Effects of Bakumondô-tô (Mai-Meu-Dong-Tang) on histamine release from and degranulation of mouse peritoneal mast cells induced by compound 48/80

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Abstract

The effects of Bakumondô-tô (Mai-Meu-Dong-Tang) were found to inhibit histamine release from and degranulation of mouse peritoneal mast cells induced by compound 48/80. This traditional Chinese medicine might be useful as the inhibitor of histamine release from and degranulation of mast cells.

Key words Bakumondô-tô, compound 48/80, degranulation, disodium cromoglycate, histamine, mast cell.

Abbreviations Co 48/80, compound 48/80 ; DSCG, disodium cromoglycate ; Bakumondô-tô (Mai-Meu-Dong-Tang), 麦門冬湯.

Introduction

In our previous reports,[1,2] we demonstrated that Syô-saiko-tô (Xiao-Chai-Hu-Tang) and Dai-saiko-tô (Da-Chai-Hu-Tang) inhibited histamine release from and degranulation of mouse peritoneal mast cells induced by compound 48/80 (Co 48/80). Saiboku-tô (Chai-Pu-Tang) and Syô-seiryu-tô (Xiao-Qing-Long-Tang), which are Saiko agents, show inhibitory activities on type I allergic reaction.[3,4] Several other Saiko agents also exhibit inhibitory effects on degranulation of mouse peritoneal mast cells induced by Co 48/80.[5]

In the present study, we tried to research the effects of Bakumondô-tô (Mai-Meu-Dong-Tang), which has been used for the treatment of bronchitis or pharyngitis,[6] on histamine release from and degranulation of mouse peritoneal mast cells induced by Co 48/80.

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 : The extract of Bakumondô-tô was obtained from Tsumura Juntendo Inc. This was a spray-drying material prepared from the coextract of Ophiopogonis Tuber, Pinelliae Tuber, Zizyphi Fructus, Glycyrrhizae Radix, Ginseng Radix and Oryzae Fructus. The extract of Bakumondô-tô was dissolved in Tyrode's solution in concentrations 0.01－10 mg/ml. Disodium cromoglycate (DSCG, Fujisawa-Fisons Co.), an inhibitor of histamine release from and degranulation of mast cells, was also dissolved in Tyrode's solution. Co 48/80 (Sigma Chemical Co.) was dissolved in Tyrode's solution in a concentration of 10 μg/ml. Other reagents were guaranteed grade.

Preparation of mast cells : Mast cells were isolated from the peritoneal cavity of mice using the modified method of Uvänes *et al.*, and suspended in Tyrode's solution to make a cell suspension of 10^6 cells/ml.

Assay of histamine release and degranula-

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tion : 0.2 ml of Bakumondô-tô or DSCG solution was added to 1.6 ml of mast cell suspension. After incubation for 10 min at 37℃, 0.2 ml of Co 48/80 solution was added and further incubated for 10 min.

Microscopic observation of degranulation of mouse peritoneal mast cells : The mast cells were fixed in methanol after incubation with Co 48/80 in the presence or absence of the drugs and stained with toluidine-blue. The degranulated cells were counted in about 100 stained cells by light microscopy.

Electron microscopic observation of degranulation of mouse peritoneal mast cells : The incubated mixture was centrifuged at 1400× *g* for 5 min at 4℃. The cell pellets were fixed in 1% glutaraldehyde at 4℃ for 1 hr and centrifuged at 200× *g* for 10 min. After washing the precipitate with 0.1% cacodylate buffer (pH 7.2), this was fixed again in 2% osmium tetroxide in cacodylate buffer (pH 7.2) for 1 hr at 4℃. After dehydration by cold ethanols the mixture treated with propylene oxide and embedded in Quetanol 812. Ultrathin section was made from the fixed cells by using Porter Blum MT-11B Ultramicrotome, and stained with uranyl acetate and lead citrate for the observation with a JOEL 1200 EX electron microscopy at 80 KV.

Determination on histamine release from mouse peritoneal mast cells : The incubated mixture was centrifuged at 1400× *g* for 5 min at 4℃. Histamine in supernatant solution was determined by the method of Shore *et al*.[8] Total histamine in the mixture was determined by the same method after boiling the mixture for 10 min. The amount of released histamine was indicated as a percent against the total amount. Values are expressed as the mean ± standard error (S.E.) of 4 experiments. Statistical analysis was carried out using two-tailed Student's *t*-test.

Results and Discussion

Table I gives the results of microscopic observation of degranulation of mouse peritoneal mast cells. As shown in Table I, though Co 48/80 caused a perfect degranulation, the spontaneous degranulation was only 6%. Percent degranulation was decreased to 49% and 34% by the incubation with 1 mg/ml of Bakumondô-tô and DSCG, respectively. These results indicated that both Bakumondô-tô and DSCG exhibited a significant inhibitory effect on degranulation.

Table I Inhibitory effect of Bakumondô-tô on degranulation on mouse peritoneal mast cells induced by compound 48/80.

Test drugs		Degranulation (%)
Spontaneous		6
Control		100
Bakumondô-tô	0.001 mg/ml	81
	0.01 mg/ml	70
	0.1 mg/ml	60
	1 mg/ml	49
DSCG	0.001 mg/ml	76
	0.01 mg/ml	71
	0.1 mg/ml	47
	1 mg/ml	34

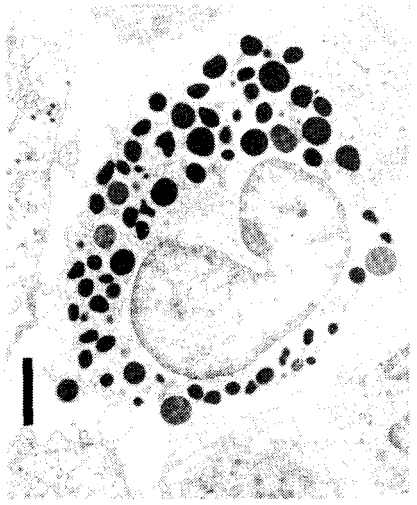

Fig. 1 Electromicrograph of mast cell treated with 1 μg/ml compound 48/80. (Bar : 2 μm, × 2500)

Figs. 1 and 2 give results of electron microscopic observations. Fig. 1 shows a typical picture of mast cell degranulation caused by Co 48/80. Majority of mast cell granules decreased in electron density and their limiting membrane disappeared. Some granules were spread out of the plasma membrane. The individual granules va-

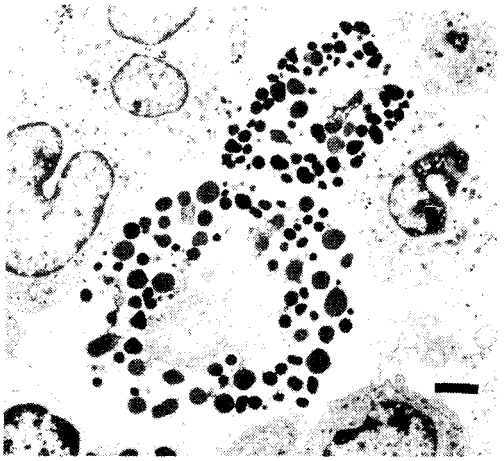

Fig. 2 Electromicrograph of mast cell treated with 1 mg/ml Bakumondô-tô and 1 μg/ml compound 48/80. (Bar : 2 μm, ×4000)

Table II Inhibitory effect of Bakumondô-tô on histamine release from mouse peritoneal mast cells induced by compound 48/80.

Test drug		Histamine release (%)
Spontaneous		4.2±0.9
Control		56.8±4.0
Bakumondô-tô	0.001 mg/ml	56.3±1.5
	0.01 mg/ml	48.5±4.4
	0.1 mg/ml	47.5±5.3
	1 mg/ml	46.0±1.7[a)]
DSCG	0.001 mg/ml	55.7±4.0
	0.01 mg/ml	53.3±1.5
	0.1 mg/ml	44.0±2.4[a)]
	1 mg/ml	43.5±1.0[a)]

Values are expressed as the Mean±S.E. of 4 experiments. a) : $p<0.05$.
Total histamine : 80.6±0.8 ng/ml.

ried in size. As shown in Fig. 2, the Co 48/80-induced degranulation (Fig. 1) was effectively inhibited by the incubation with 1 mg/ml of Bakumondô-tô. These pictures demonstrated the inhibitory effect of Bakumondô-tô on mast cell degranulation morphologically. The individual mast cell granules were high electron density and surrounded by limiting membrane. The entire surface plasma membrane displayed intact morphology. Thus, the inhibitory effect of Bakumondô-tô was also confirmed by an electron microscopic study.

As shown in Table II, 56.8±4.0% of histamine was released from mouse peritoneal mast cells by addition of 10 μg/ml of Co 48/80 alone. The spontaneous histamine release was 4.2±0.9%. Histamine release induced by Co 48/80 reduced to 46.0±0.9% by addition of 10 mg/ml of Bakumondô-tô, indicating that this drug extract has an inhibitory effect on histamine release, although the inhibitory effect of Bakumondô-tô was slightly weaker than that of DSCG.

Bakumondô-tô has been used for the treatment of bronchitis of pharyngitis. However, the mechanism of action of this drug has not been revealed. Pinelliae Tuber and Zizyphi Fructus in the constituents of Syô-saiko-tô were found to have inhibitory effects on Co 48/80-induced histamine release from and degranulation of mouse peritoneal mast cells as reported previously. Since Pinelliae Tuber and Zizyphi Fructus are also contained in Bakumondô-tô, it is possible that the effect of Bakumondô-tô on mast cells may be attributable to them. However, the effects of other herbs remain to be elucidated.

As Bakumondô-tô has been used as an exceptorant, it may act on mucosa of repiratory tract. Respiratory tract has mucosal mast cells in its mucosa. Mucosal mast cells in mucosa of respiratory tract do not respond significantly to Co 48/80 stimulation and induce little histamine release.[10] However, it is not certain whether Bakumondô-tô is effective for mucosal mast cells or not, because mouse peritoneal mast cells used in the present study are connective tissue type.

As histamine release from mast cells by Co 48/80 occurs in absence of Ca^{2+}, it is not similar to Ca^{2+}-dependent antigen-induced histamine release.[11] As indicated here, however, Bakumondô-tô exhibits an inhibitory effect on degranulation and histamine release similar to DSCG. Therefore, this traditional Chinese medicine might be useful for the treatment of type I allergic disease.

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和文抄録

麦門冬湯の compound 48/80 によるマウス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。麦門冬湯は，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。

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