*Current Perspective***Pharmacological Basis of Traditional Medicines and Health Supplements as Curatives**Takeshi Miyata^{1,*}¹Department of Presymptomatic Medical Pharmacology, Faculty of Pharmaceutical Sciences, Sojo University,
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Abstract. Traditional Oriental medicines and health supplements have been empirically used to treat various ailments but most of them have not been evaluated objectively to prove their efficacies. We have been investigating the medical benefits of traditional Oriental medicines and health supplements as alternatives and their varied actions and mechanisms by pharmacological approaches. The study on airway inflammation has shown that even a *Kampo* preparation, *Bakumondo-to*, has anti-inflammatory, anti-allergic, immunomodulatory, secretory-modulating, and metabolic regulatory actions. All of its actions are based on the restoration of normal molecular and cellular functions through DNA transcriptional regulation. In other previous studies, we showed that a health supplement, royal jelly (RJ) has weak estrogenic activity. RJ competes with 17 β -estradiol for binding to the human estrogen receptors α and β , although it is much weaker than diethylstilbestrol in binding affinity. Treatment of MCF-7 cells with RJ enhances proliferation, and concomitant treatment with tamoxifen blocked this effect. A reporter gene assay showed that RJ enhanced transcription of the luciferase gene through the estrogen-responsive element in MCF-7 cells. Furthermore, subcutaneous injection of RJ restored the expression of vascular endothelial growth factor gene in the uteri of ovariectomized rats. We suggest that the diverse pharmacological functions of RJ can be ascribed, in part, to its estrogenic effects. We hypothesize that traditional medicine, which has multiple actions, may be better than Western medicine of a single component to treat various diseases including “*Mibyō*” (pre-symptomatic disease). Our findings provide new ideas about the nature of disorders and disease-state development that involve complicated mechanisms and will contribute to novel principles to prevent diseases and establish new treatments. Adoption of the means of translational research should provide an objective background for efficacy and stimulate broader application and usage of traditional medicines and health supplements.

Keywords: traditional medicine, health supplement, steroidal hormone-like action, curative, “*Mibyō*” (presymptomatic disease)

Introduction

Traditional Oriental medicines and health supplements have been empirically used to treat various disorders but most of them have not been examined objectively to prove their efficacies.

We have been investigating the medical benefits of traditional Oriental medicines and health supplements as alternatives and their varied actions and mechanisms through the pharmacological approach. This article focuses on the steroidal hormone-like actions of *Bakumondo-to* and royal jelly.

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Glucocorticoid-like and glucocorticoid-unlike actions of *Bakumondo-to* as an airway cleansing drug

1) Characteristics of antitussive action

Bakumondo-to, a traditional Chinese medicinal prescription consisting of 6 herbs (*Ophiopogonis tuber*, *Pinelliae tuber*, *Zizyphi fructus*, *Glycyrrhizae radix*, *Ginseng radix*, and *Oryzae fructus*), has been used for the treatment of bronchitis and pharyngitis accompanying severe dry cough.

Bakumondo-to has no effect in normal animals, while it showed significant antitussive actions in bronchitic animals. Codeine, a typical narcotic antitussive remarkably reduces cough responses in normal animals but not in bronchitic animals. Thus, *Bakumondo-to* selectively shows a potent antitussive action in bronchitic animals (1, 2).

Based on this finding, the effects of *Bakumondo-to* on cough responses induced by angiotensin-converting enzyme (ACE) inhibitors were studied.

ACE inhibitors have been used to treat both hypertension and congestive cardiac failure, but persistent dry cough occurs as an adverse side effect of this treatment. In our experiments, *Bakumondo-to* but not codeine completely inhibited ACE inhibitor-induced coughing (3, 4). These effects are similar to those of steroidal anti-inflammatory drugs.

2) Mechanism of antitussive action

Cough can be induced by various mechanisms, for example, by the irritation of rapidly adapting (irritant) receptors with vagal myelinated A δ -fibers and/or C fiber receptors with unmyelinated fibers, which are located within and immediately below the epithelial cell lining. Mechanical stimulants act mainly on A δ -fibers, whereas tachykinins such as substance P mainly act on C fibers. It is well known that tachykinins are degraded by their cleavage enzyme, neutral endopeptidase (NEP). *Bakumondo-to* and ophiopogonin, a steroid saponin extracted and purified from *Bakumondo* (*Ophiopogonis tuber*), also suppressed tachykinins-induced coughs, while codeine was nearly ineffective. In this context, we found that in the airway, NEP-like activities in bronchitic animals decreased to 1/8–1/9 of those in normal healthy animals, and treatment with *Bakumondo-to* prevented this reduction of NEP-like activities. In addition, we found that *Bakumondo-to* significantly suppressed the increased afferent discharge of the superior laryngeal nerve in bronchitic animals, whereas codeine significantly augmented the increased discharge of the nerve. Therefore, *Bakumondo-to* seems to produce antitussive activities through inhibition of tachykinin receptors and restoration of NEP activity in the airway

inflammatory state (3–5).

3) Mucoregulatory and secretomotor actions

Abnormal and excessive production of airway mucus is a characteristic feature of many chronic inflammatory lung diseases. Coughs are often alleviated by the expectoration of viscous sputa. The productive cough (wet cough) as a protective reflex should not be inhibited and airway cleaning should be facilitated.

Based on these facts and from a polypharmacological point of view, we evaluated the effects of *Bakumondo-to* on airway secretion.

The normal human airway is covered with high molecular weight glycoconjugates (HMWG), which consist of O-glycosylated tandem repeats with a high percentage of serine and threonine. The airway mucous glycoproteins have a variety of protective functions, including entrapment and clearance of exogenous materials by ciliary transport, as well as humidification and lubrication of the airway mucosa. Excessive mucous production, however, characterizes airway diseases such as chronic bronchitis, asthma, cystic fibrosis, and bronchiectasis. In these diseases, the influx of polymorphonuclear leucocytes (PMNs) into the airways and the release of substance P from the peripheral endings of primary sensory neurons have also been observed. It is therefore suspected that substance P may affect the mucus secretion of airway epithelial cells through the activation of PMNs. From this standpoint, we have evaluated the mucoregulatory action of *Bakumondo-to* using the culture systems of hamster tracheal epithelial cells and co-culture of the cells with PMNs activated by substance P or by other stimulants to simulate the airway inflammatory state. *Bakumondo-to* filtrate, the hydrophobic fraction containing flavonoid and saponins, and the hydrophilic fraction containing sugars and peptides did not produce any effect on basic HMWG secretion from cultured tracheal epithelial cells. However, *Bakumondo-to* filtrate significantly suppressed the substance P-induced secretion of HMWG from tracheal epithelial cells and PMNs co-culture (5–7).

Pulmonary surfactant (PS) secreted from alveolar type II cells lowers the surface tension at the air-liquid interface in the lung and provides alveolar stability. It has been clearly demonstrated that in addition to this vital role, PS is important in airway mucociliary clearance. We found an inhibitory action of inflammatory mediators on mucociliary transport and confirmed a protective effect of PS on inhibition of mucociliary transport (7–9).

In the cells treated with *Bakumondo-to* filtrate, the basal secretion rate of PS was significantly enhanced, which is similar to the effects of β_2 -adrenoceptor

stimulants. The enhancing effect on secretion could be inhibited by pretreatment with H-89 (a protein kinase-A inhibitor) but not by propranolol (a β -adrenoceptor antagonist). We also found that *Bakumondo-to* filtrate could significantly and continuously increase intracellular Ca^{2+} levels. To simulate inflammatory states and to examine the effects of *Bakumondo-to* and ophiopogonin on the hypersecretion of pulmonary surfactant, we used co-culture systems of alveolar type II cells with PMNs activated by substance P. *Bakumondo-to* filtrate and ophiopogonin significantly inhibited both the increased secretion and the enhanced secretion by hydrogen peroxide.

These results suggest that *Bakumondo-to* has a characteristic secretory-enhancing effect on PS and normalizes hypersecretion by the inhibition of superoxide (9).

4) Effects on gene expression in airway epithelial cells

The level of β_2 -adrenoceptor mRNA in alveolar type II Cells is increased by the treatment with dexamethasone. In contrast, the β_1 -adrenoceptor mRNA level is not significantly affected by dexamethasone treatment. Unlike dexamethasone, the β_2 -adrenoceptor mRNA level in alveolar type II cells was not affected by treatment with *Bakumondo-to* filtrate, but the β_1 -adrenoceptor mRNA level was significantly increased. Although the mechanism of selective action on β_1 -adrenoceptor gene expression remains unclear, the effect may contribute to the effectiveness of *Bakumondo-to* on chronic airway diseases because both the β_1 - and β_2 -adrenoceptor subtypes mediate the secretion of PS. We further examined the effect of *Bakumondo-to* on gene expression with a specified system for glucocorticoid. Luciferase reporter plasmid containing mouse mammary tumor virus (MMTV) promoter, a glucocorticoid-sensitive promoter, was transfected to A549 human lung adenocarcinoma cells. In this system, dexamethasone clearly activated promoter transcription and the effect was completely inhibited by the simultaneous application of RU486, a glucocorticoid-receptor antagonist. In this system, although *Bakumondo-to* did not activate transcription by itself, the transcriptional activation by combined treatment with *Bakumondo-to* and dexamethasone was considerably greater than that by dexamethasone alone. Similar enhancement was observed by *Glycyrrhiza radix* and glycyrrhizin, a major component of *Glycyrrhiza radix*. These data suggest that *Bakumondo-to* increases glucocorticoid-sensitive promoter activation by enhancement of the effect of glucocorticoid and that this enhancement may be due to the effect of glycyrrhizin (10–15).

Glycyrrhizin has been reported to inhibit type II 11β -

hydroxysteroid dehydrogenase (11β -HSD2), an inactivating enzyme of glucocorticoids. The 11β -HSD2 converts glucocorticoids to their inactive 11-keto metabolites NAD-dependently. Therefore, the inhibition of 11β -HSD2 may contribute to the enhancement of the effect of dexamethasone by glycyrrhizin. The 11β -HSD2 is expressed only in tracheal, bronchial, and alveolar epithelial cells in the lung. This local expression suggests that glycyrrhizin may act only in epithelial cells without affecting other glucocorticoid-responsive cells. It is therefore possible that herbal medicines containing glycyrrhizin inhibit 11β -HSD at airway epithelial cells, resulting in the increase of GM-CSF release and decrease in mucus production.

Estrogenic effects of royal jelly

1) Interaction with estrogen receptors and endogenous gene expressions

Estrogen plays an important role in the growth, differentiation, and function of many targets including the female and male reproductive system. Estrogen also has a variety of pharmacological functions such as maintenance of bone mass, cardiovascular protection, and brain protection. Post-menopausal women are at increased risk for many health problems, such as cardiovascular diseases and osteoporosis, compared with premenopausal women or even with age-matched males (16). The most likely cause for this difference is the deficiency of the ovarian hormones, especially estrogen, in post-menopausal women.

Hormone replacement therapy (HRT) is often prescribed to suppress such estrogen-related diseases. While some salutary effects have been substantiated, HRT has recently been a subject of debate because of an increased risk of breast cancer and coronary artery disease (17, 18).

Diets rich in phytoestrogen-containing foods such as soybean products have been suggested to prevent or alleviate estrogen-related diseases and phytoestrogens are being increasingly promoted as the natural alternative to HRT (19, 20).

Royal jelly (RJ) is known as a popular and traditional food for health promotion. RJ contains a considerable amount of proteins, free amino acids, lipids, vitamins and sugars, and small amounts of steroids (21). RJ has some diverse nutritional and pharmacological functions in humans such as vasodilative and hypotensive activities, antihypercholesterolemic activity, and anti-tumor activity (22). Improvement of menopausal symptoms is also one of the salutary effects of RJ. Clinical studies in which RJ alleviated so-called autonomic imbalance in menopausal women support its

beneficial effects (23, 24). These findings suggest that RJ has estrogenic activity, but information concerning the biochemical and pharmacological evidence for the estrogenicity of RJ has not been available. Therefore, we examined the estrogenic effects of RJ using various in vitro and in vivo assay systems. The potential estrogenic activities of RJ were investigated using various approaches. RJ competed for binding of 17β -estradiol to the human estrogen receptor α and β , but its affinities were weak compared with diethylstilbesterol and phytoestrogens. The reporter gene expression assays suggested that RJ activated the estrogen receptors, leading to the enhanced transcription of a reporter gene through an estrogen-responsive element. RJ stimulated the mRNA expression of estrogen-responsive pS2 and vascular endothelial growth factor (VEGF) by increasing gene transcription in MCF-7 cells. Treatment with RJ enhanced MCF-7 cell proliferation, but concomitant treatment with tamoxifen blocked this effect. In vivo studies using ovariectomized rats showed that 17β -estradiol treatment restored VEGF expression in both uterus and brain, whereas RJ restored it in uterus but not in brain. These findings provide evidence that RJ has estrogenic activities through interaction with estrogen receptors followed by endogenous gene expressions (25, 26).

2) Effects on bone metabolism

Estrogen plays a key role in bone metabolism, particularly in women, and its deficiency is a major factor in menopause-associated bone loss and development of postmenopausal osteoporosis.

The use of dietary phytoestrogens is considered as a possible option for the prevention of osteoporosis (27, 28). In vitro studies using a mouse osteoblast-like cell line, MC3T3-E1, have shown that coumestrol, genistein, and daidzein increase alkaline phosphatase activity and enhanced mineralization, presumably through their estrogenic potency. In animal models, oral administration of soy isoflavone and pure genistein recovered bone loss caused by ovariectomy in rats and mice. Similarly, pomegranate extract containing estrogenic compounds may improve bone properties in ovariectomized mice.

RJ has diverse physiological and pharmacological functions, in particular, we have observed that it has weak estrogenic activity. The study on the effects of RJ on bone demonstrated that it stimulated the proliferation of mouse osteoblast-like MC3T3-E1 cells and also that the effect was blocked by the specific estrogen receptor antagonist ICI182,780. The addition of RJ generally enhanced collagen production in culture medium. Oral administration of RJ to normal female mice for 9 weeks increased the ash content of the tibias. DNA microarray

analysis revealed significant alteration of gene expression related to extracellular matrix formation in the femurs of mice fed with RJ. Quantitative RT-PCR confirmed the up-regulation of procollagen I $\alpha 1$ gene expression. These data suggest that RJ, as a whole or its individual components, stimulates production of type I collagen and other activities for bone formation through its action on the osteoblasts (29).

3) Active ingredients of RJ possessing estrogenic activity

We isolated four compounds from RJ that exhibit estrogenic activity as evaluated by a ligand-binding assay for the estrogen receptor (ER) β . We identified these compounds as 10-hydroxy-*trans*-2-decenoic acid, 10-hydroxydecanoic acid, *trans*-2-decenoic acid, and 24-methylenecholesterol. These compounds inhibited the binding of 17β -estradiol to ER β , although more weakly than diethylstilbestrol or phytoestrogens. However, these compounds had little or no effect on the binding of 17β -estradiol to ER α . Expression assays suggested that these compounds activated the estrogen receptors, as evidenced by the enhanced transcription of a reporter gene containing an estrogen-responsive element. Treatment of MCF-7 cells with these compounds enhanced their proliferation, but concomitant treatment with tamoxifen blocked this effect. Exposure of immature rats to these compounds by subcutaneous injection induced mild hypertrophy of the luminal epithelium of the uterus, but was not associated with an increase in uterine weight. These findings provide evidence that these compounds contribute to the estrogenic effect of RJ (30).

Summary and conclusion

From the pharmacological point of view, traditional medicines and health supplements can be classified as moderate steroid-like curatives because they consist of many kinds of active components that have various pharmacological effects similar to those of steroidal hormones. We hypothesize that traditional medicines and health supplements, which have multiple and moderate actions, may be better than Western medicine of a single component to treat various diseases including “*Mibyō*” (presymptomatic disease). As one future course of research, we believe that the efforts to seek multiple characteristic actions of traditional medicines and health supplements may lead us to new opportunities for development of ubiquitous drugs with moderate but specified actions, that is, regulation of gene expression.

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