Effects of *Saiko-ka-ryukotsu-borei-to*, a Japanese *Kampo* Medicine, on Tachycardia and Central Nervous System Stimulation Induced by Theophylline in Rats and Mice

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ABSTRACT—Effects of *Saiko-ka-ryukotsu-borei-to* (SRBT) on theophylline-induced tachycardia in anesthetized rats and theophylline-induced locomotion and convulsions in mice were examined. An intraduodenal administration of SRBT (1 g/kg) prevented theophylline (5 mg/kg, i.v.)-induced tachycardia in rats. SRBT also attenuated an increase in arterial blood pressure with a slow reduction in heart rate of rats treated with theophylline, with no influence on the plasma level of theophylline. However, SRBT did not change the beating rate of right atrium isolated from rats in the absence or presence of theophylline or isoproterenol. The locomotor activity of theophylline in mice was reduced by the treatment with SRBT. Furthermore, the latency of convulsions in mice induced by administration of theophylline at a higher dose (240 mg/kg, i.p.) was prolonged by treatment with SRBT (1 g/kg, p.o.) and seven out of fifteen mice were saved from death due to convulsions. These results suggest that theophylline-induced tachycardia and central nervous stimulation are suppressed by SRBT and that SRBT may reduce the undesirable actions of theophylline on the cardiovascular and central nervous systems.

Keywords: Theophylline, *Saiko-ka-ryukotsu-borei-to*, Heart rate, Locomotor activity, Convulsion

Theophylline is used for the treatment of chronic respiratory disease, but its undesirable actions on the cardiovascular and central nervous systems are well known. A narrow therapeutic effective range of theophylline (10–20 μ g/kg) requires a severe control of the plasma concentration. At higher plasma concentrations of theophylline, tachycardia and signs of progressive central nervous system stimulation are produced, including palpitation, restlessness, insomnia, tremors, and focal and generalized convulsions. Furthermore, after chronic administration of methylxanthines, the tolerance develops to their various behavioral actions (1, 2). Although the mechanisms of theophylline-induced phenomena are not well understood, it is suspected that blockade of adenosine receptors, inhibition of phosphodiesterase, mobilization of intracellular calcium ions, or finally, increased release of excitatory amino acids may be important (3). Some studies on experimental convulsions induced by theophylline in mice have indicated the anticonvulsant efficacy of common antiepileptic drugs

(4–8), but no effective treatment to control theophylline-induced convulsions have been yet established in clinical use.

Saiko-ka-ryukotsu-borei-to (SRBT) is a traditional Chinese herbal medicine that is widely used in a variety of clinical situations in Japan such as occasionally to treat malignant hypertension and various neurological symptoms. The pharmacological basis for the ameliorative effects of SRBT on the cardiovascular system and central nervous systems are poorly understood. We are interested in the similarity between the side effects in the treatment of chronic respiratory disease with theophylline and the indications for SRBT on the basis of traditional diagnosis.

This study was designed to evaluate the efficacy of SRBT on theophylline-induced tachycardia and central nervous system stimulation. We investigated the effects of SRBT on the chronotropic action of theophylline in rats, in vivo and in vitro, and further on the locomotions and convulsions induced by theophylline in mice.

MATERIALS AND METHODS

Materials

Theophylline was purchased from Wako Pure Chemicals (Osaka). Isoproterenol hydrochloride was obtained from Sigma (St. Louis, MO, USA). SRBT was prepared as a freeze-dried powder made of boiled water-extracts of natural products, containing the following (% w/w): *bupleurum root*, 17.5; *pinellia tuber*, 14.0; *cinnamon bark*, 10.5; *hoelen*, 10.5; *scutellaria root*, 8.8; *jujube*, 8.8; *ginseng*, 8.8; *oyster shell*, 8.8; *longgu*, 8.8; *ginger*, 3.5.

Animals

Male ddY mice (Nihon SLC, Inc., Hamamatsu) weighing 20–28 g and male Wistar rats (Nihon SLC, Inc.) weighing 240–280 g were used in this study. They were housed in a temperature- and humidity-controlled ($24 \pm 1^\circ\text{C}$, $55 \pm 10\%$) room, exposed to a 12-hr/12-hr light/dark cycle and given standard diet and tap water ad libitum before being used. Rats and mice were starved for 18–22 hr before the experiments in vivo.

Measurements of blood pressure and heart rate and measurement of theophylline in plasma in rats

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 an isothermal pad (RBC Inc., Nagoya) and/or a heating lamp. A cannula was inserted into the duodenum from stomach for SRBT 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. The experimental procedure started after at least a 30-min resting period for stabilization of blood pressure and heart rate. SRBT of 1 g/kg, which was suspended in saline containing 0.5% carboxymethylcellulose, or the vehicle was administered intraduodenally (i.d.) to rats. Theophylline was dissolved in saline and injected intravenously as a bolus of 5 mg/kg to the rats 15 min before or 15 min after administration of SRBT.

For measurement of the plasma theophylline level, 0.8 ml of blood was drawn from the carotid artery of each anesthetized rat treated with theophylline and put into a tube containing heparin. The samplings were done 5 min before and 30 min after administration of SRBT (1 g/kg, i.d.). The blood was centrifuged at $3,000 \times g$ to obtain plasma for the measurement of theophylline level. The plasma theophylline levels were measured by using an

HPLC system by the method of Tanaka et al. (9).

Beating rate of isolated right atrium of rats

Rats were killed by being stunned and bled. The right atrium was rapidly removed and placed in an organ bath (30°C) filled with Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.55 mM CaCl_2 , 1.18 mM MgSO_4 , 24.88 mM NaHCO_3 , 1.18 mM KH_2PO_4 and 11.1 mM glucose, pH 7.4) aerated with a gas 95% O_2 – 5% CO_2 . The preparation was left to equilibrate under a tension of 0.5 g and then treated with cumulative concentrations of SRBT (0.01–0.3 mg/ml) at intervals of 6–8 min in the presence and absence of theophylline (0.1 and 0.3 mM) or isoproterenol (1 and 3 nM). Isoproterenol of 1 and 3 nM are doses which that about 35 and 80% of the maximum chronotropic action on the isolated right atrium of rat, respectively. The beating rate was recorded through a cardiometer triggered by isometric contraction of the atria.

Locomotor activities in mice

Experiments were done between 9:00–14:00 hr in a room with an average temperature of $26 \pm 1^\circ\text{C}$, and each mouse was used once only. Before experiments, mice were placed singly in a novel environment, the floor of a clear perspex container ($35 \times 35 \times 16$ cm high) in a gloomy field of the Muromachi animal behavior analyzer (BTA-1; Muromachi Ind., Tokyo), and were acclimated. Thirty minutes later, mice were injected with theophylline (5, 10 and 20 mg/kg, i.p.) and immediately returned to the container in the equipment, and then locomotor activities (total distance where mice moved) were measured for 30 min. SRBT suspended in 0.5% carboxymethylcellulose was administered p.o. to mice 10 min before theophylline injection. The vehicle was administered to control animals.

Theophylline-induced convulsions in mice

Mice were injected i.p. with theophylline in a dose of 240 mg/kg, which completely induced convulsions to mice, and then observed for 60 min. Clonic and tonic convulsions as well as mortality were noted throughout this period. The convulsive tests were done in a quiet room with an average temperature of $26 \pm 1^\circ\text{C}$, between 10:00–14:00 hr. SRBT was given p.o. as a preliminary treatment to observe its effect on theophylline-induced convulsions.

Statistical analyses

Results are expressed as the mean $\pm$ S.E.M. of the number (n) of experiments. The statistical analysis of difference was done by Student's *t*-test or Welch's *t*-test for comparisons between two groups and Dunnett's test for multiple comparisons. Differences were accepted as statistically significant at *P* values < 0.05 .

RESULTS

Effects of SRBT alone on blood pressure and heart rate and effects of SRBT on theophylline-induced hypertension and tachycardia in anesthetized rats

Administration of SRBT (1 g/kg, i.d.) caused slight increases in mean arterial blood pressure and heart rate, both of whose variables were, however, not significantly different from those of the vehicle control group (Fig. 1A). Fifteen minutes later, subsequent injection of theophylline (5 mg/kg, i.v.) caused further increases in mean arterial blood pressure and heart rate, but the theophylline-increased values in heart rate of the SRBT-treated group were significantly smaller than those of the vehicle control group (Fig. 1A).

A bolus injection of theophylline (5 mg/kg, i.v.) induced increases in mean arterial blood pressure and heart rate, both of whose variables after 15 min were significantly different from the pretreatment value, and these increased levels remained for at least 60 min. Subsequent administration of SRBT (1 g/kg, i.d.) caused substantial reduction in mean arterial blood pressure so that values 30–60 min after SRBT administration were significantly different from the values produced by no further drug after the bolus of theophylline, although the values were

not completely restored to the pretreatment value. Heart rate was also slowly and partially restored by subsequent administration of SRBT (Fig. 1B).

Effect of SRBT on plasma theophylline level in rats treated with theophylline

SRBT (1 g/kg, i.d.) or the vehicle was administered to rats treated with theophylline, and the plasma theophylline levels were measured 5 min before and 30 min after SRBT (Table 1). The plasma theophylline level was reduced as time passed but administration of SRBT had no effect on the change in the plasma theophylline level.

Effects of SRBT on theophylline- and isoproterenol-stimulated beating rates of isolated right atrium of rats

SRBT (0.01–0.3 mg/ml) had no effect on the beating rate of the right atrium (basal beating rate was 194 ± 4). Theophylline at 0.1 and 0.3 mM caused increases in beating rate ($25.0 \pm 3.0\%$ and $43.7 \pm 4.5\%$ of the unstimulated beating rate, respectively). SRBT had no effect on those theophylline-stimulated beating rates. Isoproterenol at 1 and 3 nM caused increases in beating rate ($19.0 \pm 3.0\%$ and $39.6 \pm 7.5\%$ of the unstimulated beating rate, respectively). SRBT also had no effect on those isoproterenol-stimulated beating rates (Fig. 2).

Fig. 1. Effects of *Saiko-ka-ryukotsu-borei-to* (SRBT) on theophylline-induced increases in mean arterial blood pressure and heart rate. A: Effect of SRBT pre-treatment. SRBT (1.0 g/kg, i.d.) ($n=6$, $\blacktriangle$) or the vehicle ($n=3$, $\triangle$) was administered to anesthetized rats. Theophylline (5 mg/kg, i.v.) was injected into rats treated with SRBT ($n=6$, $\bullet$) or the vehicle ($n=6$, $\circ$) for 15 min. B: Effect of SRBT post-treatment. SRBT (1.0 g/kg, i.d.) ($n=6$, $\bullet$) or the vehicle ($n=6$, $\circ$) was administered to rats 15 min after injection of theophylline (5 mg/kg, i.v.). Each point represents the mean $\pm$ S.E.M. * $P < 0.05$ vs vehicle-treated rats.

Table 1. Effect of *Saiko-ka-ryukotsu-borei-to* (SRBT) on plasma theophylline levels in rats treated with theophylline (5 mg/kg, i.v.)

Treatment	n	Plasma theophylline level ($\mu\text{g/ml}$)	
		5 min before SRBT	30 min after SRBT
Vehicle control	5	221.5 $\pm$ 18.5	136.8 $\pm$ 10.8
SRBT (1 g/kg)	5	226.7 $\pm$ 10.5	141.7 $\pm$ 15.2

SRBT (1 g/kg, i.d.) or the vehicle was administered to rats 15 min after injections of theophylline (5 mg/kg, i.v.). Each datum is expressed as the mean $\pm$ S.E.M.

Effect of SRBT on locomotor activity of theophylline

When mice were placed in a novel environment, exploratory behavior appeared in mice. This behavior, however, nearly disappeared within 30 min. Thirty minutes later when the mice were generally sedate, they were administered theophylline. Theophylline (5, 10, 20 mg/kg, i.p.) produced behavioral stimulation in a dose-dependent manner. Although the treatment with another dose of theophylline (50 mg/kg) immediately resulted in a marked increase in locomotor activity, the behavioral effect gradually disappeared and was not larger than the locomotor activity elicited by 20 mg/kg theophylline for the 30 min recorded in this study, while the treatment with SRBT prevented the stimulant effect of theophylline. The effect of SRBT in a dose of 1 g/kg was largest on that of 10 mg/kg theophylline (Fig. 3A). The preventive effect of SRBT was also dose-dependent, and those of 1 and 2 g/kg of SRBT were significant on the stimulant effect of theophylline in a dose of 10 mg/kg (Fig. 3B).

Fig. 2. Effects of *Saiko-ka-ryukotsu-borei-to* (SRBT) on chronotropic actions of theophylline and isoproterenol. SRBT was administered in the absence ($\square$) and presence of theophylline (0.1 mM $\blacktriangle$, 0.3 mM $\bullet$) or isoproterenol (1 nM $\blacktriangle$, 3 nM $\bullet$). Values are represent the mean $\pm$ S.E.M. of 3 experiments. Where no error bar is shown, the error is smaller than the symbol. $\triangle$, $\circ$: Theophylline or isoproterenol alone.

Effects of SRBT on theophylline-induced convulsions in mice

At 240 mg/kg of theophylline, highly reproducible convulsions and lethality were observed. All of the mice

Fig. 3. Effects of *Saiko-ka-ryukotsu-borei-to* (SRBT) on locomotor activity of mice given theophylline. SRBT or the vehicle was p.o. injected 10 min before i.p. injection of theophylline or saline. A: 1 g/kg SRBT. B: 10 mg/kg theophylline. Open columns and dotted columns represent the locomotor activities of mice treated with vehicle and SRBT, respectively. Each value is the mean with S.E.M. from 7 mice. * $P < 0.05$ and ** $P < 0.005$ vs vehicle-treated mice.

Table 2. Effects of *Saiko-ka-ryukotsu-borei-to* (SRBT) on theophylline (240 mg/kg, i.p.)-induced convulsion and lethality in mice

Treatment	Convulsions		Lethality	
	n	Latency (min)	n	Latency (min)
Vehicle control	15/15	15.6±1.1	15/15	30.0±2.8
SRBT (g/kg)				
0.5	14/15	19.9±2.1	12/15	27.2±3.0
1.0	13/15	24.5±2.6 ^a	8/15	36.3±5.4

SRBT (p.o.) was administered 10 min before injections of theophylline. Convulsions and lethality were observed for 60 min after injections of theophylline. The data are expressed as the (number of animals showing an effect of theophylline) / (number of mice used) and the mean ± S.E.M. ^aP < 0.01 vs vehicle-treated mice.

(n=15) injected with 240 mg/kg of theophylline had severe clonic-tonic convulsions with hind limb extension and died immediately after the episode of frank convulsions. The latency to the onset of convulsions varied from 8 to 20 min, with a mean ± S.E. of 15.6 ± 1.1 min. Mice showed increased respiration, occasional tremors, incoordination and mild hyperactivity before the onset of seizures. Table 2 shows the effects of SRBT on theophylline-induced convulsions and lethality. Out of 15 mice treated with SRBT at 0.5 g/kg, 14 mice had the clonic and tonic phase of convulsion, and 12 died, upon injection with the dose of theophylline, but one mouse had tremors but not convulsions and two mice did not die. Out of 15 mice treated with SRBT at 1 g/kg, 13 mice had convulsions caused by theophylline, the latency of which was significantly prolonged from 15.6 min of the control group to 24.5 (17–36) min, and 7 mice survived the period in which lethality induced by theophylline usually occurred within the 60-min observation period.

DISCUSSION

SRBT is frequently used for patients with nervous constitutions who have psychoneurotic symptoms. It is interesting that the indications for SRBT are similar to the side effects of theophylline in clinical use. Theophylline induces undesirable actions that are mainly referable to the circulatory and central nervous systems. This study was done to evaluate the pharmacological efficacy of SRBT on theophylline-induced hypertension, tachycardia and central nervous system stimulation.

A bolus injection of theophylline produced a rise in arterial blood pressure and tachycardia in anesthetized rats. Prior administration of SRBT, which itself produced slight increases in arterial blood pressure and heart rate, partially suppressed the tachycardia by theophylline. Moreover, subsequent administration of SRBT after in-

jection of theophylline caused substantial reduction in arterial blood pressure and heart rate. The effect on blood pressure was immediately observed and the effect on heart rate appeared slowly. The plasma theophylline level of rats treated with theophylline were not influenced by SRBT. This indicates that the effects of SRBT on those hemodynamic effects of theophylline were not the result of pharmacokinetic inhibition of plasma theophylline by SRBT.

The cardiovascular properties of theophylline are known to be direct and indirect actions on vascular smooth muscle and heart rate. The former action is mainly mediated by inhibition of phosphodiesterase activity, and the latter action is mainly mediated by release of endogenous catecholamines. Our previous report indicated that the effects of theophylline on arterial blood pressure and heart rate in anesthetized rats were markedly attenuated by β -adrenergic blockade with propranolol or treatment with reserpine (10). Vestal et al. (11) found consecutive and increased catecholamine levels after aminophylline infusion and concluded that theophylline may act through release of norepinephrine from peripheral nerve terminals. Therefore, the effects of SRBT on the theophylline-induced changes in arterial blood pressure and heart rate may be due to interference in endogenous catecholamines by SRBT. It has been reported that SRBT caused a relaxation in the presence of norepinephrine in rat thoracic aorta (12). In isolated rat atrium, SRBT had no influence on the theophylline- or isoproterenol-stimulated heart rate. This result suggests that the effect of SRBT on the theophylline-induced increase in heart rate is not a result of antagonism to the direct action of theophylline on heart or β -adrenergic action of the released catecholamine. It is likely that SRBT antagonizes a mechanism of the sympathetic nervous system stimulation and thereby reduces the effect of theophylline on the heart.

It is well known that theophylline is a potent stimulant of the central nervous system, and occasionally evokes dangerous convulsions in humans (13). In this study, as shown by Choi et al. (14), theophylline induced an increase in locomotor activity, and the pretreatment with SRBT, which itself had a little motor effect on mice, decreased the motor effect of theophylline. Moreover, the higher dose of theophylline led to severe tonic-clonic seizures and lethality in mice, as shown by Czuczwar et al. (5), and prior administration of SRBT resulted either in a delay of seizure onset or in elimination of tonic episodes and a reduction in postital mortality. These results suggest that SRBT exerts a sedative effect on the central nervous system stimulation by theophylline. The mechanism of central stimulant effect of theophylline is not well understood, but adenosine receptor antagonism has been

suggested to explain the behavioral action of theophylline (14–16). In our results, although SRBT counteracted the theophylline-induced hypertension, tachycardia and the locomotor effect of theophylline, SRBT itself slightly induced increases in blood pressure and heart rate in rats and behavioral effect in mice. These results induced by theophylline and SRBT, which were not additive, bring to mind findings shown by Giménez-Llort et al. (17): both *N*-methyl-D-aspartate (NMDA) and theophylline induced motor activation in mice and, when coadministered, NMDA counteracted the effect of theophylline. Much experimental evidence supports the existence of antagonistic interactions between adenosine and NMDA receptors in the brain (18, 19). Furthermore, NMDA receptor stimulation has been described to release endogenous adenosine (20, 21), which can be a protective endogenous mechanism to avoid NMDA-induced excitotoxic effects (19). Although the sedative effect of SRBT on central nervous system stimulation by theophylline may result from interference with the entry of theophylline into the central nervous system by SRBT, SRBT as well as NMDA may release endogenous adenosine or stimulate NMDA receptors in the brain.

In summary, SRBT suppressed theophylline-induced tachycardia in anesthetized rats and theophylline-induced locomotions and convulsions in mice. In addition, in our preliminary experiment, SRBT did not inhibit the bronchodilatory effect of theophylline on acetylcholine-induced bronchoconstriction in anesthetized guinea pigs. These results may provide further insight into the pharmacological basis for the ameliorative and varied effects of SRBT as well as indicate new therapeutic approaches using Chinese medicine to reduce the side effects in the treatment of chronic respiratory disease with theophylline. We are now examining the effects of SRBT and theophylline on asthmatic model animals.

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