Bakumondo-to (Mai-Men-Dong-Tang) increases intracellular cAMP in alveolar type II cells: Bakumondo-to stimulates production and inhibits degradation of cAMP

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Abstract

Bakumondo-to (Mai-Men-Dong-Tang) is a herbal medicine which is clinically used for the treatment of bronchitis and pharyngitis accompanying severe cough. To provide the pharmacological basis of this medicine, we examined the effect of Bakumondo-to on intracellular cAMP level in alveolar type II cells. Bakumondo-to increased cAMP as well as isoprenaline in the cell, while isobutyl-methylxanthine (IBMX) did not. The combined effects of Bakumondo-to with isoprenaline and Bakumondo-to with IBMX were synergistic, as well as the combination of isoprenaline with IBMX. The fractionation of Bakumondo-to reduced the activity of increase in cAMP. These data suggest that Bakumondo-to may have both stimulatory effect of cAMP production and inhibitory effect of cAMP degradation, and that synergistic interaction between these effects may be important for the overall effect of this medicine.

Key words alveolar type II cell, Bakumondo-to, β-adrenergic receptor, cyclic AMP.

Abbreviations Bakumondo-to (Mai-Men-Dong-Tang), 麦門冬湯; cAMP, adenosine 3', 5'-cyclic monophosphate; DMEM, Dulbecco's modified Eagle's medium; IBMX, isobutyl-methylxanthine.

Introduction

Bakumondo-to (Mai-Men-Dong-Tang) which is composed of *Ophiopogonis Tuber, Pinelliae Tuber, Zizyphi Fructus, Glycyrrhiza Radix, Ginseng Radix* and *Oryzae Fructus,* has been used for the treatment of bronchitis and pharyngitis accompanying severe cough. Although the clinical usage of this herbal medicine has increased, the pharmacological basis has not been established. On the other hand, alveolar type II cell produces and secretes pulmonary surfactant which is composed of phospholipids and apoproteins.[1] The increase in pulmonary surfactant secretion lowers the surface tension at the air-liquid interface in the lung and provides alveolar stability.[1] In addition to this vital role, pulmonary surfactant activates alveolar macrophages to prevent airway infection,[2] and stimulates mucociliary transport to accelerate airway clearance.[3,4] We have reported that Bakumondo-to increases pulmonary surfactant secretion[5] and β_1-adrenoceptor mRNA expression in isolated alveolar type II cells.[6] These effects were abolished by the treatment of cAMP-dependent protein kinase inhibitor.[5,6] These data suggested that cAMP-dependent signaling is involved in the effect of Bakumondo-to. In the present study, therefore, we examined the effect of Bakumondo-to on cAMP level in alveolar type II cells.

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Materials and Methods

Materials : The rats were purchased from Kyudo Farm (Fukuoka, Japan). Extract of Bakumondo-to (TJ-29) was a gift from Tsumura Co. Ltd. (Tokyo, Japan). Isoprenaline and isobutyl-methylxanthine (IBMX) were purchased from Sigma (St Louis, MO, USA). Fetal bovine serum was from JHR Bioscience (Lenexa, KS, USA).

Bakumondo-to was fractionated into four fractions, as shown in Fig. 1. Bakumondo-to (TJ-29) was precipitated with 80 % EtOH and filtered. The filtrate was applied to hydrophobic chromatography with Diaion HP20-P gel (Mitsubishi Chem., Japan). The extract bound to the gel was then eluted with 1) distilled water, 2) 50 % MeOH, 3) MeOH, and 4) acetone. The yield of each fraction was 16 % (water), 4.1 % (50% MeOH), 1.2 % (MeOH) and 0.013 % (acetone), respectively.

Alveolar type II cells isolation and culture : Alveolar type II cells were isolated from the lungs of adult pathogen-free male Wistar rats (180-200 g), as described previously.[7] Briefly, trypsin was used to dissociate the cells from the lung tissues. The resultant cell suspension was incubated on rat IgG-coated plastic petri dishes for 30 min, to remove the non-type II cells. The isolated alveolar type II cells were suspended at 5.0×10^5 cells/ml in Dulbecco's modified Eagle's medium (DMEM) containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 % fetal bovine serum. The cells were dispensed onto plastic culture dishes at a density of 2×10^5 cells/cm^2 and cultured in 5 % CO_2/95 % air at 37°C.

cAMP assay : The cells were cultured for 24 h, after which the medium was removed and the cells were washed three times with fresh DMEM without FBS or antibiotics. Fresh DMEM was then added, and the cells were returned to the incubator. After a 30-min preincubation in the fresh medium, drugs or solvent vehicle were added, and the incubation was continued for various times, after which the medium was aspirated and the cells were extracted with ice-cold 0.1 N HCl. The extract was immediately frozen, and lyophilized. The sample was reconstituted and acetylated, and the cAMP content was determined as described by the radioimmunoassay kit manufacturer.[7]

Others : Data are presented as the mean ± S.E.M. Duncan's multiple-range test was used for statistical analysis. $P<0.05$ was considered to be significant.

Results

Isoprenaline is the most effective stimulant for cAMP formation in alveolar type II cells.[7,8] Therefore, we first examined the effect of isoprenaline. As shown in Fig. 2, isoprenaline caused a rapid increase in cAMP level that peaked at 1.5 min, then slowly declined, almost reaching control levels by 60 min

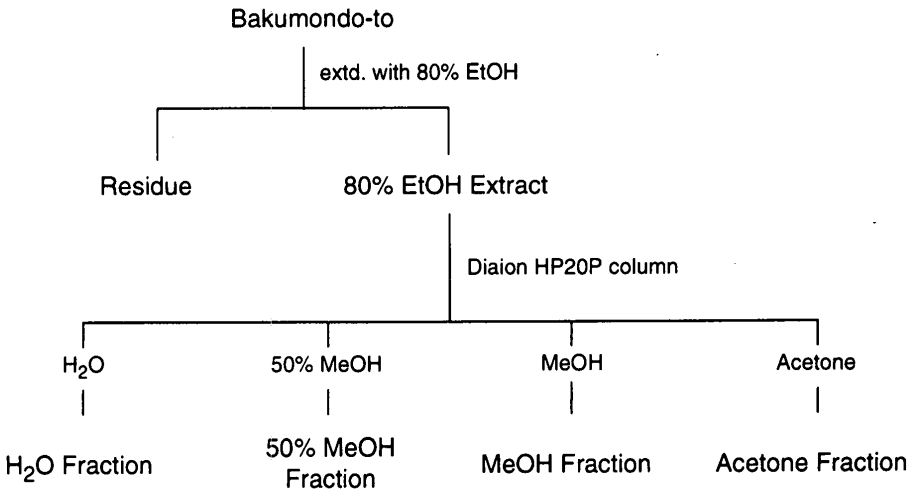

Fig. 1 Fractionation of Bakumondo-to.

(Fig. 2). In contrast, isobutyl-methylxanthine (IBMX) did not increase the cAMP level (Fig. 2). Then we examined the effect of Bakumondo-to. Bakumondo-to (10 mg/ml) increased cAMP level in the cells, but the maximum response of this medicine was reached at 15 min. In addition, the cAMP level in Bakumondo-to-treated cells was significantly greater than that in control cells at least 90 min. The increase in cAMP level by Bakumondo-to was concentration-dependent (Fig. 3).

We next examined the combined effect of Bakumondo-to with isoprenaline or with IBMX. IBMX potentiated the effect of isoprenaline or Bakumondo-to, although IBMX by itself did not increase the cAMP level in type II cells (Fig. 4). On the other hand, the combined effect of Bakumondo-to and iso-

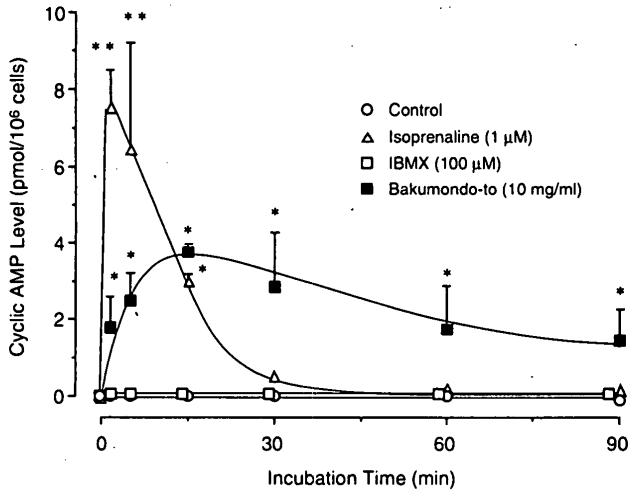

Fig. 2 Time-course of the effect of Bakumondo-to, isoprenaline and IBMX on cAMP level in alveolar type II cells.
Alveolar type II cells were isolated from rats and cultured overnight. The medium was then removed and the cells were incubated in a fresh medium for 30 min. Bakumondo-to (10 mg/ml), isoprenaline (10 μM) or IBMX (100 μM) were then added and the incubation continued for indicated time, after which cAMP content was measured. The data are mean ± S.E.M. from 4 experiments. * and * are significantly different from the control group at $p<0.05$ and <0.01, respectively.

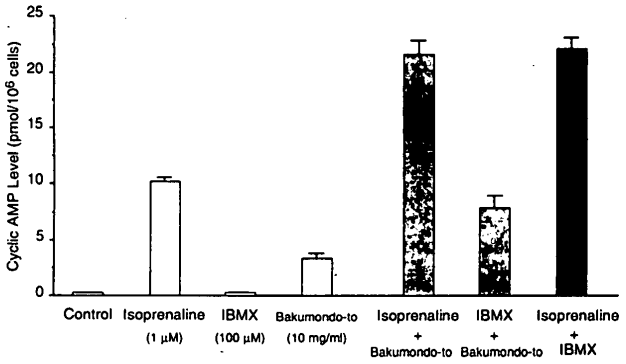

Fig. 4 Combined effect of Bakumondo-to with isoprenaline or with IBMX on cAMP level in alveolar type II cells.
Cells were incubated with indicated drugs for 15 min, after which cAMP content was measured. The data are mean ± S.E.M. from 4 experiments.

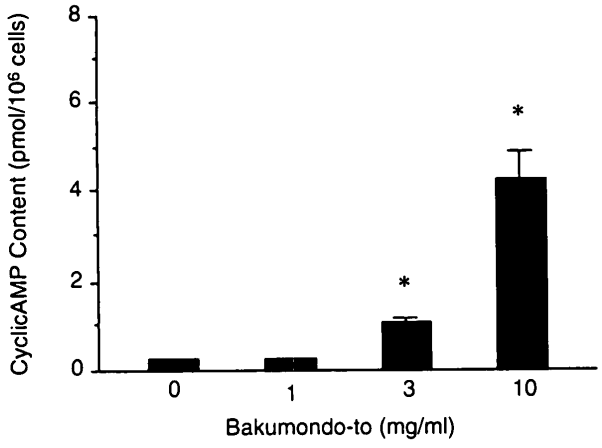

Fig. 3 Concentration-dependent effect of Bakumondo-to on cAMP level in alveolar type II cells.
Cells were incubated with indicated concentration of Bakumondo-to for 15 min, after which cAMP content was measured. The data are mean ± S.E.M. from 4 experiments. * is significantly different from the control group.

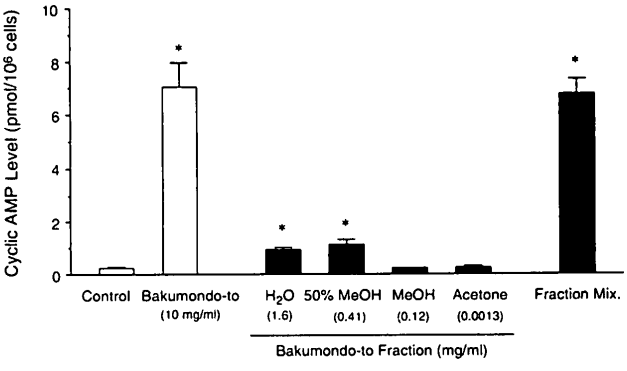

Fig. 5 Effect of Bakumondo-to fractions on cAMP on cAMP level in alveolar type II cells.
Bakumondo-to was fractionated into water-, 50% MeOH -, MeOH- and acetone-eluted fractions as described in materials and methods. Alveolar type II cells were incubated with indicated fraction or their mixture for 15 min, after which cAMP content was measured. The data are mean ± S.E.M. from 4 experiments. * is significantly different from the control group.

prenaline was also much greater than the sum of their effects, which was similar to the synergism between isoprenaline and IBMX (Fig. 4).

Finally, we fractionated Bakumondo-to, and examined the effects of fractions on cAMP level in type II cells. Concentration of each fraction was adjusted to the calculated amount contained in intact Bakumondo-to (10 mg/ml), according to the yield percentage of the fractionation. Distilled water- and 50% MeOH-eluted fraction caused a small increase in cAMP level, whereas MeOH- or acetone-eluted fraction did not (Fig. 5). The sum of the effects of these fractions was considerably less than the effect of Bakumondo-to (10 mg/ml). However, when these all fractions were added together to the cells, the increase in cAMP was almost the same as the effect of intact Bakumondo-to (Fig. 5).

Discussion

The cAMP-dependent signaling system is an important regulator in various cell types. Formation of cAMP is controlled by a cascade consisting of receptors, the stimulatory G-proteins and the adenylate cyclase. The degradation of cAMP is catalyzed by phosphodiesterase, therefore, the inhibitor of this enzyme can increase cAMP level in physiological condition. In the present study, isoprenaline increased cAMP level in isolated type II cells, while IBMX did not. This result is consistent with previous findings on β-adrenergic agonists, and various xanthine derivatives.[7,9] Therefore, it is indicated that only cAMP production stimulator but not degradation inhibitor by itself can increase the cAMP level in type II cells. In the present study, Bakumondo-to by itself increased cAMP level. Therefore, we assumed that Bakumondo-to stimulates cAMP production as well as isoprenaline. This idea was also supported by the finding that the effect of Bakumondo-to was potentiated by the combined application with IBMX. However, the increase in cAMP by Bakumondo-to was not antagonized by pretreatment of propranolol, a β-adrenergic antagonist (data not shown). Therefore, Bakumondo-to may act at receptors other than β-receptors. β_1- and β_2- adrenergic,[10,11] and A_{2B}- adenosine receptors[12] have been identified in type II cells to stimulate cAMP production. The current data suggested that A_{2B}-adenosine receptor may be a likely candidate which mediates the action of Bakumondo-to. However, we cannot exclude the possibility that Bakumondo-to acts at unknown receptors in type II cells.

There were differences between the time course of the effect of Bakumondo-to and that of isoprenaline. Isoprenaline rapidly increased cAMP with the maximum effect at 1.5 min, then declined and reached control level within 60 min. In contrast, the effect of Bakumondo-to reached a maximum effect at 15 min, then slowly declined. The effect was significant for at least 90 min. It has been reported that the combined effect of two different cAMP producing drugs at submaximum concentration in type II cells were less than additive.[12] In the current study, however, the combined effect of isoprenaline with Bakumondo-to was greater than additive. This synergism between isoprenaline and Bakumondo-to was similar to that of the combination of IBMX and isoprenaline. In addition, Suzuki *et al.* reported that Bakumondo-to has phosphodiesterase inhibitory activity in cell-free enzyme assay.[13] Therefore, we assumed that Bakumondo-to not only stimulates cAMP production but also inhibits the degradation of cAMP.

It is likely that the stimulation of cAMP production and inhibition of cAMP degradation are due to different components. This idea is supported by the data on fractionated Bakumondo-to. The water- and 50 % MeOH-eluted fractions increased cAMP level by itself, but their effects were considerably less than that of intact Bakumondo-to. The sum of the effect of each fraction was also less than that of intact Bakumondo-to. However, simultaneous application of all four fractions caused an increase in cAMP level as well as intact Bakumondo-to. Taken together, we assumed that water- and 50 % MeOH-eluted fractions may stimulate cAMP production and that MeOH- and/or acetone-eluted fractions may inhibit cAMP degradation.

Conclusion

Bakumondo-to increased cAMP level in cultured alveolar type II cells. This effect may be dependent on

both stimulation of cAMP production and inhibition of cAMP degradation. Each effect seems to be due to different components which can be separated with hydrophobic chromatography. This synergistic interaction between different components in Bakumondo-to may be important in overall pharmacological activity of this herbal medicine.

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和文抄録

麦門冬湯は激しい咳を伴う咽頭炎や気管支炎に用いられる漢方方剤である。本研究では，麦門冬湯の薬効の機序を薬理学的に解明することを目的とし，肺胞II型上皮細胞内のcAMP量に対する作用を検討した。麦門冬湯は単独でisoprenalineと同様にcAMP量を増加させたが，isobutyl-methylzanthine (IBMX) では著明な作用を示さなかった。麦門冬湯とisoprenalineおよび麦門冬湯とIBMXの併用はいずれも相乗的であり，isoprenalineとIBMXの併用時の作用と類似であった。麦門冬湯を分画するとcAMP増加作用は著明に減弱した．これらの結果から，麦門冬湯はcAMP産生促進作用と分解抑制作用の両作用を合せ持つこと，また，これらの作用をもつ複数の成分の相乗的相互作用が重要であることが考えられた。

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