Review J. Trad. Med. 22(Suppl.1), 138－144, 2005

New antiviral agents from traditional medicines

Masahiko KUROKAWA,[a] and Kimiyasu SHIRAKI*[b]

[a]*Department of Biochemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Yoshino, Nobeoka, Miyazaki 882-8508, Japan.* [b]*Department of Virology , Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-0194, Japan.* *(Accepted April 11, 2005.)*

We summarized the flow of our research on the development of antiviral traditional medicines, which was a collaboration with the late Professor Tsuneo Namba. Even if traditional medicines and compounds purified from them have strong antiviral activity *in vitro*, we believe they are just inhibitors and not medicines if they have no therapeutic efficacy *in vivo*. Therefore our study is based on the confirmation of the oral therapeutic efficacy with traditional dosages in humans. We first screened the *in vitro*-antiviral activity of typically and easily available traditional medicines that are currently used for the treatment of various chronic diseases in China, India and Japan. Then we selected the extracts of traditional medicines with prophylactic and therapeutic antiviral activity in animal infection models. By verifying the antiviral activity of extracts *in vivo*, their new indications for the use of viral infection would be established. In consequence, the drinking of the extracts of traditional medicines like a daily tea or coffee may be used as prophylaxis and therapy for diseases caused by viral infection and improve the quality of life. Here, we describe how the antiviral extracts of traditional medicines and active compounds involved in them were explored and characterized, based on a view that antiviral activity should be active in vivo.

Key words Antiviral activity, traditional medicines, herpes simplex virus, cytomegalovirus, prophylactic treatment, therapeutic treatment.

I Introduction

Efficacy of traditional medicines for diseases has been historically established by trial on humans. Many effective drugs, such as aspirin, morphine, atropine, reserpine, ephedrine, digitoxin, *etc.*, were developed from traditional medicines including natural products. Thus, the development of such drugs may have resulted from the recognition of the efficacy of traditional medicines. In this respect, traditional medicines might not be considered as therapeutic agents for diseases caused by specific virus infection for a long time. In traditional therapy, thus, less traditional medicines are used as antiviral agents against specific viral infection, such as acyclovir (ACV) which anti-herpes simplex virus (HSV) activity was scientifically proved.

Many antiviral agents, mainly synthetic nucleoside analogs, have been developed and practically used for the treatment of virus infection in immunodeficient patients such as AIDS and organ-transplanted patients. However the long-term administration of the antiviral agents has caused the appearance of the resistant virus strains. The development of new antiviral agents with various kinds of antiviral actions is now required. Thus we focused on traditional medicines including plants, insects, animal organs *etc.* rather than synthetic nucleoside analogs, because traditional medicines have their own metabolites originating from natural products and some of them may recognize the differences between viral and host metabolism resulting in antiviral activity. The traditional medicines may be useful sources to explore new antiviral agents with different antiviral actions from those of known antiviral agents.

Many traditional medicines have been historically used as hot water-extracts for the treatment of various diseases, like a cup of tea. Such oral use is very popular in China and Japan. Also, information on their appropriate use and adverse reactions has been historically accumulated. Therefore if the antiviral therapeutic efficacy of traditional medicines is verified *in vivo*, we would be able to use them prophilactically and therapeutically for the treatment of viral infection by drinking them as coffee or tea. Further, if we can isolate and identify antiviral compounds from the traditional medicines, the purified compounds would be also available as antiviral agents, like western medicines. Here we focus on the hot water-extracts of traditional medicines that are orally administered and describe how the direct antiviral activity of the extracts were verified, and then how the active compounds in the extracts were prepared and identified from the extracts.

II Antiviral activity *in vitro*

Hot water-extracts of traditional medicines contain many kinds of compounds. It is difficult to estimate their antiviral concentrations *in vitro*. Thus, we established the concentrations of compounds to be examined in an *in vitro*-assay based on the assumption that all antiviral active compounds that were administered orally are absorbed *in vivo*.[1] For example, glucose is one of the typical compounds that are rapidly and easily absorbed from alimentary tracts in human. Its maximal serum concentration is reported to increase by approximately 300 mg/dl (300 μg/ml) after its 100

*To whom correspondence should be addressed. e-mail : kshiraki@ms.toyama-mpu.ac.jp

g (10 g) oral administration in humans with diabetes mellitus.[2] Some components in the extracts may be absorbed as rapidly and efficiently as glucose. Since the extracts (approximately 3-30 g) prepared from approximately 30-100 g of dried traditional medicines have been orally administered in humans, a dose (100 μg/ml) of the extracts in *in vitro*-assay may correspond to their putative concentrations in serum after oral administration. Further higher concentrations (such as 300 or 500 μg/ml) would be helpful to survey the possible antiviral activity of compounds in the extracts that were not selected at 100 μg/ml. Therefore, *in vitro*-assay using these doses is designed to examine antiviral activity at the putative concentration of substances in serum and none of the possible antiviral substances for oral use would be lost in this assay. Concentrations more than those would not be available for an *in vitro*-antiviral assay, because the higher concentrations do not correspond to the concentrations to reach *in vivo*.

Based on this view, we screened the anti-HSV type 1 (HSV-1), poliovirus and measles virus activities of 142 (so far, more than 400) extracts of traditional medicines at 100 and 300 or 500 μg/ml using a plaque reduction assay, which are historically used in China, India, and Japan.[1] We found anti-HSV-1, anti-poliovirus and anti-measles virus extracts as shown in Table 1. The plaque reduction assay is a useful method for searching substances, such as ACV, which act directly in infected cells and exhibits direct antiviral activity after absorption *in vivo*.[3] Thus, the *in vitro*-assay may not be available for substances that act as precursor drugs or biological response modifiers (BRM).[4,5] Finally we found that five among 142 extracts of traditional medicines inhibited the plaque formation of both HSV-1 and poliovirus; one extract was effective against both HSV-1 and measles virus; three were active to HSV-1 only. All HSV-1-inhibitory extracts other than the nine extracts described above were also effective against both poliovirus and measles virus. Thus thirty two extracts were classified into 4 groups based on antiviral activities for poliovirus and measles virus.[1] Since the three viruses have different structures and replication systems, the four groups were suggested to include extracts showing different modes of antiviral action.

Four of the thirty two extracts showed cytotoxicity in plaque reduction assay. However, two among the four were finally found to exhibit significant therapeutic efficacy *in vivo*. This suggests that antiviral components in the extracts were selectively absorbed from alimentary tracts *in vivo* and exerted an HSV-1-inhibitory effect that was not associated with toxicity. Extracts contain various kinds of compounds. Some compounds may be absorbed from alimentary tracts, and others may not. Even if the antiviral compounds are absorbed, their concentrations *in vivo* vary possibly by the rate of absorption, metabolism, and excretion. Therefore we selected all thirty two extracts as possible anti-HSV-1 extracts (Fig. 1).

Table 1 Traditional medicines with anti-HSV-1 activity in vitro

Anti-HSV-1	32/142[1)]
Anti-poliovirus	55/142
Anti-measles virus	30/142

[1)]Ratio of antiviral traditional medicines in the 142 examined *in vitro*.

III Antiviral therapeutic activity *in vivo*

Animal infection experiments are very important to determine whether or not substances possess therapeutic antiviral efficacy *in vivo*. In *in vivo*-antiviral assay, antiviral efficacy is evaluated by assessing the development of symptoms caused by viral infection, virus yields in organs and mortality of infected animals. Thus, an animal infection model reflecting the efficacy of substances in humans should be properly chosen. For that reason, the following would be required, 1) the selection of animals to which the virus to be examined can infect and its infection way, 2) an animal model to be used reflects the course of virus infection in human, 3) quantitative evaluation of therapeutic efficacy on virus infection, 4) use of animal models that are accepted internationally.

Therapeutic efficacies of thirty two anti-HSV-1 extracts selected by *in vitro*-assay were examined in a

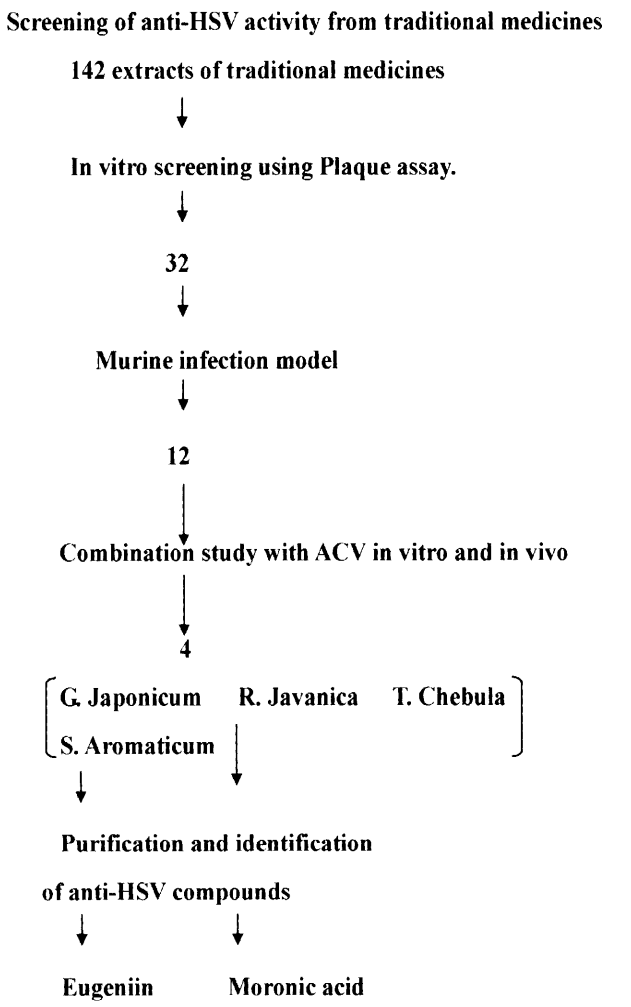

Fig. 1 Development of anti-HSV-1 agents from traditional medicines.

cutaneous HSV-1 infection model in mice (Fig. 2).[1] This murine model is very useful to evaluate the therapeutic efficacy of extracts on not only mortality but also the development of herpetic skin lesions as seen in humans. In this model, the treatment of 5 mg/kg of ACV significantly prolonged mean survival times and delayed the development of skin lesions although there was no significant reduction of mortality. The dosages of extracts used for this murine model was deduced by the dose for human use based on the body surface area. Twelve extracts with therapeutic anti-HSV-1 activities were finally selected in a cutaneous HSV-1 infection model (Fig. 1). The efficacies of the extracts were repeatable in the different lots of extracts. Those selected extracts showed therapeutic anti-HSV-1 activity similar to that of ACV at 5 mg/kg corresponding to a dose in mice deduced from human use (Table 2).[1,6] Thus, the extracts could be also effective for humans. Any toxicity of the extracts was not observed in mortality and weight loss. Also, the anti-HSV-1 activity of 12 extracts selected was confirmed to be absorbed from alimentary tracts into the serum of guinea pigs administered orally with the extracts.[7] In order to search extracts with *in vivo*-antiviral activity, this serum-pharmacological assay may be useful as a screening procedure of extracts before animal experiments.[7]

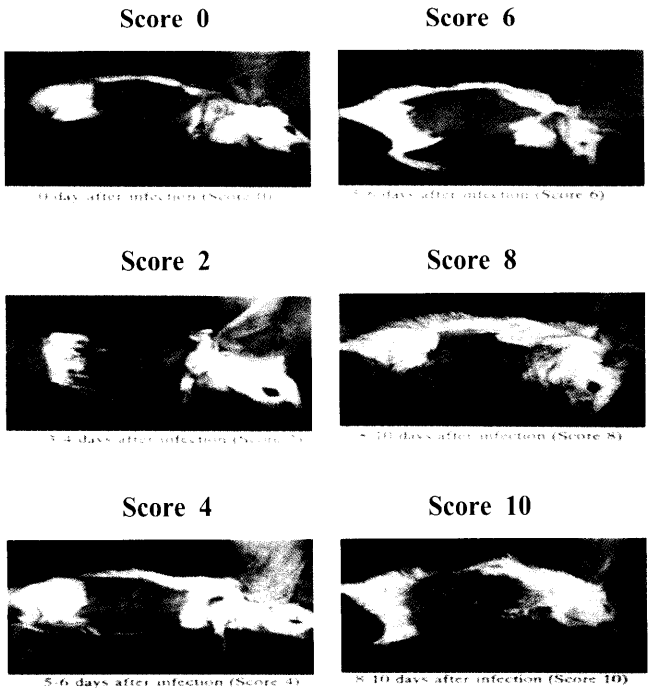

Fig. 2 The development of herpetic skin lesions in murine cutaneous HSV-1 infection model. Skin lesions were scored as follows: 0, no lesion; 2, vesicles in local region; 4, erosion and/or ulceration in local region; 6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and death.

IV Mode of antiviral action

ACV is a selective antiherpetic agent[8-11] and has been widely used for the treatment of HSV and varicella-zoster virus infection.[12-16] Combined treatments of two drugs with different modes of anti-HSV action have been shown to increase the therapeutic anti-HSV activity expected from the treatment with each drug against HSV infection.[17-24] We found that 12 extracts of traditional medicines have therapeutic anti-HSV type 1 (HSV-1) activity in mice infected with HSV-1 cutaneously.[1] These traditional medicines can be obtained easily in Japan and China. Their extracts have been directly and orally used for the treatment of various human diseases.[25,26] The application and dosage of these herbal extracts for each disease have been historically selected and established by their efficacies and adverse reactions in human use. Thus the extracts that exhibited anti-HSV-1 activity *in vivo* may supplement the anti-HSV-1 activity of ACV and augment clinically the therapeutic efficacy of ACV at their conventional doses without major adverse reactions. The combination of ACV with extracts may be beneficial for the treatment of human HSV-1 infection. Therefore we characterized the anti-HSV-1 activity of extracts by examining their combined effects of ACV for human use of extracts, themselves.[27]

Among the 12 extract selected *in vivo* assay, 4 extracts [Geum japonicum THUNB. (*G. japonicum*), Rhus javanica L. (*R. javanica*), Syzygium aromaticum (L.) MERR. et PERRY (*S. aromaticum*), Terminalia chebula RETZUS (*T. chebula*)] in combination with ACV reduced virus yields in the brain and skin more strongly than acyclovir alone (Fig. 1).[27] The combination of ACV with extracts exhibited stronger anti-HSV-1 activity in the brain than in the skin, in contrast to acyclovir treatment by itself. This effectiveness of extracts in the brain was confirmed in mice infected cuteneously with HSV type 2 (HSV-2) or phosphonoacetic acid (PAA)-resistant HSV-1 (ACV-PAA-resistant HSV-1).[28] Any treatments with ACV and/or extracts were not significantly toxic in mice. Combination of ACV with historically used traditional medicines showed strong combined

Table 2 Effect of R. javanica extract and ACV on murine cutaneous HSV-1 infection model.

Treatment	Mean time (days±SD)[1]			Mortality
	Vesicles	Zosteriform	Survival time	
Control (Water)	3.39±0.15	5.15±0.38	6.92±0.64	13/14
ACV (2.5 mg/kg)	3.62±0.51	5.02±0.87	7.73±1.49	11/14
ACV (5 mg/kg)	4.08±0.29[2]	5.70±0.48[2]	9.00±1.33[2]	9/12
R. javanica (5 mg/kg)	4.10±0.99[2]	6.50±0.54[2]	8.33±1.23	9/10

[1] Mean time at which vesicles, zosteriform, death were first observed after infection.
[2] $P < 0.05$

therapeutic anti-HSV-1 activity in mice, especially reduction of virus yield in the brain. Further, the 4 extracts were confirmed to inhibit the DNA synthesis of HSV-2 or ACV-PAA-resistant HSV-1 strains in Vero cells.[28] These results indicated that the 4 extracts possess a different mode of anti-HSV-1 action from that of ACV. The dosages of these extracts have been established in traditional therapy and used safely for humans for several thousand years. The dose (mg/kg/day) of extracts in mice was calculated from the dose (mg/kg/day) in human and was not toxic in mice as expected from the experiences in humans use. Thus the conventional use of 4 herbal extracts would be applicable as anti-HSV-1 medicines for augmenting therapeutic anti-HSV-1 efficacy of ACV in humans.

V Prophylactic and therapeutic anti-HSV activity

A long-term oral administration of the extracts of traditional medicines has been utilized for the treatment of chronic diseases such as chronic pharyngolaryngitis, gastric and duodenal ulcer, empyema, etc. using traditional therapy. It would be expected that their long-term administration may be safely and cautiously managed based on the information accumulated historically. In these points of view, we examined the prophylactic efficacy of extracts against recurrent viral diseases in mice and guinea pigs.[29,30]

HSV replicates locally in epithelial cells of skin after primary infection and is transported via peripheral nerves to central neural tissues such as trigeminal ganglia. Latently infected virus is activated and grows in the neural tissues after physical stimuli such as UV irradiation and then the produced virus is transported to skin via peripheral nerves and causes skin lesions. Mice intradermally infected with HSV-1 in the pinna were commonly used as an experimantal model for such recurrent HSV-1 infection.[29] Also, we used a genital infection model in guinea pigs, which is the best animal model for spontaneous recurrent HSV-2 genital diseases[31-33] to evaluate the efficacy of extracts against recurrent HSV infection.

The prophylactic treatment with 4 extracts arrested the progression of recurrent HSV-1 diseases after UV irradiation in mice (Fig. 3).[29] In spontaneous recurrent HSV-2 genital model in guinea pigs, the representative skin lesions of latently infected-guinea pigs are shown in Fig. 4. *R. javanica* was prophylactically effective against spontaneous recurrent genital lesions caused by HSV-2 in guinea pigs without apparent toxicity. Its efficacy was confirmed by the two independent crossover experiments of extracts and water-administration (Fig. 5).[30] Thus, the prophylactic long-term use of 4 extracts was verified to be effective against recurrent HSV diseases in mice and guinea pigs.

All 4 herbal extracts exhibited therapeutic anti-HSV-1 activity in a cutaneous HSV-1 infection model in mice and strengthen the anti-HSV activity of ACV.[1,27,28] Thus they would be able to be continuously used as therapeutic agents in ACV therapy after prophylaxis and the combinations of ACV with extracts may be expected to enhance anti-HSV activity of ACV in humans. Since the extracts also exhibited strong antiviral activity against ACV-PAA-resistant HSV-1 mutants and HSV-2,[28] the continuous prophylactic and therapeutic treatments with extracts may be effective in reducing the growth of ACV-resistant HSV that may occurs during ACV therapy. Thus, the prophylactic and therapeutic efficacy of extracts that we selected from *in vitro*-screening was verified in animal infection models.

VI Prophylactic and therapeutic anti-cytomegalovirus (CMV) activity

CMV infection is one of the troublesome infections in immunocompromised patients, especially, transplant recipients and patients with AIDS.[34-38] Symptomatic CMV infection has been successfully treated with ganciclovir, but the appearance of ganciclovir-resistant viruses is a current problem in the treatment of immunocompromised patients with CMV infection. As interstitial pneumonia is one of the major target of antiviral chemotherapy, we evaluated the anti-CMV activity *in vivo* of 4 herbal extracts (*G. japonicum, R. javanica, S. aromaticum,* and *T. chebula*) by

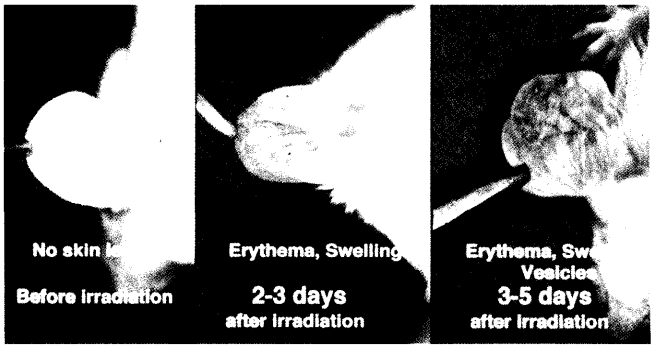

Fig. 3 Skin lesions of pinna after UV irradiation. Mice were infected intradermally with HSV-1 in the upper surface of the right pinna as primary infection. Four months later, the upper surface of right pinna originally infected was exposed to ultraviolet (UV) B light.

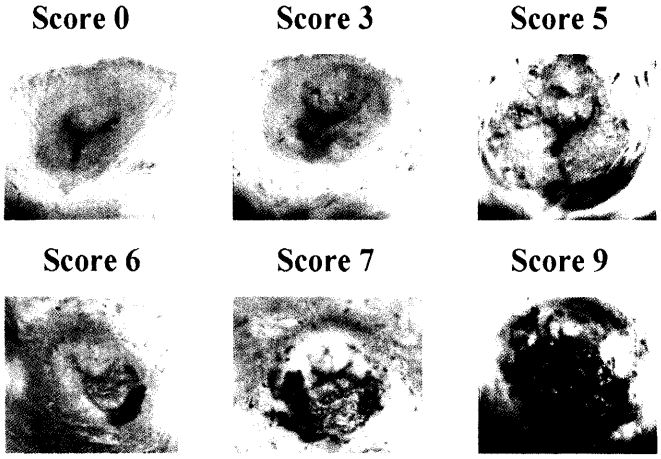

Fig. 4 Genital skin lesions in guinea pigs were scored at 3 to 5 months after primary infection as follows: 0, no lesion; 1, swelling; 2, weak erythema (less than 50% of genital skin); 3, weak erythema with swelling; 4, strong erythema (more than 50% of genital skin); 5, strong erythema with swelling; 6, strong erythema with vesicles and/or crust; 7, strong erythema with erosion and crust; 8, strong erythema with erosion; 9, erosion or ulcer with crust.

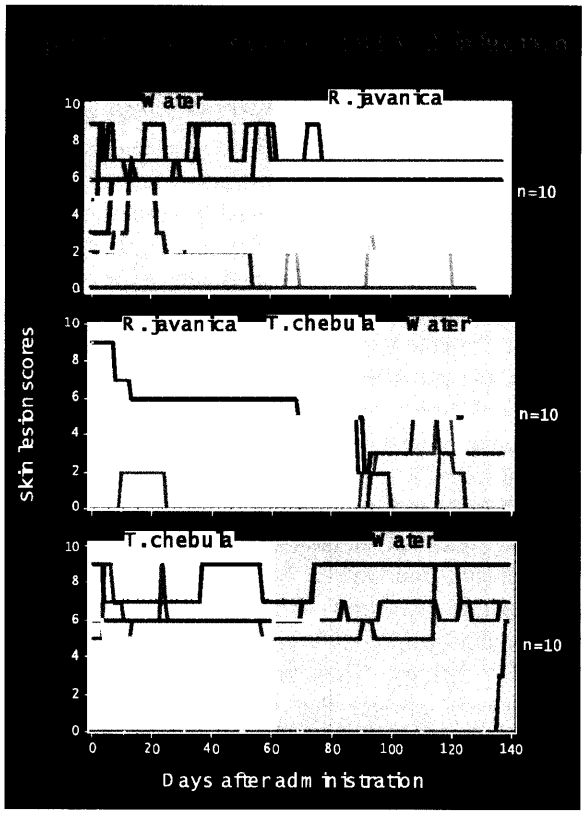

Fig. 5 Prophylactic effects of extracts of traditional medicines on the spontaneous recurrent HSV-2 infection. Guinea pigs were intravaginally infected with HSV-2. Three month later, the latently infected guinea pigs were divided into 2 groups. Each group contained 10 guinea pigs (n = 10). *R. javanica* (625 mg/kg/day) was supplied within drinking water. Crossover experiments were performed and T. Chebula was used as an inactive extract. Guinea pigs were administered with *R. Javanica* for 2 months (day 0 to day 60) and then the administration was changed to water after the first 2 months or guinea pigs were administered with water for 2 months (day 0 to day 60) and then the administration was changed to *T. chebula* (day 60 to day 90) and water after the first 2 months. The development of skin lesions was observed daily and the severity of the lesions was scored as shown in Fig.4. Each line shows changes of skin score of one guinea pig in each group. Many spikes indicate that recurrent infection occurs frequently.

using the lung infection[39,40] in mice immunosuppressed by cyclosporine.[41,42] The 4 extracts dose-dependently inhibited the growth of human and mouse CMV in the plaque reduction assay.[43] In the immunosuppressed mice, *G. japonicum, S. aromaticum,* and *T. chebula* significantly exhibited prophylactic efficacy compared with water-administered mice.[43] Especially, efficacy of oral treatment with 750 mg/kg/day of *G. japonicum*-extract was similar to that of the intraperitoneal administration with 2 mg/kg/day of ganciclovir in increasing the body weight of infected mice and reducing the virus yield in the lungs.[43] Thus the 3 extracts were proven to be effective for the prophylactic treatment of not only HSV infection but also CMV infection.

VII Identification and characterization of anti-HSV compounds

As many agents such as aspirin, morphine, digoxin, ephedrine, etc. have been developed from natural sources, novel antiviral compounds may be also developed from traditional medicines. We have proved the anti-HSV activity of the extracts by clarifying the antiviral actions of the purified compounds. Anti-HSV compounds were purified from extracts using different chromatographic fractionations guided by anti-HSV activity. The fractionations guided by antiviral activity was very effective to prepare active compounds against HSV.[44] This way was also an effective way to obtain the fraction of *Prunella vulgaris* spikes that inhibits human immunodeficiency virus type 1 replication at reverse transcription.[45]

Finally eugeniin (Figs. 1 and 6) was purified as an anti-HSV compound from the different species of plants, *G. japonicum* and *S. aromaticum,* of the 4 herbal extracts.[44] This is a surprise because the two herbs are different plant species. Also, this confirmed the validity, accuracy and feasibility of our screening and assay systems, when the active compound is in the extract. This compound was characterized to inhibit not only the growth of wild type HSV-1 but also ACV-resistant HSV-1, PAA-resistant HSV-1 and wild HSV-2. A major target site of inhibitory action of eugeniin was found to be viral DNA synthesis. It showed higher

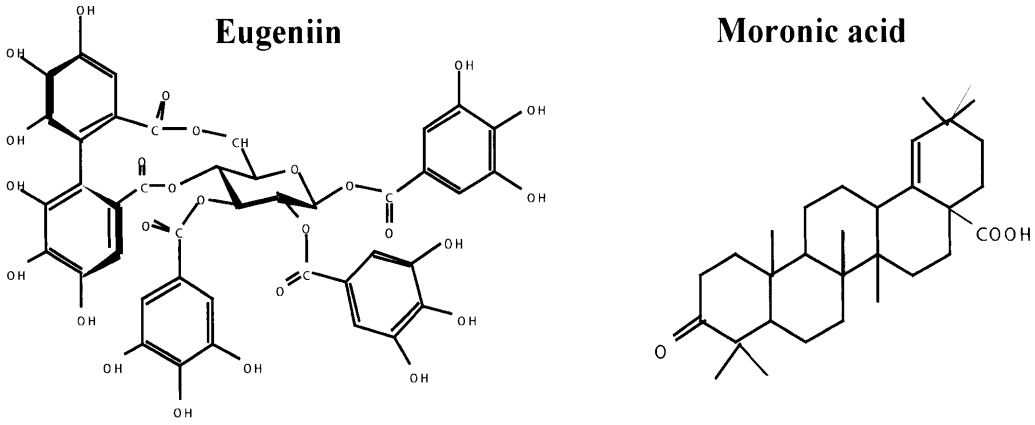

Fig. 6 Chemical structures of eugeniin and moronic acid.

specificity for the inhibition of HSV-1 DNA polymerase activity than that of cellular DNA polymerase activities. Its specificity for HSV-1 DNA polymerase as well as its therapeutic index was higher than those of 9-β-D-arabinofuranosyladenine, Vidarabine, that has been clinically used for the treatment of herpetic disease.[46-48] The inhibitory action of eugeniin for HSV-1 DNA polymerase was found to be non-competitive, such as a non-nucleoside inhibitor of reverse transcriptase of HIV-1, Nevirapine.[44]

Further, we have purified and identified an anti-HSV compound, moronic acid (Figs. 1 and 6), from one (*R. javanica*) of the 4 herbal extracts that alleviated spontaneous and ultraviolet-induced recurrent HSV-2 genital disease in guinea pigs.[50] Moronic acid showed stronger anti-HSV-1 activity in the brain of HSV-1 infected mice than in the skin similar to the 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. This compound as well as eugeniin exhibited therapeutic anti-HSV-1 and ACV-PAA-resistant HSV-1 activity in a cutaneous HSV infection model in mice.[49,50] We verified the therapeutic anti-HSV activity of extracts by identifying the antiviral actions and therapeutic activity of the purified compounds.

VIII Conclusion

We believe that the antiviral activity of natural products should be verified to be effective *in vivo* to obtain the right evaluation as antiviral agents. There have been many studies to evaluate the antiviral substances prepared from natural products over the years. However, less antiviral substances found from natural products have been clinically used. In this report we introduced how traditional medicines that show direct antiviral activity was explored and how the therapeutic activity was characterized and verified. In fact, we could find antiviral traditional medicines and some active compounds from them as candidates for new antiviral agents. We hope that our antiviral screening processes based on the view that antiviral activity should be active *in vivo* would be helpful for the development of antiviral agents from traditional medicines including natural products.

Acknowledgments

These studies presented here were in collaboration with the late Professor Tsuneo Namba. We thank Dr. M. Hattori (Toyama Medical and Pharmaceutical University) for his helpful discussion. We thank Dr. H. Sato, Mr. Y. Yoshida, Ms. T. Okuda, Ms. Y. Hama, and Ms. Y. Takanami for their excellent technical and editorial assistance.

References

1) Kurokawa, M., Ochiai, H., Nagasaka, K., Neki, M., Xu, H., Kadota, S., Sutardjo, S., Matsumoto, T., Namba, T. and Shiraki, K.: Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measeles virus *in vitro* and their therapeutic efficadies for HSV-1 infection in mice. *Antiviral Res.,* **22**, 175-188, 1993.
2) Kosaka, K., Hagura, R. and Kuzuya, T.: Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. *Diabetes,* **26**, 944-952, 1977.
3) De Miranda, P., Krasny, H. C., Page, D. A. and Elion ,G. B.: The disposition of acyclovir in different species. *J. Pharmacol. Exp. Ther.,* **219**, 309-315, 1981.
4) Nagasaka, K., Kurokawa, M., Imakita, M., Terasawa, K. and Shiraki, K.: Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. *J. Med. Virol.,* **46**, 28-34, 1995.
5) Li, Z.H., Kurokawa, M., Sato, H., Tatsumi, Y. and Shiraki, K.: Efficacy of Kampo medicines on cutaneous herpes simplex virus type 1 infection in mice. *J. Trad. Med.,* **14**, 192-198, 1997.
6) Kurokawa, M., Hase, K., Xu, H.-X., Yamamura, J., Koyasu, M., Satoh, H., Kadota, S., Hozumi, T.,Namba, T. and Shiraki, K.: A novel procedure for the identification of a fraction with anti-herpes simplex virus type 1 activity *in vivo* from hot-water extract of traditional medicines, *Geum Japonicum Thunb. J. Med. Pharm. Soc. WAKAN-YAKU,* **10**, 195-203, 1993.
7) Kurokawa, M., Oyama, H., Hozumi, T., Namba, T., Nakano, M. and Shiraki, K.: Assay for antiviral activity of herbal extracts using their absorbed sera. *Chem. Pharm. Bull.,* **44**, 1270-1272, 1996.
8) Elion, G. B., Furman, P. A., Fyfe, J. A., De Miranda, P., Beauchamp, L. and Schaeffer, H. J.: Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. *Proc. Natl. Acad. Sci. USA,* **12**, 5716-5720, 1977.
9) Fyfe, J. A., Keller, P. M., Furman, P. A., Miller, R. L. and Elion, G. B.: Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound 9-(2-hydroxyethoxymethyl)guanine. *J. Biol. Chem.,* **253**, 8721-8727, 1978.
10) Furman, P. A., St Clair, M. H. and Spector, T.: Acyclovir triphosphate is a suitable inactivator of the herpes virus DNA polymerase. *J. Biol. Chem.,* **259**, 9575-9579, 1984.
11) Shiraki, K., Yamanishi, K. and Takahashi, M.: Susceptibility to acyclovir of Oka-strain varicella vaccine and vaccine-derived viruses isolated from immunocompromised patients. *J. Infect. Dis.,* **150**, 306-307, 1984.
12) Dunkle, L. M., Arvin, A. M., Whitley, R. J., Rotbart, H. A., Feder, H. M. Jr., Feldman, S., Gershon, A. A., Levy, M. L., Hayden, G. F., McGuirt, P. V., Harris, J. and Balfour, H. H. Jr.: A controlled trial of acyclovir for chickenpox in normal children. *N. Engl. J. Med.,* **325**, 1539-1544, 1991.
13) Meyers, J. D., Wade, J. C., Mitchell, C. D., Saral, R., Lietman, P. S., Durack, D. T., Levin, M. J., Segreti, A. C. and Balfour, H. H.: Multicenter collaborative trial of intraveneous acyclovir for treatment of mucocutaneous herpes simplex virus infection in the immunocompromised host. *Am. J. Med.,* **73**, 229-235, 1982.
14) Whitley, R. J., Alford, C. A., Hirsch, M. S., Schooley, R. T., Luby, J. P., Aoki, F. Y., Hanley, D., Nahmias, A. J., Soong, S.-J. and the NIAID Collaborative Antiviral Study Group: Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N. Engl. J. Med.,* **314**, 144-149, 1986.
15) Whitley, R., Arvin, A., Prober, C., Burchett, S., Corey, L., Powell, D., Plotkin, S., Starr, S., Alford, C., Connor, J., Jacobs, R., Nahmias, A., Soong, S.-J. and the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group: A controlled trial comparing vidarabine with acyclovir in neonatal herpes simplex virus infection. *N. Engl. J. Med.,* **324**, 444-449, 1991.

16) Whitley, R. J., Gnann, J. W. Jr., Hinthorn, D., Liu, C., Pollard, R. B., Hayden, F., Mertz, G. J., Oxman, M., Soong, S.-J. and the NIAID Collaborative Antiviral Study Group: Disseminated herpes zoster in the immunocompromised host: A comparative trial of acyclovir and vidarabine. *N. Engl. J. Med.,* **165**, 450-455, 1992.
17) Ayisi, N. K., Gupta, S. V. and Babiuk, L. A.: Efficacy of 5-methoxymethyl-2'-deoxyuridine in combination with arabinosyladenine for the treatment of primary herpes simplex genital infection of mice and guinea pigs. *Antiviral Res.,* **6**, 33-47, 1986.
18) Fraser-Smith, E. B., Eppstein, D. A., Marsh, Y. V. and Matthews, T. R.: Enhanced efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl)guanine in combination with gamma interferon against herpes simplex virus type 2 in mice. *Antiviral Res.,* **5**, 137-144, 1985.
19) Ellis, M. N., Lobe, D. C. and Spector, T.: Synergistic therapy by acyclovir and A1110U for mice orofacially infected with herpes simplex viruses. *Antimicrob. Agents Chemother.,* **33**, 1691-1696, 1989.
20) Hilfenhaus, R. J., De Clercq, E., Kohler, R., Geursen, R. and Seiler, F.: Combined antiviral effects of acyclovir or bromovinyldeoxyuridine and human immunoglobulin in herpes simplex virus infected mice. *Antiviral Res.,* **7**, 227-235, 1987.
21) Lobe, D. C., Spector, T. and Ellis, M. N.: Synergistic topical therapy by acyclovir and A1110U for herpes simplex virus induced zosteriform rash in mice. *Antiviral Res.,* **15**, 87-100, 1991.
22) Pancheva, S. N.: Potentiating effect of ribavirin on the anti-herpes activity of acyclovir. *Antiviral Res.,* **16**, 151-161, 1991.
23) Schinazi, R. F., Peters, J., Williams, C. C., Chance, D. and Nahmias, A. J.: Effect of combinations of acyclovir with vidarabine or its 5'-monophosphate on herpes simplex viruses in cell culture and in mice. *Antimicrob. Agents Chemother.,* **22**, 499-507, 1982.
24) Smith, K. O., Galloway, K., Ogilvie, K. K. and Cheriyan, U. O.: Synergism among BIOLF-62, phosphonoformate, and other antiherpetic compounds. *Antimicrob. Agents and Chemother.,* **22**, 1026-1030, 1982.
25) Jiangxu New Medical College. Dictionary of Chinese Medicinal Materials. Shanghai, China: Shanghai Science and Technology Press, 1978. (in Chinese).
26) Terasawa, K.: Kampo. K. K. Standard McIntyre. Tokyo, 1993.
27) Kurokawa, M., Nagasaka, K., Hirabayashi, T., Uyama, S., Sato, H., Kageyama, T., Kadota, S., Ohyama, H., Hozumi, T., Namba, T. and Shiraki, K.: Efficacy of traditional herbal medicines in combination with acyclovir against herpes simplex virus type 1 infection *in vitro* and *in vivo. Antiviral Res.,* **27**, 19-37, 1995.
28) Kurokawa, M., Sato, H., Ohyama, H., Hozumi, T., Namba, T., Kawana, T. and Shiraki, K.: Effects of traditional herbal medicines against herpes simplex virus (HSV) type 2 and acyclovir-resistant HSV type 1 *in vitro* and *in vivo. J. Trad. Med.,* **12**, 187-194, 1995.
29) Kurokawa, M., Nakano, M., Ohyama, H., Hozumi, T.,Namba, T. and Shiraki, K.: 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, 1997.
30) Nakano, M., Kurokawa, M., Hozumi, T., Saito A., Ida, M., Morohashi, M, Namba, T., Kawana, T. and Shiraki, K.: Suppression of recurrent genital herpes simplex virus type 2 infection by *Rhus Javanica* in guinea pigs. *Antiviral Res.,* **39**, 25-33, 1998.
31) Stanberry, L. R.: Animal model of ultraviolet-radiation-induced recurrent herpes simplex virus infection. *J. Med. Virol.,* **28**, 125-128, 1989.
32) Stanberry, L. R.: Recurrent genital herpes in the guinea pig augmented by ultraviolet irradiation: effects of treatment with acyclovir. *Antiviral Res.,* **13**, 227-236, 1990a.
33) Stanberry, L. R.: Capsaicin interferes with the centrifugal spread of virus in primary and recurrent genital herpes simplex virus infection. *J. Infect. Dis.,* **162**, 29-34, 1990b.
34) Ho, M.: Viral infection after transplantation in man. *Arch. Virol.,* **55**, 1-24, 1977.
35) Marker, S. C., Howard, R. J., Simmons, R. L., Kalis, J. M., Conelly, D. P., Najarian, J. S. and Balfour, Jr., H. H.: Cytomegalovirus infection: a quantitative prospective study of 320 consecutive renal transplants. *Surgery,* **89**, 660-671, 1981.
36) Betts, R. F. and Hanshaw, J. B.: Cytomegalovirus (CMV) in the compromised host(s). *Ann. Rev. Med.,* **28**, 103-110, 1977.
37) Jacobson, M. A. and Mills, J.: Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. *Ann. Intern. Med.,* **108**, 585-594, 1988.
38) Zaia, J. A.: Prevention and treatment of cytomegalovirus pneumonia in transplant recipients. *Clin. Infect. Dis.,* **17**, 5392-5399, 1993.
39) Osborn, J. E.: CMV-herpesvirus of mice. In: H. L. Foster, J. G. Fox and J. D. Small (Eds), The Mouse in Biomedical Research, Vol. 2, pp. 267-292. Academic press, Now York, 1982.
40) Shanley, J. D. and Pesanti, E. L.: The relation of viral replication to interstitial pneumonitis in murine cytomegalovirus lung infection. *J. Infect. Dis.,* **151**, 454-458, 1985.
41) Land, W.: Optimal use of Sandimmun in Organ Transplantation. Springer-Verlag, Berlin, 1987.
42) Wilson, E. J., Medearis, Jr., D. N., Hansen, L. A. and Rubin, R. H.: 9-(1-3-dihydroxy-2-propoxymethyl)guanine prevents death but not immunity in murine cytomegalovirus infected normal and immunosuppressed BALB/c mice. Antimicrob. *Agents Chemother.,* **31**, 1017-1020, 1987.
43) Yukawa, T.A., Kurokawa, M., Sato, H., Yoshida, Y., Kageyama, S., Namba, T., Imakita, M., Hozumi, T. and Shiraki,K.: Prophylactic treatment of cytomegalovirus infection with traditional herbal extracts. *Antiviral Res.,* **32**, 63-70, 1996.
44) Kurokawa, M., Hozumi, T., Basnet, P., Nakano, M., Kadota, S., Namba, T, Kawana, T. and Shiraki, K.: Purification and characterization of eugeniin as an anti-herpes virus compound from *Geum japonicum* and *Syzygium aromaticum. J. Pharmacol. Exp. Ther.,* **284**, 728-735, 1998.
45) Kageyama, Kurokawa, M. and Shiraki, K.: Extract of prunella vulgaris spikes inhibits HIV replication at reverse transcription *in vitro* and can be absorbed from intestine *in vivo. Antivir. Chem. Chemoth.,* **11**, 157-164, 2000.
46) Coen, D. M., Furman, P. A., Geleo, P. T. and Schaffer, P. A.: Mutations in the herpes simplex virus DNA polymerase gene can confer resistance to 9-β-D-arabinofuranosyladenine. *J. Virol.,* **41**, 909-918, 1982.
47) Seidlin, M. and Straus, S. E.: Treatment of mucocutaneous herpes simplex infections. *Clin. Dermatol.,* **2**, 100-116, 1984.
48) Whitley, R. J., Alford, C. A., Hirsch, M. S., Schooley, R. T., Luby, J. P., Aoki, F. Y., Hanley, D., Nahmoas, A. J. and Soong, S.-J.: Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N. Engl. J. Med.,* **314**, 144-149, 1986.
49) Kurokawa, M., Hozumi, T., Tsurita, M., Kadota, S., Namba, T. and Shiraki, K.: Biological characterization of eugeniin as an anti-herpes simplex virus type 1 compound *in vitro* and *in vivo. J. Pharmacol. Exp. Ther.,* **297**, 372-379, 2000.
50) Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T. and Shiraki, K.: Anti-herpes simplex virus activity of moronic acid purified from Rhus javanica *in vitro* and *in vivo. J. Pharmacol. Exp. Ther.,* **289**, 72-78, 1999.