Chronotropic and Inotropic Effects of Kampo Extracts in the Canine Isolated, Blood-Perfused Heart Preparations

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Abstract—Cardiac effects of drugs used for circulatory disorders in traditional Japanese medicine based on ancient Chinese medicine (Kampo Medicine): Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, Toki-shakuyaku-san, Shimbu-to, Moku-boi-to, Ryo-kei-jutsu-kan-to, Sha-kanzo-to, Keishi-ninjin-to, Toki-to and Ryo-kan-kyo-mi-shin-ge-nin-to were investigated using canine isolated, blood-perfused sinoatrial node and papillary muscle preparations. Single injections of small doses of Oren-gedoku-to, Moku-boi-to and Ryo-kan-kyo-mi-shin-ge-nin-to (0.1 to 3 mg) dose-dependently increased the sinoatrial rate and the developed tension of papillary muscle, while other drugs showed almost no effect on these parameters. All the drugs had almost no effect on the blood flow through the nutrient arteries of each preparation. The positive chronotropic and inotropic effects induced by Oren-gedoku-to, Moku-boi-to and Ryo-kan-kyo-mi-shin-ge-nin-to did not show tachyphylaxis and were not affected after pharmacological denervation by tetrodotoxin treatment or by reserpine pretreatment, but were significantly suppressed by atenolol. These results indicate that these three drugs act as beta-adrenoceptor agonists to produce clinically useful cardiac effects.

Combination therapy using modern drugs and traditional Japanese drugs which are based on ancient Chinese medicine (Kampo Medicine) has been reevaluated as a better drug treatment system, and many clinical trials have proved its effectiveness (1–5). One of the advantages of combination therapy is to increase the efficacy and also to reduce various kinds of unwanted side effects of modern drugs by additional use of Kampo Medicines (1, 4). Because there is little literature on this combination therapy of modern drugs and Kampo Medicines, it is therefore necessary to clarify the pharmacological profile and mechanism of action of Kampo Medicine to the extent known for drugs of modern medicine, especially with regards to the cardiovascular effects (6).

The purpose of the present study was to characterize the cardiac effects of Kampo prescriptions used for patients with circulatory disorders such as congestive heart failure and valvular heart disease. Though there are numerous crude components of natural origin, Kampo extracts have been used as mixtures based on several famous traditional Chinese books of Kampo Medicine. We therefore examined these Kampo Medicines in their originally prescribed forms to determine if they exert some noticeable cardiovascular effects in isolated cardiac preparations. We used the canine isolated, blood-perfused sinoatrial node preparation and papillary muscle preparation which allows for precise evaluation of drug effects on the sinoatrial rate (SAR) and the developed tension (DT) of the papillary muscle as well as effects on the coronary blood flow (CBF) (7–9).

Materials and Methods
Experiments were carried out using sinoatrial node preparations and papillary muscle preparations perfused with heparinized arterial blood of donor dogs (7–9). A schematic circulation diagram is shown in Fig. 1.

Blood-perfused heart preparations: The
isolated heart preparations were obtained from mongrel dogs of either sex, weighing approximately 10 kg. Dogs were anesthetized with pentobarbital sodium (Tokyo Kasei), 30 mg/kg, i.v., and then they were given heparin calcium (Mitsui Seiyaku), 500 U/kg, i.v. The heart was excised after exsanguination and plunged into cold Tyrode's solution kept at 4°C.

The sinoatrial node preparation consisted of the entire right atrium. The sinus node artery was cannulated through the right coronary artery. Bipolar recording electrodes were sutured onto the right atrium close to the sinoatrial node.

The papillary muscle preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery, the nutrient artery to the papillary muscle, was directly cannulated. Bipolar electrodes were sutured onto the His bundle region close to the...
tricuspid valves. The papillary muscle preparation was driven through the electrodes with rectangular electric pulses of 1–3 V (about 20% above the threshold voltage) and 5 msec duration at a frequency of 120 beats/min using a stimulator (Dia Medical System DHM-226-3) and an isolation unit (Dia Medical System DPS-110).

Reserpine pretreated preparations: Twenty-four hours before the experiment, reserpine (Daiichi Seiyaku) was injected subcutaneously at a dose of 5 mg/kg into a small mongrel dog. Using its heart, the preparations were made in the same way.

Donor dogs: Adult mongrel dogs of either sex, weighing 14–27 kg, were used as donor dogs which were anesthetized initially with sodium pentobarbital, 30 mg/kg, i.v., and then supplemented with 50 mg when necessary. At the start of cross circulation, heparin calcium, 500 U/kg, i.v., was given, and 200 U/kg, i.v., was supplemented every hour. Respiration was controlled with a dog respirator (Harvard 607), and the systemic blood pressure and heart rate were monitored continuously with a polygraph (NEC San-ei 361-6).

Cross circulation: Both preparations were placed in double-wall glass jackets maintained 38°C by circulating warm water and were perfused with arterial blood from the carotid artery of the donor dog. Perfusion pressure was kept at 100 mmHg with a peristaltic pump (Cole-Parmer 7553-00) and a Starling's pneumatic resistance placed parallel to the perfusion system. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and were returned to the jugular vein of the donor dog.

Parameters: SAR was measured with a cardiotachograph (NEC San-ei 1321) triggered by the atrial electrogram. DT of the papillary muscle, preloaded with a 2-g weight, was measured isometrically using a force displacement transducer (Dia Medical System DRM-100S). CBF through the anterior septal artery of the papillary muscle preparation was measured with an electromagnetic flowmeter (Nihon Kohden MVF-1100). These three parameters were recorded on a rectilinear recorder (NEC San-ei 8K231S).

Experimental protocol: After equilibration of 1 hr. 1 µg of acetylcholine (ACh) was injected into each nutrient artery of both preparations. When responses to ACh became steady, ten preparations of Kampo extracts (0.1, 0.3, 1, 3 mg), 0.9% saline (10, 30 µl) and the distilled water (4, 12, 40, 120 µl) were injected into each artery with a microsyringe (Ito) for a period of 4 sec. Maximal changes in SAR, DT and CBF after drug administration were expressed as a percent of their basal value before injection.

Drugs: The following ten kinds of Kampo extracts were prepared: Saiko-ka-ryukotsu-borei-to (TJ-12), Oren-gedoku-to (TJ-15), Toki-shakuyaku-san (TJ-23), Shibu-to (TJ-30), Moku-boi-to (TJ-36), Ryo-kei-jutsu-kan-to (TJ-39), Sha-kanzo-to (TJ-64), Keishi-ninjin-to (TJ-82), Toki-to (TJ-102) and Ryo-kan-kyo-mi-shin-ge-nin-to (TJ-119). One gram of each extract was dissolved or suspended in 40 ml of distilled water, mixed for 2 hr, and centrifuged for 5 min at 3000 r.p.m. The top clear part of the fluid was passed through a filter with a pore size of 0.45 µm to get the desired solution of 25 mg/ml. Tetrodotoxin (TTX) (Sigma), atenolol (Sigma), I-isoproterenol hydrochloride (ISP) (Nikken Kagaku) and tyramine (Sigma) were dissolved in 0.9% saline at concentrations of 100 µg/ml, 1 mg/ml, 100 ng/ml and 100 µg/ml, respectively.

Statistical analysis: The data are presented as means±S.E. The paired t-test was used, and a P value of 0.05 or less was considered significant.

Results

One hour after the start of the blood perfusion, the sinoatrial preparation showed spontaneous SAR of 94±5 beats/min (n=9), while the papillary muscle preparation showed DT of 5.4±0.9 g (n=7) and CBF of 5.3±1.4 ml/min (n=7), which persisted unchanged over 8 hr until the end of the experiments. Effects of drugs were reversible and disappeared within 2 to 5 min after a single shot of the maximum doses.

Effects of 10 kinds of Kampo extracts on SAR, DT and CBF: The dose-response relationships for the % increase of SAR and DT are shown in Figs. 2 and 3, respectively. TJ-
15, TJ-36 and TJ-119 dose-dependently increased SAR and DT, but the other drugs, 0.9% saline and the distilled water had almost no effects on SAR and DT. The positive chronotropic effects of the 3 drugs were almost equal to that of 1 ng of ISP, while the positive inotropic effects were almost equal to that of 0.3 ng of ISP (Fig. 5). The dose-response relationships for the % increase of CBF are shown in Fig. 4. All drugs increased CBF dose-dependently; however, these effects were not stronger than those produced by distilled water alone.

Effects of TTX and atenolol on Kampo extracts induced responses: Because the blocking effects of a single injection of TTX (1 μg) and atenolol (10 μg) persisted for 20–30 min (7–9), each extract was administered within 10 min after treatment. Effects of TTX on TJ-15 (1 mg), TJ-36 (0.3 mg) and TJ-119 (1 mg) induced positive chronotropic and inotropic effects are shown in Figs. 6 and 7. After treatment with TTX, the positive chronotropic and inotropic responses were not significantly modified and did not show any tachyphylaxis with repetitive injections of the 3 drugs. Effects of atenolol on TJ-15 (1 mg), TJ-36 (0.3 mg) and TJ-119 (1 mg) induced responses are shown in Figs. 5, 6 and 7, respectively. The chronotropic and inotropic
responses of TJ-15, TJ-36 and TJ-119 as well as ISP were significantly inhibited by pretreatment with atenolol (10 μg).

**Effects of Kampo extracts and tyramine in the reserpine pretreated preparations:** In the reserpine untreated preparations, tyramine showed potent positive chronotropic and inotropic effects. Typical traces are shown in Fig. 8A. On the other hand, in the reserpine pretreated preparations, the effects of tyramine were sufficiently eliminated, but chronotropic and inotropic responses to the Kampo extracts were observed (Fig. 8B), which were almost the same as those observed in the reserpine-untreated preparations (Fig. 5).

**Discussion**

In the present experiment, TJ-15, TJ-36 and TJ-119 out of ten Kampo extracts produced dose-dependent positive chronotropic and inotropic effects. There were no tachyphylaxes, and the effects were not modified

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**Fig. 4.** Dose-response curves for increase in coronary blood flow to Kampo extracts injected into the anterior septal artery.

**Fig. 5.** Actual traces of the blocking effects of atenolol (10 μg) on ISP, TJ-15, TJ-36 and TJ-119 induced positive chronotropism in the sinoatrial node (upper panel) and positive inotropism in the papillary muscle preparation (lower panel).
Injection of TTX (1 μg) into these preparations and administration of reserpine into the dog (5 mg/kg) for the preparations have been reported to block the neural release of catecholamines of the cardiac autonomic nerves, producing pharmacological cardiac denervation without affecting cardiac muscles (7, 8). Therefore, it is considered that the effects induced by injections of TJ-15, TJ-36 and TJ-119 are due to the direct action on the isolated heart preparations and not due to the norepinephrine release from the postganglionic nerve terminals. A single injection of 10 μg atenolol, which inhibited the response of ISP, completely blocked the positive chronotropic and inotropic response of TJ-15, TJ-36 and TJ-119. It is thus likely that the effects of TJ-15, TJ-36 and TJ-119 are mediated by the beta-adrenergic mechanism. These present results are consistent with a previous report that TJ-36 is a positive inotropic agent (6).

All of the Kampo extracts used in this experiment were extracted into distilled water. Injection of each extract solution increased CBF dose-dependently, but the effect of distilled water alone was much larger. Therefore, it is considered that the increase in CBF

Fig. 6. Blocking effects of TTX (1 μg) and atenolol (10 μg) on TJ-15, TJ-36 and TJ-119 induced positive chronotropism in the sinoatrial node preparation. **P<0.01, significantly different from each control value.

Fig. 7. Blocking effects of TTX (1 μg) and atenolol (10 μg) on TJ-15, TJ-36 and TJ-119 induced positive inotropism in the papillary muscle preparation. *P<0.05, **P<0.01, significantly different from each control value.
of Kampo extracts and showed that TJ-15, TJ-36 and TJ-119 are beta-adrenoceptor agonists and may be clinically useful through this mechanism. At the present time, however, it remains to be elucidated by what components these three Kampo extracts produce these positive chronotropic and inotropic effects. On the other hand, TJ-12, TJ-23, TJ-30, TJ-39, TJ-64, TJ-82 and TJ-102 showed almost no effects on any variable measured; however, the efficacy of metabolites and hydrophobic components cannot be denied from the results of this experiment.

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