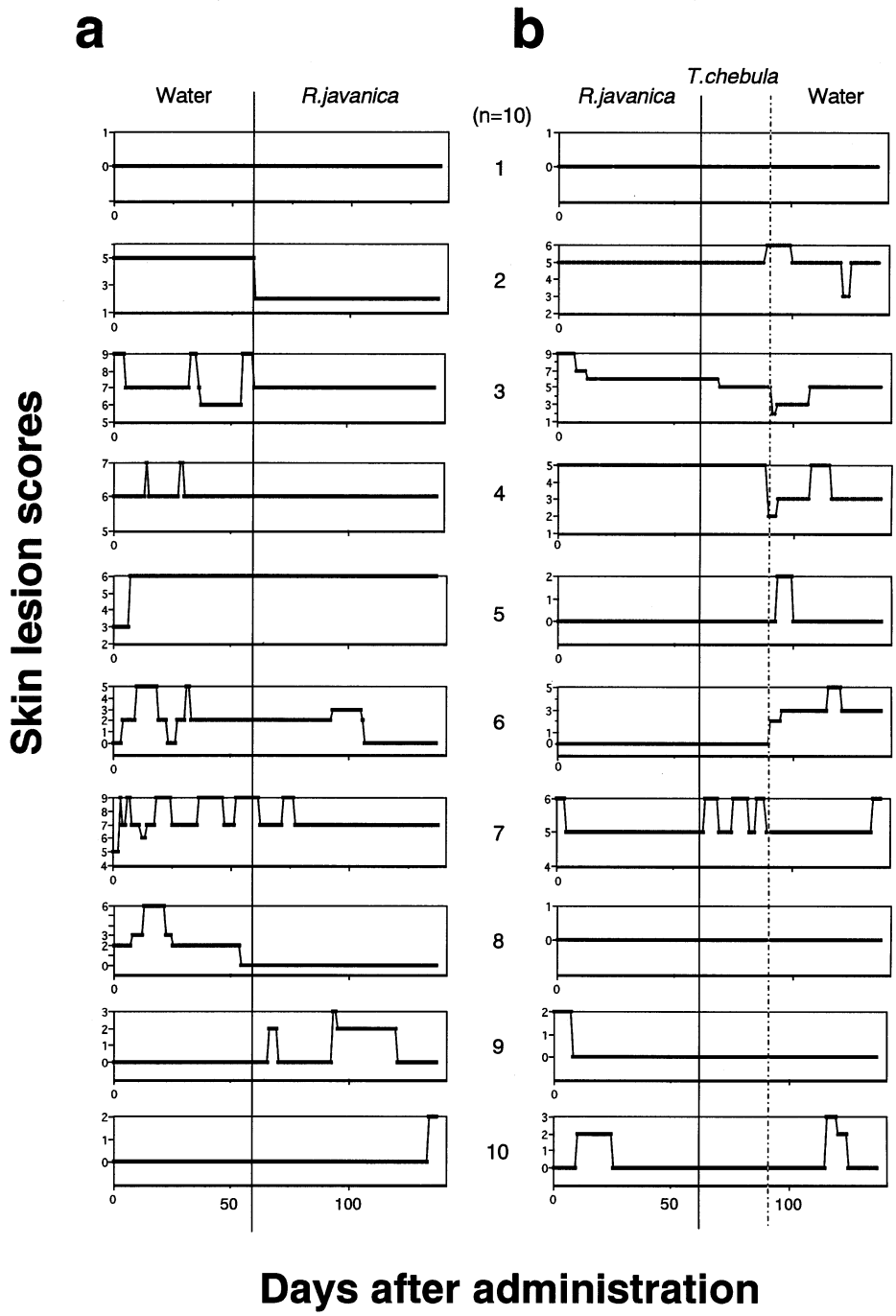
a
b
Water
R.javanica
R.javanica
T.chebula
Water
(n=10)
1
2
3
4
5
6
7
8
9
10
Skin lesion scores
50
100
50
100
Days after administration

Fig. 2.

Table 1
Prophylactic efficacy of *R. javanica* on spontaneous recurrent HSV-2 disease

Number of experiments	Group	1st and 2nd administration (days) for crossover experiments	No of guinea pigs with vesicles (sum of days of appearance of vesicles/sum of days observed for all guinea pigs in each group)[d]	Incidence[a]	Severity[b]	Mean frequency[c]
				Guinea pig	Days	
1	A	I: 1st. water (60 days)	—[e]	6/10	17.4 ± 24.3	1.3 ± 1.6
		II: 2nd. *R. javanica* (75 days)	—	4/10	1.3 ± 4.1	0.5 ± 0.
	B	III: 3rd. *R. javanica* (60 days)	—	1/10[f] ($P<0.05$ vs I and IV)	0.0 ± 0.0[g] ($P<0.05$ vs I)	0.1 ± 0.3[g] ($P<0.05$ vs I and IV)
		IV: 2nd. *T. chebula* (30 days)+water (45 days)	—	7/10	3.2 ± 7.4	1.1 ± 1.2
2	A	I: 1st. water (60 days)	3[h](10/492)[f] ($P<0.01$ vs II and III) ($P<0.05$ vs IV)	5/10	8.5 ± 15.9	0.7 ± 0.8 0.1 ± 0.4
		II: 2nd. *R. javania* (60 days)	0(0/420)		0.0 ± 0.0[g] ($P<0.05$ vs I and IV)	
	B	III: 1st. *R. javanica* (60 days)	0(0/563)	1/10[j] ($P<0.05$ vs IV)	2.5 ± 7.9	0.1 ± 0.3[g] ($P<0.05$ vs I and IV)
		IV: 2nd. Water (60 days)	1[h] (1/420)	4/7[i]	9.4 ± 15.8	2.4 ± 3.3

Prophylactic efficacy of herbal extracts was examined against spontaneous recurrent HSV-2 infection in guinea pigs. For crossover experiments, ten latently infected guinea pigs with various skin lesions were orally administered with water or *R. javanica* (625 mg/kg per day) for 60 days (1st administration) and then these administration was changed to *R. javanica* or water (or *T. chebula* followed by water, respectively, for the next 60 (Expt. 2) or 75 (Expt. 1) days (2nd administration). The exacerbation and amelioration of skin lesions were observed daily and scored as described in the text. Expt. 1 represents the results of Fig. 2.

[a] Number of guinea pigs with exacerbation of skin lesions over the total no. of guinea pigs.

[b] Mean lesion days ± S.D. for guinea pigs that had severe skin lesions (score 6–9) and showed continued exacerbation and amelioratin of the severe lesions. Guinea pigs without severe lesions (score 6–9) were not included.

[c] Mean frequency ± S.D. for the number of recurrent disease (continued exacerbation and amelioration of skin lesions) per guinea pig in a spontaneous model.

[d] In A-I of Expt. 2, three guinea pigs were alive for 11, 23 and 38 days and all others were alive for 60 days (492 days). In B-III of Expt. 2, three guinea pigs were alive for 47, 47,and 49 days and all of the others were alive fot 60 days (563 days).

[e] Not determined.

[f] Statistical significance by Fischer's test.

[g] Statistical significance by Mann–Whitney *U*-test.

[h] The number of guinea pigs with new vesicles for *R. javanica* administration (0/17, the sum of b and c) was significantly lower than that for water administration (4/17, the sum of a and d) by the χ^2 test.

[i] Since three guinea pigs in each group were dead guring 1st administration, the number of remaining guinea pigs were used as total number of guinea pigs for 2nd administration.

[j] Statistical significance by the χ^2 test.

3. Results

3.1. Prophylactic efficacy of R. javanica on spontaneous recurrent disease

R. javanica was examined for its prophylactic efficacy on spontaneous recurrent HSV-2 infection in guinea pigs. Fig. 1 shows representative spontaneous recurrent HSV-2 skin lesions on the external genitalia 1.5 months after primary infection. We observed genital skin lesions for at least 1.5 months after primary infection and certified the onset of healing of the skin lesions caused by primary genital herpes. In order to distinguish the appearance of recurrent skin lesions from primary skin lesions in spontaneous recurrent experiments, we used guinea pigs in which the healing status lasted at least for more than a month before the crossover experiments. In Fig. 2, each line represents one guinea pig from each group and the peaks of each lines represent the continued exacerbation and amelioration of skin lesions during the administration period. Although some guinea pigs did not develop recurrence after primary infection, all guinea pigs were confirmed to be infected with HSV-2 by the presence of antibody in their sera (data not shown). It was obvious that *R. javanica* reduced the number of times that skin lesions were continuously exacerbated and ameliorated (the number of peaks in each line in Fig. 2). As shown in Table 1, *R. javanica* significantly reduced both the number of guinea pigs with new vesicle formations and the period that the vesicles were observed. Also, *R. javanica* significantly reduced the incidence, severity and/or mean frequency of recurrent skin lesions as compared with water-administered guinea pigs before the crossover administration ($P < 0.05$). When compared after the crossover administration in each group, *R. javanica* was significantly effective in reducing the incidence, severity and/or mean frequency of recurrent skin lesions as compared with guinea pigs with water administration ($P < 0.05$). The exacerbation of skin lesions did not occur immediately after the discontinuation of *R. javanica*. After the crossover administration, *R. javanica* significantly reduced the severity of recurrent skin lesions as compared with water-administered guinea pigs ($P < 0.05$). The herbal extracts used did not cause weight loss in latently infected guinea pigs as compared with the water-administered guinea pigs (data not shown). The prophylactic efficacy was confirmed by repeating the crossover experiment (Table 1, experiments 1 and 2). Thus, the prophylactic treatment with *R. javanica* significantly alleviated recurrent genital skin disease in guinea pigs without apparent toxicity.

Table 2
Prophylactic effects of *R. javanica* on recurrent HSV-2 infection by UV-irradiation

Treatment	Incidence[a]	Severity[b]	
	Guinea pig	Days	*P*-value
Water	5/5	7.2 ± 2.5	
R. javanica	5/5	3.6 ± 1.5	<0.05[c]

Recurrent disease in latently HSV-2 infected guinea pigs was induced by UV-irradiation on 3 months after primary infection. Herbal extracts (625 mg/kg per day) or water orally administered to HSV-2 infected guinea pigs for 10 days starting 5 days before UV-irradiation and recurrent skin lesions were observed daily and scored as described in the text.
[a] Incidence was expressed as the number of guinea pigs with severe erythema with swelling and/or vesicles over the total number of guinea pigs.
[b] Severity was expressed as the mean days $\pm$ S.D. for severe erythema with swelling and/or vesicles.
[c] Data were evaluated using Mann–Whitney's *U*-test (vs water administered guinea pigs).

3.2. Prophylactic efficacy of R. javanica on UV-induced recurrent disease

Prophylactic efficacy of *R. javanica* was confirmed with UV-induced recurrent HSV-2 disease on the external genitalia of guinea pigs. In water-administered guinea pigs, erythema and swelling were observed for 2 days after irradiation. These symptoms became severe and showed strong erythema (more than 50% of genital skin) with swelling and/or vesicles 3–5 days after irradiation. Histological analysis of these genital skins provided evidence for both the existence of viral antigen and lack of eosinophilic change of epidermis typically caused by UV-irradiation was observed (data not shown). Prophylaxis with *R. javanica* was significantly effective in shortening

the duration of severe skin lesions as compared with water-administered guinea pigs ($P < 0.05$, Table 2). Thus, *R. javanica* exhibited prophylactic efficacy on genital lesions in a UV-induced recurrent infection model in guinea pigs.

4. Discussion

R. javanica was prophylactically effective against spontaneous recurrent genital lesions caused by HSV-2 in guinea pigs. Its efficacy was confirmed by the two independent crossover experiments of *R. javanica*- and water-administration. *R. javanica* also alleviated UV-induced recurrent genital lesions. We have previously shown that the doses of herbal extracts corresponding to human use are also successful in attaining therapeutic anti-HSV-1 activity without toxicity in mice (Kurokawa et al., 1993, 1995a,b) and prophylactically effective against recurrent HSV-1 disease in mouse pina (Kurokawa et al., 1997). Thus, the prophylaxis with *R. javanica* against recurrent HSV disease was verified in both mice and guinea pigs. During this study, we have noticed that the genital skin lesions we observed are somewhat different from those as reported by Stanberry (1989, 1990a,b). Before starting the guinea pig experiments we selected ITO 1262 strain of HSV-2 for a murine cutaneous infection model, because this strain induced the most typical vesicular lesions in the skin of mice among the four HSV-2 strains examined (Yoshida et al., 1996). Based on this, we expected that this strain may induce typical vesicular lesions in guinea pigs. However, the vesicular lesions as well as some skin lesions induced by ITO 1262 strain in our guinea pig model were not the typical, recurrent genital skin lesions shown by Stanberry (1989, 1990a,b). Thus, we used different scoring system for the evaluation of recurrent skin lesions from that as reported by Stanberry (1989, 1990a,b).

Efficacy of *R. javanica* against HSV infection is suggested to result from its potent anti-HSV-2 activity rather than an anti-immflamatory activity (Kurokawa et al., 1993, 1995a,b, 1996). ACV and phosphonoacetic acid (PAA)-resistant HSV-1 mutants and HSV-2 as well as a wild HSV-1 strain are similarly susceptible to the hot-water extract of *R. javanica* (Kurokawa et al., 1995b), suggesting that its extract contains a certain anti-HSV compound which shows different anti-HSV action from that of ACV and PAA. We have previously selected four herbal extracts from 142 herbal medicines, which exhibited oral therapeutic antiviral activity against cutaneous HSV-1 infection in mice and augmented therapeutic efficacy of ACV (Kurokawa et al., 1993, 1995a). Among the four herbal medicines, eugeniin was coincidentally purified as a major anti-HSV compound from *Geum japonicum* Thunb. and *Syzygium aromaticum* (L.) MERR. et PERRY (Kurokawa et al., 1998). Identification of such an anti-HSV compound may allow us to clarify the mechanism of anti-HSV action of herbal extracts and standardize their anti-HSV activity in view of their quality control. These studies are now under way.

Long-term prophylaxis of genital herpes with ACV reduced the incidence of recurrence for a short time after its cessation of ACV administration (Straus et al., 1988). Likewise, in our crossover experiments the recurrent infection did not occur immediately after the discontinuation of *R. javanica* (Fig. 2). *R. javanica* has been shown to augment therapeutic anti-HSV activity of ACV in mice (Kurokawa et al., 1995a,b). Thus, it may augment the anti-HSV activity of ACV when the apparent recurrent infection may occur. Since *R. javanica* exhibited antiviral activity against ACV-resistant HSV and synergistic effect in combination with ACV based on different anti-HSV action of *R. javanica* from ACV (Kurokawa et al., 1993, 1995a,b), prophylaxis with *R. javanica* may be effective in reducing the appearance of ACV-resistant HSV during ACV therapy. Such use of R. javanica may reduce the use of ACV and preserve the efficacy of ACV for the future. Recently we identified an anti-HSV-1 compound from the extract of *R. javanica* and the analysis of the mechanism of its anti-HSV activity is now in progress.

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References

Corey, L., Nahmias, A.J., Guinan, M.E., Benedetti, J.K., Critchlow, C.W., Holmes, K.K., 1982. A trial of topical acyclovir in genital herpes simplex virus infections. New Engl. J. Med. 306, 1313–1319.

Douglas, J.M., Critchlow, C., Benedetti, J., Mertz, G.J., Connor, J.D., Hintz, M.A., Fahnlander, A., Remington, M., Winter, C., Corey, L., 1984. A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. New Engl. J. Med. 310, 1551–1556.

Erlich, K.S., Mills, J., Chatis, P., Mertz, G.J., Busch, D.F., Follansbee, S.E., Grant, R.M., Crumpacker, C.S., 1989. Acyclovir-resistant herpes simplex virus infections in patients with the acquired immunodeficiency syndrome. New Engl. J. Med. 320, 293–296.

Jiangxu New Medical College, 1978. Dictionary of Chinese Medicinal Materials. Shanghai Science and Technology Press, Shanghai (in Chinese).

Kurokawa, M., Ochiai, H., Nagasaka, K., Neki, M., Xu, H., Kadota, S., Sutardjo, S., Matsumoto, T., Namba, T., Shiraki, K., 1993. Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measles virus in vitro and their therapeutic efficacies for HSV-1 infection in mice. Antiviral Res. 22, 175–188.

Kurokawa, M., Nagasaka, K., Hirabayashi, T., Uyama, S., Sato, H., Kageyama, T., Kadota, S., Ohyama, H., Hozumi, T., Namba, T., Shiraki, K., 1995a. Efficacy of traditional herb medicines in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral Res. 27, 19–37.

Kurokawa, M., Sato, H., Ohyama, H., Hozumi, T., Namba, T., Kawana, T., Shiraki, K., 1995b. Effects of traditional herb medicines against herpes simplex virus (HSV) type 2 and acyclovir-resistant HSV type 1 in vitro and in vivo. J. Traditional Med. 12, 187–194.

Kurokawa, M., Ohyama, H., Hozumi, T., Namba, T., Nakano, M., Shiraki, K., 1996. Assay for antiviral activity of herbal extracts using their absorbed sera. Chem. Pharm. Bull. 44, 1270–1272.

Kurokawa, M., Nakano, M., Ohyama, H., Hozumi, T., Namba, T., Shiraki, K., 1997. Prophylactic efficacy of traditional herbal medicines against recurrent herpes simplex virus type 1 infection from latently infected ganglia in mice. J. Dermatol. Sci. 14, 76–84.

Kurokawa, M., Hozumi, T., Basnet, P., Nakano, M., Kadota, S., Namba, T., Kawana, T., Shiraki, K., 1998. Anti-herpesvirus activity of eugeniin purified from Geum japonicum with oral therapeutic activity in mice. J. Pharmacol. Exp. Ther. 284, 728–735.

Nagasaka, K., Kurokawa, M., Imakita, M., Terasawa, K., Shiraki, K., 1995. Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. J. Med. Virol. 46, 28–34.

Norris, S.A., Kessler, H.A., Fife, K.H., 1988. Severe progressive herpetic whitlow caused by an acyclovir-resistant virus in a patient with AIDS. J. Infect. Dis. 157, 209–210.

Pass, R.F., Whitley, R.J., Whelchel, J.D., Diethelm, A.G., Reynolds, D.W., Alford, C.A., 1979. Identification of patients with increased risk of infection with herpes simplex virus after renal transplantation. J. Infect. Dis. 140, 487–492.

Rand, K.H., Rasmussen, L.E., Pollard, R.B., Arvin, A., Merigan, T.C., 1977. Cellular immunity and herpesvirus infections in cardiac transplant patients. New Engl. J. Med. 296, 1372–1377.

Reusser, P., Cordonnier, C., Einsele, H., Engelhard, D., Link, H., Locasciulli, A., Ljungman, P., 1996. For the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation (EBMT). European survey of herpesvirus resistance to antiviral drugs in bone marrow transplant recipients. Bone Marrow Transplant 17, 813–817.

Stanberry, L.R., 1989. Animal model of ultraviolet-radiation-induced recurrent herpes simplex virus infection. J. Med. Virol. 28, 125–128.

Stanberry, L.R., 1990a. Recurrent genital herpes in the guinea pig augmented by ultraviolet irradiation: effects of treatment with acyclovir. Antiviral Res. 13, 227–236.

Stanberry, L.R., 1990b. Capsaicin interferes with the centrifugal spread of virus in primary and recurrent genital herpes simplex virus infection. J. Infect. Dis. 162, 29–34.

Straus, S.E., Croen, K.D., Sawyer, M.H., Freifeld, A.G., Felser, J.M., Dale, J.K., Smith, H.A., Hallahan, C., Lehrman, S.N., 1988. Acyclovir suppression of frequently recurring genital herpes. J. Am. Med. Assoc. 260, 2227–2230.

Yoshida, Y., Yamamura, J., Sato, H., Koyasu, M., Obara, Y., Sekiguchi, H., Kawana, T., Shiraki, K., 1996. Efficacy of Cafon gel on cutaneous infection with herpes simplex virus (HSV)-2 and acyclovir-resistant HSV in mice. J. Dermatol. Sci. 13, 237–241.