Effects of Syô-saiko-tô (Xiao-Chai-Hu-Tang), Dai-saiko-tô (Da-Chai-Hu-Tang) and Saiko-ka-ryûkotu-borei-tô (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang) on degranulation of and release of histamine from mouse peritoneal mast cells induced by compound 48/80

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Abstract

The effects of Syô-saiko-tô (Xiao-Chai-Hu-Tang), Dai-saiko-tô (Da-Chai-Hu-Tang) and Saiko-ka-ryûkoku-borei-tô (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang) inhibited on degranulation of and release of histamine from mouse peritoneal mast cells induced by compound 48/80 were investigated. Inhibitory effects of Syô-saiko-tô and Dai-saiko-tô were found to be strong both on degranulation of and release of histamine from mouse peritoneal mast cells. But Saiko-ka-ryûkotu-borei-tô was found to have few inhibitory effects. Inhibitory effects of Syô-saiko-tô and Dai-saiko-tô were comparable to disodium cromoglycate that is a known inhibitor to degranulation of and release of histamine from mast cells. Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus and Zingiberis Rhizoma are common herbs in Syô-saiko-tô, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô. Saiko-ka-ryûkotu-borei-tô contains Fossilia Ossis Mastodi and Ostreae Testa that have calcium salts. From these results, it is suggested that Bupleuri Radix, Pinelliae Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus and Zingiberis Rhizoma may have inhibitory effects on degranulation of and release from mouse peritoneal mast cells. Few inhibitory effects of Saiko-ka-ryûkotu-borei-tô may be caused by calcium salts contained in Fossilia Ossis Mastodi and Ostreae Testa.

Key words compound 48/80, Dai-saiko-tô, degranulation, disodium cromoglycate, histamine, mast cell, Saiko-ka-ryûkotu-borei-tô, Syô-saiko-tô

Abbreviations Co 48/80, compound 48/80 ; DSCG, disodium cromoglycate ; Dai-saiko-tô (Da-Chai-Hu-Tang), 大柴胡湯 ; Saiko-ka-ryûkotu-borei-tô (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang), 柴胡加竜骨牡蠣湯 ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯

Introduction

Syô-saiko-tô (Xiao-Chai-Hu-Tang) was found to have inhibitory effects on degranulation in mouse peritoneal mast cells induced by compound 48/80 (Co 48/80) morphologically.[1] Such effects appeared with other saiko-agents ; Saiko-keisi-tô (Chai-Hu-Gui-Zhi-Tang), Saikan-tô (Chai-Xian-Tang), Dai-saiko-tô (Da-Chai-Hu-Tang), and Zyû-mi-haidoku-tô (Shi-Wei-Bai-Du-Tang). But Saiko-ka-ryûkotu-borei-tô (Chai-Hu-Jia-Long-Gu-Mu-Li-Tang) could not have such effects.[2]

Syô-saiko-to, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô have Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus and Zingiberis Rhizoma as constitutional herbs. In this study, we investigated the differences of inhibitory effects on degranulation of and release of histamine from mouse peritoneal mast cells induced by Co 48/80.

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Materials and Methods

Animals : Male ddY mice weighing 25−30 g were purchased from Shizuoka Laboratory Animal Center. They were housed in an animal room at room temperature ; about 25℃, humidity : about 60% relative humidity.

Materials : Table I shows the constitutional herbs in Syô-saiko-tô, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô. The extracts of saiko-agents were obtained by spray-drying the water-extracts from Tsumura Juntendo Co., Ltd. These herbs are drugs listed in Pharmacopeia of Japan No.11. The solution of these extracts was prepared to contain 30, 10, 5 and 1 mg/ml with Tyrode's solution. The solution of disodium cromoglycate (DSCG, Fujisawa-Fisons Co.), that is an inhibitor to degranulation of and release of histamine from mast cells, was prepared to contain 30, 10, 5 and 1 mg/ml with Tyrode's solution. The solution of Co 48/80 (Sigma Chemical Co.) was prepared to contain 50 or 10 μg/ml with Tyrode's solution. One hundred percent degranulation in peritoneal mast cells was induced by 50 μg/ml solution of Co 48/80 on assay system described below. A 48.8±3.6% release of histamine from peritoneal mast cells was induced by 10 μg/ml solution of Co 48/80 on assay system described below. Other reagents were of special grade.

Preparation of mast cells : Mast cells were isolated from the peritoneal cavity of mice using the modified method of Uvänes *et al*.[3] Suspension of mast cells was prepared to contain 10^6 cells/ml with Tyrode's solution.

Morphological assay on degranulation of mouse peritoneal mast cells : The assay was followed by the method reported by Toda *et al*.[2] 0.2 ml of solution containing 30, 10 and 5 mg/ml of saiko-agent or DSCG was added to 1.6 ml of suspension of mast cells. To the mixture incubated for 10 min was added 0.2 ml of solution containing 50 μg/ml of Co 48/80. The mixture was also incubated for 10 min at 37 ℃. The smears of this mixture were fixed in methanol and stained with toluidine blue. The cells of about 100 stained mast cells were examined in light microscopy and the distributions of mast cells were classfied into four groups (group I : normal, group II : appearance in formation of space at periphery of granule and little degranulation, group III : appearance in formation of vacuole and marked degranulation, group IV : destruction of mast cell membrane) according to method of

Table I Constitutional herbs in Syô-saiko-tô, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô.

Herbs	Crude drugs tested (g)		
	Syô-saiko-tô	Dai-saiko-tô	Saiko-ka-ryû-kotu-borei-tô
Bupleuri Radix	7.0	6.0	5.0
Pinelliae Tuber	5.0	4.0	4.0
Scutellariae Radix	3.0	3.0	2.5
Zizyphi Fructus	3.0	3.0	2.5
Zingiberis Rhizoma	1.0	1.0	1.0
Amoni Semen		3.0	
Aurantii Fructus Immaturus		2.0	
Rhei Rhizoma		1.0	2.5
Ginseng Radix	3.0		
Glycyrrhizae Radix	2.0		
Cinnamomi Cortex			3.0
Holen			3.0
Fossilia Ossis Mastodi			2.5
Ostreae Testa			2.5

Herbs are drugs listed in the Pharmacopeia of Japan.

Sakamoto *et al*.[4]

Assay on release of histamine from mouse peritoneal mast cells : 0.2 ml of solution containing 10, 5 and 1 mg/ml of saiko-agent or DSCG was added to 1.6 ml of suspension of mast cells. The mixture was incubated for 10 min at 37 ℃. To the mixture incubated for 10 min was also added solution containing 10 μg/ml of Co 48/80. The mixture was also incubated for 10 min at 37 ℃. After incubation, the mixture was centrifuged at 1400×g for 5 min at 4 ℃. Histamine in supernatant solution was determined by the method of Shore *et al*.[5] Total histamine in the mixture had been determined by the method of Shore *et al*. after the mixture was boiled for 10 min. The release of histamine from peritoneal mast cells was represented by the ratio of total histamine in mixture to the content in supernatant solution. Values are expressed as the mean± standard errors of 4 experiments. Statistical analysis depended on the use of t-test.

Results and Discussion

As shown in Table II, 100 degranulation of mast cells was observed by Co 48/80, inducer of degranulation, when no saiko-agents were added. Syô-saiko-tô, Dai-saiko-tô and DSCG were found to have inhibitory effects on degranulation even in lower concentration. The inhibitory effects of 10 mg/ml of Syô-saiko-tô on degranulation were classified according to their grade of morphological changes ; group I : 18%, group II : 25 %, group III : 47%, and group IV : 10%. The inhibitory effects of 10 mg/ml of Dai-saiko-tô were classified as follows ; group I : 17%, group II : 23%, group III : 25%, and group IV : 35%. Such effects were comparable to that of DSCG. Little effect on the inhibition of degranulation was demonstrated by 30 mg/ml solution of Saiko-ka-ryûkotsu-borei-tô as shown in Table II.

As shown in Table III, 48.8±3.6% of release from mouse peritoneal mast cells was found in absence of saiko-agents. As 19.4±1.5% of release of histamine from mouse peritoneal mast cells was found on addition of 10 mg/ml of Syô-saiko-tô and 20.3±1.7% of that of Dai-saiko-tô, both saiko-agents were shown to have inhibitory effects on release of histamine from mouse peritoneal mast cells. Both inhibitory effects were comparable to that of DSCG. But release of histamine from mouse peritoneal mast cells on addition of 5 and 1 mg/ml of Syô-saiko-tô or Dai-saiko-tô was similar to that in the absence of saiko-agents. As release of histamine from mouse peritoneal mast cells on addition of 10, 5 and 1 mg/ml of Saiko-ka-ryûkotu-borei-tô was similar to that in the absence of saiko-agents, this showed to have few inhibitory effects on release of histamine from mouse peritoneal mast cells.

Syô-saiko-tô and Dai-saiko-tô, that were found to be inhibitors on the degranulation of and release of histamine from mouse peritoneal mast cells, contained Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus and Zingiberis Rhizoma as common herbs. As their herbs found to have inhibitory effects on allergy, Syô-saiko-tô and Dai-saiko-tô may have such inhibitory effects.[6] Other herbs except such common herbs are Ginseng Radix and Glycyrrhizae Radix in Syô-saiko-tô, and Amoni Semen, Aurantii Fructus Immaturus and Rhei Rhizoma in Dai-saiko-to. The inhibitory effects of water extracts of Ginseng Radix and Glycyrrhizae Radix were found to be weak on the degranulation of mouse peritoneal mast cells.[2] The inhibtory ratio of group I on addition of 30 mg/ml of Syô-saiko-tô was less than that of Dai-saiko-tô on the degranulation of mouse peritoneal mast cells in Table II. Such a result may depend on inhibitory effects of water extracts of Ginseng Radix and Glycyrrhizae Radix that are contained in Syô-saiko-tô. Fossilia Ossis Mastodi and Ostreae Testa in Saiko-ka-ryûkotu-borei-tô contain calcium salts in quantity. It was found that the release from rat peritoneal mast cells induced by Co 48/80 with Ca^{++} is inhibited by ethylenediaminetetraacetic acid.[7] It was reported that Co 48/80 has several oligomers, which differ in the Ca^{++}-dependent histamine releaser and Ca^{++}-independent histamine releaser.[8] So few inhibitory effects of Saiko-ka-ryûkotu-borei-tô depend on the interaction between calcium salts in Fossilia Ossis Mastodi and Ostreae Testa, and a com-

Table II Inhibitory effects of Syô-saiko-tô, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô on degranulation in mouse peritoneal mast cells induced by compound 48/80.

Test drugs		Group (%)			
		I	II	III	IV
None		88	12	0	0
Compound 48/80 (50 μg/ml)		0	0	54	46
Compound 48/80 (50 μg/ml)+Syô-saiko-tô	(30 mg/ml)	25	38	17	20
	(10 mg/ml)	18	25	47	10
	(5 mg/ml)	0	0	44	56
Compound 48/80 (50 μg/ml)+Dai-saiko-tô	(30 mg/ml)	48	36	15	1
	(10 mg/ml)	17	23	25	35
	(5 mg/ml)	5	11	37	47
Compound 48/80 (50 μg/ml)+Saiko-ka-ryûkotu-borei-tô	(30 mg/ml)	2	25	42	31
	(10 mg/ml)	0	14	38	48
	(5 mg/ml)	0	0	35	65
Compound 48/80 (50 μg/ml)+Disodium cromoglycate	(30 mg/ml)	64	33	3	0
	(10 mg/ml)	27	56	15	2
	(5 mg/ml)	25	57	15	3

Group I : normal.
Group II : appearance in formation of space at periphery of granule and little degranulation.
Group III : appearance in formation of vacuole and marked degranulation.
Group IV : destruction of mast cell membrane.
Various experimental group : about 100 mast cells.

Table III Inhibitory effects of Syô-saiko-to, Dai-saiko-tô and Saiko-ka-ryûkotu-borei-tô on release of histamine from mouse peritoneal mast cells induced by compound 48/80.

Test drugs		Histamine release (%)	
None		1.9±0.1	
Compound 48/80 (10 μg/ml)		48.8±3.6	
Compound 48/80 (10 μg/ml)+Syô-saiko-tô	(10 mg/ml)	19.4±1.5	a)
	(5 mg/ml)	28.6±2.3	b)
	(1 mg/ml)	34.1±0.9	N.S.
Compound 48/80 (10 μg/ml)+Dai-saiko-tô	(10 mg/ml)	20.3±1.7	a)
	(5 mg/ml)	30.9±0.9	N.S.
	(1 mg/ml)	33.6±0.4	N.S.
Compound 48/80 (10 μg/ml)+Saiko-ka-ryûkotu-borei-tô	(10 mg/ml)	36.2±1.1	N.S.
	(5 mg/ml)	35.6±1.1	N.S.
	(1 mg/ml)	39.9±0.4	N.S.
Compound 48/80 (10 μg/ml)+Disodium cromoglycate	(10 mg/ml)	17.2±2.1	a)
	(5 mg/ml)	18.2±0.9	a)
	(1 mg/ml)	22.8±1.3	b)

Values are expressed as the Mean±S.E. of 4 experiments.
a) : $p<0.01$, b) : $p<0.05$, N.S. : not significant.

ponent of Co 48/80 that is Ca^{++}-independent histamine releaser.

Saiko-agents were found to differ in their inhibtory actions on degranulation of and release of histamine from mouse peritoneal mast cells. Such differences may suggest a dependence on constituents of herbs in saiko-agents. The conformed experiments of interactions between such herbs and Ca^{++} are now progressing.

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和文抄録

小柴胡湯，大柴胡湯，柴胡加竜骨牡蠣湯の compound 48/80 によるマウス腹腔内肥満細胞からのヒスタミン遊離及び脱顆粒に対する作用について検討した。小柴胡湯，大柴胡湯は，肥満細胞からのヒスタミンの遊離及び脱顆粒を強く抑制した。柴胡加竜骨牡蠣湯のヒスタミン遊離及び脱顆粒に対する抑制は，弱かった。小柴胡湯，大柴胡湯のそれらの作用は，ヒスタミン遊離及び脱顆粒抑制剤である disodium cromoglycate に匹敵するものであった。これら三種の柴胡剤の構成生薬の内，柴胡，大棗，生姜，黄芩，半夏が共通している。小柴胡湯，大柴胡湯のそのような強い抑制作用は，これらの生薬によるものと思われる。柴胡加竜骨牡蠣湯にはカルシウム塩を多量に含有している竜骨や牡蠣が含まれていることから，この抑制作用が弱いのは竜骨や牡蠣によるものと思われる。

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