ELSEVIER

Effects of Kakkon-to and Sairei-to on Experimental Elevation of Aqueous Flare in Pigmented Rabbits

Hiroki Yano, Shigeyoshi Hiraki and Seiji Hayasaka

Department of Ophthalmology,
Toyama Medical and Pharmaceutical University, Toyama, Japan

Purpose: To evaluate the possible inhibitory effects of Kakkon-to and Sairei-to, traditional Sino-Japanese herbal medicines, on experimental aqueous flare elevation in pigmented rabbits.

Methods: Anterior uveitis was induced either by an application of prostaglandin E_2 (PGE_2), 10 μg/mL, to the cornea, or an intravenous injection of lipopolysaccharides (LPS), 0.5 μg/kg, in an ear vein. Dose dependency of experimental uveitis induced by LPS (0.1, 0.25, 0.5, or 1.0 μg/kg) was also determined. For pretreatment, about 150 g/day of food containing Kakkon-to (1% w/w) or Sairei-to (0.6% or 2% w/w) was given to two groups of animals for 5 days before experimental uveitis was induced. A third group of animals underwent pretreatment with betamethasone, 130 μg/kg, injection into an ear vein 4 hours before experimental uveitis was induced. A fourth group of rabbits with no herbal medicine or betamethasone pretreatment served as controls. Aqueous flare was measured using a laser flare-cell meter. Aqueous flare intensity was expressed as the area under the curve (AUC) in arbitrary units.

Results: The increase in aqueous flare induced by LPS was dose-dependent. The AUC of PGE_2 (10 μg/mL) and LPS (0.5 μg/mL) induced aqueous flare elevations were 1,119 and 4,950 arbitrary units, respectively. Kakkon-to (AUC, 1,055) and Sairei-to (AUC, 965) did not inhibit the aqueous flare elevation induced by PGE_2. β-Methasone did inhibit the elevation (AUC, 271). Kakkon-to (AUC, 4,495) did not suppress the aqueous flare elevation induced by LPS. Both 0.6% and 2% Sairei-to (AUC, 2,478, and 978) and β-methasone (AUC, 443) did suppress the aqueous flare elevation induced by LPS significantly ($P < .05$).

Conclusion: Sairei-to could have an inhibitory effect on experimental anterior uveitis induced by LPS. **Jpn J Ophthalmol 1999;43:279–284** © 1999 Japanese Ophthalmological Society

Key Words: Experimental anterior uveitis, Kakkon-to (Ge-Gen-Tang), lipopolysaccharides, prostaglandin E_2, Sairei-to (Cai-Ling-Tang).

Introduction

Traditional herbal, or Kampo, medicines have been used clinically in China for about 3,000 years and in Japan for about 1,000 years. The hot water extract of a mixture of herbs is commonly administered orally for several days as therapy for human physical disorders. In Japan, herbal medicines are available in granular form, and approved as therapeutic drugs. Kakkon-to has been indicated for the treatment of the common cold and acute febrile disease in humans. Sairei-to is frequently used to treat diarrhea, edema, and acute gastroenteritis, and was recently reported to be useful in the treatment of Behçet's disease, sarcoid uveitis, and Vogt–Koyanagi–Harada disease.[1–3] To our knowledge, the effects of these traditional herbal medicines on experimental uveitis have not been reported previously.

To produce experimental elevation of aqueous flare, we topically applied prostaglandin E_2 (PGE_2) with the use of a glass cylinder.[4,5] The intravenous injection of lipopolysaccharides (LPS) was also performed to induce anterior uveitis.[6,7] In the present study, we examined the effects of these traditional herbal medicines on the experimental elevation of aqueous flare induced by PGE_2 or LPS.

Received: January 8, 1998
Correspondence and reprint requests to: Hiroki YANO, MD, Department of Ophthalmology, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Jpn J Ophthalmol 43, 279–284 (1999)

Published by Elsevier Science Inc.

0021-5155/99/$–see front matter
PII S0021-5155(99)00019-2

Jpn J Ophthalmol
Vol 43: 279–284, 1999

Materials and Methods

Animals

Seventy-eight Japanese mongrel pigmented male rabbits weighing 2.0–3.0 kg were used. The animals were housed under 12-hour dark and 12-hour light conditions, and were fed food and water ad libitum during the experiment. The experiment was performed following the strict recommendations of the Animal Rights and the Association for Research in Vision and Ophthalmology. For PGE_2 administration, only 1 eye of each animal was used for one experiment. After LPS injection, both eyes of each animal were measured to obtain a mean value of aqueous flare for one experiment.

Chemicals and Preparation of Herbal Medicines

PGE_2 (Funakoshi Chemicals, Tokyo) was dissolved in 100% ethanol, and stored at −70°C. PGE_2 solutions were diluted to 5% ethanol with 0.9% NaCl aqueous solution just before use. LPS from *Escherichia coli*, serotype 055:B5 (Sigma Chemical, St. Louis, MO, USA), was dissolved in 0.9% NaCl aqueous solution just before use. β-Methasone phosphate was purchased from Shionogi Pharmaceutical Company (Osaka).

Kakkon-to (Ge-Gen-Tang in Chinese) extract powders and Sairei-to (Cai-Ling-Tang in Chinese) extract powders were gifts from Tsumura & Company, Ltd. (Tokyo). Kakkon-to is a mixture of the extracts of seven medicinal herbs (Table 1). The herbs were boiled in water and the aqueous extract was lyophilized to obtain the powder. Sairei-to is a mixture of the extracts of 12 medicinal herbs (Table 2), which were prepared in powder form as described above. Food containing either Kakkon-to powder (1% w/w) or Sairei-to powder (2% w/w) was prepared by Sankyo Lab Service Corporation (Tokyo) for rabbit consumption.

Table 1. Seven Medicinal Herbs in Kakkon-to

Medicinal Herbs	Source of Medicinal Plants
Puerariae radix	*Pueraria lobata* Ohwi
Zizyphi fructus	*Zizyphus jujuba* Miller *var. inermis* Rehder
Ephedrae herba	*Ephedra sinica* Stapf
Glycyrrhizae radix	*Glycyrrhiza uralensis* Fischer *Glycyrrhiza glabra* L.
Cinnamomi cortex	*Cinnamomum cassia* Blume
Paeoniae radix	*Paeonia Lactiflora* Pallas
Zingiberis rhizoma	*Zingiber officinale* Roscoe

Pretreatment of Animals

The animals in the two herbal medicine groups were fed Kakkon-to– or Sairei-to–treated food (140–160 g/day, mean = 150 g/day) for 5 days. The weight of food consumed was measured every day. After 5 days of the herbal medicine diet, experimental uveitis was induced in the two groups of herbal medicine rabbits. Four hours before the induction of experimental uveitis, β-methasone (130 μg/kg) was injected into an ear vein in a third group of rabbits with no other form of pretreatment. Animals without herbal medicine or β-methasone pretreatment formed a fourth group, as controls.

PGE_2 Induction of Uveitis

Transcorneal diffusion of PGE_2 was carried out, as described previously.[4,5] After topical anesthesia was

Table 2. Twelve Medicinal Herbs in Sairei-to

Medicinal Herbs	Source of Medicinal Plants
Bupleuri radix	*Bupleurum falcatum* L.
Alismatis rhizoma	*Alisma orientale* Juzepczuk
Pinelliae tuber	*Pinellia ternata* Breitenbach
Scutellariae radix	*Scutellaria baicalensis* Georgi
Atractylodis lanceae rhizoma	*Atractylodes lancea* D.C. *Atractylodes chinensis* Koidzumi
Zizyphi fructus	*Zizyphus jujuba* Miller *var.inermis* Rehder
Polyprous	*Polyporus umbellatus* Fries
Ginseng radix	*Panax ginseng* C.A. Meyer
Hoelen	*Poria cocos* Wolf
Glycyrrhizae radix	*Glycyrrhiza uralensis* Fischer *Glycyrrhiza glabra* L.
Cinnamomi cortex	*Cinnamomum cassia* Blume
Zingiberis rhizoma	*Zingiber officinale* Roscoe

applied, a glass cylinder (11 mm in diameter) was placed upon the rabbit cornea. Then 600 μL of PGE_2 (10 μg/mL) solution was put into the cylinder and applied to the cornea. After 4 minutes, the solution was pipetted out and the cylinder was removed. The corneal surface and conjunctival sac were rinsed with 20 mL of 0.9% saline.

LPS Induction of Uveitis and Dose-Dependency Study

Uveitis was induced in the unanesthetized experimental animals by injection of LPS (0.5 μg/kg) into an ear vein. To determine dose-dependence, an injection of LPS (0.1, 0.25, 0.5, or 1.0 μg/kg) was administered into an ear vein of unanesthetized rabbits without herbal medicine or β-methasone pretreatment.

Aqueous Flare Measurement

Aqueous flare (photon counts/microsecond) was measured in the animals using a flare-cell meter (FC-1000; Kowa, Tokyo), according to the method of Sawa et al.[8] Five measurements were performed at each time point and the mean value was calculated. Aqueous flare intensity was expressed as the area under the curve (AUC) in arbitrary units.

Statistical Analysis

The results were expressed as mean value ± standard deviation. Statistical analysis was performed using Dunn's procedure for multiple comparisons of mean values. $P < .05$ was considered statistically significant.

Results

The animals consumed 140–160 g/day of the food with or without herbal medicine. There was no difference in the volume of the food and water that was taken by the pretreated animals and the controls. The body weights were similar in the animals pretreated with Kakkon-to or Sairei-to, and the controls.

Changes in aqueous flare after topical application of PGE_2 to the cornea are shown in Figure 1. After the topical application of PGE_2, aqueous flare increased to a peak at 90 minutes, then gradually decreased and returned to baseline levels after approximately 8 hours. The mean AUC of PGE_2-induced aqueous flare elevations was 1,119 arbitrary units.

The AUC of PGE_2-induced aqueous flare elevation in the pretreatment groups is shown in Figure 2. The mean AUC of the eyes pretreated with 1% Kakkon-to was 1,055; with 2% Sairei-to, the AUC was 965. These values were not significantly different from the control values.

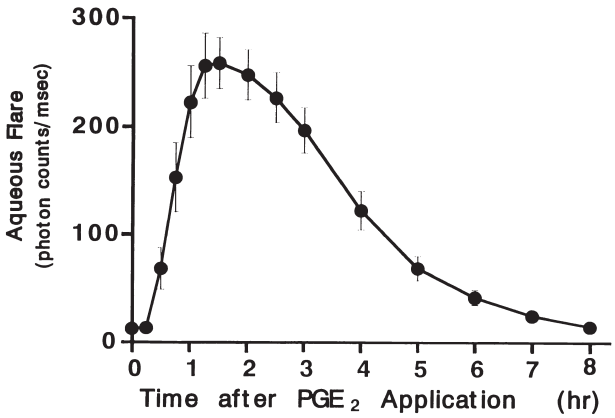

Figure 1. Changes in aqueous flare after topical application of PGE_2. PGE_2 solution (10 μg/mL) was applied to cornea using glass cylinder. Mean ± standard deviations are plotted (n = 9).

The mean AUC value for the eyes pretreated with β-methasone was 271, which was significantly lower ($P < .01$) than the value in control eyes.

Changes in aqueous flare after LPS injection are shown in Figure 3. After intravenous injection of LPS (0.1–1.0 μg/kg), the aqueous flare increased to a

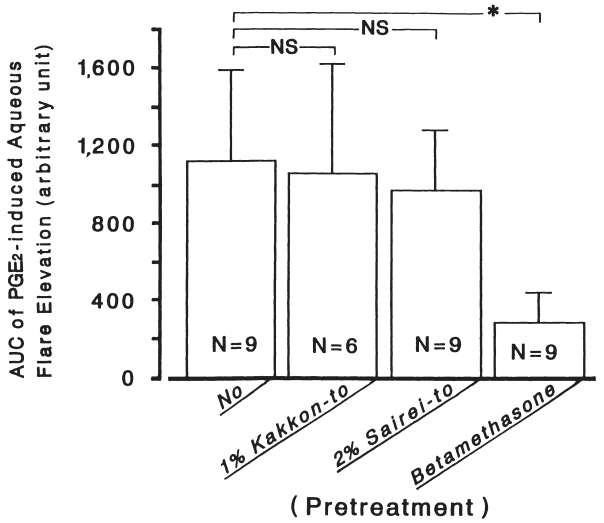

Figure 2. Area under the curve (AUC) of PGE_2-induced aqueous flare elevation and pretreatment. Rabbits were pretreated with about 150 g/day of food containing Kakkon-to (1% w/w) or Sairei-to (2% w/w) for 5 days, or intravenous injections of β-methasone (130 μg/kg) 4 hours before induction of experimental uveitis. Animals without any pretreatment served as controls. Mean ± standard deviations are plotted. *$P < .05$, NS: not significant.

Jpn J Ophthalmol
Vol 43: 279–284, 1999

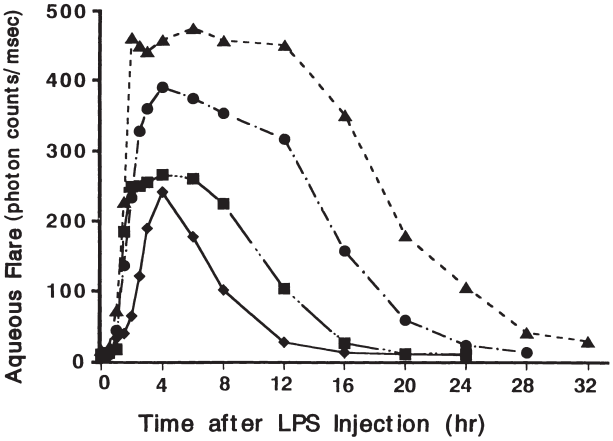

Figure 3. Changes in aqueous flare after intravenous injection into ear vein of lipopolysaccharides (LPS). LPS (◆: 0.1 μg/kg, ■: 0.25 μg/kg; ●: 0.5 μg/kg; ▲: 1.0 μg/kg.

peak between 3–12 hours, then gradually decreased and returned to baseline levels 16–32 hours later. The mean AUC of the 0.5 μg/kg LPS-induced aqueous flares was 4,950. The increase in the AUC caused by LPS was dose-dependent, as shown in Figure 4.

The AUC of 0.5 μg/kg LPS-induced aqueous flare in the pretreated animals is shown in Figure 5. The mean AUC of the eyes pretreated with 1% Kakkon-to was 4,495, which was not significantly different from the aqueous flare in eyes without the pretreatment. The mean AUC of the eyes pretreated with 0.6% Sairei-to was 2,478; with 2% Sairei-to, the

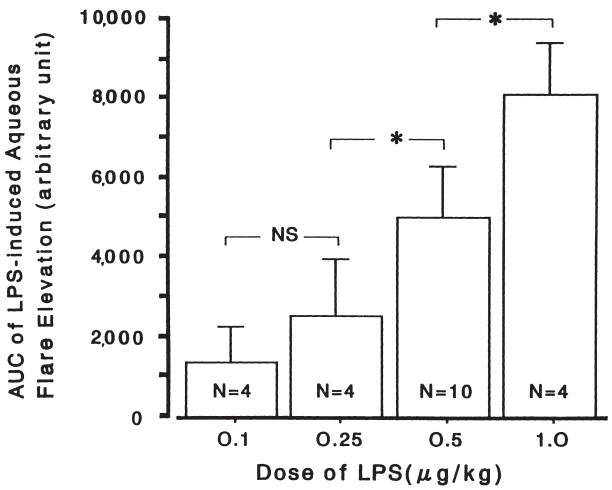

Figure 4. Area under the curve (AUC) of lipopolysaccharide (LPS)-induced aqueous flare elevation. Various concentrations (0.1–1.0 μg/kg) of LPS were injected. Mean ± standard deviations are plotted. $*P < .05$.

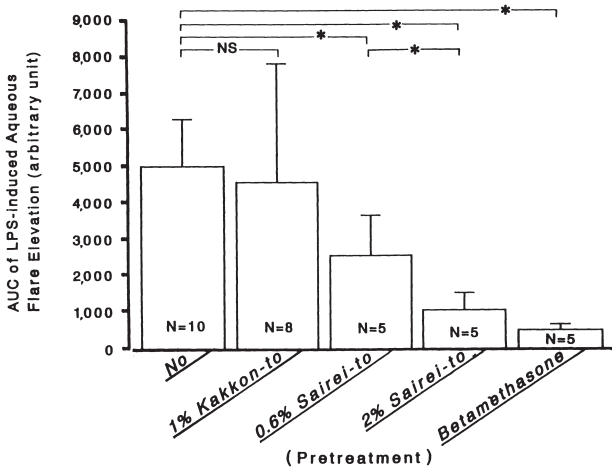

Figure 5. Area under the curve (AUC) of lipopolysaccharide (LPS)-induced aqueous flare elevation and pretreatment. LPS (0.5 μg/kg) was injected. Rabbits were pretreated with about 150 g/day of food containing Kakkon-to (1% w/w) or Sairei-to (0.6% or 2% w/w) for 5 days, with or without intravenous injection of β-methasone (130 μg/kg) 4 hours before experimental uveitis. Animals without any pretreatment served as controls.

AUC was 977. Both values were significantly lower than the control AUC. This inhibition by Sairei-to of the aqueous flare elevation induced by LPS was dose-dependent.

With β-methasone pretreatment, the AUC was 443, which was also significantly lower than in eyes without pretreatment.

Discussion

Many kinds of traditional herbal medicines have been safely used for the treatment of various diseases in China and Japan. In some medicines, the methods of preparation, dosage, and indication have been established, and the incidence of adverse effects is known to be relatively low. Several active components, such as ephedrine, glycyrrhizin, and gingerol, have been extracted from the herbal medicines.[9] Kimura[9] has suggested that the blended effect of crude drugs is characteristic of traditional herbal medicines; however, the extract efficacy of Kampo medicines on uveitis has not been confirmed by animal experiments, and no double-masked clinical tests have been performed in the ophthalmology field.

There have been reports of Kakkon-to being used in mice for treating Arthus reaction and suppressing anti-sheep red blood cell antibody formation.[10] There are also reports of its use in mice as an additive treatment for herpes simplex virus type 1 and

for suppressing interleukin-1α production.[11–14] Sairei-to has been reported effective in mice in stimulating a wide spectrum of intracellular phenomena,[15–18] and has also been reported effective in the clinical treatment of uveitis.[1–3] Because Kakkon-to and Sairei-to have demonstrated antiinflammatory activity, and because these two herbal medicines have been used frequently in Japan, we selected Kakkon-to and Sairei-to from the many kinds of traditional herbal medicines for use in the present study.

In our unpublished preliminary studies, rabbits drank varying volumes of hot-water extracts of Kakkon-to and Sairei-to. Therefore, lyophilized powders of the extracts were mixed into the food in the present study. The experimental animals consumed almost as much of the food containing Kakkon-to or Sairei-to as controls ate of normal food.

In our unpublished preliminary studies, 150 g/day of the food containing 0.1% (w/w) Kakkon-to or 0.2% (w/w) Sairei-to was given to the rabbits. However, no inhibitory effects were found on experimental flare elevation. In the present study, rabbits ate 150 g/day of the food containing 1% (w/w) Kakkon-to or 0.6% or 2% (w/w) Sairei-to, which were calculated to correspond roughly to 0.6, 0.36, or 1.2 g/day · kg, respectively, of the two herbal medicines. Typical doses of Kakkon-to and Sairei-to for human use are 3.75 and 6.0 g/day · 50 kg, 0.075 and 0.12 g/day · kg, respectively. Thus, the doses of Kakkon-to (1% w/w) and Sairei-to (2% w/w) in the present study may be about 10-fold higher than the ordinary human dosages. Shiga et al.[10] and Ozaki[11] used 10- to 30-fold higher than the ordinary clinical dosage of herbal medicine in their animal experiments. The oral LD_{50} of Sairei-to in the rat was estimated to be more than 8 g/kg.[19] In the present study, therefore, 1% Kakkon-to and 0.6% or 2% Sairei-to were used.

β-Methasone (130 μg/kg), intravenously injected, was less than the median lethal dose (LD_{50}, 1,300 mg/kg) for intravenous β-methasone in the rabbit, and is almost the same as the clinical dose.

β-Methasone inhibited aqueous flare elevation induced by both PGE_2 and LPS, whereas Sairei-to suppressed only the aqueous flare elevation induced by LPS, not by PGE_2. Kakkon-to did not suppress the elevation induced by either PGE_2 or LPS. It is possible that the mechanisms of elevated aqueous flare induced by PGE_2 and LPS may be different.

Planck et al,[20] Yoshida et al,[21] and Vos et al[22] reported that gene expression of interleukin-1α, interleukin-1β, and tumor necrosis factor-α in rat ocular tissues was induced by systemic LPS injection. Yamashiki et al[15] showed that Sairei-to induced the interleukin-1 receptor antagonist dose-dependently in cultures of peripheral blood mononuclear cells collected from healthy persons. Although cytokine levels were not determined in the present study, it is possible that the LPS-induced aqueous flare elevation may be mediated by interleukin-1, and that Sairei-to may stimulate the interleukin-1 receptor antagonist, resulting in inhibition of the flare elevation in the pigmented rabbits.

We believed that Sairei-to may have an inhibitory effect on some forms of experimental uveitis.

The authors would like to thank Prof. Katsutoshi Terasawa, Department of Japanese Oriental Medicine at Toyama Medical and Pharmaceutical University, for his advice, and Tsumura & Company, Ltd, for giving us Kakkon-to and Sairei-to. This study is supported in part by a grant from the Ministry of Education, Science, Sports and Culture (No. 08672011), Japan.

References

1. Ogasawara K, Takeda M. Effect of Saireito, a Chinese medicine, on uveitis. Atarashii Ganka (J Eye) 1989;6:439–43.
2. Miki S, Fujita Y, Tanouchi Y, et al. Clinical studies of Japanese kampo medicine "Sairei-to" on uveitis. Atarashii Ganka (J Eye) 1990;7:439–43, 601–4.
3. Kato Y, Harada W, Zako M, et al. Effects on herbal medicine Sairei-to on sarcoid uveitis. Ganka Kiyo Nihou Ganka Kiyo (Folia Ophthalmol Jpn) 1995;46:732–4.
4. Kaji Y, Hiraki S, Hirata H. The effect of calcium antagonists on prostaglandin E_2 reaction in rabbit eyes. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1994;98:825–31.
5. Hirata H, Hiraki S, Kaji Y, et al. The effect of transcorneal administration of prostaglandin E_2 on rabbit eyes. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1994;98:927–34.
6. Parks DJ, Cheung MK, Chan CC, et al. The role of nitric oxide uveitis. Arch Ophthalmol 1994;112:544–6.
7. Rosenbaum JT, Boney RS. Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis. Arch Ophthalmol 1992;110:547–9.
8. Sawa M, Tsurimaki Y, Tsuru T, et al. New quantitative method to determine protein concentration and cell number in aqueous in vivo. Jpn J Ophthalmol 1988;32:132–42.
9. Kimura M. The molecular patho-pharmacological studies for novel drug design by a principle of drug action in Japanese traditional Sino-medicine system. Yakugaku Zasshi 1997;117:133–54.
10. Shiga T, Komatsu Y, Hosoya E. Effects of Kakkon-to on the active Arthus and the delayed type hypersensitivity in mice. Wakan Iyakugaku Kaishi (J Med Pharm Soc Wakan-Yaku) 1988;5:41–5.
11. Ozaki Y. Studies on anti-inflammatory effect of Japanese Oriental medicines (Kampo medicines) used to treat inflammatory diseases. Biol Pharm Bull 1995;18:559–62.
12. Nagasaka K, Kurokawa M, Imakita M, et al. Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. J Med Virol 1995;46:28–34.
13. Kurokawa M, Imakita M, Kumeda CA, et al. Kakkon-to suppressed interleukin-1α production responsive to interferon and alleviated influenza infection in mice. J Trad Med 1996;13:201–9.

Jpn J Ophthalmol
Vol 43: 279–284, 1999

14. Kurokawa M, Nakano M, Ohyama H, et al. Prophylactic efficacy of traditional herbal medicine against recurrent herpes simplex virus type 1 infection from latently infected ganglia in mice. J Dermatol Sci 1997;14:76–84.

15. Yamashiki M, Nishimura A, Kimura M, et al. Effects of herbal medicine "Sairei-to" (TJ-114) on in vitro interleukin-1 receptor antagonist production in peripheral blood mononuclear cells. Curr Ther Res 1993;54:232–40.

16. Nakano Y, Suda T, Tozawa F, et al. Sairei-to (a Chinese herbal drug) stimulated secretion and synthesis of pituitary ACTH are mediated hypothalamic corticotropin-releasing factor. Neurosci Lett 1993;160:93–5.

17. Iwai I, Suda T, Yozawa F, et al. Stimulatory effects of Saireito on proopiomelanocortin gene expression in the rat anterior pituitary gland. Neurosci Lett 1993;157:37–40.

18. Takenaka T, Okitzu-Negishi S, Hashira S, et al. The dichotomy effect of Saireito on the cytokine production from peritoneal macrophages of mice. Ensho (Jpn J Inflamm) 1994;14:371–77.

19. Kanitani M, Maemura S, Yamada S, et al. A single oral dose toxicity study and a 13-week repeated dose study with a 4-week recovery period of Tsumura Sairei-to (TJ-114) in rats. Yakuri to Chiryou (Jpn Pharmacol Ther) 1995;23:S-2057–73.

20. Planck SR, Huang XN, Robertson JE, et al. Cytokine mRNA levels in rat ocular tissues after systemic endotoxin treatment. Invest Ophthalmol Vis Sci 1994;35:924–30.

21. Yoshida M, Yoshimura N, Hangai M, et al. Interleukin-1α, interleukin-1β, and tumor necrosis factor gene expression in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 1994; 35: 1107–13.

22. Vos AF, Klaren VN, Kijlstre A. Expression of multiple cytokines and IL-1RA in the uvea and retina during endotoxin-induced uveitis in the rat. Invest Ophthalmol Vis Sci 1994;35: 3873–83.