Interferon inducing activity of Hochu-ekki-to in mice

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Abstract

We have investigated the interferon (IFN)-inducing activity of Hochu-ekki-to (HET) in mice in comparison with that of poly I : C, a well-known IFN inducer. HET (62.5 to 2000 mg/kg) and poly I : C (2 mg/kg) were administrated by intraperitoneal and intravenous injection, respectively. In the sera from poly I : C-injected mice, the antiviral activity by the induced IFN peaked at 2 hours after injection, versus 48 hours for HET-injected mice. The antiviral activity was demonstrated using murine L929 cells but not a canine kidney-derived cell line, indicating that the activity shows host-species specificity. The optimum dose of HET for IFN induction was 250 mg/kg, at which the highest antiviral activity was 160 units (U)/ml corresponding to 400 international units (IU)/ml IFN-alpha. When the inducing activity of each component in HET was examined, only Saiko (62.5 mg/kg) induced IFN at 12 hours after injection, and the highest antiviral activity was 320 U/ml corresponding to 800 IU/ml IFN-alpha. Poly I : C-induced IFNs required both anti-murine IFNs-alpha and beta antibodies to neutralize the activity, whereas HET- and Saiko-induced IFNs were neutralized only by anti-murine IFN-alpha antibody but not by anti-murine IFN-beta or -gamma antibody. Although we could not eliminate the possibility of IFN-beta and/or -gamma induction by HET at the undetectable level in our assay system, it was evident that HET induced primarily IFN-alpha. This feature was the most distinctive from that of poly I : C which induced both IFNs-alpha and beta.

Key words interferon inducer, mice, Hochu-ekki-to, Saiko, poly I : C, antiviral activity, murine interferon-alpha.

Abbreviations CPE, cytopathogenic effect ; FBS, fetal bovine serum ; HET, Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益気湯 ; IFN, interferon ; Juzen-taiho-to (Shi-Quan-Da-Bu-Tang), 十全大補湯 ; mAB, monoclonal antibody ; MEM, minimal essential medium ; PBS, phosphate-buffered saline ; poly I : C, polyriboinosinic-polyribocytidylic acid ; SST, Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Since the discovery of interferon (IFN) as a virus inhibitory factor,[1] it has been observed that IFNs are host-coded proteins and thereby host-species specific.[2] Many studies on the biological and immunological characterization have led to the classification of both human and murine IFNs into alpha, beta and gamma species.[3] Recent evidences suggest that IFNs act upon cellular differentiation and immunomodulation, and thereby IFNs are considered to be one of lymphokines or monokines which play an important role on the expression and development of host defense mechanisms.[4-7]

Kampo formulations (traditional Chinese medicines) are used frequently for the treatment of chronic diseases in contrast to the western medicines. Hochu-ekki-to (HET) has been used

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from old times for chronic diseases due to the deficiency of vital energy (*'Ki'* in Chinese medical concept), and for infectious diseases *etc.* in patients with a weak constitution. In clinical studies, HET has been applied to the patients in various immunological disorders (weak immunity, immunosuppressive state and autoimmune diseases).[8] Recently, it has been shown that HET possesses various immunomodulating activities in experimental studies, *i. e.*, activation of macrophages,[9] effects on human natural killer cell activity,[10] and anti-tumor effects.[11] IFNs also possess the above biological activities, and it is probably supposed that HET might induce IFNs. However, laboratory studies on IFN-inducing activity of HET have not yet been reported.

In the present study, we demonstrated IFN-inducing activity of HET and its components in mice. Here we show that HET and Saiko induce primarily IFN-alpha in sharp contrast to poly I : C which induces both IFNs-alpha and -beta. This is the first report of HET as an IFN inducer.

Materials and Methods

Mice : An outbred specific-pathogen-free strain of ICR female mice aged 8 weeks were obtained from Japan SLC Co. Ltd. (Hamamatsu, Japan). Five mice were used for each experimental group.

Cells and virus : Murine L929 cells and Madin-Darby canine kidney (MDCK) cells were grown in Eagles's minimal essential medium (MEM) supplemented with 10% heat-inactivated (56℃ for 30 min) fetal bovine serum (FBS), penicillin G (100 U/ml) and streptomycin (100 μg/ml). VERO cells, an established cell line from the kidney of the African green monkey, were also grown in the same medium. These cells were maintained as a monolayer culture in a humidified atmosphere containing 5% CO_2 at 37℃.

Encephalomyocarditis virus (EMCV), which was kindly supplied by Chiharu Kishimoto (Toyama Medical and Pharmaceutical University, Japan) was grown in monolayer cultures of VERO cells. Virus stock solution was prepared as described elsewhere[12] and stored in small portions at −80℃. The 50% tissue culture infectious dose ($TCID_{50}$) of the virus stock solution was 10^4/0.1 ml assayed on L929 cells.

HET, its component and poly I : C : The germ-free lyophilized powder of HET and 10 components included in HET were kindly supplied from Tsumura & Co. (Tokyo, Japan). The constitution of HET is listed in Table I. Poly I : C was obtained from Sigma (St. Louis, USA). After weighing under aseptic conditions, these drugs were dissolved in sterile phosphate-buffered saline (PBS) and treated with ultrasonication for 1 min (except poly I : C).

IFN induction in vivo : 0.5 ml of HET were given by intraperitoneal (*i.p.*) injection at various doses (62.5 to 2000 mg/kg). Its component was given in the same way (31.25 to 500 mg/kg). As a positive control, 0.2 ml of poly I : C (2 mg/kg) were given by intravenous (*i.v.*) injection according to the method of Suzuki *et al*.[13] At the

Table I Constitution of Hochu-ekki-to.

Components		Amount	Origin
Astragari Radix	(Ogi)	4.0 g	Japan
Atractylodis Lanceae Rhizoma	(Sojutsu)	4.0 g	China
Ginseng Radix	(Ninjin)	4.0 g	Korea
Angelicae Radix	(Toki)	3.0 g	Japan
Bupleuri Radix	(Saiko)	2.0 g	Japan
Zizyphi Fructus	(Taiso)	2.0 g	China
Aurantii Nobilis	(Chinpi)	2.0 g	Japan
Glycyrrhizae Radix	(Kanzo)	1.5 g	China
Cimicifuge Rhizoma	(Shoma)	1.0 g	China
Zingiberis Rhizoma	(Shokyo)	0.5 g	China

indicated times after injection, blood samples were obtained from axillary vessels and kept at 4℃ for 12 hr. Thereafter, the sera were collected after centrifugation at 7000 rpm for 10 min and stored at −80℃ until used.

IFN titration : IFN titration was carried out according to the method of cytopathogenic effect (CPE) assay as reported elsewhere[13 15] with slight modifications. Briefly, sera were initially diluted 10-fold with MEM supplemented with 2% FBS and then serial two-fold dilutions in 50 μl of the same medium were prepared in each row of 96-well microplates (Corning, USA). The last well of each row (total 12 wells) was filled with 50 μl of the medium to serve as virus and cell controls (6 wells each). Thereafter, 100 μl of suspension of L929 cells (6×10^4 cells/well) were added to each well and the plates were incubated at 37℃ for 24 hr in a CO_2 incubator. After removing the medium, 100 μl of EMCV suspension containing virus titer of 100 $TCID_{50}$ were added to each well except the cell control wells and the plates were further incubated in the same way until the virus control wells displayed CPE of 100%. Thereafter, the remaining cells were fixed with acetone-methanol (1 : 3 v/v) for 5 min and stained with 0.1% crystal violet solution in 20% ethanol for 10 min. IFN titer was read as the highest reciprocal serum dilution at which 50% of cell monolayers was protected from the CPE determined by visual examinations of the monolayers with the aid of microscopy. Unless otherwise stated, IFN titer was expressed as units (U)/ml based on the above determination. To test host-species specificity of antiviral activity, MDCK cells were used instead of L929 cells. Moreover, reference standard murine IFNs-alpha, -beta (both obtained from PAESEL LOREI, Frankfurt, Germany) and -gamma (from Genzyme, Boston, USA) were included in each assay to standardize the IFN titer.

Neutralizing test of IFNs : To determine the types of induced IFN, neutralizing tests were carried out using monoclonal antibodies (mABs) against murine IFNs-alpha, -beta (both obtained from Yamasa Shoyu Co., Ltd, Tokyo, Japan) and -gamma (Holland Biotechnology, Leiden, Neitherland). The serum specimens were incubated with an equal volume of mAB (neutralizing titer : more than 320 IU/ml to the respective reference standard in this assay system) at 37℃ for 1 hr. Thereafter, the remaining antiviral activity was titrated in the same way as above. In some experiments, this test was performed in the simultaneous presence of two different mABs.

Statistical analysis : Mann-Whitney test was used to evaluate significant difference between the independent two groups.

Results

IFN-inducing activity of HET

To confirm that the sensitivity of our assay system used in this study was comparable to that reported by Suzuki *et al.*,[13] sera from poly I : C injected mice were initially titrated. As shown in Fig. 1, antiviral activity could be detected in all sera from poly I : C-injected mice but not in sera from untreated mice. The antiviral activity was maximum at 2 hr after injection (mean 960 U/ml ; peak 1280 U/ml). These data demonstrated that our assay system showed almost the same sensitivity as the above report.[13]

We therefore examined antiviral activity in sera collected at various periods after *i.p.* injection of HET at doses of 62.5 to 2000 mg/kg. As a result, in a group which received 250 mg/kg of HET, a significant increase in antiviral activity (mean 60 U/ml ; peak 160 U/ml) was observed at 48 hr after injection, but not earlier or later than 48 hr (Fig. 2). At this period after injection, antiviral activity (mean 30 U/ml ; peak 80 U/ml) was also detectable in sera from mice injected with 500 mg/kg of HET, but the effect of other doses was negligible (Fig. 3). Moreover, antiviral activity was not detected in sera collected at other than 48 hr at any dose (data not shown).

Host-species specificity of induced antiviral activity

To test host-species specificity of the detected antiviral activity, the same assay carried out using MDCK cells. As shown in Fig. 4, the antiviral activity induced by the injection of 250 or 500 mg/kg of HET was reduced to the control level, confirming that antiviral activity induced

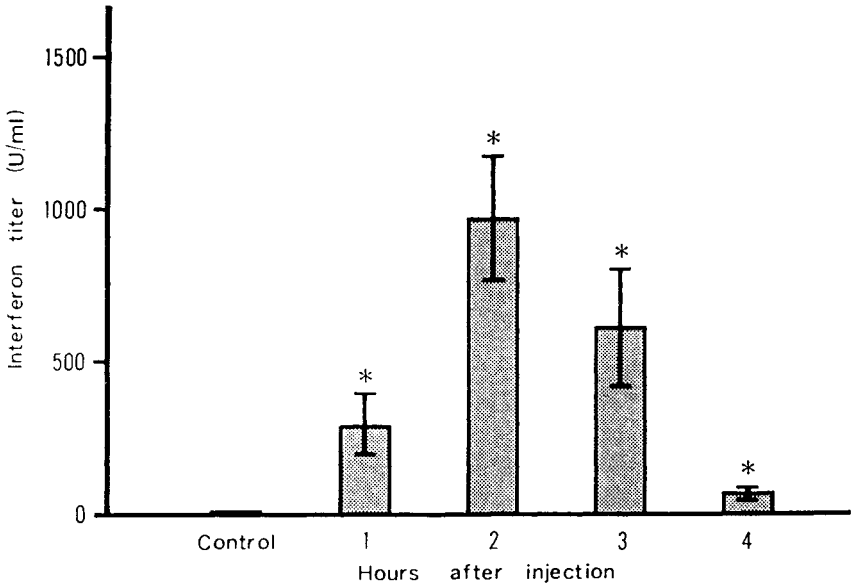

Fig. 1 Time-related IFN induction by poly I : C. Poly I : C was given with *i.v.* injection at a dose of 2 mg/kg. Untreated mice served as a control. At the indicated times after injection (abscissa), sera were collected to assay antiviral activity. IFN titer (ordinate) was expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar) which assayed on L929 cells. The definition of antiviral activity was described in the text. Asterisks indicate significant difference from the control ($p<0.01$).

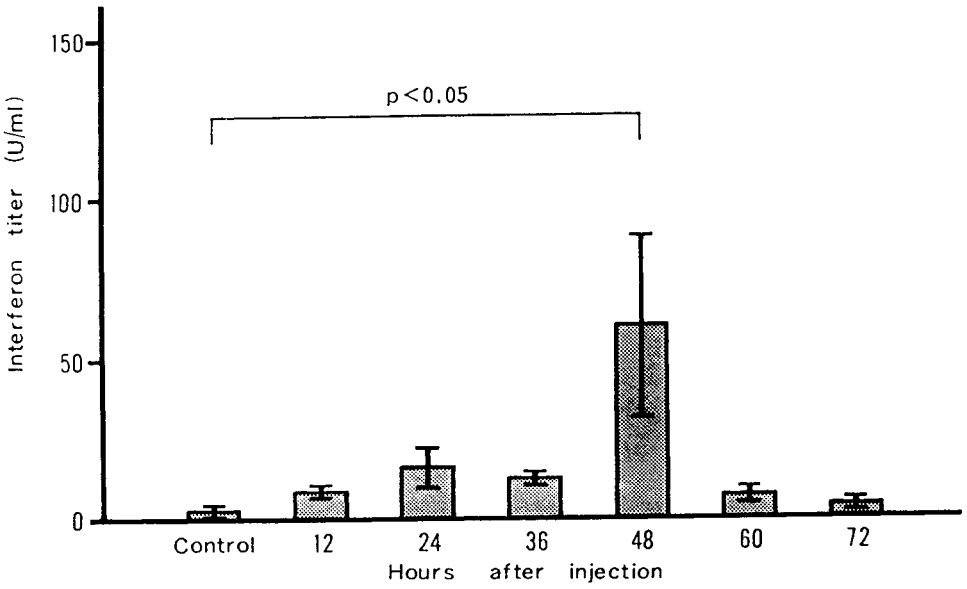

Fig. 2 Time-related IFN induction by HET. HET was given with *i.p.* injection at a dose of 250 mg/kg. Untreated mice served as a control. At the indicated times after injection (abscissa), sera were collected to assay their antiviral activity on L929 cells. At each experimental point, IFN titer (ordinate) was expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar). IFN titer at 48 hr showed significant difference from the control ($p<0.05$).

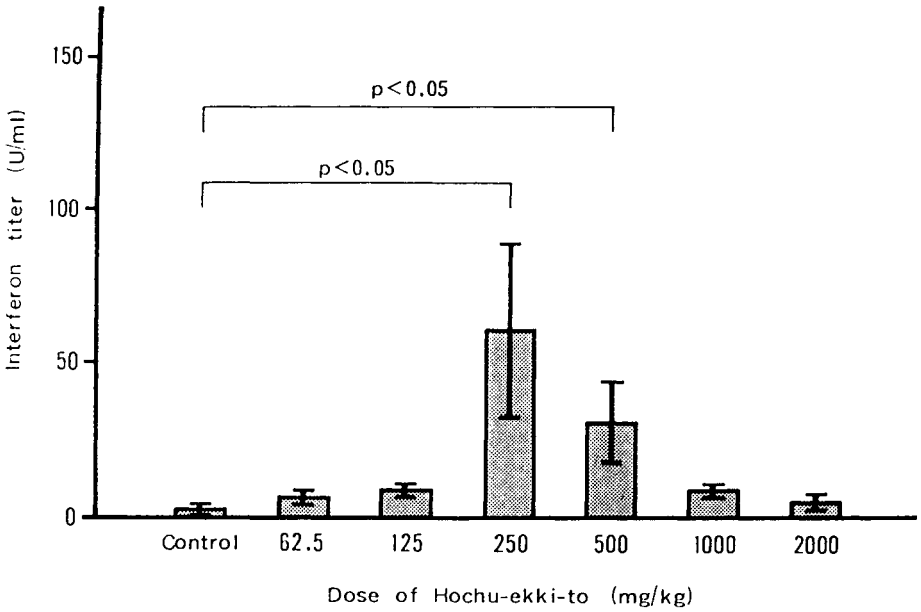

Fig. 3 Dose-related IFN induction by HET. HET was given with *i.p.* injection at the indicated doses (abscissa). Untreated mice served as a control. At 48 hr after injection, sera were collected to assay their antiviral activity on L929 cells. At each experimental point, IFN titer (ordinate) was expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar). IFN titer at 250 and 500 mg/kg showed significant difference from the control ($p<0.05$).

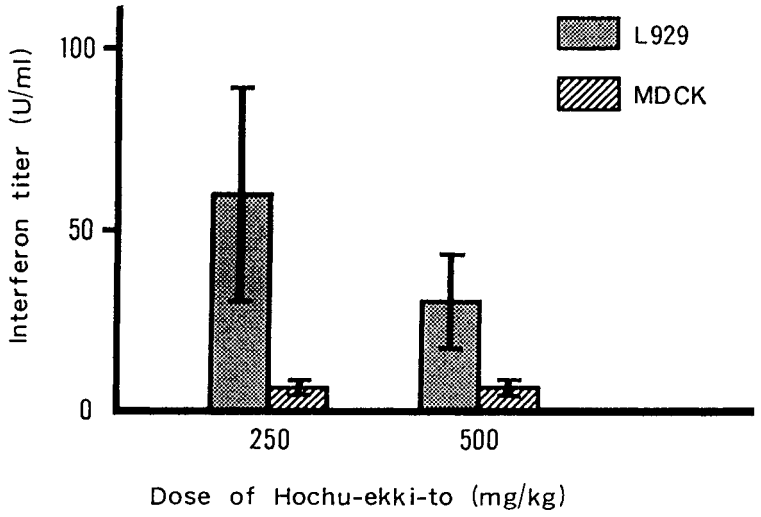

Fig. 4 Host-species specificity of antiviral activity induced by HET. Mice were given HET with *i.p.* injection at a dose of 250 or 500 mg/kg (abscissa) and sera were collected at 48 hr after injection. IFN titer (ordinate) in sera was titrated on L929 cells or canine MDCK cells and expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar).

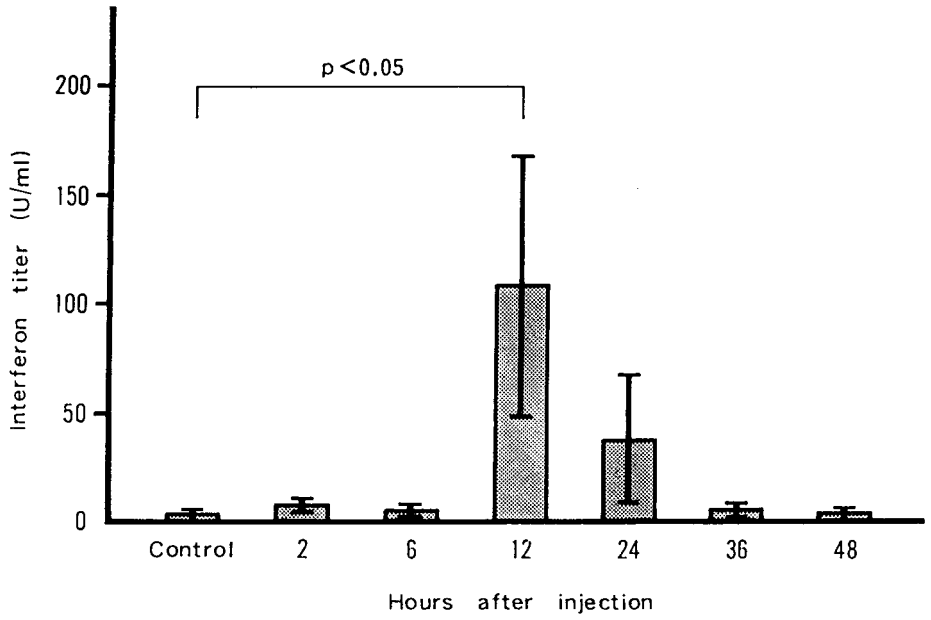

Fig. 5 Time-related IFN induction by Saiko. Saiko was given with *i.p.* injection at a dose of 62.5 mg/kg. Untreated mice served as a control. At the indicated times after injection (abscissa), sera were collected to assay their antiviral activivity on L929 cells. At each experimental point, IFN titer (ordinate) was expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar). IFN titer at 12 hr showed significant difference from the control ($p<0.05$).

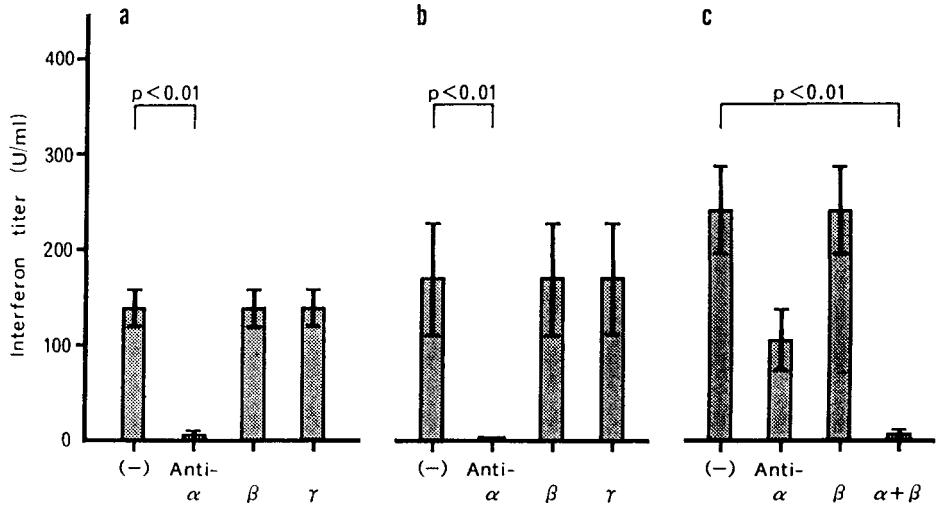

Fig. 6 Neutralization test of IFNs induced by HET, Saiko, and poly I : C by murine IFN type-specific mABs. Serum samples collected from HET(a), Saiko(b), and poly I : C(c) injected mice were treated with each mAB alone or anti-(alpha+beta) mABs at 37℃ for 60 min. Samples without mABs were served as a control and designed "(−)". Thereafter, remaining IFN titers were assayed and expressed as the mean antiviral activity (U/ml) of five mice (thick bar) ± SEM (thin bar). Significant differences from the control were observed by anti-IFN-alpha in a and b, and anti-IFN-alpha+beta in c, respectively ($p<0.01$).

by HET is host-species specific.

IFN-inducing activity of each component included in HET

HET consists of 10 components as listed in Table I. Thus, we examined which components acted as an IFN inducing agent. Among the components, only Saiko (62.5 mg/kg) could induce antiviral activities at 12 hr after injection (mean 108 U/ml ; peak 320 U/ml) to a comparable level to that induced by HET (Fig. 5). In this case, the duration period for induction became somewhat shorter (compare 12 and 48 hr for Saiko and HET, respectively). Sojutsu and Shoma showed some activities (not significant) at 48 hr, but other components showed no activities to induce IFN (data not shown).

Neutralization test of the induced antiviral activity by mABs against murine IFNs-alpha, -beta and -gamma

Based on the host-species specificity, it was suggested that the antiviral activity was exerted by IFN. Hence, we attempted to identify the type of induced IFN by a neutralizing test using IFN type-specific mABs. As shown in Fig. 6a-c, HET- and Saiko-induced IFNs were completely neutralized by anti-IFN-alpha mAB. However, both mABs against IFNs-beta and -gamma could not neutralize its activity. On the other hand, poly I : C-induced IFNs could be neutralized only in the simultaneous presence of both mABs against IFNs-alpha and -beta. Anti-IFN-alpha or -beta alone could not neutralize the activity completely.

Based on these findings, we attempted to standardize the IFN titers induced by HET and Saiko to international units (IU/ml) using reference standard IFN-alpha. As a result, 1 U/ml of induced IFN corresponded to 2.5 IU/ml of reference standard IFN-alpha in our assay system. Therefore, 160 and 320 U/ml, which was the maximum IFN titers induced by HET and Saiko, could be expressed as 400 and 800 IU/ml IFN-alpha, respectively.

Discussion

With reference to others' reports, immunomodulating activities of Kampo formulations including HET can be summarized as follows : the induction of polyclonal antibodies in peripheral blood mononuclear cells,[16] the activation of macrophages,[9] the augmentation of natural killer or lymphokine activated killer cell cytotoxicity,[10,17,18] and anti-tumor effects.[11,19] Because IFNs are recognized as one of the important immunomodulating cytokines,[4-7] it allows us to postulate that the immunomodulating activity of HET may be intimately associated with the induction of IFNs. Indeed, several reports have shown the IFN-inducing activity of Kampo formulations. In the case of human, Sho-saiko-to (SST) induced IFN-alpha[20] and -gamma.[21] Juzen-taiho-to enhanced the production of phytohemagglutinin-induced IFN-gamma.[22] In the case of mice, SST induced IFN-alpha+beta.[23] Naito *et al.*[24] reported that 17 Kampo formulations (except HET) induced murine IFN-like substance, but they made no identification of murine IFNs themselves. However, laboratory studies on IFN inducing activity of HET has not yet been reported. In this study, we demonstrated the IFN-inducing activity of HET in mice for the first time.

It has been reported that poly I : C induced IFNs-alpha and beta powerfully which peaks at 2-3 hr and disappears at 6-8 hr after either *i.v.* or *i.p.* injection[13,25,26] consistent with this study (Fig. 1). At the neutralization test of poly I : C-induced IFNs (Fig. 6c), anti-IFN-beta mAB could not reduce the IFN titer (comparable to the control level), although sufficient amounts of each mAB were used to this test. This finding might be explained that the residual IFNs after the treatment of this mAB was enough to prime L929 cells in wells of high dilutions (low contents of residual IFNs), and that the following challenge of EMCV induced newly high titer IFNs from L929 cells by this priming effect. Thus, we estimated that the IFN titer at this test might be expressed as antiviral activities of cells treated by both residual IFNs (low diluted wells) and newly induced IFNs (high diluted wells).

The highest IFN titer induced by HET was only 1/8 of that induced by poly I : C, and HET

required a much longer period for induction than poly I : C in this study. However, these results in HET were comparable to those in other Kampo formulations studied in mice.[23,24] Moreover, it is shown that even an extremely low amount (less than 10 U/ml) of IFN is enough to induce cellular responses.[27] Thus, we can speculate that HET is actually an IFN inducer *in vivo*.

Although we could not eliminate the possibility of IFNs-beta and/or -gamma induction by HET at the undetectable level in our assay system, it was evident that HET induced primarily IFN-alpha (Fig. 6a). This feature was the most distinctive from that of poly I : C which induced both IFNs-alpha and beta. All murine cells reported previously have produced both IFN-alpha and -beta in response to poly I : C or Newcastle disease virus (NDV). Moreover, the existence of murine cells producing IFNs-alpha or -beta independently have remained unknown. HET, thereby, may be useful for obtaining better insights into the distinct mechanisms of IFN-alpha induction on cellular and inducer levels in mice.

Among the components included in HET, Saiko also induced primarily IFN-alpha (Fig. 6b). Although HET and SST share Saiko as one of their components, several reports demonstrated that SST induced IFNs-alpha, -gamma, and -alpha+beta, in human and mice.[20,21,23] Because SST includes Pinelliae Tuber (Hange) and Scutellariae Radix (Ogon) which HET does not include, this constitutional difference might influence the induction of plural IFN subtypes in SST.

It is interesting to know that the duration time for induction was somewhat longer by HET compared with that by Saiko alone. This might be explained with several mechanisms. First, intraperitoneal environment may delay the absorption of HET into the circulation system. Secondly, IFN inducing agents (possibly included in Saiko) may be converted more slowly into active forms when given as HET. Thirdly, although the inducing effects of Sojutsu and Shoma were weak (data not shown), these components might act as IFN inducers at a later time (48 hr) in response to the priming effect of Saiko exhibited at an earlier time (12 hr). However, further studies are needed to clarify this issue.

It was shown that HET and Saiko induced IFN only in the optimum dose range (250-500 mg/kg and 62.5 mg/kg, respectively). One explanation for this might be that some agents in HET and Saiko act as inducers while others show suppressive effects. Thus, IFN induction may occur in the optimum dose range depending on a balance between enhancing and suppressive effects of the agents included in HET and Saiko. Abe *et al.*[28] also reported that Sairei-to improved nephrosis rats at the optimal dose. This phenomenon might be a characteristic of Kampo formulations.

Finally, we might explain that activation of macrophages and augmentation of natural killer cell cytotoxicity were mediated by HET-induced IFN-alpha. Considering this immunostimulating potency, we can confidently apply HET in clinical cases.

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和文抄録

マウスにおける補中益気湯のインターフェロン（IFN）誘発作用を，既知のIFN誘発物質であるpoly I：Cとの比較により検討した。補中益気湯は各種濃度に希釈して腹腔内投与し，poly I：Cは2 mg/kgを静脈内投与した。マウス血清中の抗ウイルス活性は，poly I：Cが投与後1－4時間で認められたのに対し，補中益気湯では48時間後にのみ認められた。また，48時間後に抗ウイルス活性を検出した補中益気湯の投与濃度は250-500 mg/kgであった。これらの抗ウイルス活性は，マウスL929細胞で検出されたが，犬腎由来細胞では検出されず種特異性を有していた。補中益気湯の構成生薬10種類についても各種濃度で腹腔内投与したが，柴胡のみ（62.5 mg/kg）が投与12時間後に抗ウイルス活性を検出した。抗マウスIFN α，β，及びγモノクロ

ナール抗体による抗ウイルス活性中和試験では，poly I：C誘発IFNが抗 α，β 抗体の両者の存在が必要なのに対して，補中益気湯及び柴胡誘発IFNは抗 α 抗体のみで活性が中和された。同時に行った標準IFN α の検定により国際単位換算を行い，その作用は最高値で補中益気湯：400 IU/ml，柴胡：800 IU/mlと換算された。これらの結果から，補中益気湯及び柴胡は，IFN α と β 双方を誘発するpoly I：Cとは極めて対照的な，IFN α に対する特異的な誘発物質であることが示唆された。

Reference

1) Issacs, A,. Lindenmann, J：Virus interference. I. The interferon. *Pros. R. Soc. Lond (Biol).* **147**, 258-267, 1957.
2) Lindahl-Magnusson, P., Leary P., and Gresser I.：Interferon and cell division VI. inhibitory effect of interferon on the multiplication of mouse embryo and mouse kidney cells in primary culture. *Proc. Soc. Exp. Biol. Med.* **138**, 1044-1050,1971.
3) Stewart II, W.E.：Committee on Interferon Nomenclature. *Nature* **286**, 110, 1980.
4) Pindak, F.F.：Protection of mice against bacterial infection by interferon inducers. *Infect. Immun.* **1**, 271-273, 1970.
5) Herman, R., and S. Baron：Effect of interferon inducers on the intracellular growth of the protozoan parasite, Leishmania donovani. *Nature* **226**, 168-170, 1970.
6) Umenai, T., Suzuki, F., and Ishida, N.：Effect of an interferon inducer, 9-Methylstreptotimidone, on Candida albicans infection in mice. *Antimicrob. Agents Chemother.* **13**, 939-943, 1978.
7) Yabrov, A.A.：Interferon：anticancer agent of multifarious activity. In Interferon and Nonspecific Resistance Edited by A.A. Yabrov. Human Science Press, New York, p128, 1980.
8) Terasawa, K.：A textbook of Japanese Oriental (Kampoh) medicine, Igaku Shoin, Tokyo, pp. 16-20, 1990.
9) Kataoka, T., Akagawa, K., Tokunaga, T., and Nagao, S.：Activation of macrophage with Hochu-ekki-to. *Jpn. J. Cancer Chemother.* **16**, 1490-1493, 1989.
10) Ohono, S.：Effects of a traditional chinese medicine 'Hochu-ekki-to' on natural killer activity. *Allergy* **37**, 107-114, 1988.
11) Ito, H.：Study of anti-tumor effect of Japanese Kampo medicine. *Biotherapy* **3**, 760-766, 1989.
12) Stebbing, N., C.A Grantham, and N.H. Carey：Antiviral activity of single-stranded homopolynucleotides against encephalomyocarditis virus and Semliki forest virus in adult mice without interferon induction. *J. Gen. Virol.* **30**, 21-39, 1976.
13) Suzuki, F., Okabe, H., Todaka, T., Hayashi, K., Koide, F., Maeda, H., and Ishida, N.：Heat-killed lactobacillus casei, LC9018, as an interferon inducer. *Jpn. J. Bacteriol.* **43**, 821-827, 1988.
14) Rubinstein, S., P.C. Familletti, and S. Pestka：Convenient assay of interferons. *J. Virology* **37**, 755-758, 1981.
15) Suzuki, F., and R.B. Pollard：Alteration of interferon production in a mouse of thermal injury. *J. Immun.* **129**, 1806-1810, 1982.
16) Ikemoto, Y., Mizoguchi, Y., Arai, T., Yamamoto, S., and Morisawa, S.：Effects of Syo-saiko-to (Xiao-Chai-Hu-Tang) and Dai-saiko-to (Da-Chai-Hu-Tang) on antibody response in vitro. *J. Med. Pham. Soc. WAKAN-YAKU* **1**, 235-242, 1984.
17) Mizoguchi, Y., Fujinobu, Y., Kobayashi, K., Yamamoto, S., and Morisawa, S.：The effects of Xiao-Chai-Hu-Tang (Syo-saiko-to) on natural killer (NK) cell activity. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 184-188, 1986.
18) Mizoguchi, Y., Sakagami, Y., Miyajima, K., and Yamamoto, S.：The effects of Xiao-Chai-Hu-Tang (Syo-saiko-to) on lymphokine activated killer (LAK) cell activity. *Allergy* **35**, 1119-1121, 1986.
19) Haranaka, K., Satomi, N., Sakurai, A., Haranaka, R., Kosoto, H., and Kobayashi, M.：Antitumor activities and tumor necrosis factor producibility of traditional Chinese medicine and crude drugs. *J. Trad. Sino-Jpn. Med.* **6**, 85-91, 1985.
20) Nakajima, O., Koga, K., Fujii, K., and Ami, K.：Interferon-α induction and clinical efficacy on chronic hepatitis subjects by Sho-saiko-to. *J. Med. Pharm. Sci.* **10**, 687-693, 1983.
21) Kakumu, S., and Fuji A.：The effects of Sho-saiko-to on induction of interferon-γ by peripheral blood mononuclear cells. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 219-222, 1987.
22) Sakagani, Y., Mizoguchi Y., Miyajima, K., Kuboi, H., and Kobayashi, K.：Antitumor activity of Shi-Quan-Da-Bu-Tang and its effect on interferon-γ and interleukin-2 production. *Allergy* **37**, 57-60, 1988.
23) Kawakita, T., Nakai, S., Kumazawa, Y., Miura, O., Yumioka, E., and Nomoto, K.：induction of interferon after administration of a traditional Chinese medicine, Xiao-Chai-Hu-Tang (Sho-saiko-to). *Int. J. Immunopharm.* **12**, 515-521, 1990.
24) Naito, S., Tominage, H., Tanaka, M., Yoshihara, H., and Nakamura, K.：Chinese indigenous medicine as inducers of interferon-like substances in mice. *Jpn. Pharmacol. Ther.* **16**, 9-15, 1988.
25) Hayashi, Y., Nakada, K., Aso, H., Suzuki, F., and Ishida N.：Interferon inducing activity of SNMC. *Jpn. Pharmacol. Ther.* **12**, 3861-3864, 1979.
26) Sudo, T.：Preparation and quantification of IFN mRNA. *Protein, Nucleic acid and Enzyme ISSN 0371-8565* **25**, 146-156, 1981.
27) Rubin, B.Y., Gupta, S.L.：Differential efficacies of

human type I and type II interferon as antiviral and antiproliferative agents. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5928-5932, 1980.

28) Abe, H., Orita, M., Konishi, H., and Arichi, S. : Effects of Sairei-to on aminonucleoside nephrosis in the rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 24-30, 1986.