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

Yoichiro ISOHAMA,² Kana KURITA,² Hirofumi KAI,¹ Kazuo TAKAHAMA,³ and Takeshi MIYATA⁴

¹Department of Pharmacological Sciences, Faculty of Pharmaceutical Sciences, Kumamoto University
²Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Kumamoto University

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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,⁵ and stimulates mucociliary transport to accelerate airway clearance.⁵⁳ We have reported that Bakumondo-to increases pulmonary surfactant secretion ⁵ and β₁-adrenoceptor mRNA expression in isolated alveolar type II cells.⁶ These effects were abolished by the treatment of cAMP-dependent protein kinase inhibitor.⁵ 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.
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). Isoproterenol 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. 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 x 10^6 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 x 10^6 cells/cm² and cultured in 5% CO2/95% 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.

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

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

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**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-

![Graph showing the effect of Bakumondo-to on cAMP level.](image)

**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 p<0.01, respectively.

![Graph showing the combined effect of Bakumondo-to with isoprenaline or IBMX.](image)

**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.

![Graph showing the effect of Bakumondo-to fractions.](image)

**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, 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.
renaline 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 deriva-
tives.7,10 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 iso-
prenaline. 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 antago-
nized by pretreatment of propranolol, a β-adrenergic
antagonist (data not shown). Therefore, Bakumondo-
to may act at receptors other than β-receptors. β- and
β2-adrenergic,10,11 and A2β- adenosine receptor
12 have been identified in type II cells to stimu-
late cAMP production. The current data suggested
that A2β-adenosine receptor may be a likely candidate
which mediates the action of Bakumondo-to. How-
ever, we cannot exclude the possibility that Bakumon-
do-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 iso-
prenaline. 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 signifi-
cant 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, how-
ever, the combined effect of isoprenaline with Ba-
numondo-to was greater than additive. This syner-
gism between isoprenaline and Bakumondo-to was
similar to that of the combination of IBMX and iso-
prenaline. 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 produc-
tion 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 Ba-
numondo-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 fra-
cions 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

麦門冬湯は凄しい咳を伴う咽喉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-methylxanthine (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わせ持つこと、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。

References

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