Cardiac Effects of Clinically Available Kampo Medicine Assessed With Canine Isolated, Blood-Perfused Heart Preparations

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ABSTRACT—Cardiac effects of 10 kinds of clinically available Kampo medicines were investigated: Kakkon-to (TJ-1), Dai-saiko-to (TJ-8), Boi-ogi-to (TJ-20), Chorei-to (TJ-40), Rokumi-gan (TJ-87), Tsudo-san (TJ-105), Gosha-jinki-gan (TJ-107), San’o-shashin-to (TJ-113), Sairei-to (TJ-114) and Inchin-gorei-san (TJ-117). Chronotropic and inotropic effects were studied using canine isolated, blood-perfused heart preparations, while subcellular mechanisms were analyzed by measuring the drug-induced changes of the adenylate cyclase activity in the canine ventricular membrane preparation. Intracoronary injections of TJ-1, TJ-20, TJ-105 and TJ-113 increased the sinoatrial rate and developed tension of papillary muscle in a dose-related manner, which was significantly attenuated by the pretreatment of the preparations with β-blocker propranolol. Meanwhile, the other extracts hardly affected these parameters. TJ-1, TJ-20 and TJ-113 increased the adenylate cyclase activity in a dose-related manner, but their potency was significantly less compared with that by an equivalent concentration of isoproterenol. Moreover, TJ-105 did not increase the adenylate cyclase activity. These results suggest that the positive chronotropic and inotropic effects of TJ-1, TJ-20, TJ-105 and TJ-113 may be exerted through the direct stimulation of the β-adrenoceptor and/or the norepinephrine release from the postganglionic nerve terminals in the heart.

Keywords: Kampo, Alternative medicine, Heart, Ephedrine, Adenylate cyclase

Kampo drugs are blended herbal medicines made from numerous crude components of natural origin. Appropriate doses are prescribed for the specific conditions of each patient according to a unique empiric system peculiar to Kampo diagnosis. Recently, Kampo as an alternative medicine has become more popular worldwide (1, 2). Indeed, in the United States as well as in Japan, Kampo is widely used as a complementary therapy in Western medicine (1, 2). Moreover in Japan, physicians can use Kampo drugs under the medical insurance system. However, pharmacological evidence for their ameliorative and adverse effects is still limited (3).

To begin to explore their mechanisms of action, we first randomly selected 10 kinds of clinically available Kampo medicines in their originally prescribed forms (Table 1) and assessed whether they may exert any cardiac effects using canine isolated, blood-perfused heart preparations (4, 5). This model has been known to be suitable for the precise evaluation of drug effects on sinoatrial automaticity and ventricular contraction. As some Kampo extracts exerted cardiotimulatory action in the isolated heart preparation, we next assessed the effects of Kampo medicines on the adenylate cyclase activity to estimate the drug-induced subcellular responses using a highly sensitive enzymatic fluorometric assay technique (6, 7).

MATERIALS AND METHODS

All experimentation was performed in accordance with the rules and regulations of the Committee for Research at Yamanashi Medical University, which are equivalent to those of The Japanese Pharmacological Society. Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University.

Drugs

The Kampo extracts (Kakkon-to (TJ-1), Dai-saiko-to (TJ-8), Boi-ogi-to (TJ-20), Chorei-to (TJ-40), Rokumi-gan (TJ-87), Tsudo-san (TJ-105), Gosha-jinki-gan (TJ-107), San’o-shashin-to (TJ-113), Sairei-to (TJ-114) and Inchin-gorei-san (TJ-117)) were generously provided by

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Tsumura Co., Ltd. (Tokyo) as freeze-dried powder made of boiled water-extracts of natural products. One gram of each powder was dissolved or suspended in 40 ml of distilled water, mixed for 2 h at room temperature, and centrifuged for 5 min at 10,000 g. The top clear part of the fluid was passed through a filter with a pore size of 0.22 μm to get the desired solution of 25 mg/ml. The following drugs were purchased: pentobarbital sodium (Tokyo-Kasei, Tokyo) and heparin calcium (Mitsui, Tokyo). All other enzymes and substrates were bought from Sigma Chemical Company (St. Louis, MO, USA).

Table 1. Names of Kampo extracts and their clinical application used in this study

| Japanese                  | Chinese                  | Typical clinical application*                  |
|---------------------------|--------------------------|-----------------------------------------------|
| Kakkon-to                 | Ge-Gen-Tang              | Cold                                           |
| Dai-salko-to              | Da-Chai-Hu-Tang          | Cholelithiasis, Hypertension                   |
| Boi-ogi-to                | Fang-Yi-Huang-Qi-Tang    | Nephritis, Edema                               |
| Chorei-to                 | Zhu-Ling-Tang            | Hematuria, Diarrhea                            |
| Rokumi-gan                | Liu-Wei-Wan              | Pollakisuria, Dysuria                          |
| Tsu-do-san                | Tong-Dao-San             | Constipation, Lumbago                          |
| Gosha-jinki-gan           | Niu-Che-Shen-Qi-Wan      | Lumbago, Dysuria                               |
| San’o-shashin-to          | San-Huang-Xie-Xin-Tang    | Nasal bleeding, Constipation                   |
| Saiei-to                  | Chai-Ling-Tang           | Acute gastroenteritis, Edema                   |
| Inchin-gorei-san          | Yin-Chen-Wu-Ling-San     | Nausea, Vomiting                               |

*Cited from drug information attached to the commercially available Tsumura Kampo Medicines.

Experiment 1: Effects on the sinoatrial automaticity and ventricular contraction

Experiments were carried out using the canine isolated sinoatrial node and papillary muscle preparations cross-circulated with heparinized arterial blood of the donor dog (4, 5).

Isolated heart preparations: The preparation was obtained from a beagle dog (CSK Research Park, Nagano) of either sex, weighing approximately 10 kg. The dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and supplemented with 4 – 5 mg/kg per hour. After intubation, dogs were artificially ventilated with room air (SN-480-3; Shinano, Tokyo). The systemic blood pressure and surface lead II ECG were monitored using a polygraph system (RM-6000; Nihon Kohden, Tokyo). At the start of cross-circulation, heparin calcium (500 U/kg, i.v.) was given followed by an additional dose of 200 U/kg per hour.

Cross-circulation: The preparations were placed in a double-wall glass jacket maintained at 38°C by circulating warm water and were perfused with arterial blood from the carotid artery of the blood-donor dog. Perfusion pressure was kept at 120 mmHg with a peristaltic pump (7553-00; Cole-Parmer, Chicago, IL, USA) and Starling’s pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the blood-donor dog.

Parameters: The spontaneously beating rate of the sinoatrial node preparation (i.e., sinoatrial rate) was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by the atrial electrogram. The papillary muscle preparation was electrically driven through the stimulating electrodes at a cycle length of 500 ms using a stimulator (SEN-7203, Nihon Kohden) and an isolation unit (SS-201J, Nihon Kohden). The stimulation pulses were rectangular in shape, 1 – 2 V amplitude (about 20% above the threshold voltage), and of 5-ms duration. Developed tension of the papillary muscle under a resting tension of 2 g was measured isometrically using a force displacement transducer (DRM-200S; Dia Medical, Tokyo) and an amplifier (DRM-T20, Dia Medical). The coronary blood flow through the nutrient arteries of each preparation was continuously monitored with an electromagnetic flowmeter (MFV-3200, Nihon Kohden).
Experimental protocol: Once the preparations were stabilized, Kampo extracts in doses of 0.1–3.0 mg or vehicle solution (distilled water) were injected into each nutrient artery using a small microsyringe in volumes of 4–120 μl over 4 s. Physiological recordings were performed for 10 min after each dose. Because a relatively small amount of a drug was administered to the preparations compared to those needed in a whole animal model, multiple drug doses were studied in the same preparation. The effluent blood through each preparation immediately after the drug injection was discarded to eliminate the drug effects on the donor dog. Having assessed the effects of the drugs and vehicle on each parameter, the effects on the donor dog. Having assessed the effects of the drugs and vehicle on each parameter, the β-adrenoceptor antagonist propranolol in a dose of 10 μg was administered to each preparation. Then, TJ-1 (3 mg), TJ-20 (0.3 mg), TJ-105 (3 mg) or TJ-113 (3 mg) was administered, and the effects on each parameter were compared with those before the propranolol treatment. The β-adrenoceptor agonist isoproterenol (1 ng) was used as a reference drug.

Experiment 2: Effects on the adenylate cyclase activity
Production of the plasma membrane preparation: When each protocol in Experiment 1 was finished, the heart of the donor dog was excised and immediately placed in ice-cold SET buffer (0.25 mol/l sucrose, 0.1 mmol/l EDTA, 5.0 mmol/l Tris-acetate; pH 7.4). The apex region of the left ventricle, weighing 2–3 g, was trimmed. The sample was homogenized in 5 vol of SET buffer. The homogenate was filtered (Nitex filter, Tetko, CA, USA) and centrifuged at 10,000 × g for 5 min at 4°C. The pellet was resuspended in SET buffer and the mixture was centrifuged three more times. Protein analysis was performed using a commercially available protein assay reagent (Pierce, Rockford, IL, USA). The membrane suspension was diluted with SET buffer to a concentration of 3–5 mg protein/ml, and it was stored at −80°C until enzyme activity was measured.

Enzymatic fluorometric assay of adenylate cyclase activity: The adenylate cyclase activity of the membrane preparation was measured with an enzymatic fluorometric assay technique (6). Fifty microliters of reaction mix (100 mmol/l Tris-acetate, pH 7.4; 20 mmol/l KCl; 10 mmol/l MgCl2; 20 mmol/l phosphoenolpyruvate; 2 mmol/l ATP; 20 μmol/l GTP; 2 mmol/l dithiothreitol; 0.4 mg/l bovine serum albumin; 100 μmol/l 3-isobutyl-1-methylxanthine (IBMX); 100 μg/ml pyruvate kinase) was added to each microcentrifugation tube in duplicate with or without either 0.2 to 2 mg/ml of TJ-1, TJ-20, TJ-105, TJ-113 or 2 × 10−6 mol/l of isoproterenol. These drug concentrations could reflect the doses of experiment 1, since the coronary blood flow through the preparations were about 2–4 ml/min and peak cardiac effects of the extracts were observed within 1 min. Next, the membrane suspension in a volume of 50 μl was added to each tube. The reaction mixture and membrane suspension, both before and after being mixed, was maintained at 4°C to ensure the same starting time for all assay tubes. The reaction was initiated by placing the tubes in a water bath maintained at 37°C. After 30 min, the reaction was terminated by heating at 95°C for 5 min. The mixture was vortexed 3 times and centrifuged at 10,000 × g for 5 min. A 5-μl aliquot of the supernatant was transferred to a 10 × 75-mm disposable assay tube (Iwaki Lab Ware, Tokyo) in triplicate. For the cyclic AMP standard, 5 μl of a known amount of cyclic AMP was added to the tubes. The cyclic AMP concentration was assayed using the enzymatic fluorometric method as previously described (7).

Statistics
The data are presented as the mean ± S.E.M. The statistical comparisons of mean values in Experiment 1 were carried out by the paired t-test, while those in Experiment 2 were performed with one-way repeated-measures analysis of variance (ANOVA) followed by Contrast. A P-value of less than 0.05 was considered significant.

RESULTS

Experiment 1: Effects on the sinoatrial automaticity and ventricular contraction
One hour after the start of cross-circulation, the sinoatrial preparation showed spontaneous regular automaticity of 86 ± 2 beats/min and coronary blood flow of 2.0 ± 0.2 ml/min (n = 4). Meanwhile, the papillary muscle preparation showed a developed tension of 3.5 ± 0.3 g and coronary blood flow of 4.1 ± 0.3 ml/min (n = 4). Administration of TJ-1, TJ-20, TJ-105 and TJ-113 increased the sinoatrial rate and the developed tension of papillary muscle in a dose-related manner, whereas the other extracts as well as the vehicle distilled water hardly affected these two variables. The results are summarized in Fig. 1. Typical tracings of the positive chronotropic and inotropic effects of TJ-1 (3 mg), TJ-20 (0.3 mg), TJ-105 (3 mg), TJ-113 (3 mg) and isoproterenol (1 ng) are shown in Fig. 2. The onset speed of these cardiotimulatory effects was relatively faster for TJ-20, TJ-105 and isoproterenol, while it was slow for TJ-1 and TJ-113.

Since the β-blocking action of intracorony injection of propranolol (10 μg) has been known to persist for more than 30 min (8), each extract exerting significant positive chronotropic and inotropic effects was administered within 15 min after the treatment. The positive chronotropic and inotropic effects induced by TJ-1, TJ-20, TJ-105, TJ-113 and isoproterenol were significantly attenuated by the pretreatment of the preparations with propranolol. Typical tracings of the effects of propranolol on TJ-1 (3 mg), TJ-20 (0.3 mg), TJ-105 (3 mg), TJ-113 (3 mg) and isoproterenol
(1 ng)-induced responses are depicted in Fig. 2, and the results are summarized in Fig. 3.

**Experiment 2: Effects on the adenylate cyclase activity**

The effects of TJ-1, TJ-20, TJ-105, TJ-113 and isoproterenol on the adenylate cyclase activity in the ventricular membrane preparation are summarized in Fig. 4. Basal and isoproterenol-stimulated adenylate cyclase activities (cyclic AMP production, pmol·min⁻¹·mg protein⁻¹, n = 4) were 16.3 ± 1.0 and 27.4 ± 2.8, respectively. TJ-1, TJ-20, and TJ-113 (0.1 to 1 mg/ml) increased the adenylate cyclase activity in a dose-related manner (n = 4), but each increment by these Kampo extracts was significantly less compared with that by the equivalent concentration of isoproterenol. On the other hand, TJ-105 tended to increase the adenylate cyclase activity; however, this change did not reach statistical significance (n = 4).
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Fig. 2. Typical tracings showing the blocking effects of propranolol (10 μg) on the isoproterenol (Iso, 1 ng)-, TJ-20 (0.3 mg)-, TJ-105 (3 mg)-, TJ-113 (3 mg)- and TJ-1 (3 mg)-induced responses. Typical tracings of the sinoatrial rate (SAR) (A) and the developed tension (DT) of papillary muscle (B). The positive chronotropic and inotropic effects of the drugs were effectively attenuated by pre-treatment with propranolol.

Fig. 3. Summary of the blocking effects of propranolol (10 μg) on TJ-1 (3 mg)-, TJ-20 (0.3 mg)-, TJ-105 (3 mg)-, TJ-113 (3 mg)- and isoproterenol (Iso, 1 ng)-induced responses. Increases (%) in the sinoatrial rate (SAR) (A) and the developed tension (DT) of papillary muscle (B) were effectively attenuated by the pre-treatment of the preparation with propranolol. *P<0.05, significantly different from each pre-propranolol responses. Open rectangles: changes by the Kampo extracts before the propranolol treatment. Closed rectangles: changes by the Kampo extracts after the propranolol treatment.
Different from the basal activity. This may be exerted through the chronotropic and inotropic effects of TJ-1, TJ-20, TJ-105 and TJ-113 compared with propranolol. These results indicate that the positive chronotropic effects were significantly attenuated by the pre-treatment with these Kampo medicines.

DISCUSSION

Given the limited information regarding the cardiac effects of Kampo medicine, which is one of the major alternative therapies to Western medicine (1, 2), we first assessed the chronotropic and inotropic effects of 10 kinds of clinically available Kampo extracts using canine isolated, blood-perfused heart preparations. As clearly shown in the results, TJ-1, TJ-20, TJ-105 and TJ-113 out of the 10 Kampo medicines exerted positive chronotropic and inotropic effects, which were significantly attenuated by the pre-treatment of the preparations with the β-blocker propranolol. These results indicate that the positive chronotropic and inotropic effects of TJ-1, TJ-20, TJ-105 and TJ-113 may be exerted through the β-adrenoceptor-dependent pathway in the heart, which has not been reported elsewhere. Similar pharmacological profiles have been reported by us for Oren-gedoku-to (TJ-15), Moku-boi-to (TJ-36) and Ryo-kan-kyo-mi-shin-ge-nin-to (TJ-119) (9).

The following two potential mechanisms can be estimated from the results of Experiment 1. First, the effects induced by TJ-1, TJ-20, TJ-105 and TJ-113 can be due to the direct stimulatory action of each extract on the β-adrenoceptor in the isolated heart preparations. Second, the effects may be indirectly induced by the norepinephrine release from the postganglionic nerve terminals in the heart preparations (5), which could stimulate the β-adrenoceptors. To better clarify the potential roles of these mechanisms, we assessed the effects of TJ-1, TJ-20, TJ-105 and TJ-113 on the adenylate cyclase activity using canine ventricular membrane preparations that lack intact cardiac autonomic nerves. TJ-1, TJ-20 and TJ-113 increased the adenylate cyclase activity in a dose-related manner, but their potency was significantly less compared with that by an equivalent concentration of isoproterenol, suggesting that these extracts may exert cardiotimulatory actions via both direct and indirect mechanisms. Moreover, TJ-105 did not increase the adenylate cyclase activity, indicating that cardiac actions of TJ-105 may solely depend on the indirect mechanism.

Another important finding is the relation between the doses of Kampo extracts needed to cause the observed changes in this study and those required for exerting their clinical efficacy. In our previous experiments with the same type of canine models as used in this study, we examined several agents that induce chronotropic and inotropic effects (8-11). It can be roughly estimated from those studies that effects of intracoronary administration of 1 mg of a drug on these preparations will correspond to those of 100 mg/kg, p.o., in vivo. As the Kampo extracts are clinically prescribed in doses of 1.5 to 7.5 g/day, the doses used in the isolated heart preparations (0.1 to 3.0 mg) are considered to be close in dosage to those clinically used.

It should be also noted that each Kampo extract used in this study consists of several crude drugs. Among them, some components are known to possess modulatory effects on the cardiac function, which are possibly related to the β-adrenoceptor dependent pathway (12). For example, Astragali radix (Ougi) in TJ-20 and TJ-114, Zizyphi fructus (Taiso) in TJ-1, TJ-8, TJ-20 and TJ-114, and Rhei rhizoma (Daio) in TJ-8, TJ-105 and TJ-113 have been reported to increase serum cyclic AMP level via β-adrenoceptor stimulation after p.o. administration in some animal models such as mice (12, 13). In addition, Zingiberis rhizoma (Shoukyo) in TJ-1, TJ-8, TJ-20 and TJ-114 has been shown to exert positive chronotropic and inotropic effects in the isolated rat atrium (12, 14). It should be noted that the amount of these crude drugs are relatively less in TJ-8 and TJ-114 compared with those of TJ-1, TJ-20, TJ-105 and TJ-113. Coptidis rhizoma (Oren) in TJ-113 is known to inhibit isoproterenol- or chola toxin-induced activation of adenylate cyclase (12), whereas Ephedrae herba (Mao) in TJ-1 contains l-ephedrine, which acts primarily through the release of stored catecholamines and has some direct actions on adrenoceptors (15). This previous knowledge can at least in part explain the present results, but further study is required to fully understand which components of the crude drugs of Kampo medicine are essential for current observation. Moreover, the effects of the metabolites as well as hydrophobic components should be assessed using other experimental designs.

In summary, the present study showed that TJ-1, TJ-20, TJ-105 and TJ-113 possess positive chronotropic and

![Graph](Fig. 4. Effects of Kampo extracts (TJ-1, TJ-20, TJ-105 and TJ-113) and isoproterenol on the adenylate cyclase activity of the canine ventricular membrane preparations (n = 4). *P<0.05, significantly different from the basal activity.)
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inotropic actions, which may be exerted through the direct stimulation of the \( \beta \)-adrenoceptor and/or the norepinephrine release from the postganglionic nerve terminals in the heart. Although the clinical application of each Kampo extract may not be explained by the current results, TJ-1, TJ-20, TJ-105 and TJ-113 can potentiate the effects of cardiotonic agents, including phosphodiesterase III inhibitors, adenylate cyclase stimulators and \( \beta \)-adrenoceptor agonists. Thus, caution has to be paid when using such combination therapy in clinical practice.

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