Effects of 9 Kampo Medicines Clinically Used in Hypertension on Hemodynamic Changes Induced by Theophylline in Rats

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We examined the effects of 9 kinds of Kampo medicines, which are clinically used for the treatment of hypertension, on anesthetized rats with increases in arterial blood pressure, heart rate and peripheral blood flow induced by theophylline (5 mg/kg, i.v.) that were partially or completely mediated by endogenous catecholamines. Each Kampo medicine (1 g/kg) was intraduodenally administered. Shinbu-to caused a severe disturbance of the arterial blood pressure. Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, San'o-shashin-to and Dai-jyoki-to had hypotensive effects, while Hachimi-jio-gan, Gosha-jinki-gan, Dai-saiko-to and Choto-san did not have such an effect. Moreover, Saiko-ka-ryukotsu-borei-to attenuated the heart rate. In Oren-gedoku-to, San'o-shashin-to and Dai-jyoki-to, a reduction in peripheral blood flow was observed. These results suggest that Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, San'o-shashin-to and Dai-jyoki-to are ameliorative to the hypertension in sympathetic system dominance and Shinbu-to is occasionally dangerous to it.

Key words Kampo medicines; theophylline; hypotensive effect; blood pressure; heart rate; peripheral blood flow

Some Kampo medicines are clinically used to treat hypertension and the various simultaneous symptoms, including chronic headache, painful tension of the shoulder and cervical muscles, hot flush, insomnia, and palpitation. However, the pharmacological basis for the ameliorative effects of the Kampo medicines on circulation and cardiovascular systems is poorly understood. Kampo medicines are blended herbal medicines made from several crude medicines and the appropriate prescription is chosen according to the specific conditions of each patient in the system of Kampo. A new evaluation system for some efficacy of Kampo medicines as a whole, considering the pathophysiology concept, has been expected.

In the case of essential hypertension, whose cause is unknown, the sympathetic system is often hypertonic. Mild hypertension includes almost 3/4 of hypertensives, and most of these patients have increased heart rate, stroke volume, and cardiac output but show normal or only slightly elevated total peripheral resistance. These hemodynamic findings indicate a hyperkinetic circulation with increased adrenergic drive to the heart and peripheral vessels. We have previously indicated that a bolus injection of theophylline produced a rise in arterial blood pressure in an anesthetized rat, with increases in heart rate and peripheral blood flow in the rat ear, and the effects of theophylline were consecutive and markedly attenuated by the β -adrenergic blockade with propranolol or the treatment with reserpine.¹⁾ Vestal *et al.*²⁾ have found consecutive and increased catecholamine levels after aminophylline (theophylline ethylenediamine) infusion, and concluded that theophylline may act through release of norepinephrine from peripheral nerve terminals. Therefore, the theophylline-treated rat might be assumed as a model of the hypertension with the evidence of adrenergic stimulation.

In this study, we have investigated the effects of 9 kinds of Kampo medicines (Hachimi-jio-gan, Gosha-jinki-gan, Shinbu-to, Dai-saiko-to, Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, San'o-shashin-to, Dai-joki-to and Choto-san), which are clinically used for the treatment of hypertension,

on increases in blood pressure, heart rate and peripheral blood flow induced by theophylline in anesthetized rats in order to compare the hypotensive effects of these Kampo medicines.

MATERIALS AND METHODS

Materials Theophylline was purchased from Wako Pure Chemicals (Osaka). Hachimi-jio-gan, Gosha-jinki-gan, Shinbu-to, Dai-saiko-to, Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, San'o-shashin-to, Dai-joki-to and Choto-san were products of Tsumura & Co., prepared as freeze-dried powder made of boiled water-extracts of natural products. Each Kampo medicine is composed of the following natural products in fixed proportions: 1) Hachimi-jio-gan; Rehmanniae Radix (6.0 g), Corni Fructus (3.0 g), Dioscoreae Rhizoma (3.0 g), Alismatis Rhizoma (3.0 g), Hoelen (3.0 g), Moutan Cortex (2.5 g), Cinnamomi Cortex (1.0 g), Aconiti Tuber (0.5 g). 2) Gosha-jinki-gan; Rehmanniae Radix (5.0 g), Achyranthis Radix (3.0 g), Corni Fructus (3.0 g), Dioscoreae Rhizoma (3.0 g), Plantaginis Semen (3.0 g), Alismatis Rhizoma (3.0 g), Hoelen (3.0 g), Moutan Cortex (3.0 g), Cinnamomi Cortex (1.0 g), Aconiti Tuber (1.0 g). 3) Shinbu-to; Hoelen (4.0 g), Paeoniae Radix (3.0 g), Atractylodis Lanceae Rhizoma (3.0 g), Zingiberis Rhizoma (1.5 g), Aconiti Tuber (0.5 g). 4) Dai-saiko-to; Bupleuri Radix (6.0 g), Pinelliae Tuber (4.0 g), Paeoniae Radix (3.0 g), Zizyphi Fructus (3.0 g), Aurantii Fructus Immaturus (2.0 g), Zingiberis Rhizoma (1.0 g), Rhei Rhizoma (1.0 g), Scutellariae Radix (3.0 g). 5) Saiko-ka-ryukotsu-borei-to; Bupleuri Radix (5.0 g), Scutellariae Radix (2.5 g), Pinelliae Tuber (4.0 g), Cinnamomi Cortex (3.0 g), Ostreae Testa (2.5 g), Hoelen (3.0 g), Zizyphi Fructus (2.5 g), Ginseng Radix (2.5 g), Fossilii Ovis Mastodi (2.5 g), Zingiberis Rhizoma (1.0 g). 6) Oren-gedoku-to; Scutellariae Radix (3.0 g), Coptidis Rhizoma (2.0 g), Gardeniae Fructus (2.0 g), Phellodendri Cortex (1.5 g). 7) San'o-Shashin-to; Scutellariae Radix (3.0 g), Coptidis Rhizoma (3.0 g), Rhei Rhizoma (3.0 g). 8) Dai-joki-to; Magnoliae Cortex (5.0 g),

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Aurantii Fructus Immaturus (3.0 g), Rhei Rhizoma (2.0 g), Natrium Sulfuricum (1.3 g). 9) Choto-san; Gypsum Fibrosum (5.0 g), Angelicae Radix (3.0 g), Ophiopogonis Tuber (3.0 g), Pinelliae Tuber (3.0 g), Hoelen (3.0 g), Ginseng Radix (2.0 g), Ledebouriellae Radix (2.0 g), Glycyrrhizae Radix (1.0 g), Zingiberis Rhizoma (1.0 g), Uncariae Ramulus et Uncus (3.0 g), Chrysanthemi Flos (2.0 g).

Animals Male Wistar rats (Nihon SLC, Inc., Hamamatsu) weighing 240–280 g were used in this study. After fasting for 18–22 h before experiments, rats were anesthetized with urethane (800 mg/kg, i.p., supplemented as required) and α -chloralose (80 mg/kg, i.p.). Rectal temperature was maintained at 37–38 °C with a isothermal pad (RBC, Inc., Nagoya) and/or a heating lamp.

Measurements of Hemodynamics and Experimental Procedure A cannula was inserted into the duodenum from the stomach for Kampo medicine administration. The left carotid artery and vein were catheterized for blood pressure measurement and intravenous injections, respectively. Blood pressure and heart rate were monitored continuously with a pressure transducer (MP5200; Baxter, Tokyo) and recorded on a recorder (WR3701; Graphtec, Yokohama). The heart rate was counted with a cardiometer (AT-601G; Nihon Kohden Co., Tokyo) triggered by blood pressure pulses. Peripheral blood flow of the rat's ear was continuously monitored by laser-Doppler flowmetry, using a laser-Doppler flowmeter LBF-221 (Biochemical Science Co., Kanazawa), as described in the previous paper.¹⁾

The experimental procedure started after an at least 30-min resting period for stabilization of blood pressure, heart rate, body temperature and cutaneous blood flow. Theophylline was dissolved in saline and injected intravenously as a bolus of 5 mg/kg to the rats. This dose of theophylline produces significant and continuous increases in mean arterial blood pressure, heart rate and mean peripheral blood flow in rats.¹⁾ Usually, daily doses of the present Kampo medicines in the treatment for human is 1.5–4.5 g (0.5–1.5 g three times a day). It seems that 1 g Kampo medicine for human corresponds to about 1 g/kg for a rat, presuming from their some drug susceptibilities. So Kampo medicine of 1 g/kg, which was suspended in saline containing 0.5% carboxymethylcellulose, or the vehicle was administered intraduodenally (i.d.) to rats 15 min after injection of theophylline. For Shinbu-to and San'o-Shasin-to, furthermore, the dose of 0.5 and 0.25 g/kg were examined. The hemodynamic measurements were recorded at least 45 min after administration of Kampo medicine. No effect of the vehicle volume on the hemodynamics was observed in anesthetized rats.

Statistical Analysis Results are expressed as the mean $\pm$ S.E.M. of mean arterial blood pressure, heart rate, and change in mean peripheral blood flow expressed as percentages of the baseline values of the number (*n*) of experiments. The statistical analysis of difference was done by Dunnett's test for multiple comparisons. Differences were accepted as statistically significant at *p* values < 0.05 or < 0.005.

RESULTS AND DISCUSSION

A bolus injection of theophylline (5 mg/kg, i.v.) produced a rise in arterial blood pressure, with increases in heart rate

and peripheral blood flow in anesthetized rats. These increased variables after 15 min were significantly different from the pre-treatment values and remained so for at least 60 min. The cardiovascular properties of theophylline are known to be direct and indirect actions on vascular smooth muscle and heart. The former action is mainly mediated by inhibition of phosphodiesterase activity and the latter action is mainly due to release of endogenous catecholamines. The theophylline-induced increases in arterial blood pressure and heart rate, which were attenuated by pre-treatment with reserpine,¹⁾ therefore, might be partially due to actions of the endogenous catecholamines on vascular smooth muscle and heart. While, the flow increase by theophylline, as we previously reported, is due to an independent effect on the heart and is mediated by adrenergic and nitrgenic pathways.¹⁾

Each Kampo medicine (1 g/kg) was administered to rats 15 min after theophylline injection. Effects of Kampo medicines on the theophylline-induced increases in hemodynamics are shown in Table 1.

Both Hachimi-jio-gan and Gosha-jinki-gan had little influence on arterial blood pressure, heart rate and mean peripheral blood flow in theophylline-treated rats.

Shinbu-to (1 g/kg) caused a reduction of mean arterial blood pressure, although the rats suffered severe disturbance of arterial blood pressure 15–30 min after the administration. Consequently, 2 out of 7 rats died within 60 min. Shinbu-to of 0.5 g/kg had no hypotensive effect, although 4 out of 7 rats suffered disturbance of arterial blood pressure without a lethality 30–60 min after the administration. Shinbu-to of 0.5 g/kg had little influence on arterial blood pressure within the 60-min observation period (data not shown). Aconiti Tuber, a component of Shinbu-to, is well known to be toxic on heart. It was reported that aconitine, a component of Aconiti Tuber, causes release of endogenous catecholamins centrally, followed by neurogenic pulmonary edema and ventricular arrhythmias.^{3,4)} Shinbu-to is clinically used to treat weak patients with marked disturbance of the body metabolism, and there is a warning against treatment with this medicine to patients in high metabolism.

Dai-saiko-to caused a reduction in mean arterial blood pressure, although the hypotensive effect was small.

Saiko-ka-ryukotsu-borei-to caused reductions in not only mean arterial blood pressure but also heart rate without a reduction in the peripheral blood flow in theophylline-treated rats. The rapid effect on arterial blood pressure was observed after the administration and the effect on heart rate appeared slowly. The effect of Saiko-ka-ryukotsu-borei-to on arterial blood pressure may result from antagonism to endogenous catecholamine since it had no influence on arterial blood pressure and heart rate in theophylline-nontreated rat (Table 2). It has been reported that Saiko-ka-ryukotsu-borei-to caused a relaxation in the presence of norepinephrine in rat thoracic aorta.⁵⁾ It is well known that theophylline is a potent stimulant of the central nervous system. We have previously reported that Saiko-ka-ryukotsu-borei-to reduced theophylline-enhanced locomotor activity of mice and prolonged the latency of convulsions by theophylline in mice.⁶⁾ In addition, Saiko-ka-ryukotsu-borei-to is widely used in a variety of clinical situations to treat various neurological symptoms besides hypertension. The effect of Saiko-ka-ryukotsu-borei-to on heart rate is probably mediated by a sedative effect on

Table 1. Effects of Kampo Medicines on Theophylline-Induced Increases in Mean Arterial Blood Pressure (MABP), Heart Rate (HR) and Pheripheral Blood Flow (PBF) of Rats

	<i>n</i>	time ^{a)} (min)	MABP (mmHg)	HR (beats/min)	PBF ^{b)} (%)
Control	7	Basal	93.5±3.3	403±10	100
		0	116.1±1.8	470±12	130±9.3
		15	115.1±3.8	473±12	132±6.2
		30	116.8±3.1	477±12	125±8.5
		45	117.3±3.7	480±12	124±6.7
Hachimi-jio-gan	7	Basal	92.1±3.2	394±12	100
		0	113.6±2.6	474±6	147±1.8
		15	109.6±3.4	469±7	121±9.8
		30	106.0±3.8	465±8	127±3.6
		45	108.3±3.3	463±11	129±3.6
Gosha-jinki-gan	6	Basal	88.0±2.0	383±4	100
		0	107.6±4.0	470±19	136±18.6
		15	111.2±5.0	462±25	121±17.0
		30	115.4±5.4	461±23	127±11.2
		45	120.0±3.5	462±16	129±13.0
Shinbu-to	7	Basal	88.6±2.6	397±5	100
		0	110.3±6.1	512±9	128±6.0
		15	102.6±6.8	489±14	124±15.2
		30	96.6±6.2**	478±16	134±20.1
		45	96.1±4.5**	462±7	141±24.0
Dai-saiko-to	7	Basal	90.0±3.2	390±8	100
		0	110.7±2.6	493±6	147±10.5
		15	100.4±3.4	485±10	121±8.5
		30	101.4±3.8	481±11	127±10.5
		45	102.9±3.3	478±10	129±4.5
Saiko-ka-ryukotsu-borei-to	7	Basal	89.7±4.6	397±14	100
		0	110.8±5.5	512±11	129±8.0
		15	103.5±6.7	489±13	139±8.7
		30	96.0±5.2*	478±19	132±10.0
		45	94.7±2.7**	462±13*	114±4.0
Oren-gedoku-to	7	Basal	96.0±5.9	393±12	100
		0	117.4±5.4	486±6	133±8.5
		15	98.4±6.1*	485±7	94±6.1**
		30	98.4±7.7	471±8	105±8.0
		45	101.7±7.3	470±11	107±8.2
San'o-shashin-to	7	Basal	94.3±1.2	375±8	100
		0	115.6±1.9	469±7	139±8.5
		15	101.4±3.7*	462±6	100±6.2**
		30	97.4±2.7**	450±7	88±5.6**
		45	101.7±2.3**	439±9	90±3.9**
Dai-jiyoki-to	7	Basal	93.3±2.3	399±9	100
		0	111.9±5.0	461±4	143±7.0
		15	92.7±4.0**	459±7	92±6.7*
		30	95.0±2.7**	452±9	105±5.5
		45	100.3±1.8**	453±12	110±8.5
Choto-san	7	Basal	94.6±2.6	391±11	100
		0	116.1±2.4	470±12	135±6.1
		15	111.0±3.7	463±10	101±7.7*
		30	105.0±5.8	448±15	114±4.0
		45	107.3±4.6	444±14	114±4.0

Each Kampo medicine (1 g/kg) was intraduodenally administered to rats 15 min after injection of theophylline (5 mg/kg, i.v.). a) Time after administration of Kampo medicine are expressed, and "Basal" is just before injection of theophylline. All of values of MABP, HR and PBF in each group at 0 min are significantly different from their basal values. b) PBF were expressed as percentage of the baseline values. **p*<0.05, ***p*<0.005 versus vehicle control.

the central nervous system stimulation. Therefore, the results obtained from this study suggest that Saiko-ka-ryukotsu-borei-to is useful to treat hypertension in the sympathetic system dominance with palpitation, following little reduction of peripheral blood flow.

Oren-gedoku-to, San'o-shashin-to and Dai-jiyoki-to caused reductions in mean arterial blood pressure but did not in

Table 2. Effects of Kampo Medicines on MABP, HR and PBF of Rats

	<i>n</i>	Time (min)	MABP (mmHg)	HR (beats/min)	PBF ^{a)} (%)
Control	3	0	91.7±1.7	390±10	100
		15	91.0±2.1	393±12	98±1.7
		30	91.0±1.0	393±12	101±2.1
		45	91.7±1.7	397±15	100±2.9
Saiko-ka-ryukotsu-borei-to	5	0	92.8±1.9	393±12	100
		15	98.8±2.4	412±12	94±5.3
		30	96.8±3.0	414±9	99±2.1
		45	96.0±4.2	409±7	107±8.0
Oren-gedoku-to	5	0	90.8±1.8	384±12	100
		15	91.0±1.1	399±14	78±6.2*
		30	89.6±2.1	392±18	83±5.2*
		45	93.8±3.2	393±21	90±4.7
San'o-shashin-to	7	0	95.7±3.5	388±10	100
		15	93.1±4.3	391±11	84±4.4*
		30	95.0±1.5	404±10	87±5.8
		45	95.0±2.0	408±11	95±8.8
Dai-jiyoki-to	5	0	96.0±2.4	381±14	100
		15	98.0±2.0	379±12	68±5.6**
		30	96.4±1.0	380±13	74±5.0*
		45	99.0±2.1	379±15	92±5.6

Each Kampo medicine (1 g/kg) was intraduodenally administered to rats. a) PBF were expressed as percentage of the baseline values. **p*<0.05, ***p*<0.005 versus vehicle control.

heart rate in theophylline-treated rats, although these Kampo medicines had no influence on arterial blood in theophylline-nontreated rats (Table 2). The hypotensive effects of these 3 Kampo medicines were produced in a dose-dependent manner. The effect of San'o-shashin-to, which is composed of *Scutellariae Radix*, *Coptidis Rhizoma* and *Rhei Rhizoma*, is shown in Fig. 1. These results suggest that Oren-gedoku-to, San'o-shashin-to and Dai-jiyoki-to are also useful to treat hypertension in the sympathetic system dominance. Oren-gedoku-to, Sano-shashin-to and Dai-jiyoki-to are all clinically used to treat considerably or relatively strong patients with symptoms such as feeling of uprising heat, restlessness, insomnia, irritability and psychic symptoms. Since Kampo medicines are blended herbal medicines made from several crude medicines that may possess complex interactions, it is generally uncertain what crude drugs are responsible for their biological actions. However, *Scutellariae Radix*, which is a common constituent of Oren-gedoku-to and San'o-shashin-to in addition to Dai-saiko-to and Saiko-ka-ryukotsu-borei-to, possesses sedative actions by inhibition of adenylate cyclase activity,⁷⁾ and *Rehi Rhizoma*, a common constituent of San'o-shashin-to and Dai-jiyoki-to in addition to Dai-saiko-to, possesses a psychotropic action by depression of norenergic and dopaminergic nerve activities.⁸⁾ Therefore, it seems that *Scutellariae Radix* and/or *Rehi Rhizoma* play(s) a main role in suppressing the theophylline-induced increase in arterial blood pressure by these Kampo medicines. Moreover, it has been reported that Oren-gedoku-to and *Scutellariae Radix* caused a relaxation in rat thoracic aorta and their effects are not mediated by soluble guanylate cyclase or β -adrenoceptor.⁹⁾ The effects of Oren-gedoku-to, San'o-shashin-to and Dai-jiyoki-to on arterial blood pressure may be partially due to interference in release of endogenous catecholamines.

Moreover, Oren-gedoku-to, San'o-shashin-to and Dai-jiyoki-to caused reductions in mean peripheral blood flow in

Fig. 1. Effects of San'o-shashin-to on Theophylline-Induced Increases in Mean Arterial Blood Pressure (MABP), Heart Rate (HR) and Peripheral Blood Flow (PBF) in Ear of Anesthetized Rats

San'o-shashin-to of 1 g/kg (●), 0.5 g/kg (○), 0.25 g/kg (▲) and 0.125 g/kg (△) or the vehicle (□) was intraduodenally administered to rats 15 min after injection of theophylline (5 mg/kg, i.v.). PBF were expressed as percentage of the baseline values. All of values of MABP, HR and PBF at 0 min are significantly different from their theophylline pre-treatment values. Each point represents the mean $\pm$ S.E.M. of 4–7 rats. * $p < 0.05$ vs. vehicle-treated rats.

theophylline-treated rats, although these Kampo medicines reduced peripheral blood flow but not arterial blood pressure in theophylline-nontreated rats (Table 2). These findings suggest that the changes in peripheral blood flow are independent of the reduction in arterial blood pressure. The reduction of peripheral blood flow may be occurring by a modulating mechanism, except for the adrenergic pathway. Although it is uncertain what mechanisms mediate these Kampo medicines-produced flow changes, the reduction in peripheral

blood flow must be available for local status of heat or hemorrhage. The results in this study seem to be related to some indications of Oren-gedoku-to and Sano-shashin-to, such as feeling of uprising heat, red face, tendency for hemorrhage, hematemesis, and epistaxis.

Choto-san had little influence on arterial blood pressure and heart rate of theophylline-treated rats, although mean peripheral blood flow was reduced by this medicine. The values of mean peripheral blood flow 15 min after Choto-san administration were significantly different from the values of the vehicle control rats. However, in theophylline-nontreated rats, this medicine had little influence on either arterial blood pressure or peripheral blood flow (data not shown). Our previous report indicated that the flow increase by theophylline is mediated by the nitrgic pathway through stimulation of the adrenergic nerve.¹⁾ Choto-san may act on vascular function by modulating a NO-dependent mechanism in vascular smooth muscle cells or NO as the major nitrgic transmitter in the peripheral nervous system.¹⁾

In conclusion, the present findings suggest that Saiko-ka-ryukotsu-borei-to, Oren-gedoku-to, San'o-shashin-to and Dai-ryoki-to are ameliorative to the hypertension in sympathetic system dominance and Shinbu-to is occasionally dangerous to it.

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