

Effects of traditional herbal medicine and anti-allergic agents on cell growth and histamine content in human basophilic leukemia cell line KU 812F

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Abstract

Our previous study demonstrated the inhibitory effects of Sho-seiryu-to (T19), Saiboku-to (T96), Seihai-to (T90), baicalein and ketotifen on the growth of human basophils derived from umbilical cord bloods. In this study, we examined the effects of these traditional herbal medicines and various other anti-allergic agents on the proliferation and the differentiation of human basophilic leukemia cell line KU 812F.

The effects of these agents were evaluated by alcian blue-safranin staining and spectrofluorometric assay of histamine content using 4-day cultures of KU 812F cells with the highest differentiation rate. A suppressive effect on the growth of KU 812F at a concentration of 10^{-4} – 10 mg/ml was found, the order of potency being $T96=T19 > Bakumondo-to (T29)=T90$. Saikosaponins Sa, Sd, baicalein, azelastine, anlexanox, and disodium cromoglycate also suppressed growth at $1\sim 10\ \mu\text{M}$, while saikosaponin Sc, ginsenosides Rb1 and Rg1, ketotifen and tranilast did not show any effects even at $10\ \mu\text{M}$. The suppressive effect on histamine content caused by these agents was more remarkable when compared using only alcian blue positive cells rather than total cells, suggesting a selective inhibition.

These results suggest that traditional herbal drugs may affect allergic diseases by suppressing the growth and the differentiation of basophils/mast cells.

Key words Kampo medicine, basophilic leukemia cell, anti-allergic drug.

Abbreviations DSCG, disodium cromoglycate ; FCS, fetal calf serum ; T19, Sho-seiryu-to (Xiao-Qing-Long-Tang), 小青竜湯 ; T29, Bakumondo-to (Mai-Men-Dong-Tang), 麥門冬湯 ; T90, Seihai-to (Qing-Fei-Tang), 清肺湯 ; T96, Saiboku-to (Chai-Pu-Tang), 柴朴湯.

Introduction

It is known that in allergic diseases, the count of basophilic leukocytes¹⁾ and their precursors²⁾ in the peripheral blood is elevated. Therefore, an important therapy for allergic diseases seems to be the control of the proliferation of excessive basophilic leukocytes or mast cells. However, there are no reports on the study of any such agents in the literature. We previously reported that herbal remedies, crude drug prepara-

tions, and anti-allergic agents inhibited the *in vitro* proliferation of basophilic leukocytes derived from human umbilical cord blood.³⁾

To obtain a better understanding of this we examined the effects of these agents on the *in vitro* proliferation and the differentiation of human basophilic cell line KU 812F cells, which has the capability of differentiating mature basophil-like cells under serum-free conditions.⁴⁾ Valent *et al.* reported that human interleukin-3 induced a significant increase in formation of metachromatically granules and the

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histamine content through a high affinity receptor of this cell line.⁵⁾ Furthermore, when this cell line was stimulated by phorbol myristate, the activity of histidine decarboxylase, a histamine-forming enzyme, increased 1.8-fold, resulting in the increase of the histamine content as a matured cell.⁶⁾

Materials and Methods

Cell cultures: We used the KU 812F cell line provided by Dr. Fukuda of the Department of Pathology, Niigata University. These cells have the ability to differentiate into basophilic-like cells and to increase the content of intracellular histamine in a serum-free culture.⁴⁾ KU 812F cells were cultured in a RPMI 1640 (Grand Island Biological Co., Grand Island, NY) with 10 % fetal calf serum (FCS; Flow Laboratories, McLean, VA), resuspended in a serum-free RPMI 1640 to a concentration of $1-3 \times 10^6$ cells/ml, and then cultured in microplates (Coster, Cambridge, MA) containing 12 or 24 wells.^{6,7)}

Various agents were added in three 10-fold dilutions to this culture.³⁾ The agents used in this study were Syo-seiryu-to (Xiao-Qing-Long-Tang, T19), Bakumondo-to (Mai-Men-Dong-Tang, T29), Seihai-to (Qing-Fei-Tang, T90), Saiboku-to (Chai-Pu-Tang, T96) extract powder (Tsumura Co., Tokyo, Japan), baicalein, saikosaponin, Sa, Sc, Sd, ginsenosides Rb1, Rg1 (Wako Pure Chemical Industry Co., Tokyo, Japan), azelastin (Esai Pharmaceutical Co., Tokyo, Japan), anlexanox (Takeda Pharmaceutical Co., Tokyo, Japan), disodium cromoglycate (DSCG, Fujisawa Pharmaceutical Co., Tokyo, Japan), ketotifen (Sandoz Pharmaceutical Co., Switzerland), tranilast (Kissei Pharmaceutical Co., Tokyo, Japan).

Cell Morphology: Cell counts and viabilities were determined using 0.2 % trypan blue dye exclusion. Basophilic cells were identified by cytochemical staining with Wright-Giemsa or alcian blue-safranin as described previously.^{3,8)} Alcian blue is a specific basophil stain because this cationic dye can bind to the sulfate groups of basophil granules.⁹⁾ Briefly, air-dry smears were prepared by cytocentrifuge (Shandon Co., England), fixed with Carnoy's fluid and stained with 1 % alcian blue in 0.5 M HCl before counterstaining with 0.5 % safranin O in 0.125 M HCl. The number

of basophilic cells in each culture was determined by counting more than 200 cells per smear.

Histamine assay: A histamine assay was performed using automated spectrofluorometric methods. After adding 6 % perchloric acid to the cell suspensions, the histamine contents of separated supernatants were determined using an autoanalyser (Technique; Tokyo, Japan).

Results

The effects of various agents were compared using 4-day cultures which have been shown to have the highest differentiation rate.

As shown in Fig. 1, a suppressive effect on the cell growth of KU 812F was found at a concentration of 10^{-4} – 10 mg/ml in aqueous extracts. The order of potency being $T96 = T19 > T29 = T90$. Baicalein and the saikosaponins Sa and Sd, the active compounds of the herbal drug preparation, suppressed the growth of KU

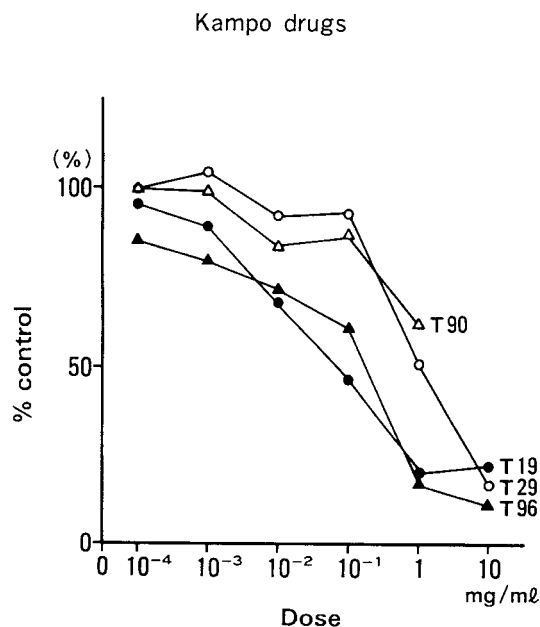


Fig. 1 Effects of traditional herbal medicines on the growth of basophilic leukemia cell line KU 812F. Cells were incubated for 4 days with or without drugs at a concentration of $0.1 \mu\text{g} \sim 10 \text{ mg/ml}$ of Sho-seiryu-to (T19), Bakumondo-to (T29), Seihai-to (T90) and Saiboku-to (T96). The ordinate indicates % of control cell number that was cultured without drugs for 4 days.

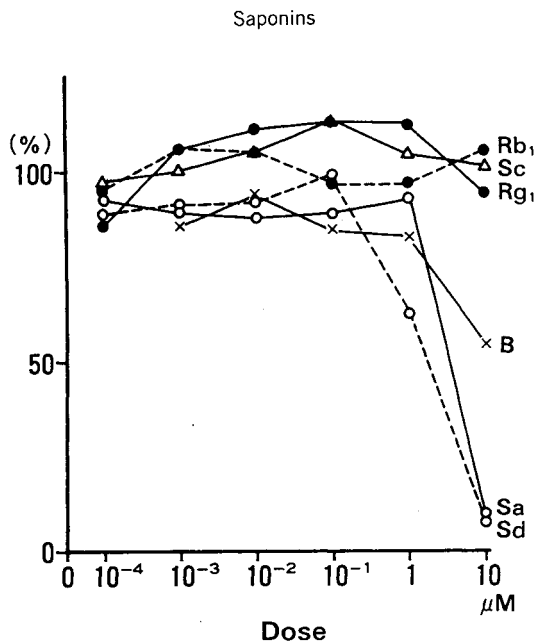


Fig. 2 Effects of saponins on the growth of basophilic leukemia cell line KU 812F. Cells were incubated for 4 days with or without drugs at a concentration of $0.1 \sim 10 \mu\text{M}$ of ginsenosides Rb₁ and Rg₁, saikosaponins Sa, Sc and Sd, and baicalein (B).

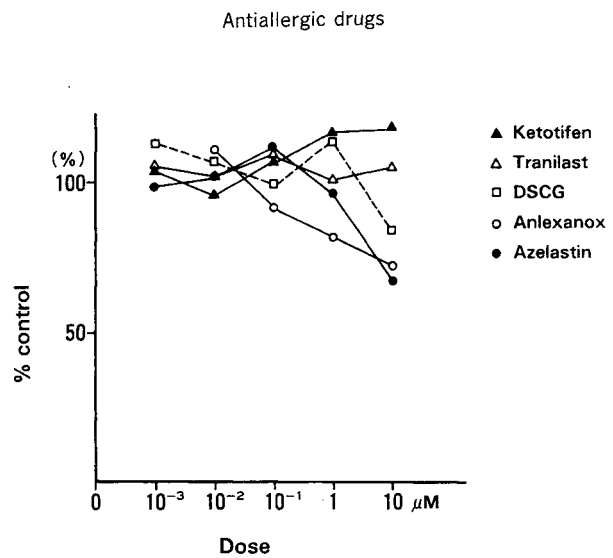


Fig. 3 Effects of anti-allergic drugs on the growth of basophilic leukemia cell line KU 812F. Cells were incubated for 4 days with or without drugs at a concentration of $0.1 \sim 10 \mu\text{M}$ of ketotifen, tranilast, disodium cromoglycate (DSCG), anlexanox and azelastin.

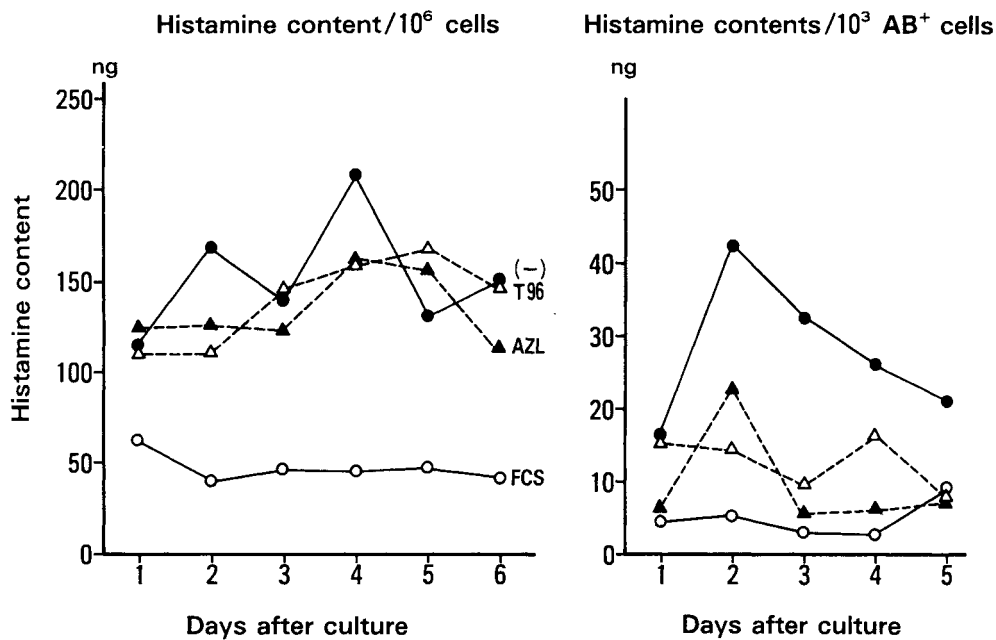


Fig. 4 Effects of anti-allergic drugs on the time course of histamine content/ 10^6 cells (A) and histamine content/ 10^3 alcian blue (AB) positive cells of KU 812F cultured with or without fetal calf serum (FCS, 10%), azelastin (AZL, $10 \mu\text{M}$) or Saiboku-to (T96, 1 mg/ml). Histamine was measured by spectrofluorometric assay.

812F at 1–10 μM , but saikosaponin Sc and ginsenosides Rb1, Rg1, did not show effective results, even at 10 μM (Fig. 2). Among the anti-allergic agents, azelastine, anlexanox and DSCG showed suppressive effects on the cell growth at 1–10 μM , but ketotifen and tranilase did not show any effects, even at 10 μM (Fig. 3).

Fig. 4 shows the time course of the effectiveness of these drugs on the histamine content of KU 812F. When the histamine content was determined using total cells (10^6), the effectiveness of these drugs was unremarkable although there was a significant difference between serum-containing cultures and serum-free cultures. In contrast, when the histamine content of just alcian blue positive cells was determined (B), T96 and azelastin had a remarkable suppressive effect.

Discussion

In this study, it was demonstrated that herbal or crude drug preparations with anti-allergic action inhibited the proliferation and the differentiation of basophilic leukemia cell line KU 812F. The most potent action was shown by herbal and crude drugs such as Saiboku-to and Sho-seiryu-to, which are often administered to patients with bronchial asthma for prophylaxis. These results were similar to those from a previous experiment which used human umbilical cord blood.³⁾ While comparable suppression was caused by the addition of anlexanox and azelastin as anti-allergic agents, no action was recognized for ketotifen and tranilast, and DSCG showed a slight action. Since ketotifen had a suppressive effect in a previous experiment utilizing human umbilical cord blood, it is necessary to investigate whether this discrepancy was due to differences between normal cells and leukemic cells or to the heterogeneity of the basophil/mast cell lineage.

As for the effects of the components of crude drugs that we examined in this study, suppressive actions by saikosaponins Sa, Sd and baicalein were recognized but no action was seen in the case of saikosaponin Sc and ginsenosides Rb1 and Rg1. These suppressive effects seem not due to cytotoxic effects because neither 2 mg/ml of T90 nor 100 μM of

baicalein affected the viability and lactate dehydrogenase releasing of human alveolar macrophages.¹⁰⁾

From these limited results it can be assumed that the suppressive effect of herbal or crude drug preparation results in part from the presence of Bupleuri Radix or Scutellariae Radix which contains saikosaponins or baicalein, respectively. Various saponins other than Rb1 and Rg1, however, were identified in ginsenosides, and Rh2 is reported to have an action that inhibits the proliferation and differentiation of melanoma cells.¹¹⁾ Therefore, it is necessary to examine the effects of ginsenosides other than Rb1 and Rg1 on KU 812F cells.

As mentioned above, the basophil/mast cell number is known to be elevated in patients with allergic diseases. It is thought that the specific suppression of excessive growth of these cells should be useful in the control allergic diseases. The *in vivo* effects of these drugs should be examined, especially on lung mast cells/basophils.

A study using a guinea pig asthmatic model in which the effects of traditional herbal medicines on lung inflammatory cells and cytokines are being investigated is now in progress.

和文抄録

以前我々は、小青龍湯、柴朴湯、清肺湯、バイカレインおよびケトチフェンがヒト臍帯血由来好塩基球の増殖分化を抑制することを示した。今回はヒト好塩基球性白血細胞株 KU812F を用いてこれら漢方製剤と抗アレルギー薬の効果を検討した。効果の比較は本細胞の分化率が最も高い培養4日目に行った。アルシャン・ブルー染色とヒスタミン含量により分化程度を測定した。

その結果、柴朴湯＝小青龍湯＞麦門冬湯＝清肺湯の順に 10^{-4} ～10 mg/ml の濃度で抑制効果を示した。柴胡サポニン Sa, Sd, バイカレイン, アゼラスチン, アンレクサノックス, DSCG は 1～10 μM の量で抑制効果が認められたが、柴胡サポニン Sc, 人參サポニン Rb1, Rg1, ケトチフェン, トラニラストは 10 μM でも効果はみられなかった。これらの抑制効果は全細胞あたりより、アルシャンブルー陽性細胞あたりで比較した場合顕著であり、効果の選択性が示唆された。以上の結果より、漢方薬は好塩基球/肥満細胞の増殖、分化を抑制することによりアレルギー疾患に効果を発揮している可能性が示唆された。

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