0014-4754/94/080774-06$1.50 + 0.20

The antiviral effect of Keishi-ni-eppi-ichi-to, a traditional Chinese herbal medicine, on influenza $A_2(H_2N_2)$ virus infection in mice

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Received 23 November 1994; accepted 8 April 1994

Abstract. The antiviral effect of Keishi-ni-eppi-ichi-to (TJS-064), a traditional Chinese herbal medicine, was investigated in mice infected with influenza $A_2(H_2N_2)$ virus. When mice exposed to a 5 LD_{50} dose of the virus were treated orally with a 70 mg/kg dose of TJS-064 1 day before and 1 day and 4 days after the infection, 100% survived over a 25-day experimental period. At the end of this period all the control mice, treated with saline alone, had died; their mean survival time in days (MSD) was 11.2 days. When mice infected with a 10 LD_{50} dose of the virus were treated with TJS-064, the MSD was >17.4 days and there was a 50% survival rate, while the control group had a MSD of 8.7 days and a 0% survival rate. No significant antiviral effect of TJS-064 was observed when the agent was administered orally to mice infected with a 100 LD_{50} or larger dose of influenza virus. Pulmonary consolidations, virus titers in lung tissues and HAI titers in sera of infected mice treated with TJS-064 were all significantly lower than those of infected mice treated with saline. Interferon activities were detected in sera of mice treated with the agent at a dose of 100 mg/kg orally. Since viricidal and viristatic activities of the agent against influenza virus were not demonstrated, the antiviral effects of TJS-064 may be expressed through the host's antiviral functions including interferon production.
Key words. Traditional Chinese herbal medicine; antiviral effect; influenza virus; a mouse model.

Introduction

Each year many people are infected by influenza viruses, and although influenza is not life threatening for most people it is debilitating and requires 2–4 days of bed rest to recover. In addition, influenza infection causes substantial morbidity among school-age children and excess mortality among the elderly during each influenza season[1]. Recent reports[2] described an increase in the number of patients that have been hospitalized or died from acute pneumonia or chronic cardiopulmonary disease or other conditions that can be induced by influenza virus infection. Although vaccination for influenza viruses is effective in improving the resistance of individuals to infection[1], it is only effective against particular viruses and is dependent upon the type of viral antigens vaccinated[1]. However, the type of influenza virus prevalent in the coming year is unpredictable. Furthermore, although therapeutic agents such as amantadine[3] and ribavirin[4] seem to reduce the intensity of the infection, these drugs have not been shown to be safe or to have clinical potential[5,6]. Therefore, new antiviral strategies against influenza are necessary to control the infection.

Traditional Chinese herbal medicines are crude drugs containing extracts from 3 to 10 or more species of medicinal plants in a specific combination[7,8]. Several kinds of traditional Chinese herbal medicines have been used clinically in Japan and the Far East as therapeutic agents for cancer, bacterial infections, viral infections, and many other aliments[7,8]. In Japan, more than 120 Chinese herbal medicines (Japanese name: Kampo medicine) are currently available by doctor's prescription. In these traditional Chinese herbal therapies, the underlying principle is to improve the resistance of the patients through the action of these compounds or to restore the patient to a normal physiological state, rather than to attack the invading viruses[7,8]. Keishi-ni-eppi-ichi-to (TJS-064) is one of these Chinese medicines composed of extracts from the following 6 medicinal plants and 1 pH stabilizer: *Cinnamomi* cortex, *Paeoniae* radix, *Glycyrrhizae* radix, *Ephedrae* herba, *Zingiberis* rhizoma, *Zizyphi* fructus and plaster at a ratio of 5:5:5:5:2:6:6. Some of these components are known to have anti-bacterial activities (*Cinnamomi* cortex, *Paeoniae* radix), antiviral activities (*Ephedrae* herba), anti-inflammatory activities (*Glycyrrhizae* radix, *Zingiberis* rhizoma) or immunopotentiating activities (*Glycyrrhizae* radix)[8]. However, the prescription of one product alone is rare. These medicinal plant extracts are said to exert a synergistic effect. In this paper, we examined the antiviral activities of TJS-064 in mice infected with a lethal amount of influenza $A_2(H_2N_2)$ virus.

Materials and methods

Mice and viruses. Seven-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, Maine, USA) were used

throughout the experiment. These mice were infected with a mouse-adapted Kumamoto strain of influenza $A_2(H_2N_2)$ virus[9]. The virus was maintained mouse to mouse as described previously[9]. Before being used for the infection, the virus was propagated once in the allantoic cavity of embryonated eggs. The titer of the allantoic fluid in a 50% egg infections dose (EID_{50}) was 2.6 x 10^8/ml, which corresponded to a 10^4 LD_{50} when it was inhaled by mice in our standard infectious procedure[10]. The Indiana strain of vesicular stomatitis virus (VSV) was grown in monolayer cultures of L-Galveston cells and stored at −70 °C until use. The 50% tissue culture infectious dose ($TCID_{50}$) of the VSV was $10^{7.5}$/ml in the same cells.

TJS-064. TJS-064 was supplied by Tsumura & Co., Tokyo Japan. With sonication, TJS-064 was dissolved in saline at a concentration of 7 mg/ml, then it was diluted with saline to appropriated concentrations. The solution of TJS-064 was administered orally through a 20 gauge feeding needle into the throat of mice 1 day before and 1 and 4 days after infection with influenza A_2 virus. In this experiment, a 70 mg/kg dose of TJS-064 was administered to mice, in accordance with our preliminary experiments[11]. In Japan the agent is used clinically at this dosage.

Virus inoculation procedures. Mice were infected with the virus by inhalation using a glass vaporephirine type nebulizer spraying 10 ml of diluted allantoic fluid over 30 min[10]. The mice were in a caged rotating container into which the nebulizer was inserted. This procedure results in each mouse receiving 20 µl of the virus solution[10].

Virus titer in mouse lungs. Infected mouse lungs were disrupted using a glass homogenizer (Wheaton) to make a 10% suspension in medium. After centrifugation at 1580 × *g* for 10 min, supernatants were serially diluted ten-fold and inoculated into groups of four embryonated chicken eggs. The allantoic fluids were tested for hemagglutinin activity after incubation at 36 °C for 72 h[12]. The EID_{50} value was calculated according to Reed and Muench[13].

Calculation of lung consolidation score. The number of lung consolidations induced by the infection was determined as described previously[9]. Mice were killed and bled on the appropriate day after the inhalation of influenza virus. Before the lungs were removed, they were irrigated by injecting 1/100 M phosphate buffer (pH 7.2) in to the heart to remove red blood cells. The consolidation scores were an average of the scores obtained from each mouse. The five possible scores that each mouse could be rated were: 0 = survival without pulmonary consolidation, 1 = 25–50% consolidations, 2 = 50–74% consolidations, 3 = 75–99% consolidations, 4 = 100% consolidations or death.

HA inhibition (HAI) test. The techniques recommended by the committee on Standard Serological Procedures in Influenza Studies were followed to determine the antibody response in sera of infected mice[10].

Viricidal and viristatic tests. To test for viricidal activity 10 to 1000 µg/ml of TJS-064 in 1 ml of medium was incubated with 2.6 × 10^6 EID_{50} dose of influenza A_2 for 1 h at 37 °C. After the incubation, the remaining viral activity was titered on MDCK cells cultured in Eagle's Minimum Essential Medium supplemented with 2% fetal bovine serum, 2 mM L-glutamine, and antibiotics. Medium was used as a negative control and 10% formalin was used as a positive control. To test the agent for viristatic activity, a 2.6 × 10^4 $TCID_{50}$ dose of virus was added to MDCK cells. The cells were incubated for 1 h at 37 °C. After the incubation TJS-064 was added at a dose of 20 or 200 µg/ml. Forty-eight h after treatment the virus growth was evaluated by CPE methods as described previously[14].

Interferon (IFN) induction. Mice treated with TJS-064 had blood drawn every 6 h. After the blood samples were kept at 4 °C, serum samples were prepared by centrifugation. The IFN titration was performed as described previously[15], using a plaque reduction assay in L-Galveston cells infected with VSV. The laboratory standard and reference standard of IFN were included in all assays. In our assay system, 1 IU of IFN as determined by reference standard (G-002-905-511) equalled approximately 0.81 U of IFN activity. To characterize the antiviral activities detected in sera of mice the serum specimens were treated either with anti-IFN γ mAb[15] or anti-IFNα/β antiserum[16], as described previously[15]. In this experiment, 40 U of IFN samples were incubated at 37 °C for 1 h with the IFN antibody (500 IFN neutralization units) at a final dilution of 1:10. In addition, the IFN-inducing activity of the agent was tested in mice previously treated with anti-IFNγ mAb (5000 neutralizing units/mouse), as described previously[15].

Experimental design. Mice exposed to the influenza virus at doses of 5-1000 LD_{50} were given 70 mg/kg of TJS-064 1 day before and 1 day and 4 days after the infection. The antiviral effects of TJS-064 were evaluated on: 1) survival rate, 2) mean survival time in days (MSD), 3) the virus growth in lung tissues, 4) lung consolidation scores, and 5) antibody production in sera. Results obtained in treated groups were compared with those of control groups treated with saline. The survival rate was calculated 25 days after the infection when all control mice that were infected with influenza virus at doses of 5-1000 LD_{50} had died. The MSD was calculated as the average number of days that the mice in each group survived. The antiviral agent Virazole was used as a positive control[5,6]. Mice exposed to various doses of the virus were treated i.p. with 50 mg/kg of Virazole prophylactically and therapeutically 24, 3 and 1 h before, 1 and 3 h after infection, and then twice daily for 4 days. The mice given Virazole therapeutically

were treated with 50 mg/kg at 1 and 3 h after the infection and then twice daily for 4 days.

In order to determine the effect of TJS-064 on the HAI titer in sera, 40 mice exposed to a 5 LD_{50} dose of the virus were divided into two groups. One group was treated with the agent at a dose of 70 mg/kg 1 day before, and 1 and 4 days after the infection and the other group was treated with saline using the same schedule. Four, 5, 6 and 7 days after the infection, 5 mice from each group were killed and serum specimens were pooled. The HAI titer was assayed using the techniques recommended by the committee on Standard Serological Procedures in Influenza Studies. To determine the effect of TJS-064 on the growth of influenza virus in lungs, 60 mice were infected with a 10 LD_{50} dose of influenza virus and divided into two groups. One group was treated with the agent as described above, while the other group served as the control. One to 6 days after the infection, 5 mice from each group were killed and their lung tissues removed. The virus titer in each set of lungs was determined in embryonated eggs by the Reed and Muench method[13]. Similarly, to demonstrate the effect of TJS-064 on the development of lung consolidations, 50 mice infected with a 10 LD_{50} dose of influenza virus were divided into two groups. One group was treated with the agent, the other with saline using the same schedule. The score of lung consolidations induced by the infection was determined according to the Horsfall method with a minor modification[9]. IFN-inducing activity of the agent was examined in mice. IFN activities were determined in L-Galveston cells infected with VSV by the plaque reduction method.

Statistical analysis. All data was analyzed as follows: percent survival by X^2 analysis; MSD, viral growth and lung consolidations by Student's *t*-test. If the p value was below 0.05 we considered the results significant.

Results

In the first series of experiments mice were exposed to high doses of influenza virus and treated orally with TJS-064 at a dose of 70 mg/kg 1 day before and 1 and 4 days after the infection. When mice were infected with a 1000 LD_{50} dose of influenza A_2 virus, the treated group had a MSD of 5.7 days and a 0% survival rate compared to the controls which had an MSD of 5.9 days and a 0% survival rate (table 1). The agent produced no significant increase in the MSD or the number of survivors as compared with that of controls. On the other hand, the treated mice that were infected with a 100 LD_{50} dose of the virus showed a small but significant increase in the MSD (9.5 days) over that of the control group (6.7 days, $p < 0.05$). However, the percent survival of treated mice did not increase: both groups had 100% mortality rates (table 1). The positive controls treated with Virazole had similar results. The 100 LD_{50} group treated with Virazole had an MSD of 9.9 days, and both positive control groups had a 0% survival rate. Overall in these groups no antiviral effect of TJS-064 was demonstrated except for a small protective effect on the MSD of mice infected with a 100 LD_{50} dose of the influenza virus.

Table 1. Antiviral activity of TJS-064 in mice infected with high doses of the influenza $A_2(H_2N_2)$ virus

Virus dose (LD_{50})	Treatment[a]	No. of mice	MSD[b] (days)	Survival[c] (%)
1000	Saline	15	5.9	0
	Virazole	10	5.8	0
	TJS-064	10	5.7	0
100	Saline	15	6.7	0
	Virazole	10	9.9[d]	0
	TJS-064	10	9.5[d]	0

[a]Mice infected with influenza virus were treated orally with saline (0.2 ml/mouse) or TJS-064 at a dose of 70 mg/kg 1 day before, 1 and 4 days after the infection. Virazole was administered i.p. at a dose of 50 mg/kg to mice 24, 3, 1 h before, 1 and 3 h after infection, then twice daily for 4 days.
[b]MSD during the 25-day experimental period.
[c]Percentage of mice surviving 25 days after the infection.
[d]Student's *t*-test, $p < 0.05$.

In the next series of experiments mice were exposed to low doses of influenza virus and treated orally with the same dose and schedule of TJS-064 as described above. Results obtained are shown in figure 1A to figure 1C. After treatment with TJS-064, the group (20 mice) that had been infected with a 20 LD_{50} dose of influenza virus had a 20% survival rate over 25 days ($p < 0.05$), while all mice in the control group (20 mice) died within 12 days of the infection. When 20 mice treated with TJS-064 were given a 10 LD_{50} dose of influenza virus, they had a 50% survival rate over 25 days ($p < 0.001$) and the MSD for the treated group was >17.4 days ($p < 0.001$) (fig. 1B). In contrast, the control group (20 mice) exposed to the 10 LD_{50} dose of the virus had a MSD of 8.7 days and 0% survival rate over 14 days (fig. 1B). When mice were infected with a 5 LD_{50} dose of the virus, the control group (20 mice) had a 0% survival rate over 15 days, while TJS-064-treated mice (20 mice) had a 100% survival rate over 25 days (fig. 1C). These results suggest that the antiviral activity of TJS-064 against the infection of influenza virus depends upon the dose of the virus inhaled.

Three groups of mice were exposed to a 10 LD_{50} dose of influenza virus. The first group of 20 mice was treated with the agent at a dose of 70 mg/kg 1 day before, and 1 and 4 days after the infection (prophylactic and therapeutic treatment). The second group of 20 mice was treated with the same dose of the agent 1, 2 and 3 days after the infection (therapeutic treatment). The third group of 40 mice treated with saline served as a control. A fourth group of 20 mice was treated with Virazole prophylactically and therapeutically. A fifth

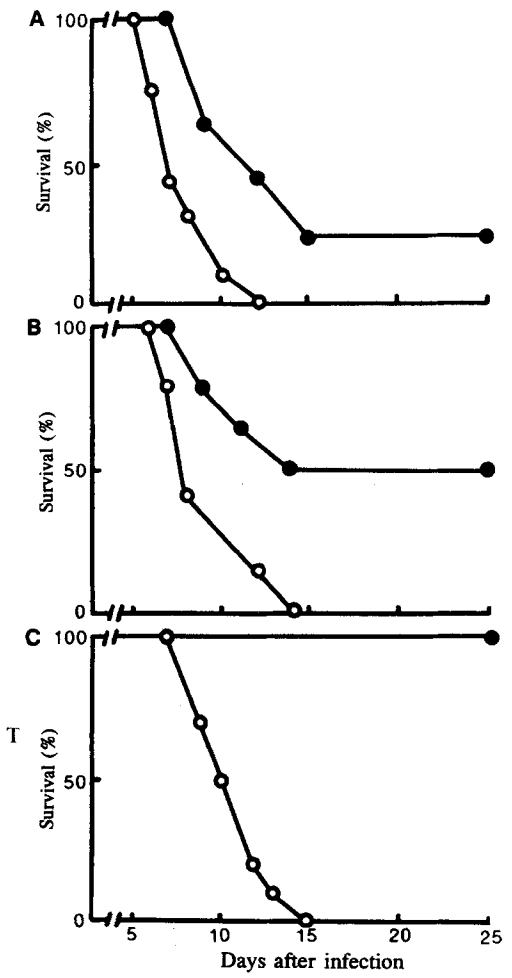

Figure 1. Effect of TJS-064 on the survival of mice infected with influenza. Effect of TJS-064 on the survival of mice exposed to a *A*, 20 LD_{50} *B*, 10 LD_{50} or *C*, 5 LD_{50} doses of influenza A_2 virus. The control groups (40 mice) received saline (0.5 ml/mouse; ○) and treated group (20 mice) received TJS-064 (70 mg/kg; ●) orally 1 day before and 1 and 4 days after the infection with the virus.

group of 20 mice was treated therapeutically with Virazole. As shown in table 2, 50% of mice survived in the first group when they were treated with the agent prophylactically and therapeutically. When the therapeutic treatment was given to mice, they (second group) had a 40% survival rate, while the controls (third group) had a 0% survival rate over 15 days. These results suggest that TJS-064 was effective both prophylactically and therapeutically in mice infected with influenza virus. The effect of TJS-064 treatments on the HAI titer was investigated using mice given a 5 LD_{50} dose of influenza A_2 virus. A 15-fold decrease in the HAI titer was found in day 7 sera of the treated group (70 mg/kg, orally, 1 day before and 1 and 4 days after the infection) when compared with the HAI titer in day 7 sera of the control group treated with saline (fig. 2A).

Next the effect of TJS-064 on the growth of influenza virus in lung tissues was examined. Two groups of mice (30 each) exposed to a 10 LD_{50} dose of the virus were

Table 2. Effect of different schedules of TJS-064 treatment in mice infected with influenza virus

Drug	Treatment[a]	No. of mice	MSD (days)[b]	Survival (%)[c]
TJS-064	Pro & Ther	20	>18.1[d]	50[e]
TJS-064	Ther	20	>16.8[d]	40[e]
Virazole	Pro & Ther	20	20.5	60[e]
Virazole	Ther	20	16.9	30[e]
Saline	Pro & Ther	40	8.8	0

[a]Mice that inhaled a 10 LD_{50} dose of influenza $A_2(H_2N_2)$ virus were treated orally with TJS-064 in two different ways. The first group was treated prophylactically and therapeutically (Pro & Ther) with 70 mg TJS-064/kg 1 day before, 1 and 4 days after the infection. The second group was treated just therapeutically (Ther) with the same dose 1, 2, and 3 days after the infection. As a positive control Virazole was given prophylactically and therapeutically at a dose of 50 mg/kg 24, 3, 1 h before, 1 and 3 h after infection then twice daily for 4 days. The second positive control group was given Virazole therapeutically. This group was treated i.p. with 50 mg/kg 1 and 3 h after the infection and then twice daily for 4 days. As a negative control 40 mice exposed to the same amount of influenza virus were treated with saline 1 day before, 1, 2, 3 and 4 days after the infection.
[b]Mean survival days during the 25-day experimental period.
[c]Percentage of mice surviving 25 days after the infection.
[d]Student's *t*-test, $p < 0.001$.
[e]X^2 analysis, $p < 0.001$.

used in this experiment. One group was treated with TJS-064 and the other served as the control. As shown in figure 2B, the virus titer of the treated group was 10 times lower than that of the control by the second day. On the third day the virus titers of the treated group started to drop while the control group had titers that remained at $5-10 \times 10^6$ EID_{50}/lungs from day 2 to day 6 (fig. 2B). The effect of TJS-064 on the development of lung consolidations in mice exposed to the virus was tested. Sixty mice exposed to a 10 LD_{50} dose of influenza virus were divided into two groups. One group was treated orally with the agent, and the other group treated with saline served as the control. For evaluation of the lung consolidation, 5 mice in each group were killed consecutively 7, 9, 10, 11, 12 and 15 days after the infection. Over a 15 day period the treated group never had a mean score greater than 1, while the control group's score reached 4 (the maximum) by day 13.

The viricidal test showed that TJS-064 did not inhibit the infectious activity of the virus, while a 10% formalin solution eliminated the viral activity completely. In the viristatic test, non-cytotoxic concentrations of the agent did not inhibit the growth of the virus in vitro. This suggests that TJS-064 has neither viristatic nor viricidal activities. The IFN-inducing activity of the agent was determined by the plaque reduction method using L-Galveston cells and VSV[15]. Serum specimens from mice treated with TJS-064 (100 mg/kg, p.o.) were taken every 6 h and were assayed for their antiviral activities. The IFN activity was assayed in the serum of mice 18 h after the administration of TJS-064 (105 U/ml) and reached its peak (252 U/ml) after 24 h (fig. 3). The activity

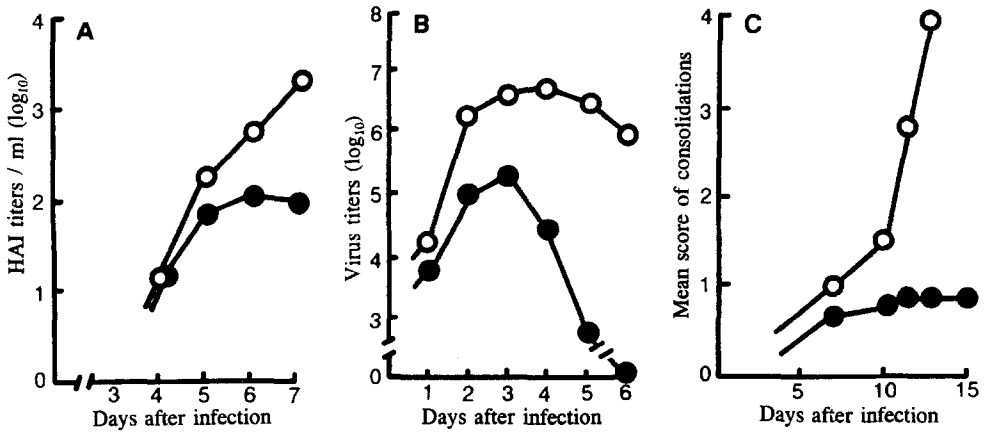

Figure 2. Effect of TJS-064 on HIA titers in sera, virus titers in lungs, and the number of pulmonary consolidations of infected mice. A Effect of TJS-064 on the HAI titer in sera of mice infected with influenza A_2 virus. HAI titer in (pooled) sera of mice infected with a 5 LD_{50} dose of influenza A_2 virus was assayed using the standard serological procedures for influenza studies.
B Effect of TJS-064 on the growth of influenza virus in lung tissues. The lung virus titer of TJS-064-treated mice infected with a 10 LD_{50} dose of influenza A_2 virus was evaluated, and compared with that of controls treated with saline. The lung tissue samples were prepared as described in 'Materials and Methods'. The virus titer in this figure is expressed as an EID_{50}/lung.
C Effect of TJS-064 on the development of lung consolidations. The number of lung consolidation scores in mice infected with a 10 LD_{50} dose of influenza A_2 virus were calculated as described in the text. In these three experiments, mice were treated with saline (○) or TJS-064 (●) orally.

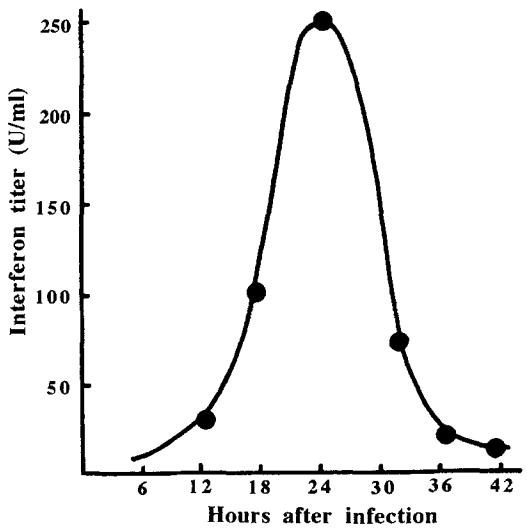

Figure 3. The production of IFN in serum of mice treated with TJS-064. A 100 mg/kg dose of TJS-064 was administered orally to mice at various intervals. After the stimulation, serum specimens were obtained from these mice and were assayed for their IFN activities in L-Galveston cells infected with VSV.

gradually disappeared by 36 h after treatment. The majority of serum IFN induced in mice by the agent was characterized as gamma IFN (IFNγ), because it was neutralized by anti-IFNγ mAb, but not by anti-IFNα/β antiserum. In addition IFN was not produced in the sera of mice given anti-IFNγ mAb (5000 IFNγ neutralizing U/kg) before treatment with TJS-064 (data not shown).

We have examined the synergistic effects of various plant extracts contained in Keishi-ni-eppi-ichi-to in mice exposed to influenza A_2 virus. Each component was tested individually. *Cinnamomi* cortex (10 mg/kg, p.o.) and *Zizyphi* fructus (12 mg/kg, p.o.) demonstrated no improvement in the MSD or survival rate of infected mice. *Paeoniae* radix (10 mg/kg, p.o.), *Glycyrrhizae* radix (10 mg/kg, p.o.) *Ephedrae* herba (10 mg/kg, p.o.), and *Zingiberis* rhizoma (4 mg/kg, p.o.) all showed a small increase in the MSD of infected mice. In contrast, the mixture of the 4 extracts derived from *Paeoniae* radix, *Glycyrrhizae* radix, *Ephedrae* herba, and *Zingiberis* rhizoma and the mixture from all 6 extracts showed significant antiviral activities illustrated by an increase in the MSD and survival rate. Those results suggest that the antiviral effect of Keishi-ni-eppi-ichi-to in mice infected with influenza A_2 virus may be a result of the synergistic effects of its components (except *Cinnamomi* cortex and *Zizyphi* fructus).

Discussion

The present study demonstrates that mice infected with influenza A_2 virus were protected by oral administration of TJS-064. The protective effect of TJS-064 against influenza virus infection of mice was shown by the increase in the survival rate, the extension of mean survival time, a suppression of virus growth in the lung, a decrease of the HAI titer in sera, and an inhibition of lung consolidation development. The effectiveness of TJS-064 was dependent upon the amount of the virus used to infect the mice. The agent showed protective effects in mice infected with 20 LD_{50} to 5 LD_{50} doses of influenza virus. However, no increase in survival effect was demonstrated in mice infected with doses of influenza virus at 100 LD_{50} or greater. In addition to prophylactic/therapeutic antiviral effects, TJS-064 showed a therapeutic antiviral effect in mice infected with this virus which was comparable to the results of Virazole[14].

The agent seems to have reduced growth, therefore reducing the antibody (HAI) titers. The reduced viral growth also reduces the number of lymphocytes infiltrating the lungs[9,17], thus reducing the number of lung consolidations. We have previously reported[9,17] that the consolidations in lungs of infected mice are caused by the infiltration of small lymphocytes. Most of these lymphocytes were found to be T-cells[15].

Many natural and synthetic immunopotentiators, including a) IFN inducers such as polyriboinosinic-polyribocytidylic acid[18], dextran phosphate[19], 9-methylstreptimidone[20,21] and tirolone[22], b) immunotherapeutic agents for cancer such as antibiotics (myroridin)[23], bacterial cells (OK-432)[24,25], extracts from basidiomycetes lentinan[26], extracts from bacteria[27–30] and synthetic compounds (Ge-132)[30] inhibit viral infections in vivo. Although the mechanisms of antiviral action of these immunopotentiating agents have not been clearly defined, it is usually accepted that IFN induction is involved. The IFN-inducing activities of various cancer immunotherapeutic agents have also been described[31]. The IFN induced by cancer immunotherapeutic agents plays a very important role in their antitumor activities[30,31]. It has also been suggested that the protective action of some of these materials may be due to the activation of reticuloendothelial systems[24–31] and induction of various other cytokines[32,33]. The IFN-inducing activity of the agent was demonstrated when it was administered orally to mice. Since the agent has no direct viricidal or viristatic activities against the influenza virus, the antiviral actives of TJS-064 may be expressed through the host's own antiviral functions such as IFN.

Although TJS-064 is a crude drug, it is formulated by the specific combination of 6 medicinal plant extracts and a pH stabilizer. The extracts from the individual medicinal plants have various biological and pharmacological activities. *Cinnamomi* cortex has been shown to have antibacterial properties and be a vasodilator[8]. *Paeoniae* radix is described to have some antibacterial effects[8]. *Glycyrrhizae* radix is an antiviral and anti-inflammatory agent[8]. *Ephedrae* herba has been found to reduce body temperature and to have antiviral properties[8]. *Zingiberis* rhizoma has been described as an anti-inflammatory and antibacterial agent[8]. Some of the main components of these medicinal plants used in Keishi-ni-eppi-ichi-to have been isolated chemically. However, most components extracted from medicinal plants do not show strong therapeutic activities when administered individually to patients. Various components or crude extracts are said to exert a synergistic effect.

Acknowledgments. The authors would like to thank Tsumura & Co., Tokyo, Japan for their generous donation of Keishi-ni-eppi-ichi-to used in these experiments.

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