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Evaluation of efficacy of Kakkon-to on influenza infection

Masahiko KUROKAWA

Department of Virology, Toyama Medical and Pharmaceutical University

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Abstract

Kakkon-to (葛根湯) has been used for the alleviation of symptoms such as fever, headache, muscle pain, etc. in the early phase of influenza infection. The therapeutic efficacy of Kakkon-to was confirmed in an intranasal influenza infection model in mice and the mode of action was analyzed. Oral administration of Kakkon-to caused the early recovery of body weight in infected mice, retarded the development of pneumonia and decreased mortality of infected mice as compared with water-administered groups. This treatment also suppressed fever 1 to 2 days after infection. Kakkon-to was verified to be effective in alleviating symptoms caused by influenza infection in mice. Since Kakkon-to treatment did not affect the growth of influenza virus in the lungs, its effects on the production of cytokines as immune mediators against influenza infection were examined. Kakkon-to treatment did not affect interferon (IFN) activity and the levels of interleukin (IL)-2, tumor necrosis factor-α and IFN-γ in serum after infection as compared with water administration. However, Kakkon-to significantly suppressed the rise of IL-1 α level in serum and the bronchoalveolar lavage fluid of lungs compared with untreated mice. When fever abated in infected mice treated with Kakkon-to, IL-1 α level also decreased in serum and was maintained at the level of uninfected mice. Such mode of antipyretic action was different from that of aspirin and was confirmed using the compounds identified from Kakkon-to. Thus, the alleviation of pneumonia and antipyretic action by Kakkon-to was possibly based on the suppression of IL-1 α production induced by IFN in influenza. Since IL-1 α is characteristically produced in the early phase of influenza infection, it could be understood that the use of Kakkon-to has advantages in the early phase of influenza infection.

Key words Kakkon-to, influenza infection, pneumonia, fever, cytokine, interleukin-1 α.

Abbreviations COX, cyclooxygenase ; COX-PGE_2, cyclooxygenase activity and prostagrandin E_2 production ; ELISA, enzyme-linked immunosorbent assay ; HSV, herpes simplex virus ; IL, interleukin ; IFN, interferon ; PG, prostagrandin ; TNF, tumor necrosis factor.

1. Introduction

Influenza virus infection causes headache, muscle pains, malaise, etc. accompanied with high fever after 2-3 days incubation and sometimes follows severe complications such as pneumonia and encephalopathy. Kakkon-to (葛根湯), a traditional herbal medicine, is composed of 7 medicinal herbs, *Pueraria pseudo-hirsuta* TANG et WANG (Radix), *Ephedra sinica* STAPF (Cortex), *Zizyphus jujuba* MILL. (Fruit), *Cinnamomum cassia* BLUME (Cortex), *Paeonia lactiflora* PALL (Radix), *Glycyrrhiza uralensis* FISCH. (Radix), and *Zingiber officinale* ROSC. (Rhizome).[1,2] This herbal medicine has been used for the alleviation of symptoms in influenza infection and the common cold since ancient times in China and more than 20 million doses are prescribed annually in Japan. In traditional therapy, Kakkon-to has been managed in the eary phase of influenza infection. This application is differ-

〒930-0194 富山市杉谷2630
富山医科薬科大学医学部ウイルス学教室　黒川昌彦
2630 Sugitani, Toyama 930-0194, Japan

和漢医薬学雑誌 **15**, 33－39, 1998

ent from those of Sho-saiko-to (小柴胡湯) and Hochu-ekki-to (補中益気湯) in that they are not applied in the early phases. However we have no scientific reasons why such traditional use has been histologically selected in humans, because the efficacy of Kakkon-to and its pharmacological and biochemical bases of action have not been well understood in influenza infection.

We have been utilizing experimental animal models to develop a new treatment of viral infection.[3-6] In our previous study, Kakkon-to did not inhibit the growth of herpes simplex virus (HSV) *in vitro* but it exhibited therapeutic efficacy in HSV-infected mice and reduced their mortality.[6] The efficacy of Kakkon-to was demonstrated to result from the augmentation of delayed type hypersensitivity to HSV.[6] A murine infection model has been useful in analyzing the mode of action of traditional medicines *in vivo*. We utilized an intranasal influenza virus infection model in mice to evaluate the therapeutic efficacy of Kakkon-to. This model has been used as a human model for influenza virus infection since influenza virus produces typical pneumonia.[7,8] The pneumonia observed in the infected mice is caused by host immune response against influenza virus infection[9-11] and histopathological findings in infected murine pulmonary tissues are similar to the pathological changes during human infection.[12] Based on this infection model, we have developed a fever production system using a mouse strain with high susceptibility to interferon (IFN), because IFN is known to induce fever.[13] This model using DBA/2 Cr mice was shown to be the most suitable model for analyzing the mechanism of fever production among 7 mouse strains.[13] We have demonstrated the cascade of fever production in influenza infection using the murine model as follows : influenza virus infection, elevated IFN activity, interleukin (IL)-1 α production, elevated cyclooxygenase (COX) activity and prostagrandin (PG) E_2 production (COX-PGE_2), fever induction.[13]

In this study using the murine models, we showed that Kakkon-to treatment alleviated pneumonia and reduced fever by different mode of antipyretic action from aspirin in influenza infection.[14] The alleviation of pneumonia and novel antipyretic action by Kakkon-to were suggested to originate in the suppression of responsive IL-1 α production subsequent to IFN production after infection.[14] Further, we identified the compounds with antipyretic activity from Kakkon-to. The mode of antipyretic activity was confirmed to be due to the suppression of interferon-induced IL-1 α production and different from that of aspirin. Based on the results, we discussed the efficacy of Kakkon-to in the early phase of influenza infection.

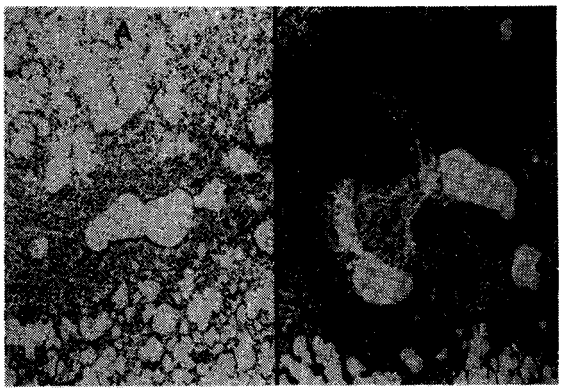
2. Therapeutic efficacy of Kakkon-to in mice

Therapeutic efficacy of Kakkon-to was evaluated in an intranasal influenza virus infection model in mice. Female ICR or DBA/2 Cr mice were intranasally infected or mock-infected with influenza virus, A/PR/8/34 (H1N1), under ether anesthesia. Kakkon-to or water was administered orally to the mice three times daily (approximately 8 hr interval) for 7 days starting a day before infection. In influenza virus-infected mice, the body weight decreased markedly later than 2 days after infection and the consolidation of lungs was obviously observed. However, Kakkon-to treatment delayed the decrease of body weight and caused the early recovery of body weight. The development of consolidation was also retarded in Kakkon-to-treated mice. The pathologic changes of lungs became evident microscopically on day 4 after infection and progressed later. The epithelium of bronchi and bronchioles showed infiltration of inflammatory cells and necrosis, and was overlaid by mucopurulent materials. These pathologic changes of the lungs were milder in Kakkon-to-treated mice than in water-administered mice (Figs. 1a and 1b). Kakkon-to treatment reduced the mortality of infected mice as one of the representative results (Fig. 2). Thus, the treatment was confirmed to exhibit therapeutic efficacy in alleviating pneumonia in an influenza infection model in mice.

3. Effect of Kakkon-to on production of cytokines in infected mice

Although pneumonia was milder in Kakkon-to-treated mice than in water-treated mice, virus yields were similar in the lungs of Kakkon-to- and water-treated mice (Fig. 3). The alleviation of pneumonia by

(a)



(b)

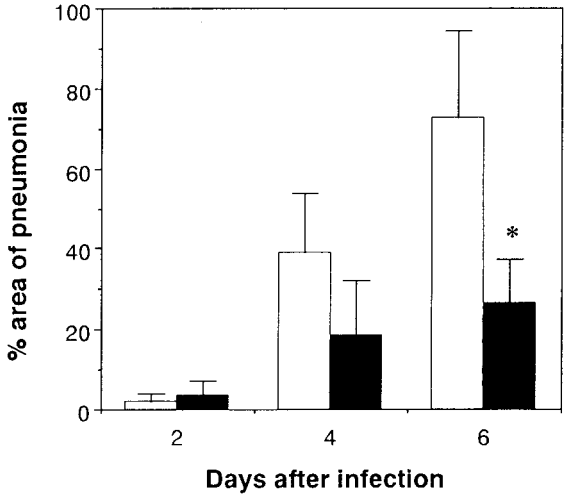


Fig. 1 a) Histopathological analysis of lungs in influenza virus-infected DBA/2 Cr mice. The lungs of Kakkon-to-treated (A) and untreated (B) mice were removed on day 4 after infection. The resected lung was fixed in 10 % formalin solution, dehydrated and embedded in paraffin. Four-micrometer thick sections were cut and stained with hematoxylin and eosin. b) Effect of Kakkon-to on the development of pneumonia in mice infected with influenza virus. ICR mice were infected intranasally with influenza virus, and Kakkon-to and water were orally administered. The lungs were removed from 3 to 4 mice in each group on days 2, 4 and 6 after infection. Open and closed columns show water- and Kakkon-to-administered groups, respectively. Asterisk indicates significant difference from water-administered group, $p<0.05$ by the Student's t-test. (cited from Ref. 14)

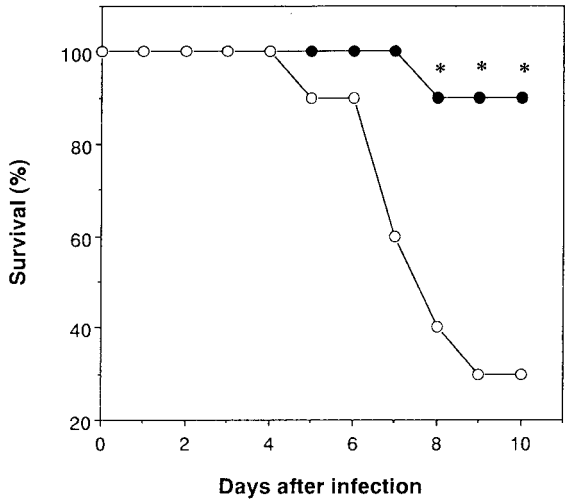


Fig. 2 Survival time of influenza virus-infected mice. ICR mice were intranasally infected with influenza virus. Kakkon-to (closed circles) and water (open circles) were orally administered. The mice were used in each group. Asterisk indicates significant difference from water-administered group, $p<0.05$ by the Fisher's exact test. (cited from Ref. 14)

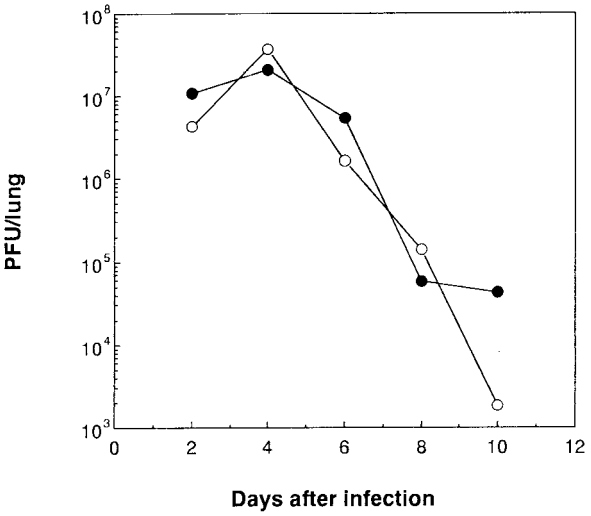


Fig. 3 Effect of Kakkon-to on the growth of influenza virus in lungs. ICR mice were infected intranasally with influenza virus, and Kakkon-to (closed circles) and water (open circles) were orally administered. The lungs were removed from 3 mice in each group on days 2, 4, 6, 8 and 10 after infection and homogenized. Virus titer in the supernatant of the homogenate was determined by the plaque assay and the mean values of 3 lungs were plotted. (cited from Ref. 14)

Kakkon-to treatment may be due to the modification of an immunopathological response against influenza infection rather than the direct cytopathic effect of viral replication. Thus, the effect of Kakkon-to on the production of cytokines including IFN as an immune mediator was examined in mice infected with influenza virus. IFN activity increased in serum on day 2

Table I Amounts of IL-1α in the bronchoalveolar lavage fluid of lungs and serum from mice treated with or without Kakkon-to*

Expt. No.	Days after infection	IL-1α (mean+SD), pg/ml			
		Uninfected mice		Infected mice	
		Water-treated	Kakkon-to-treated	Water-treated	Kakkon-to-treated
1.Serum (n=2 or 3)[a]					
	2	40.4±11.6[b]	42.8±22.8	122.4±18.8[b]	62.4±25.2[d]
	4	16.4±10.8[b]	22.0±7.2	46.4±5.2[b]	45.2±29.6
	6	24.0[b,c]	8.0[c]	30.8±16.4[b]	54.4±8.0
Lavage (n=3)					
	2	7.6±13.0[b]	4.8±3.0	65.2±23.6[b]	9.2±15.8[d]
	4	6.4±5.8[b]	0.8±1.4	45.6±40.4[b]	10.8±4.8
	6	0.0±0.0[b]	0.2±0.2	16.0±14.0[b]	6.0±10.4
2.Serum (n=3)					
	2	13.3±23.1	31.0±37.7	191.3±91.0	26.0±22.5[d]
	4			26.7±30.6	0.0±0.0
	6			31.0±37.7	26.7±46.2
Lavage (n=3)					
	2			32.6±11.8	4.6±7.9[d]
	4			22.8±20.2	5.4±2.4
	6			8.0±7.0	3.0±5.2
3.Serum (n=7 to 10)					
	2			80.2±39.4	44.8±14.2[d]
Lavage (n=2)					
	2	13.0[b,c]	5.0[c]	152.6[b,c]	64.1[c]
	4		5.6[c]	62.0[b,c]	80.6[c]
	6		5.0[c]	39.6[b,c]	15.6[c]
4.Serum (n=10)					
	2			139.2±28.8	87.3±34.8[e]

*IL-1 α levels in the bronchoalveolar lavage fluid of lungs and serum prepared from ICR mice were compared in Kakkon-to (750 mg/kg/day) and untreated mice.
[a]Parentheses indicate the number of mice used in each group.
[b]Values were cited from ref. 13.
[c]Mean of 2 samples.
[d]$p<0.05$ vs. infected mice with water administration.
[e]$p<0.01$ vs. infected mice with water administration.
(cited from Ref. 14)

after infection. However, Kakkon-to treatment did not affect the increase of IFN activity. Among cytokines examined, only IL-1 α level increased in both bronchoalveolar lavage fluid of lungs and serum mainly 2 days after infection (Table I). However, Kakkon-to treatment reduced IL-1 α level in the bronchoalveolar lavage fluid and serum on day 2 after infection significantly and preserved its level at that in uninfected mice (Table I), whereas the levels of other cytokines (IL-2, tumor necrosis factor-α and IFN-γ) examined were not affected by Kakkon-to treatment. Therefore, Kakkon-to treatment permitted the increase of IFN activity but suppressed IL-1 α production responsive to IFN.

4. Effect of Kakkon-to on fever production

IL-1 α, as an endogenous pyrogen, is an immune mediator and produced only in the early stage after infection in the lungs of infected mice.[9] Therefore the local production of IL-1 α may be due to cellular infiltration in lungs of infected mice. The level of IL-

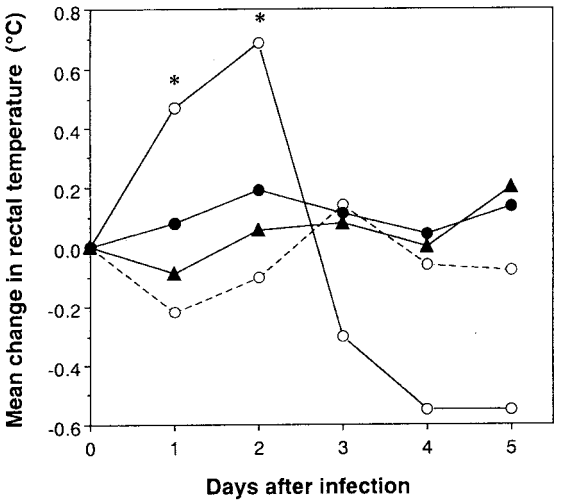


Fig. 4 Effects of Kakkon-to on the mean rectal temperatures of DBA/2 Cr mice. The mice were mock-infected (broken lines) or infected (solid lines) intranasally with influenza virus. Kakkon-to (closed circles), aspirin (closed triangle) or water (open circles) was orally administered as described in the text and the rectal temperature was measured every morning. Five to 7 mice were used in each group. Asterisk indicates significant difference from water-administered, aspirin-administered and mock-infected groups, $p<0.05$ by the Student's t-test. (cited from Ref. 14)

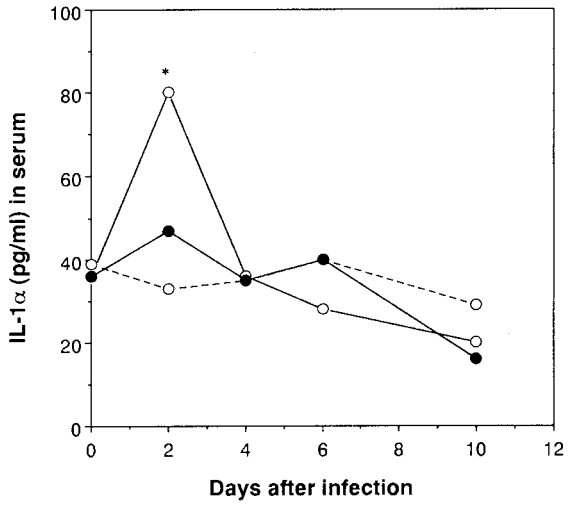


Fig. 5 Effects of Kakkon-to on the amount of IL-1 α in serum of mice. The mice were mock-infected (broken lines) or infected (solid lines) intranasally with influenza virus. Kakkon-to (closed circles) or water (open circles) was orally administered as described in the text. Whole blood was collected from 5-7 mice in each group on days 0, 2, 4, 6 and 10 after infection and serum was prepared. The concentrations of IL-1 α in serum were determined by the ELISA method. Asterisk indicates significant difference from Kakkon-to-administered and mock-infected groups, $p<0.05$ by the Student's t-test. (cited from Ref. 14)

1 α also rose in serum prominently as compared with other cytokines examined. Since circulating endogenous pyrogens are suggested to interact with organum vasculosum laminae terminalis and induce febrile response,[15-17] IL-1 α may be a possible endogenous pyrogen that initiates fever in influenza infection. When fever was prominently produced on day 2 after infection (Fig. 4), IL-1 α level rose significantly in serum of infected mice (Fig. 5). However, Kakkon-to treatment as well as aspirin treatment reduced fever significantly (Fig. 4) and reduced IL-1 α concentration to the level of uninfected mice in the serum (Fig. 5). Thus fever was well correlated in the IL-1 α level in serum and the reduction of IL-1 α production responsive to IFN production by Kakkon-to probably caused the suppression of fever.

5. Antipyretic activity of Kakkon-to

We have previously shown that the fever production is induced in influenza virus-infected mice by the following cascade : elevated IFN activity, IL-1 α production, elevated COX-PGE_2, as summarized in Fig. 6. Kakkon-to permitted the increase of IFN activity after infection but suppressed IL-1 α production responsive to IFN, resulting in reduction in fever. Aspirin, a representative antipyretic agent [18-21] has

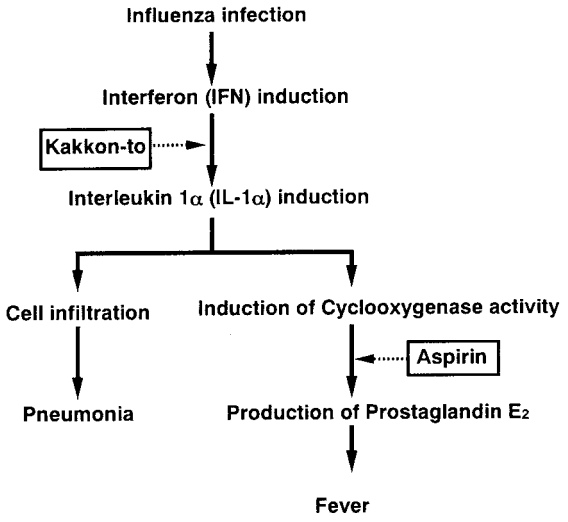


Fig. 6 Possible action of Kakkon-to on fever and pneumonia in influenza. (cited from Ref. 14)

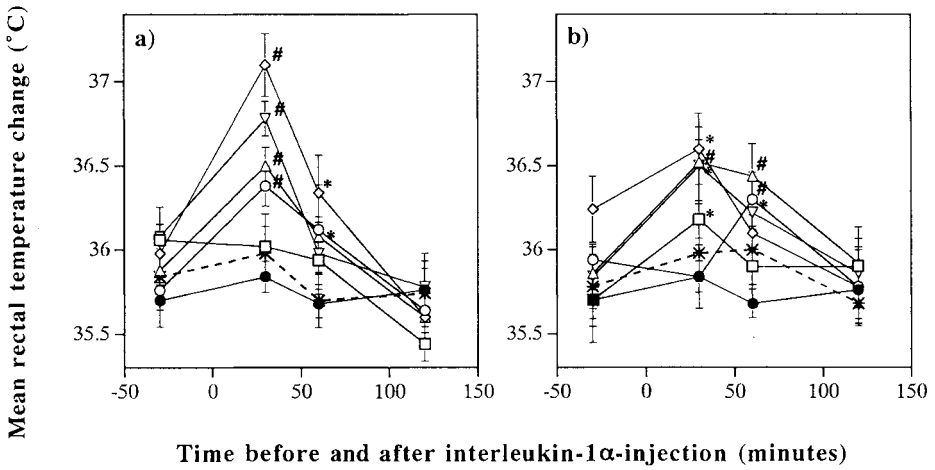


Fig. 7 Antipyretic activity of aspirin and cinnamyl derivatives and related compounds in mice injected intravenously with interleukin-1 α (a), 20 ng/mouse, b), 150 ng/mouse). Aspirin (···*···) and 4 compounds (□, 7-hydroxycoumarin ; ◇, 4-allylanisole ; △, cinnamic acid ethylester ; ▽, acetic acid cinnamylester) were administered orally to DBA/2 Cr mice (5 mice in each group) and interleukin-1 α was intravenously injected to the mice. In water-administered mice, interleukin-1 α (○) and saline (●) were injected. The rectal temperature was monitored at 30 min before and 30, 60 and 120 min after injection. $^{*}p<0.05$ vs. water-administered mice with saline-injection. $^{\#}p<0.01$ vs. water-administered mice with saline-injection. Horizontal bars indicate the range of standard error. (cited from Ref. 22)

been shown to permit the increase of IFN activity and IL-1 α production but reduces COX-PGE_2 by inhibiting COX activity.[13] Therefore, the inhibitory step of Kakkon-to was suggested to be prior to that of aspirin in the cascade (Fig. 6). Thus, Kakkon-to has a different mode of antipyretic action from aspirin and one of the major antipyretic actions is due to the suppression of IL-1 α production responsive to IFN in infected mice. As shown in Fig. 7, aspirin showed antipyretic activity in intravenously IL-1 α-injected mice but the compounds identified as antipyretic compounds from the extracts of Kakkon-to did not, as expected.[22] The difference between modes of antipyretic action of aspirin and Kakkon-to was confirmed.

6. Alleviation of pneumonia and defervescence by the suppression of IL-1α-production in Kakkon-to-treated mice

Kakkon-to treatment was effective in retarding the development of pneumonia and prolonging the survival of mice. This treatment also alleviated fever as a major morbidity caused by influenza infection in the acute phase, and the novel antipyretic action of Kakkon-to was confirmed in febrile mice infected with influenza virus. These beneficial effects of Kakkon-to on pneumonia and fever correlated with the suppression of responsive IL-1 α production subsequent to IFN production in infected mice as described above. The suppression of responsive IL-1 α production by Kakkon-to may be one of the major actions leading the alleviation of pneumonia and defervescence in infected mice. IL-1 α production possibly plays an important role in the defense system against influenza infection.[9] In spite of the reduction of IL-1 α production by Kakkon-to, pneumonia was alleviated without reducing virus yields in the lungs and the mortality of infected mice decreased. Kakkon-to treatment did not deteriorate the defense system even suppressing IL-1 α production in infected mice. Therefore IL-1 α production responsive to IFN may be an overreaction of the defense system in influenza infection. Kakkon-to was effective in limiting the overreation in the pathogenesis of influenza infection possibly by suppressing responsive IL-1 α production.

7. Conclusion

In conclusion, the suppression of overreaction for

responsive IL-1 α production by Kakkon-to was one of the major actions leading the alleviation of pneumonia and defervescence in the early phase of influenza infection in mice. Kakkon-to has been used for the alleviation of symptoms in the early phase of influenza infection in traditional therapy. This application is different from those of Sho-saiko-to and Hochu-ekki-to that may not be applied in the acute phase. These herbal medicines can activate the cellular immune system in influenza virus infection and strengthen the defense system in the convalescence. However, since the action of Kakkon-to is suppressive for influenza infection, its use may not be suitable in convalescence and for persons who have a weak constitution. This application has been done in traditional therapy. Such benefical use was justified in this study.

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References

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

2) Nagasaka, K., Kurokawa, M., Imakita, M. 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.

3) Kurokawa, M., Hase, K., Xu, H X., Yamamura, Y., Koyasu, M., Sato, 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. *Journal of Medical and Pharmaceutical Society for WAKAN-YAKU,* **10**, 195-203, 1993.

4) 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 herb medicines in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. *Antiviral Research,* **27**, 19-37, 1995.

5) Kurokawa, M., Ochiai, H., Kazuhiko, N., Neki, M., Xu, H., Kadota, S., Sutardjo, S., Matsumoto, T., Namba, T. and Shiraki, K.: Antiviral traditional medicines against herpes simplex virus (HSV-1), polio virus, and measles virus in vitro and their therapeutic effecacies for HSV-1 infection in mice. *Antiviral Research* **22**, 175-188, 1993.

6) Nagasaka, K., Kurokawa, M., Imakita, M. and Shiraki, K.: Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. *Journal of Medical Virology* **46**, 28-34, 1995.

7) Tashiro, M., Ciborowski, P., Klenk, H.-D., Pulverer, G. and Rott, R.: Role of Staphylococcus aureus in the development of inflenza pneumonia. *Nature,* London **325**, 536-537, 1987.

8) Tashiro, M., Ciborowski, P., Reinacher, M., Pulverer, G., Klenk, H.-D. and Rott, R.: Synergistic role of staphylococcal proteases in the induction of influenza virus pathogenicity. *Virology* **157**, 421-430, 1987.

9) Hennet, T., Ziltener, H. J., Frei, K. and Peterhans, E.: A kinetic study of immune mediators in the lungs of mice infected with influenza A virus. *Journal of Immunology* **149**, 932-939, 1992.

10) Hurd, J. and Heath, R. B.: Effect of cyclophosphamide on infections in mice caused by virulent and avirulent strains of influenza virus. *Infection and Immunity* **11**, 886-889, 1975.

11) Sullivan, J. L., Mayner, R. E, Barry, D. W. and Ennis, F. A.: Influenza virus infection in nude mice. *Journal of Infectious Diseases* **133**, 91-94, 1976.

12) Hert, J. F., Mulder, J., Masurel, N. and Kuip, L.V.D.: Studies on the pathogenesis of influenza virus pneumonia in mice. *Journal of Pathology and Bacteriology* **83**, 207-217, 1962.

13) Kurokawa, M., Imakita, M., Kumeda, C.A. and Shiraki, K.: Cascade of fever production in mice infected with influenza virus. *J. Med. Virol.,* **50**, 152-158, 1996.

14) Kurokawa, M., Imakita, M., Kumeda, C.A., Yukawa, T.A. and Shiraki, K. : Kakkon-to suppressed interleukin-1α production responsive to interferon and alleviated influenza infection in mice. *J. Traditional Med.* **13**, 201-209, 1996.

15) Blatteis, C.M.: Role of the OVLT in the febrile response to circulating pyrogens. *Progress in Brain Research* **91**, 409-412, 1992.

16) Hashimoto, M., Ishikawa, Y., Yokota, S., Goto, F., Bando, T., Sakakibara, Y. and Iriki, M.: Action site of circulating interleukin-1 on the rabbit brain. *Brain Research* **540**, 217-223, 1991.

17) Saper, C.B. and Breder, C.D.: Endogenous pyrogens in the CNS : role in the febrile response. *Progress in Brain Research* **93**, 419-429, 1992.

18) Bodel, P., Reynolds, C.F. and Atkins, E.: Lack of effect of salicylate on pyrogen release from human blood leukocytes in vitro. *Yale J. Biol. and Med.* **46**, 190-195, 1973.

19) Clark, W.G. and Moyer, S.G.: The effects of acetaminophen and sodium salicylate on the release and activity of leukocytic pyrogen in the cat. *J. Pharm. and Exp. Therap.* **181**, 183-191, 1972.

20) Flower, R. J. and Vane, J. R.: Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature* **240**, 410-411, 1972.

21) Insel, P. A.: Analgesic-antipyretics and antiinflammatory agents; drugs employed in the treatment of rheumatoid arthritis and gout. In : Goodman and Gilman's. The Pharmacological Basis of Therapeutics. Pergamon Press., New York, 1992.

22) Kurokawa, M., Kumeda, C.A., Yamamura, J., Kamiyama, T. and Shiraki, K.: Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice. *Eur. J. Pharm.* in Press.