Anti-allergic effect of Maô-busi-saisin-tô and active principles of *Aliasarum sieboldi*

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Abstract

The anti-allergic effect of Maô-busi-saisin-tô (Ma-Huang-Fu-Zi-Xi-Xin-Tang), a Chinese blended medicine, used for the treatment of bronchitis and bronchial asthma was examined using the Schultz-Dale (SD) reaction in sensitized guinea-pig bronchial muscle. Active components in the anti-allergic effect were found to be Saisin and Maô. Further examination of Saisin revealed that elemicine is one of its active principles.

Key words Chinese blended medicine, Maô-busi-saisin-tô, anti-allergic effect, Schultz-Dale reaction, Saisin, elemicine

Abbreviations HPLC, high-performance liquid chromatography ; Maô-busi-saisin-tô (Ma-Huang-Fu-Zi-Xi-Xin-Tang), 麻黄附子細辛湯 ; SD, Shultz-Dale ; Syô-seiryû-tô (Xiao-Qing-Long-Tang), 小青竜湯 ; TLC, thin layer chromatography

Introduction

Maô-busi-saisin-tô, a Chinese blended medicine appeared in Shôkanron, has been frequently used for the treatment of asthmatic bronchitis, bronchial asthma, and rhinitis. Through a screening examination using Schultz-Dale (SD) reaction on sensitized guinea-pig bronchial muscle, it was found that the extract of Maô-busi-saisin-tô and Saisin and Maô which are prepared in the medicine exhibited an anti-allergic activity.

The experiment, therefore, was undertaken in order to examine the active principles of Saisin. Elemicine which is contained in Saisin was found to possess an anti-allergic effect and its results are described here.

Materials and Methods

Powdered extract of Maô-busi-saisin-tô was obtained from a mixture of Maô (*Ephedra sinica* LINNE, Ephedrae Herba, Chinese, 4 g), Saisin (*Asiasarum sieboldi* F. MAEKAWA, Asiasari Radix, Chinese, 3 g) and Houbusi (*Aconitum carmichaeli* DEBX., Aconiti Tuber, Chinese, 1 g), purchased from the market in Osaka. They were coarsely cut, steeped as prescribed[1] and filtered. The filtrates were freeze-dried after being concentrated under a reduced pressure at below 50 ℃. The powdered extract was found to contain *l*-ephedrine (0.0803%) based on high-performance liquid chromatography (HPLC) analyses.[2] Further in examining the effect of individual herbs in the mixture, powdered 50%-methanol extracts which exhibited similar thin layer chromatographic (TLC) patterns were used because of

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ease of extraction procedure. In brief, individual medicinal herbs were soaked in about 3 volumes of 50% methanol for 3 days at room temperature. After filtration, the solvent was evaporated under a reduced pressure at below 50 ℃, and the remaining filtrates were freeze-dried. In addition, Saisin was soaked in 10 volumes of acetone for 3 days, filtered at room temperature, and the extract obtained after removal of the solvent under a reduced pressure at below 30 ℃. Each powdered extract and the extract were kept in a desiccator until use.

1) Methods of isolation and purification of active principles in Saisin : The acetone extract of Saisin (*Aliasarum sieboldi*) was obtained from 3 kg of Saisin as described above (yield ; 3.12%). The actone extract was fractionated, through column chromatography (eluant ; benzene : acetone = 10 : 1→acetone) using silica gel as absorbents, into 8 fractions (Fr. I ~ Fr. VIII). One of the fractions, which was found to be active in the SD reaction, was further purified in column chromatography (eluant ; benzene : acetone = 5 : 1) and was found to be elemicine, based on various physical data (Figs. 1 and 2).

2) Schultz-Dale (SD) reaction : Hartley guinea-pigs (Kitayama Labes), weighing about 250 g, were administered of egg albumin (Wako Junyaku) intraperitoneal injection (100 mg/kg, i. p.) and intramuscular injection (100 mg/kg, i.m.)

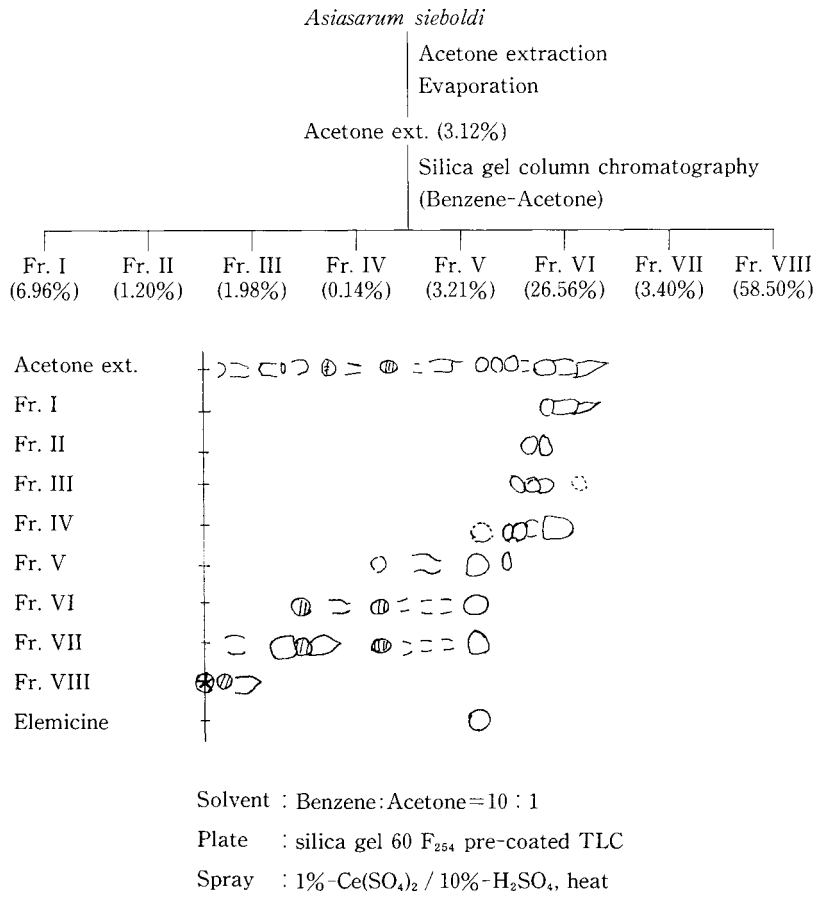

Fig. 1 Flow diagram of fractionation of *Asiasarum sieboldi* acetone ext.

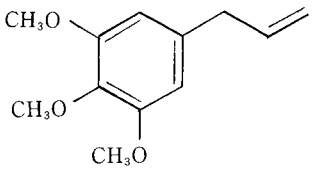

Fig. 2 Chemical structure of elemicine.

and an additional sensitization was applied 3 days thereafter. Experiments were performed 3–4 weeks later. Each guinea-pig was killed by a blow on the back of the head and bled from the carotid artery. The laryngeal area through the chest was cut open and the trachea was exposed. The trachea between the epiglottis and the tracheal branch was isolated. The trachea was cleaned of fat and connective tissues in a petri dish filled with a modified Krebs medium consisting of ; NaCl : 120.4, KCl : 4.8, $MgSO_4$: 1.3, $CaCl_2$: 1.2, KH_2PO_4 : 1.2, $NaHCO_3$: 25.2, glucose : 5.8 (mM). The trachea was cut into rings (about 1.5 mm wide), and 3 rings were tied in series with cotton threads. One side of the tissue preparation was fixed in an organ bath containing a modified Krebs medium at 37 ℃ and was aerated with a 95% O_2-5% CO_2 gas mixture. The other side of the ring was connected by a silk thread to a transducer (Nihon Denki San'ei, 45196A) with an initial loading tension of 1 g. The tissue preparation was rinsed with the medium 30 min thereafter and left for another 30 min for stabilization. The final loading tension was kept at 1 g. After stabilization, histamine HCl (Wako Junyaku) at 10^{-5} M was added to the bath and the contractile response was recorded by a polygraph (Nihon Denki San'ei). The tissue preparation was rinsed with the modified Krebs solution after the contraction reached the maximum level. After stabilization, each drug was added to the bath 10 min prior to an addition of egg albumin (5×10^{-2} mg/ml). Inhibition percentage in SD reaction was calculated as follows :

$$\text{Inhibition } \% = \left(1 - \frac{A'/H'}{A/H}\right) \times 100$$

A : contractile response to egg albumin in the control group

H : contractile response to histamine in the control group

A′ : contractile response to egg albumin in the test group

H′ : contractile response to histamine in test group

3-I) Inhibitory effect of elemicne on the response to histamine : Isolated bronchial muscle of normal guinea-pig was prepared by the same manner as described in Section 2). The inhibitory effect of elemicine, which inhibited SD reaction, was examined on the response to histamine in organ bath.

3-II) The relaxing effect of elemicine in the bronchial muscle : Isolated bronchial muscles were prepared as described in Section 2). Relaxing effects of *dl*-isoproterenol HCl (Sigma) were compared to that of elemicine in organ bath. Tranilast (Rizaben, Kissei Pharmaceutical) was used as a reference drug.

Results

1. SD reaction

1-I) Effects of powdered extracts of Maô-busi-saisin-tô and individual herbs on SD reaction : Powdered extracts of Maô-busi-saisin-tô at 5×10^{-2} mg/ml inhibited SD reaction by 66.9 ±7.0% as shown in Fig. 3. Inhibition of SD reaction by each of the powdered extracts of individual medicinal herbs at 5×10^{-2} mg/ml was 55.6±16.7% for Maô, −2.9±9.9% for Houbusi and 80.2±7.2% for Saisin. Acetone extract of Saisin at 5×10^{-2} mg/ml also inhibited the reaction by 88.5±5.6%. In addition powdered extracts of Maô-busi-saisin-tô and Maô, and the 50%-MeOH extract of Saisin, all at 5×10^{-2} mg/ml, relaxed the sensitized bronchial muscle preparation by 0.12±0.08 g, 0.28±0.10 g, and 0.45±0.16 g, respectively.

1-II) Effects of various fractions of Saisin acetone extract on SD reaction : Inhibitory effects of

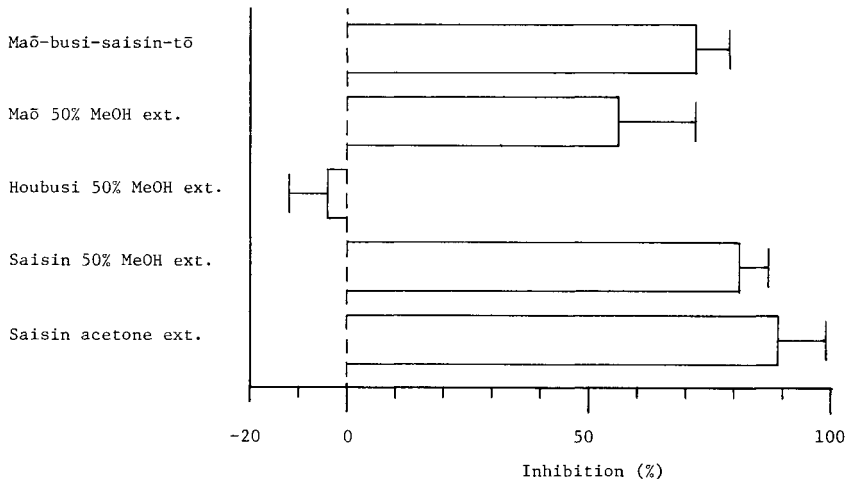

Fig. 3 Effects of Maô-busi-saisin-tô, Maô 50% MeOH ext., Houbusi 50% MeOH ext., Saisin 50% MeOH ext. and Saisin acetone ext. on the Schultz-Dale reaction in the tracheal muscle of guinea-pig which was actively sensitized with egg albumin.

Treatment with the sample was carried out at 10 min prior to challenge with egg albumin. All doses are 5×10^{-2} mg/ml. Each column represents the mean ± S.E. of 6 experiments.

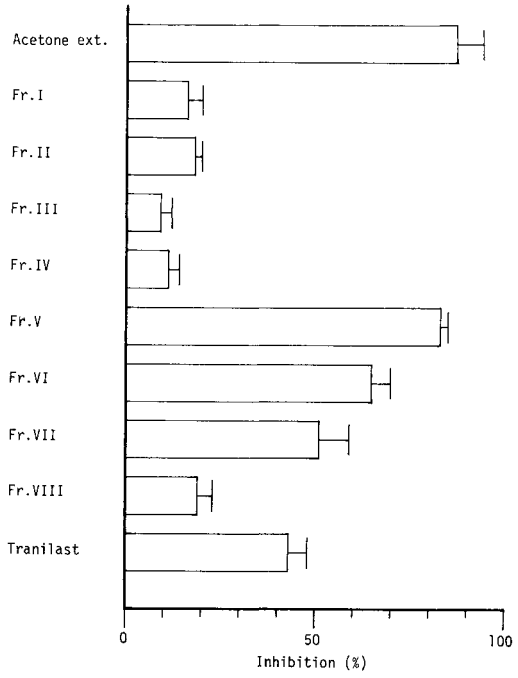

Fig. 4 Effects of fractions from Saisin acetone ext. and tranilast on the Schultz-Dale reaction in the tracheal muscle of guinea-pigs which were actively sensitized with egg albumin.

Treatment was at 10 min prior to challenge with egg albumin. Dose of fractions is 5×10^{-2} mg/ml and tranilast is 10^{-5} M. Each column represents the mean ± S.E. of 6 experiment.

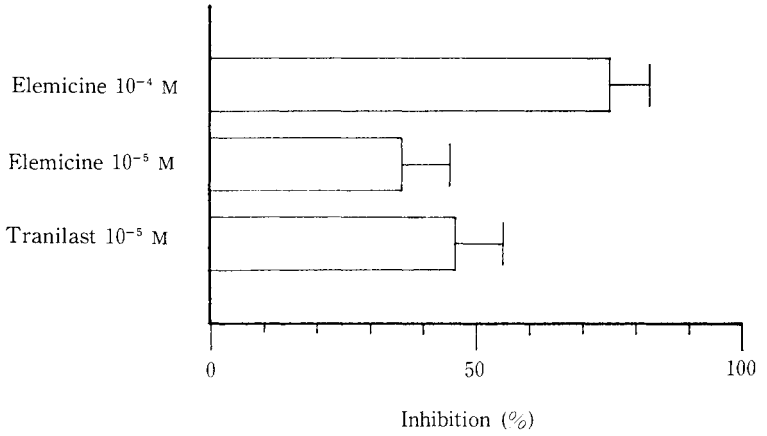

Fig. 5 Effects of elemicine and tranilast on the Schultz-Dale reaction in tracheal muscle of the guinea-pig which was actively sensitized with egg albumin.
Dose of elemicine is 10^{-4} M, 10^{-5} M and tranilast 10^{-5} M. Each column represents the mean ± S.E. of 6 experiments.

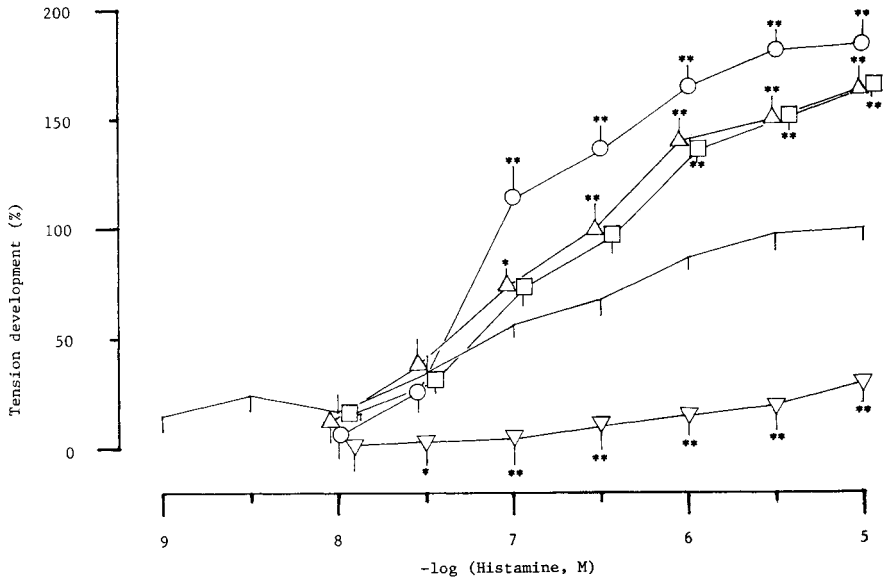

Fig. 6 Effects of elemicine, tranilast and chlorphenilamine on histamine-induced contraction in the guinea-pig tracheal muscle.
——— : Control, —○— : 10^{-4} M Elemicine, —□— : 10^{-5} M Elemicine, —△— : 10^{-5} M Tranilast, —▽— : 10^{-8} M Chlorphenilamine. Each point represents the mean ± S.E. of 6 experiments. Significantly different from control at $*p<0.05$, $**p<0.01$.

each of the fractions of Saisin on SD reaction were shown in Fig. 4. In addition, elemicine, which was purified from Fr. 5～Fr. 7, at 10^{-4} M and 10^{-5} M, and tranilast at 10^{-5} M inhibited the reaction by 65.0±8.56%, 36.1±10.00% and 45.1± 9.80%, respectively. Elemicine at 10^{-4} M and 10^{-5} M and tranilast at 10^{-5} M also relaxed the sensitized bronchial muscle preparation by 0.80± 0.21 g, 0.48±0.12 g and 0.23±0.15 g, respectively (Fig. 5).

2-I) Inhibitory effects of elemicine on the response to histamine : Histamine induced contrac-

tions of guinea-pig bronchial muscle preparations in a concentration-dependent manner. Elemicine at 10^{-4} M and 10^{-5} M and tranilast at 10^{-5} M, each of which was applied to the bath 10 min prior to the histamine application, did not inhibit the response to histamine, on the contrary, each compound significantly increased the contraction of histamine (Fig. 6).

2-II) Relaxing effect of elemicine in the bronchial muscle preparation : As compared to isoproterenol at 10^{-5} M which relaxed guinea-pig bronchial muscle preparations, elemicine at 10^{-4} M and tranilast at 10^{-5} M also caused relaxation of the tissue preparation by 30.0±8.75% and 35.5±8.95%, respectively.

Discussion

Tranilast, is an anti-allergic drug developed from active components of natural products. It is used as a membrane stabilizer to inhibit release of chemical mediators from mast cells due to antigen-antibody reactions.[3] Maô and Saisin are reported to have anti-allergic effects using SD reaction[4] but their active constituents are not yet known. In this report, effectiveness of Maô-busi-saisin-tô, a Chinese blended medicine, was confirmed based on SD reaction and finally elemicine in Saisin was found to be one of the active principles. These results show that 1) elemicine inhibited SD reaction as effectively as tranilast, which was used as a reference drug. 2) elemicine caused relaxation of normal and sensitized bronchial muscles. 3) elemicine did not have any anti-histamine action. Since elemicine had relaxing effects on normal and sensitized bronchial muscles, its mechanism of action will be further examined in the future. Both Syô-seiryû-tô (Xiao-Qing-Long-Tang) and Maô-busi-saisin-tô contain Maô and Saisin as a part of their medicinal mixtures. These Chinese blended medicines may be effective in the treatment of bronchial asthma[5] due to the bronchial dilating action through activation of β-adrenoceptors by *l*-ephedrine contained in Maô and the anti-allergic and bronchial dilating actions of elemicine contained in Saisin. In this experiment, Maô was also found to inhibit SD reaction, and therefore examination of its active principles is currently in progress.

Acknowledgement

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和文抄録

気管支炎や気管支喘息にも応用される漢方方剤麻黄附子細辛湯の抗アレルギー作用を感作したモルモット気管支を用いてShultz-Dale反応により検討した。その結果サイシンとマオウに有効性が認められた。サイシンについてさらに活性成分を検討したところエレミシンが作用成分の1つであることが明らかとなった。

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