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Effects of *Saiboku-to* and *Saiko-ka-ryukotsu-borei-to*, Japanese *Kampo* medicines, on theophylline-induced bronchodilation in guinea-pigs

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Abstract

Saiboku-to and *Saiko-ka-ryukotsu-borei-to* (SRBT) are Japanese *Kampo* medicines used clinically for the treatment of asthma and bronchitis and for the treatment of hypertension and various neurological symptoms, respectively. Effects of *Saiboku-to* and SRBT on the bronchodilatory effect of theophylline were examined using guinea-pigs *in vitro* and *in vivo*. In isolated tracheal smooth muscle, *Saiboku-to* significantly potentiated the relaxant effect of theophylline. The potentiation by *Saiboku-to* was abolished by N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide (NO) synthesis, or ODQ, a guanylate cyclase inhibitor, and was absent in airway epithelium-removed tracheal segments, suggesting that the potentiation may include a mechanism through NO released from airway epithelium. But SRBT had little influence. In guinea-pigs under urethane anesthesia, pretreatment with SRBT (1.0 g/kg, intraduodenally) potentiated the bronchodilatory effect of theophylline (5 mg/kg, i.v.) with weak inhibition of the heart stimulation, whereas pretreatment with *Saiboku-to* had little influence on the effect of theophylline. In this study, *Saiboku-to* and SRBT potentiated the effect of theophylline *in vitro* and *in vivo*, respectively, probably through different mechanisms. We have previously reported that SRBT suppressed theophylline-induced tachycardia in rats and theophylline-induced locomotion and convulsion in mice. In the treatment of chronic respiratory disease with theophylline, concomitant treatment with SRBT may reduce the therapeutic dose and the side effects on the cardiovascular and central nervous system of theophylline.

Key words theophylline, Saiboku-to (Chai-Pu-Tang, 柴朴湯), Saiko-ka-ryukotsu-borei-to (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang, 柴胡加竜骨牡蛎湯), bronchodilation.

Introduction

Theophylline is used for the treatment of chronic respiratory disease, but its undesirable actions on the cardiovascular and central nervous systems are well known. The narrow therapeutic effective range of theophylline requires tight control of the plasma concentration. We have previously reported that *Saiko-ka-ryukotsu-borei-to* (SRBT, *Chai-Hu-Jia-Long-Gu-Mu-Li-Tang*), a traditional Japanese herbal medicine (*Kampo* medicine), which is used in a variety of clinical situations such as to treat malignant hypertension and various neurological symptoms, suppressed theophylline-induced tachycardia in anesthetized rats and theophylline-induced locomotion and convulsion in mice and suggested that SRBT may reduce the undesirable actions of theophylline on the cardiovascular and central nervous systems.[1] However, it is unknown whether SRBT affects the airway smooth muscle per se or the bronchodilatory influence of theophylline. *Saiboku-to* (*Chai-Pu-Tang*), another *Kampo* medicine, is used in the treatment of asthma and bronchitis, and the anti-allergic action of this medicine based on the suppression of type I and IV allergic reaction has been confirmed in animal experiments.[2,3] Furthermore, *Saiboku-to* has been used for glucocorticoid-dependent asthmatic patients with the aim of reducing glucocorticoid dose.[4] Recently, investigators have shown evidence of anti-inflammatory action, steroid-like activity, of theophylline.[5] In the medication of theophylline for asthmatic patients,

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Saiboku-to may also have a theophylline dose-reducing effect. In addition, it has been reported that *Saiboku-to* potentiated the isoproterenol-induced production of cyclic AMP in canine airway smooth muscle and accumulation of cyclic AMP there.[6] Therefore, the bronchodilatory effect of theophylline, a cyclic nucleotide phosphodiesterase inhibitor, could be potentiated by *Saiboku-to*.

This study evaluated whether SRBT could not reduce the bronchodilatory effect of theophylline and *Saiboku-to* could enhance the effect of theophylline, and thereby studied the possibility of minimizing the undesirable actions and reducing the therapeutic dose of theophylline for chronic respiratory disease. We examined the influence of these two *Kampo* medicines on the bronchodilatory effect of theophylline in guinea-pigs, *in vitro* and *in vivo* experiments.

Materials and Methods

Materials: SRBT (Lot No. 250012010) and *Saiboku-to* (Lot No. 260096020) prepared as freeze-dried powders made of boiled water-extracts of natural products were a gift from Tsumura & Co. (Tokyo). SRBT and *Saiboku-to* are composed of the following natural products (%, w/w): SRBT; *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) and *Ginger* (3.5). *Saiboku-to*; *Bupleurum root* (20.6), *Pinellia tuber* (14.7), *Hoelen (14.7), Magnolia bark* (8.8), *Scutellaria root* (8.8), *Jujube* (8.8), *Ginseng* (8.8), *Glycyrrhiza* (5.9), *Perilla herb* (5.9) and *Ginger* (3.0). Theophylline (Wako Pure Chemicals, Osaka), isoproterenol and carbachol (Sigma Chemical Co., St. Louis, MO), acetylcholine chloride (Ovisot™, Daiichi Pharmaceutical Co., Tokyo), and N^G-nitro-L-arginine methyl ester (L-NAME) and 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (Research Biochemicals International, Natick, MA) were purchased.

Animals: Male Hartley guinea-pigs weighing 350-400 g (Nihon SLC, Inc., Hamamatsu) were used. All protocols and procedures employed in this study were reviewed and approved by the Laboratory Animal Care and Use Committee of Hokuriku University.

Isometric tension experiments in tracheal smooth muscle: Isolated tracheal ring segments from guinea-pig were dissected free of connective tissue and the ring chains were then mounted in standard organ baths filled with Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.55, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.18, glucose 11.1 and $NaHCO_3$ 24.88) (pH 7.4), maintained at 37℃ and continuously bubbled with a 95% O_2-5% CO_2 mixture. Isoproterenol (1 μM) was then added to produce complete relaxation. After the isoproterenol was washed out, a tension of 0.5-1.0 g was applied to the preparation. Tension was measured with an isometric force transducer. After an equilibration period of 60 min, the preparations were placed in contact with carbachol (2.0 μM). A contractile response was measured as the difference between peak tension developed and resting tension. In some experiments, the airway epithelium was removed by gently rubbing the internal surface with filter paper.

First, the responses of tracheal preparations to *Saiboku-to* and SRBT were examined. Tracheal preparations were pre-contracted to approximately 80 % of maximal tension by exposure to 2.0 μM carbachol. After the carbachol-induced contractions were sustained, theophylline was added cumulatively. SRBT and *Saiboku-to* were incubated for 20 min prior to addition of carbachol. L-NAME and ODQ were incubated for 30 min prior to addition of carbachol.

In vivo determination of airway responsiveness to acetylcholine: The experiments were performed according to the method described in the previous paper.[7] After being starved for 18-22 hr before experiments, guinea-pigs were anesthetized with urethane (1.5 g/kg, i.p., supplemented as required), placed in the supine position and maintained at a body temperature of 37-38℃ with an isothermal pad (RBC, Inc., Nagoya). A cannula was inserted into the duodenum from the stomach for *Kampo* medicines. The left carotid artery and vein were catheterized for blood pressure measurement and intravenous injections, respectively. Then, the guinea-pig was ventilated artificially through a tracheal cannula at a frequency of 60 beats/min by means of a small animal ventilator (683; Harvard Apparatus, Natick, MA). The bronchoconstriction in the animal was recorded using a bronchospasm transducer (7020; Ugo Basile, Comerio-Varese, Italy) by the overflow technique of Konzett and Rossler.[8] Blood pressure and heart rate were monitored continuously with a pressure transducer (MP5200; Baxter, Tokyo). The heart rate was counted with a

cardiotachometer (AT-601G; Nihon Kohden Co., Tokyo) triggered by blood pressure pulses. All the above parameters were recorded on a recorder (WR3701; Graphtec, Yokohama). The experimental procedure started after at least a 30-min rest period for stabilization of these parameters. Acetylcholine chloride (40 μg/kg) was injected at 15 min intervals. After three similar responses to acetylcholine, the *Kampo* medicine, which was suspended in saline containing 0.5% carboxymethylcellulose, was administered intraduodenally (i.d.) to guinea-pigs 15 min before the injection of theophylline. Theophylline was dissolved in saline and injected intravenously (i.v.) as a bolus of 5.0 or 10.0 mg/kg to the guinea-pigs 5min before the next injection of acetylcholine, and the responses induced by acetylcholine were monitored at 15 min intervals at least 65 min after the injection of theophylline. No effect of the vehicle volume on the parameters was observed in guinea-pigs.

Statistical analysis: Results are expressed as the mean ± S.E. The statistical analysis of difference was performed with Dunnett's test for multiple comparisons. Differences were accepted as statistically significant at p values < 0.05.

Results

Effects of Saiboku-to and SRBT on the relaxant effect of theophylline in isolated tracheal smooth muscle

Both *Saiboku-to* and SRBT at 0.3 mg/ml had little effect on the responses of tracheal preparation. Theophylline induced a dose-dependent relaxation of the carbachol-contracted preparations. *Saiboku-to* dose-dependently potentiated the relaxant effect of theophylline and the effect of 0.3 mg/ml was significant, whereas SRBT (0.3 mg/ml) slightly potentiated the effect of theophylline (Fig. 1). Though both L-NAME (30 μM) and ODQ (1 μM) had little effect on the carbachol-induced contractions, in the presence of these inhibitors, the synergistic relaxation induced by *Saiboku-to* was abolished (Fig. 2). The potentiation by *Saiboku-to* was absent in airway epithelium-removed tracheal segments (Fig. 3).

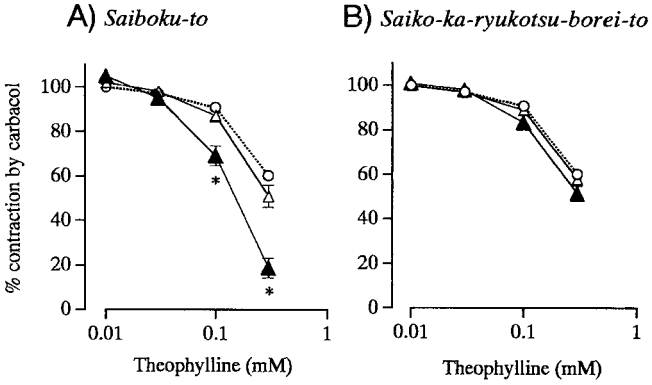

Fig. 1. Effects of *Saiboku-to* (A) and *Saiko-ka-ryukotsu-borei-to* (B) on theophylline-induced guinea-pig tracheal muscle relaxation. After treatment without (○) and with *Saiboku-to* or *Saiko-ka-ryukotsu-borei-to* at 0.3 mg/ml (▲) or 0.1 mg/ml (△) for 15 min, segments were made to contract with carbachol (2.0 μM) and theophylline was cumulatively administered. Values are means ± S.E. of 5-7 experiments. *p<0.05, significantly different from the control response.

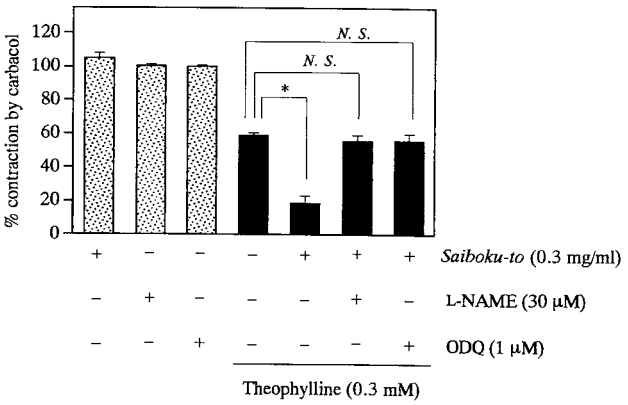

Fig. 2. Effects of *Saiboku-to* and N^G-nitro-L-arginine methyl ester (L-NAME) or ODQ on theophylline-induced guinea-pig tracheal muscle relaxation. After treatment without and with *Saiboku-to* at 0.3 mg/ml for 15 min, segments were made to contract with carbachol (2.0 μM) and theophylline (0.3 mM) was administered. L-NAME or ODQ was added to the baths 30 min prior to the addition of carbacol. Values are means ± S.E. of 5-7 experiments. *p<0.05, significantly different from the control response. N.S., not significant.

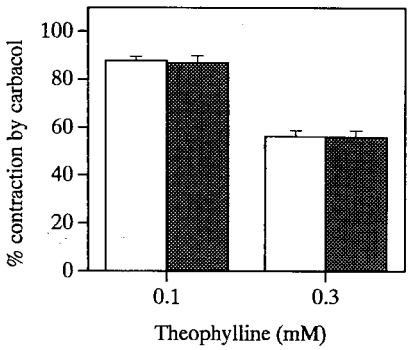

Fig. 3. Effect of *Saiboku-to* on relaxation of theophylline in airway epithelium-removed guinea-pig tracheal muscle. After treatment without (open column) and with *Saiboku-to* (dotted column) at 0.3 mg/ml for 15 min, segments were made to contract with carbachol (2.0 μM) and theophylline was cumulatively administered. Each column represents as % of basal contraction without theophylline. Values are means ± S.E. of 4 experiments.

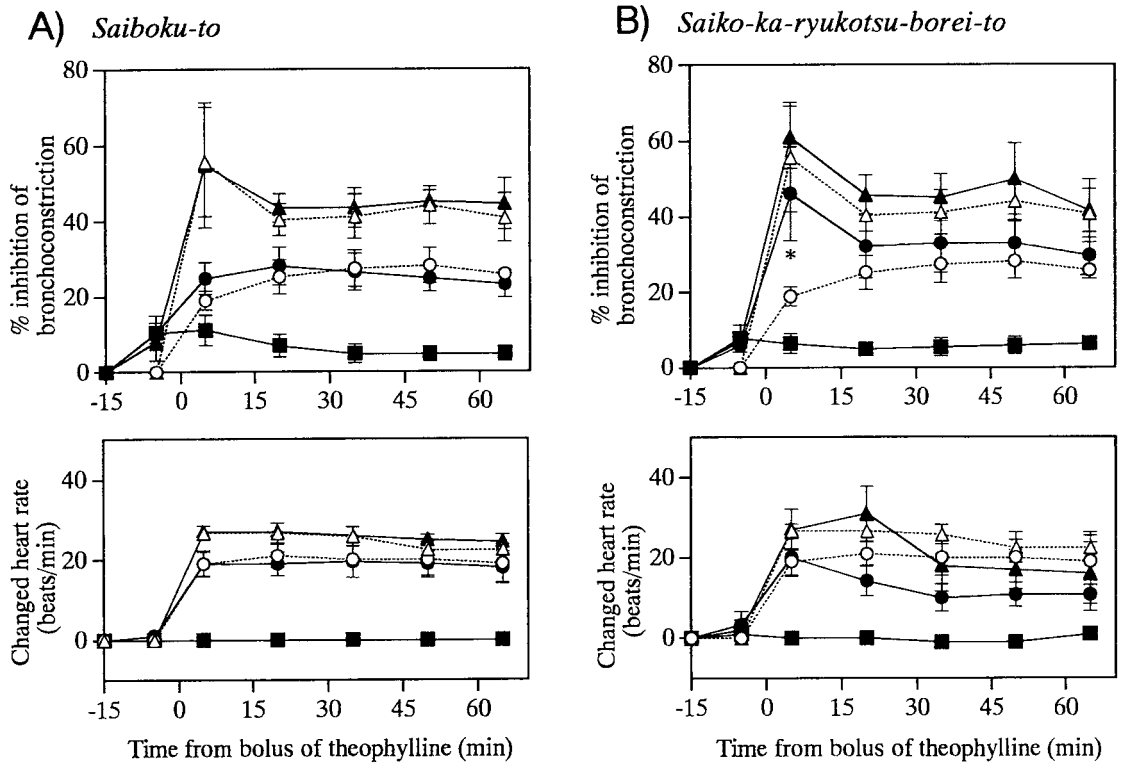

Fig. 4. Effects of *Saiboku-to* (A) and *Saiko-ka-ryukotsu-borei-to* (B) on the theophylline-induced bronchodilation (upper panel) and increases in heart rate (lower panel) in anesthetized guinea-pigs. The bronchodilation is represented as % inhibition of basal airway responsiveness to acetylcholine (40 μg/kg, i.v.). Heart rate was measured just before the acetylcholine was given. *Saiboku-to* (1.0 g/kg) and *Saiko-ka-ryukotsu-borei-to* (1.0 g/kg) were intraduodenaly administered to guinea-pigs 15 min before theophylline (n=5-7, ●, ▲) or saline (n=5, ■). Theophylline at 5 mg/kg (○, ●) and 10 mg/kg (△, ▲) was injected into guinea-pigs treated without (n=5-7, ○, △) and with *Saiboku-to* or *Saiko-ka-ryukotsu-borei-to* (●, ▲). The responsiveness to theophylline (○, △) is the same in (A) and (B). Each point represents the mean ± S. E. *$p < 0.05$ vs theophylline control group.

Effects of Saiboku-to and SRBT on airway responsiveness to acetylcholine in vivo

The mean arterial blood pressure and heart rate of anesthetized guinea-pigs was 48.2 ± 0.7 mmHg and 261 ± 3 beats/min (n=48), respectively. Bolus injection of theophylline at doses of 5.0 and 10.0 mg/kg, i.v. induced increases in the heart rate without changes in the mean arterial blood pressure. Each dose of theophylline resulted in increases in the heart rate 20 min after theophylline injections from the values at 0 min: 21.0 ± 3.3 (n=7) and 26.7 ± 1.7 (n=7) beats/min for 5.0 and 10.0 mg/kg, respectively. Theophylline also reduced the acetylcholine-induced bronchoconstriction in a dose-dependent manner. The increases in the heart rate and bronchodilations evoked by theophylline remained for at least 65 min. The effects of pretreatment with *Saiboku-to* (1.0 g/kg, i.d) and SRBT (1.0 g/kg, i.d) on these effects of theophylline were examined. *Saiboku-to* had little influence on the effects of theophylline, though *Saiboku-to* alone caused slight bronchodilation (Fig. 4A). SRBT alone had little influence on the heart rate and the bronchoconstriction, however this medicine potentiated the bronchodilatory effect of theophylline with moderate attenuation of the heart rate (Fig. 4B). The synergistic effect was significant 5 min after the injection of theophylline at doses of 5.0 mg/kg. In addition, we examined the effect of pretreatment with SRBT (1.0 g/kg, i.d) on the effect of theophylline at doses of 2.5 mg/kg. Theophylline at that dose induced slight increases in the heart rate but no changes in the acetylcholine-induced bronchoconstriction. However, the pretreatment with SRBT induced an inhibition of the bronchoconstriction 5 min after the injection of theophylline: 21.8 ± 3.8 (n=4) % (data not shown).

Discussion

It has been reported that *Saiboku-to* potentiates β-

adrenergic function.[6] In this study, *Saiboku-to* potentiated the theophylline-induced relaxation *in vitro* without a β-agonist. *Saiboku-to* by itself had little effect on the preparations in the presence and absence of carbachol. These findings indicate that *Saiboku-to* at least does not stimulate the β-adrenergic receptor and that the potentiation of β-adrenergic function by *Saiboku-to* may include a mechanism other than the inhibition of cyclic AMP phosphodiesterase activity in bronchial smooth muscle. We also found that the *Saiboku-to*-induced synergism in the relaxation produced by theophylline *in vitro* was absent in the presence of L-NAME, an inhibitor of nitric oxide (NO) synthesis, suggesting that the potentiation of *Saiboku-to* may include an NO-dependent mechanism. It has been reported that *Saiboku-to* stimulates the generation of NO in airway epithelial cells.[9] NO released from airway epithelial cells activates soluble guanylate cyclase, which increases cyclic GMP levels in bronchial smooth muscle cells. The accumulation of cyclic AMP and cyclic GMP as a result of the inhibition of phosphodiesterase by theophylline and the accumulation of cyclic GMP through NO released from epithelial cells by *Saiboku-to* may cause the synergistic effect in the relaxation of bronchial smooth muscle. This possibility is supported by the result that the potentiation by *Saiboku-to* was absent in airway epithelium-removed tracheal segments and inhibited by the guanylate cyclase inhibitor ODQ. On the other hand, SRBT did not significantly potentiate the effect of theophylline.

In guinea-pigs under urethane anesthesia, *Saiboku-to* alone immediately caused slight inhibition of the acetylcholine-induced bronchoconstriction, but on the bronchodilatory effect of theophylline the pretreatment with this medicine had little influence. The synergistic effect of *Saiboku-to* found *in vitro* did not reflect the results *in vivo* under the present conditions. To potentiate the effect of theophylline, the dose (1g/kg) of *Saiboku-to* used may be insufficient. Moreover, some pharmacokinetic variations of the active components in *Saiboku-to* may cause the invalidity *in vivo* experiments.

On the other hand, the pretreatment with SRBT potentiated the bronchodilatory effect of theophylline, although SRBT alone had little influence on the acetylcholine-induced bronchoconstriction. SRBT did not significantly potentiate the effect of theophylline *in vitro*, but we found a synergistic effect of theophylline and SRBT. The potentiation by SRBT *in vivo* may not be mediated by the direct action on bronchial smooth muscle but by other actions. Since *Kampo* medicines are blended herbal medicines composed of several crude drugs that may possess complex interactions, it is generally uncertain which crude drugs are responsible for their biological actions. However, both SRBT and *Saiboku-to* consist of ten herbs with some homology. Among them, *Cinnamon bark*, *Oyster shell* and *Longgu* are components of SRBT, but not *Saiboku-to*. Certain components contained in these three crude drugs may help to potentiate the bronchodilatory effect of theophylline *in vivo*. Though the mechanism of the observed effects of SRBT remains unclear, our results indicate that SRBT at least does not inhibit the bronchodilatory effect of theophylline that is a desirable action for the treatment of respiratory disease.

In this study, we examined the effects of *Saiboku-to* and SRBT on the bronchodilatory action of theophylline. *Saiboku-to* potentiated the effect of theophylline *in vitro,* probably through a NO-dependent mechanism, and SRBT potentiated the effect of theophylline *in vivo*. Our previous report indicated the possibility that SRBT reduces the undesirable actions of theophylline on the cardiovascular and central nervous systems,[1] and in this study we confirmed that SRBT did not inhibit the bronchodilatory effect of theophylline. The present results may provide for clinically useful combinations of *Kampo* medicines, *Saiboku-to* and SRBT, in the treatment of chronic respiratory disease with theophylline, which may reduce the therapeutic dose and the side effects of theophylline.

和文抄録

テオフィリンの気管支拡張作用に対する柴朴湯および柴胡加竜骨牡蛎湯の効果を *in vitro* および *in vivo* 実験系で検討した。モルモットより摘出した気管鎖標本において，柴朴湯はテオフィリンの気管支平滑筋弛緩作用を増強させた。この増強作用は，NO 合成阻害剤の L-NAME や guanylate cyclase 阻害剤の ODQ の前処置によって抑制された。また，気管上皮を剥離した標本ではみられなかった。一方，柴胡加竜骨牡蛎湯は，テオフィリンの気管支平滑筋弛緩作用にほとんど影響を与えなかった。ウレタン麻酔下のモルモットにおいて，柴朴湯はテオフィリンの気管支拡張作用にほとんど影響を与えなかっ

たが，柴胡加竜骨牡蛎湯は，テオフィリンの心拍増加作用を軽度に抑制するとともに，テオフィリンの気管支拡張作用を増強させた。本研究において，柴朴湯および柴胡加竜骨牡蛎湯はそれぞれ *in vitro* および *in vivo* 実験系でテオフィリンの効果を増強させた。柴朴湯による増強作用は，柴朴湯が気管上皮からの NO 遊離を促した結果と推察されるが，*in vivo* での柴胡加竜骨牡蛎湯の増強作用は，おそらく異なる作用機序によると考えられる。以前，我々は柴胡加竜骨牡蛎湯がテオフィリンの心拍亢進作用や中枢興奮作用を抑制することを報告した。したがって，柴胡加竜骨牡蛎湯は，閉塞性呼吸器疾患の治療におけるテオフィリンの循環系や中枢系に対する副作用の軽減とともにテオフィリンの投与量の減量に有用であることが示唆された。

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