Effect of Hachimi-jio-gan on Immunoglobulin A Producing Cells in Peyer's Patch by Oral Administration

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We investigated here the effect of Hachimi-jio-gan (HJ), a Japanese and Chinese herbal medicine, on immunoglobulin A (IgA) producing cells in Peyer's patch. The oral administration of HJ (0.1, 0.2, 1.0 g/kg for 2 d) enhanced the IgA producing cells almost 2-fold compared with the control group on days 4 and 5 after the administration. Furthermore, HJ augmented antigen-specific IgA (anti-SRBC IgA) production. These results suggested that HJ acted as a polyclonal B cell activator. To characterize its active ingredients, HJ was fractionated by ethanol precipitation. The crude polysaccharide fraction (EP fraction) showed the ability to augment IgA production, but low molecular weight fraction (ES fraction) did not. These results suggest that the crude polysaccharide fraction might be responsible for the augmentation of IgA production by HJ.

Key words Hachimi-jio-gan (HJ); immunoglobulin A producing cell; Peyer's patch; plaque forming cell assay; crude polysaccharide fraction

Hachimi-jio-gan (HJ), a traditional Japanese and Chinese herbal medicine, has been used for treating various senile diseases associated with aging such as prostatic hypertrophy,¹⁾ oligozoospermia²⁾ and senile colitis.³⁾ In our previous study, we reported that HJ had a physostigmine-like protective effect on cerebral anoxia,⁴⁾ and ameliorated various experimental amnesia in mice⁵⁾ as well as the scopolamine-induced cognitive disturbance of the radial maze performance.⁶⁾ Furthermore, HJ-induced increases in acetylcholine content and choline acetyltransferase activity were observed in the frontal cortex of HJ treated-rats. However, the pharmacological and clinical effects of HJ have not been thoroughly investigated.

Traditional Japanese and Chinese herbal remedies are thought to work as balancers of the homeostasis. Namely, they are believed to work through three systems: the central nervous system, the endocrine system and the immune system. To our knowledge, it has been reported that HJ affects both the central nervous system and the endocrine system; however, the effect of HJ on the immune system is still unclear. Since most traditional Japanese and Chinese herbal medicines have been used as an oral administration for treating diseases, we thought they could affect the mucosal immune system first. Peyer's patches (PP), part of the gut associated lymphoid tissues (GALT), are known to be the inductive site for immunoglobulin A (IgA) in the mucosal immune system. IgA is the predominant immunoglobulin class existing in mucosal secretions and plays an important role in infection immunity in the mucosa.

In this paper, we studied the effect of HJ on IgA production in PP by oral administration.

MATERIALS AND METHODS

Animals Male C3H mice (6–7 weeks old) purchased from Charles River, Japan, Inc. (Kanagawa, Japan) were used. The animals were housed in an air-conditioned room (24 °C) and given a commercial diet (CE-2, Nippon Clea Co., Tokyo, Japan) and water *ad libitum*. The animals

were kept for at least 6 d before being used.

Preparation and Fractionation of HJ Crude drugs of HJ were authenticated and provided by Tsumura Co., Ltd. (Tokyo, Japan). The crude drug composition of HJ is noted in Table 1. The mixture of crude drugs was boiled in 12 volumes of water and extracted for 1 h. The decoction was centrifuged, passed through a 1.7-mm mesh sieve and evaporated under 50 °C. The concentrate was spray-dried to give a powdered extract. The yield was 18%, which represents one human dose per day. For the fractionation of HJ, the powdered extract of HJ was dissolved in water and then the addition of 3 volumes of EtOH to this solution gave the precipitate and an EtOH-soluble supernatant. The precipitate was suspended in the appropriate volume of water and then lyophilized to give a powder (EP fraction, 2.0 g). The EtOH-soluble supernatant was also concentrated and then lyophilized to obtain a powder (ES fraction, 4.0 g). The total carbohydrate and uronic acid contents were determined by the phenol-sulfuric acid⁷⁾ and the sulfuric acid-carbazole methods,⁸⁾ respectively, using glucose and galacturonic acid as the respective standards. Protein was assayed by the Bradford method⁹⁾ with bovine serum albumin as the standard. These chemical analyses showed that the EP fraction contained 73.8% total sugar

Table 1. Crude Drug Composition of Hachimi-jio-gan (HJ)^{a)}

Crude drug	Plant name	Composition (g)
Rehmanniae Radix	<i>Rehmannia glutinosa</i> LIBOSH. var. <i>purpurea</i> MAKINO (Scrophulariaceae)	6.0
Corni Fructus	<i>Cornus officinalis</i> SIEB. et ZUCC. (Cornaceae)	3.0
Dioscoreae Rhizoma	<i>Dioscorea batatas</i> DENCE (Dioscoreaceae)	3.0
Alismatis Rhizoma	<i>Alisma orientale</i> JUZEP. (Alismataceae)	3.0
Poria	<i>Poria cocos</i> WOLF (Poriaceae)	3.0
Moutan Cortex	<i>Paeonia suffruticosa</i> ANDR. (Paeoniaceae)	2.5
Cinnamomi Cortex	<i>Cinnamomum cassia</i> BLUME (Lauraceae)	1.0
Aconitum Tuber	<i>Aconitum carmichaeli</i> DEBX. (Ranunculaceae)	0.5

a) As provided by Tsumura Co., Ltd. (Tokyo, Japan).

as glucose, 7.4% uronic acid as galacturonic acid, and 11.1% protein.

Preparation of Cell Suspensions from PP and Cell Culture PP were carefully excised from the wall of the small intestines and then single cell suspensions were prepared by mincing and tapping the PP fragments on a nylon screen (200 mesh) in cold RPMI-1640 medium (Irvine Scientific, Santa Ana, CA, U.S.A.). PP cells were suspended in RPMI-1640 supplemented with 10% Nuserum (Becton Dickinson Labware, Bedford, MA, U.S.A.), 100 U/ml penicillin and streptomycin (Gibco Life Technology, Inc., Brandisland, U.S.A.), 2 mM HEPES, and 50 μ M 2-mercaptoethanol (Nacalai Tesque, Inc., Kyoto, Japan) and then cultured at a density of 5×10^6 cells/200 μ l/well with 10 μ g of lipopolysaccharide (LPS, from *Escherichia coli* 011: B4, Difco laboratories (Detroit, MI, U.S.A.)) in a 24-well plastic culture plate (Falcon Plastics, NJ, U.S.A.) at 37°C for 72 h under 5% CO₂-95% air. After the culture period, PP cells were harvested and used for plaque forming cell (PFC) assay, as described by Tauchi *et al.*¹⁰⁾

PFC Assay PFCs were determined 72 h after culture by the method of Cunningham and Szenberg¹¹⁾ with a minor modification. Briefly, for the assay of anti-SRBC specific IgA producing cells, an aliquot of a mixture of 50 μ l of SRBC suspension, 50 μ l of guinea pig complement (Rockland, PA, U.S.A.), 10 μ l of diluted goat anti-mouse IgA antibody (IgG, Zymed Laboratory, Inc., San Francisco, CA, U.S.A.) and 10 μ l of PP cell suspension was packed into Cunningham-type chambers and incubated for 1 h at 37°C. After incubation, the numbers of indirect PFC in the chambers were counted under a light microscope. The numbers of anti-SRBC specific IgA producing cells were calculated by subtracting the number of direct PFC (10 μ l of medium was added to the mixture instead of anti-IgA antibody) from the number of indirect PFC. For the number of total IgA producing cells, the protein-A PFC technique was used.¹²⁾ An aliquot of a mixture of 50 μ l of the protein-A conjugated SRBC, 50 μ l of guinea pig complement, 10 μ l of PP cell suspension and 10 μ l of diluted goat anti-mouse IgA antibody was packed into a chamber and incubated for 3 h at 37°C. After incubation, the numbers of total IgA producing cells in the chambers were counted as well.

Statistical Analysis Results were given as the mean $\pm$ S.E.M.: mean values were compared by the two-tailed Student's or Aspin-Welch's *t*-test.

RESULTS

Effect of HJ or SRBC on Polyclonal Induction of IgA Producing Cells in PP Cell Culture Oral administration of SRBC (2.5×10^9 cells/mouse) did not show any effect on the polyclonal induction of IgA producing cells in PP cell culture (Fig. 1). On the other hand, oral administration of HJ on days -4 and -5 enhanced the IgA producing cells almost two-fold compared with the control.

Effect of HJ or SRBC on Induction of Anti-SRBC Specific IgA Producing Cells in PP Cell Culture SRBC significantly induced anti-SRBC IgA PFC 4 and 5 d after the oral administration (Fig. 2). HJ also induced anti-

Fig. 1. Effect of HJ on the Induction of Anti-Protein A IgA PFC in PP Cell Culture with LPS

Each column represents the mean $\pm$ S.E.M. of 5 to 6 mice. **p* < 0.05 vs. the control group.

Fig. 2. Effect of HJ on Induction of Anti-SRBC IgA PFC in PP Cell Culture with LPS

Each column represents the mean $\pm$ S.E.M. of 5 to 6 mice. **p* < 0.05, ***p* < 0.01 vs. the control group.

Fig. 3. Effect of Co-administration of SRBC and HJ on Induction of (A) Anti-Protein A and (B) Anti-SRBC IgA PFC in PP Cell Culture with LPS

SRBC and HJ were administered orally 4 and 5 d prior to the excision of PP. Each column represents the mean $\pm$ S.E.M. of 6 mice. **p* < 0.05, ***p* < 0.01 vs. the control group.

Fig. 4. Effects of Fractions of HJ on Induction of (A) Anti-Protein A and (B) Anti-SRBC IgA PFC in PP Cell Culture with LPS

HJ and its fractions were administered orally 4 and 5 d prior to the excision of PP. Each column represents the mean $\pm$ S.E.M. of 6 mice. * $p < 0.05$, ** $p < 0.01$ vs. the control group.

SRBC IgA PFC by almost double 4 and 5 d after the administration. Co-administration of HJ and SRBC did not show any differences from when they were administered orally alone (Fig. 3).

Effects of the Fraction of HJ on Induction of IgA Producing Cells in PP Cell Culture In order to determine its active components, the fractionation of HJ was carried out by ethanol precipitation. As shown in Fig. 4, ethanol precipitates (EP fraction) showed the same effect as HJ did, whereas the ethanol soluble fraction did not show the induction.

DISCUSSION

In the present paper we studied the effect of HJ on the immune system, especially the local immune system. Since most traditional Japanese and Chinese medicines are administered orally for the treatment of various diseases, the alimentary tract is thought to be the site of their first pharmacological action. It has been reported that some ingredients are metabolized by intestinal microflora to produce the active or inactive metabolites. These results let us consider the possibility that some components in herbal medicines could affect the function of the alimentary tract and *vice versa*.

Tauchi *et al.* reported that the oral administration of Shosaiko-to, a traditional Japanese and Chinese herbal medicine, induced IgA production.¹⁰⁾ Shosaiko-to is known to act as a polyclonal B cell activator and its pharmacological actions are widely studied.^{13,14)} As shown in our results, HJ also showed the polyclonal induction of IgA secreting cells in PP culture. The increment of induction of SRBC specific IgA secreting cells by HJ was less than that by SRBC. We thought the increases of SRBC specific IgA PFC might be due to the polyclonal induction of IgA secreting cells as in the case of Shosaiko-to. However, these augmentations of the induction of IgA secreting cells were transient. A single administration of HJ 4 or 5 d before the assay also showed the same results, but the degree of augmentation was slightly weak (data not shown). The oral administration of SRBC also showed the same effect. The reason these effects were transient is still obscure. Fractionation by

ethanol precipitation revealed that the EP fraction prompted the induction of IgA production. EP fraction consists mainly of polysaccharides. Many polysaccharides from medicinal plants, algae and fungi are known to have various biological activities such as immunopotentiating, anti-tumor, anti-ulcer and hypoglycemic effects.¹⁵⁾ We have reported that the crude polysaccharide fraction of Shosaiko-to plays an important role in the immunopharmacological actions of Shosaiko-to,¹⁶⁾ and that the oral administration of the crude polysaccharide fractions of Daisaiko-to, Hachimi-jio-gan and Hochu-ekki-to, in addition to Shosaiko-to, augment the macrophage function.¹⁷⁾ Furthermore, the oral administration of other prescriptions of Kampo-hozai, such as Sho-fu-san and Unsei-in, also show the polyclonal induction of IgA secreting cells in PP culture (data not shown) in the same manner. These results suggest that the induction of IgA production in the mucosal immune system might be universal in herbal medicines. Since HJ and EP fractions are plant extracts, the possibility of occasional contamination with bacterial LPS is discussed. LPS is known to be a potent polyclonal B cell mitogen. Thus, we studied the effect of oral administration of LPS (from *Escherichia coli* 011:B4) on PFC in PP culture, but we could not find any influence on IgA production (data not shown). These results ruled out the possibility of contamination with LPS on these actions of HJ and EP fraction.

The mucosal immune system has been widely investigated and the role of secreting IgA and γ , δ -T cells in infection has been reported. It has been suggested that a local immune system such as the mucosal immune system could affect the systemic immune system. Further study in the immunopharmacological action of HJ and other herbal medicines is therefore necessary.

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