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Efficacy of Sho-saiko-to and its components in treatment of experimental herpes virus infection

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Abstract

The effect of Sho-saiko-to and its components was studied in golden hamsters inoculated with herpes simplex virus type 1 (HSV-1) in cornea. When evaluated as inhibitors of HSV-1 in the animal model, Sho-saiko-to and its components with *in vitro* therapeutic indexes ≧ 10 (Pinelliae Tuber, Glycyrrhizae Radix, Scutellariae Radix and Bupleuri Radix) were found to have statistically significant efficacy at a dose of 200 mg/kg per day tested in terms of the delay of appearance of primary lesions and prolongation of survival time. Some of the infected animals treated with the extracts survived without herpes simplex encephalitis, while all animals of the control group treated with placebo (water) died by 8 days postinfection.

Key words corneal infection, *in vivo* therapy, herpes simplex encephalitis.

Abbreviations HSV-1, herpes simplex virus type 1 ; PFU, plaque forming unit ; Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Sho-saiko-to (Xiao-Chai-Hu-Tang) is a well known traditional Chinese medicine which contains various components extracted from several plants and used frequently for the treatment of chronic hepatitis and nephritis in Japan and China, although their pharmacological basis is poorly understood. In our previous paper,[1] we reported that Sho-saiko-to and its components were evaluated for the *in vitro* action on the replication of herpes simplex virus type 1 (HSV-1), using plaque forming assay system in HeLa cells. Therapeutic index (ID_{50}/ED_{50}) was 12 for Sho-saiko-to, 27 for Pinelliae Tuber, 26 for Glycyrrhizae Radix, 12 for Scutellariae Radix and 10 for Bupleuri Radix, all of those showing relatively high selectivity.

In the present study, we report the effectiveness of the oral use of Sho-saiko-to and its components with high selectivity against HSV-1 infection in the golden hamster model.

Materials and Methods

Preparation of Sho-saiko-to and its components : Powdered extracts of Sho-saiko-to and its components were obtained from Tsumura & Co. (Tokyo, Japan) and were prepared according to the method in our previous paper.[1]

Virus : HSV-1 strain HF was used for inoculation. HeLa cells grown in Eagle's minimal essential medium (MEM) supplemented with 10% calf serum were used for virus propagation and titration.

Animals : Four-week old golden hamsters weighing 50−55 g or six-week old hamsters weighing 65−70 g supplied by Shizuoka Laboratory Animal Center were used.

Application of drugs for animal treatment : For oral dosage, the drug was suspended in dis-

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tilled water, and placebo treatment was done with distilled water. Treatment was started just after inoculation with an oral application of the 0.5 ml of drug two times per day for 6 or 7 days.

Inoculation of animals and evaluation of HSV-1 infection : For infection, 10 μl of virus suspension in MEM containing 5×10^6 plaque forming units (PFU) was placed on the right eye of the hamster. For each animal 45 linear scarifications and a further 45, perpendicular to the first, were made with twenty 23-gauge needles. The development and severity of the facial lesions and survival times were recorded every day. The scores of the lesions are as follows : 0, no lesion ; 1, slight and local erosion ; 2, moderate erosion ; 3, severe erosion. The average lesion score was calculated as the mean severity for all animals in a treatment group.

Statistical analysis : The significance of differences between the results of different groups was examined by use of Wilcoxon-Mann-Whitney test.

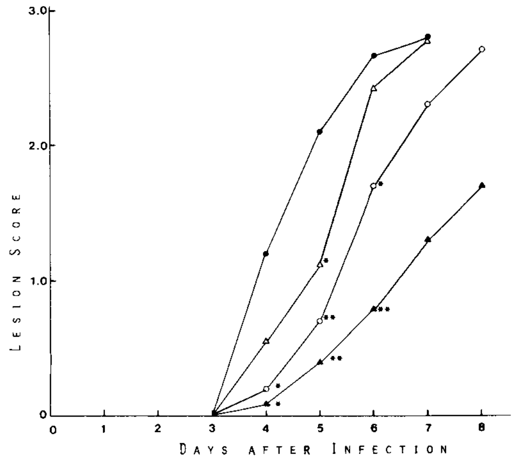

Fig. 1 Effect of Sho-saiko-to on the growth of HSV-1 in the hamster cornea.
Treatment was begun immediately after infection and was continued for 7 days. Each score is the mean of the lesions in a single experiment. ● : Control ; ○ : 40 mg/kg/day ; ▲ : 200 mg/kg/day ; △ : 2,000 mg/kg/day.
*Statistically different from placebo-treated infected hamsters ($p<0.05$).
**Statistically different from placebo-treated infected hamsters ($p<0.001$).

Results

Therapeutic efficacy of Sho-saiko-to against experimental HSV-1 corneal infection of hamsters

The efficacy of Sho-saiko-to at a dose of 40, 200 or 2,000 mg/kg body weight per day against experimental infection with HSV-1 was evaluated using 4-week old hamsters. Figure 1 shows the results of the drug on the primary facial erosions. Treatment with 40 mg/kg per day decreased significantly the average lesion score at the 4th ($p<0.05$), 5th ($p<0.001$) and 6th ($p<0.05$) days. Also, at the dose of 200 mg/kg per day, a significant reduction in the average lesion score was recorded on days 4, 5 and 6 at the level of 0.05, 0.001 and 0.001, respectively. On the other hand, at a higher dose of 2,000 mg/kg per day a statistical difference from H_2O-treated group was seen only at the 5th day ($p<0.05$).

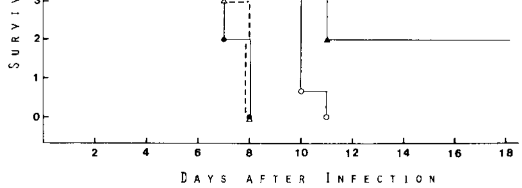

Fig. 2 Treatment of HSV-infected hamsters with Sho-saiko-to.
Each symbol is the same as in Fig. 1.

The results of the survival rate of the hamsters are presented in Fig. 2. From these data it is clear that at the highest dose (2,000 mg/kg) tested Sho-saiko-to caused no protection compared with the control group and all animals died by 10 days postinfection, while a number of late deaths were observed in the groups with the dose of 40 or 200 mg/kg per day.

Table I Effect of Sho-saiko-to on acute HSV-1 corneal infection of hamsters.

Treatment group	Survivors /Total	Mean lag phase of animals that developed facial lesions (days±S.D.)	Mean survival time of animals that died (days±S.D.)
None	0/10	4.3±0.46	6.7±0.9
40 mg/kg/day	0/10	5.4±0.92[a]	8.8±1.4[b]
200 mg/kg/day	2/10	6.3±1.19[b]	9.5±1.4[b]
2000 mg/kg/day	0/ 9	4.8±0.87	7.2±0.6

[a]Significantly different from placebo-treated infected animals ($p<0.01$).
[b]Significantly different from placebo-treated infected animals ($p<0.001$).

Table I summarizes the therapeutic efficacy of the drug when administered orally for 7 days. The efficacy was assesed in terms of the time required for the appearance of herpetic lesions and survival times. When animals were treated using doses of 40 or 200 mg/kg per day, the appearance of acute lesions took a significantly longer time ($p<0.01$ or $p<0.001$) in both groups compared with the placebo (water)-treated control group where all animals showed the lesion by 5 days postinfection. The mean survival times of the hamsters were also found to be prolonged by the drug at the doses of 40 and 200 mg/kg ($p<0.001$). At the dose of 2,000 mg/kg per day, however, no significant effect on both primary infection and survival time was observed.

Effect of Sho-saiko-to and its components on the course of HSV infection

Six-week old hamsters were treated immediately after viral infection with the suspension in distilled water of all extratcs listed in Table II at the same dose of 200 mg/kg per day for 6 days. Figure 3 shows the results where these drugs were evaluated for acute infection in animals. All extracts exerted a significant inhibitory effect on the appearance of herpetic lesions compared with the control group at 4 days postinfection ($p<0.001$ for Sho-saiko-to, Glycyrrhizae Radix and Bupleuri Radix, and $p<0.01$ for Scutellariae Radix and Pinelliae Tuber). On the 5th day all drugs except Pinelliae Tuber showed significant reduction of average lesion scores ($p<0.01$ for Sho-saiko-to and Scutellariae Radix, and, $p<0.05$ for Glycyrrhizae Radix and Bupleuri Radix), and on the 6th day only one group treated with Glycyrrhizae Radix produced a significant effect ($p<0.05$).

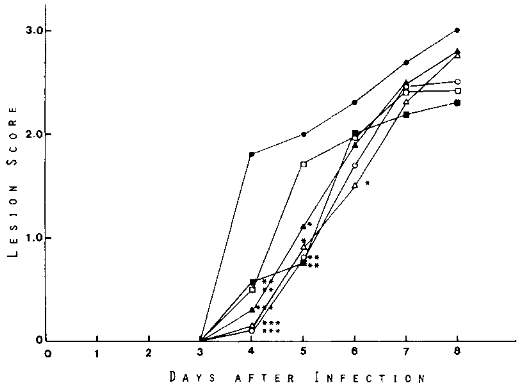

Fig. 3 Effect of Sho-saiko-to and its components on the development of facial lesions after corneal inoculation of HSV-1 into golden hamsters.

Treatment was started immediately after inoculation and consisted of 2 daily oral application of 0.5 ml of drug at a dose of 200 mg/kg/day. ● : Control ; ○ : Sho-saiko-to ; ▲ : Bupleuri Radix ; △ : Glycyrrhizae Radix ; ■ : Scutellariae Radix ; □ : Pinelliae Tuber. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

The results obtained from this experiment are summarized in Table II. HSV-infected control animals developed acute disease with a mean lag phase of 4.0 days, while Sho-saiko-to-, Bupleuri Radix-, Glycyrrhizae Radix-, Scutellariae Radix- or Pinelliae Tuber-treated animals developed the disease with a mean lag phase of 6.0 ($p<0.05$), 5.2 ($p<0.01$), 6.7 ($p<0.05$), 4.8 ($p<0.05$) or 5.0 ($p<0.01$) days, respectively. The former four extracts were shown to be significantly effective on the prolongation of the mean survival times ($p<0.05$), but Pinelliae Tuber showed no effect compared with the control group. While all

Table II Effect of Sho-saiko-to and its components on experimental HSV-1 corneal infection

Treatment group	Survivors /Total	Mean lag phase of animals that developed facial lesions (days±S.D.)	Mean survival time of animals that died (days±S.D.)
None	0/10	4.0±0.45	7.2±0.87
Sho-saiko-to	4/10	6.0±2.41[a]	8.3±1.37
Pinelliae Tuber	3/10	5.0±0.89[b]	7.7±0.70
Glycyrrhizae Radix	4/10	6.7±3.20[a]	8.8±1.95[b]
Scutellariae Radix	3/ 9	4.8±0.87[a]	10.0±1.29[b]
Bupleuri Radix	0/10	5.2±0.75[b]	8.4±1.43[a]

Hamsters were infected with 5×10^6 PFU of HSV-1 strain HF and treated perorally two times per day at a dose of 200 mg/kg/day for 6 days.
[a]Significantly different from placebo-treated animals ($p<0.05$).
[b]Significantly different from placebo-treated animals ($p<0.01$).

animals of control- and Bupleuri Radix-treated groups died, some of the animals treated with the other drugs remained alive with the survival rates of about 30−40%.

Discussion

In a hamster model used in this study, all animals of the non-treated control group died of herpes simplex encephalitis by 8 days after HSV infection. This *in vivo* assay system is suitable for the evaluation of antiviral activity as reported previously.[2)]

Sho-saiko-to showed statistically significant effect on the prolongation of both the appearance of primary disease and the survival time at a dose of 40 or 200 mg/kg per day, the latter dose corresponding to the daily dose for humans. At the highest dose tested, 2,000 mg/kg per day, while animals did not show apparent evidence of toxicity (change in activity or appetite) over the survival period, a significant difference from the control group could not be seen. This might reflect the immunosuppressive action of Sho-saiko-to at a relatively higher dose, which can result in the reactivation of viral growth in the body.[3)] Nasr *et al.*[4)] reported that unless *in vitro* antiviral activity was separated from cytotoxicity by at least seven- to eightfold, samples might not merit additional consideration. On this basis, the components of Pinelliae Tuber, Glycyrrhizae Radix, Scutellariae Radix and Bupleuri Radix were considered to merit further investigation, so that those were subjected to *in vivo* assay. The

Table III A comparison of Sho-saiko-to and its components evaluated in the *in vitro* and *in vivo* assay systems.

Extract	*In vitro* therapeutic index[a]	*In vivo* tests	
		Delay of development of lesions[b]	Prolongation of survival time[b]
Sho-saiko-to	12	Yes	Yes
Pinelliae Tuber	27	Yes	No
Glycyrrhizae Radix	26	Yes	Yes
Scutellariae Radix	12	Yes	Yes
Bupleuri Radix	10	Yes	Yes

[a]Previously reported elsewhere[1)].
[b]Extracts producing a statistically significant delay in the development of primary disease or prolongation of survival time are listed as "Yes." Extracts failing to exert significant effects are listed as "No."

results obtained from previous work[1] and present study are summarized in Table III. There is in general a positive correlation between *in vitro* therapeutic index and activity in the corneal herpes hamster model ; namely, *in vitro* therapeutic indexes≧10 correlate with activity in the animal model.

Among many antiviral drugs, acyclovir provides a potent and specific treatment for selected human herpes virus infections.[5] The *in vitro* selectivity index of acyclovir was reported to be 2,416 and the suvival rate of mice treated intraperitoneally with the doses of 100－130 mg/kg per day was 50－80%.[6] But the development of acquired resistance to this drug has been shown in clinical isolates.[7] From this problem and the lack of efficacious chemicals for severe and life-threatening herpes virus infections, the search for new, safe and more effective antiviral therapeutics has been continued.

From the data obtained in this experiment, it appears that there is potential clinical application for oral delivery of Sho-saiko-to and several components.

和文抄録

ゴールデンハムスターに単純ヘルペスウイルス1型を角膜接種後，小柴胡湯及びその単味生薬を経口投与して，ウイルス感染症に対するこれらの薬物の治療効果を検討した。ここで用いた単味生薬は，*in vitro* での治療指数10以上の半夏，甘草，黄芩，柴胡である。1日 200 mg/kg を投与したところ，いずれの薬物も初感染症状の発現を遅延させ，生存期間を延長させることがデータの統計的処理で明らかになった。蒸留水を投与した対照群の動物はすべてヘルペス脳炎で死亡したのに対して，薬物投与群では一部の動物が生残した。

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