Investigation of Decoction Procedure of Maoto Described in Shokan-ron Using Mouse Amylase Inhibitory Activity

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Abstract
The adequacy of the Maoto decoction procedure described in the Shokan-ron text was investigated using the inhibitory activity of blended Ephedra herb on amylase enzyme. This enzyme is closely related to stress, and used clinically as an indicator of stress in humans.

α-amylase in isolated mouse plasma was used in the experiment, and the enzymatic activity was measured according to the Caraway method. Two different decoction methods: the general process used today, and that described in the Shokan-ron (Ephedra herb alone is firstly infused, then another 3 crude drugs are added and it is further infused), were compared for yield of the extracts, inhibitory actions of the extracts, their crude polysaccharide fraction (one of the active components), and remaining fractions contained within each extract. The inhibitory activity of the extract was clearly promoted by the latter, ancient decoction method, compared with the former. Hence, the decoction method described in the Shokan-ron is presumed to have the aim of reducing stress to the body caused by Maoto itself, and mitigating the side effects of its component Ephedra herb. This biologically qualitative approach, reflected over the entire decoction process, is different from the simply physicochemically analytical approach which concerns itself merely with particular constituents, such as alkaloids. So, it may be beneficial to scientifically elucidate the significance of this decoction and its ingestion methods, as described by the ancient Shokan-ron and Kinki-yoryaku texts.

Key words: Ephedra herb, Maoto, Shokan-ron (Shang Han Lun), decoction, α-amylase, inhibition, hyperglycemia

要旨
従来の学術的の研究においても、マオトの薬効機序について研究が行われており、その効果の一部はストレスの軽減や抗酸化作用、免疫促進作用等とされており、実臨床的の用途として多くの薬物を利用することが知られている。しかしながら、この薬物の効果が実臨床的にどのように表われるかはまだ不明である。そこで、本研究では、マオトの薬効機序についてさらに深く理解することを目的として、マオトの成分とその効果についての研究を行った。

キーワード：薬効、麦芽酶、α-アミラーゼ、抗酸化、高血糖
I. Introduction

Most Kampo formulas used in Japan originated from ancient Chinese medical books. Occasionally, the process of infusion or dose method for individual formulas is indicated in these books. These descriptions were based on considerable experience, with the aim of reducing side effects, increasing drug efficacy, or allowing easy intake. However, scientific evaluation of these descriptions has not been sufficient, with only a few reports.\(^{1-8}\)

We previously reported on the inhibitory effect of Ephedra herb on \(\alpha\)-amylase activity in the plasma and gastrointestinal tubes of mice to be expected to improve postprandial hyperglycemia.\(^9\) In this study, we investigated the adequacy of the decoction procedure for Maoto revealed in Shokan-ron, that is, “first Ephedra herb alone should be decocted, then the rest 3 crude drugs are added and it is decocted further”, by utilizing amylase inhibitory activity of Ephedra herb as a parameter.

This enzyme is closely related to stress. Since amylase secretion from the salivary glands is increased by elevated norepinephrine release from the adrenal glands under stress stimulation and direct stimulation of the salivary glands through sympathetic innervation, an increase in amylase activity is an indicator of mental stress in humans and is already used clinically for the evaluation of stress intensity. Also, increase in the blood glucose level under stress stimulation have been reported.\(^{10,11}\)

II. Materials and methods

Materials Chopped crude drugs were purchased from Nakai-kohshindo (Kobe) and are defined by the Japanese Pharmacopoeia XV. Phenol and conc. sulfuric acid were purchased from Nacalai Tesque (Kyoto) and \(D(+)\)-glucose was obtained from Wako Pure Chemical Industry (Osaka).

Animals Five to 6 week-old male mice of the ddY strain were purchased from Nihon SLC, Hamamatsu, Japan. They were housed in plastic cages with free access to food (until 12 h before use) and water, and were kept in a room at 25±1°C, 55±5% humidity, with a 12 h light-dark cycle.

Dispensing of prescription Maoto was prepared according to the prescription for a one-day dose written in the literature,\(^12\) that is, Ephedra herb 5.0 g, Apricot kernel 5.0 g, Cinnamon bark 4.0 g, and Glycyrrhiza 1.5 g. Independently, Ephedra herb was decocted with 600 ml of water till it was concentrated to about 450 ml. Next, Apricot kernel, Cinnamon bark, and Glycyrrhiza were added together to 300 ml in the same fashion (decoction B). Each decocted solution was concentrated in vacuo and freeze-dried. Ephedra herb alone or Maoto removed Ephedra herb (decoction C) was also decocted with 600 ml of water and post-treated in the same manner to obtain each freeze-dried extract.

Crude polysaccharide fraction Referring to Terawaki et al.,\(^13\) each extracted sample was dissolved in a ten-fold volume of water, followed by the removal of insoluble material. A thirty-fold volume of ethanol was added to the solution and it was permitted to stand for 24 h at room temperature. After centrifuging at 3000 rpm for 10 min, the precipitate was collected and dried well. Supernatant liquid was concentrated in vacuo and also dried well.

Estimation of sugar content The total sugar content was estimated according to the phenol-sulfuric acid method.\(^14\) Glucose was used as the standard. A microplate reader, MPR-A4i (Tosoh), was employed for the measurement of visible absorbance (at 490 nm).

Assay of \(\alpha\)-amylase activity Cardiac blood was collected from mice with a heparin-treated cylinder and centrifuged to prepare plasma. A 0.1 ml portion of each test sample and crude polysaccharide fraction adjusted to each final reaction concentration in distilled water was added to the mouse plasma for the assay. The amylase activity was measured according to the Caraway method\(^15\) using a kit (Amylase-Test Wako, Wako Pure Chemical Industry), as described previously.\(^16\) The inhibitory activity (\%\) was calculated as \((1-B/A)\times100\), where \(A\) is the activity of the enzyme without the test solution, and \(B\) the activity of the enzyme with the test solution.

Statistics Statistical analysis was performed using Student’s \(t\)-test. A value of \(p<0.05\) was regarded as significant.

III. Results

At present medical facilities, Maoto is generally produced by the decoction of 4 crude drugs together with 500~600 ml of water till it is concentrated to a half volume (decoction A). However, according to Shokan-ron, Ephedra herb alone is firstly decocted till about 1/4 of the water volume is reduced. Next, another 3 crude drugs (Apricot kernel, Cinnamon bark, and Glycyrrhiza) are added to the infusion and it is decocted further (decoction B).
Comparison of extract yields is shown in Table 1. The yield of decoction B was slightly less than that of decoction A, being statistically different ($p<0.001$). The yield of decoction A was less than the sum of Ephedra herb alone and the mixture of the 3 crude drugs (Maoto lacking Ephedra herb, decoction C). The pH value of decoction is also one of the factors which influence the elution of components from crude drugs. But no significant difference was found between decoction A (pH 5.02±0.03) and B (pH 5.02±0.05) at 27°C.

Fig.1 shows alterations in the inhibitory activity of decoction extracts toward isolated mouse blood plasma amylase. Ephedra herb revealed an inhibitory activity of about 49.9%, whereas no activity was noted in decoction C. Therefore, it is clear that Ephedra herb only participates in the inhibitory action of Maoto. As exhibited in Fig. 1, the inhibitory activity of decoction B (66.8%) was markedly higher than decoction A (27.8%). Each final concentration of the test sample in the reaction solution was calculated as: (yield of one-day dose extract×1/10)/300 ml.

We already presumed that polysaccharides of Ephedra herb inhibit mouse α-amylase activity, and that alkaloids, principal constituents, are of no concern. The amount of crude polysaccharide fraction in each extract was therefore estimated. As depicted in Table 2, the yield of polysaccharide fraction of decoction B was slightly greater than in decoction A, being significantly different ($p<0.01$). The yield of the polysaccharide fraction of Ephedra herb alone was more than that of decoction C. As the total sugar content was conversely low, the purity of this fraction seems to be lower than the others.

Inhibitory effects of these polysaccharide fractions are presented in Fig.2. The fraction obtained from Ephedra herb inhibited the α-amylase activity at 65.8% (final concentration in reaction solution, 300 μg/ml). The polysaccharide fractions from decoctions A and B showed moderate inhibitory activities in the same reaction concentration at 38.4% and 34.2%, respectively, with no significant difference. The polysaccharide fraction from decoction C revealed no activity.

Consequently, the supernatant after the removal of polysaccharides with ethanol was examined next. The yields of supernatant extracts after evaporating the solvent are shown in Table 3. Similarly to decoction extracts, the yield of supernatant extract A was more than B (no statistical difference), and the extract derived from the decoction of Ephedra herb alone showed the lowest value. All of these extracts showed a positive reaction with geratin reagent, proving the presence of tannins.

As shown in Fig.3, supernatant extracts of A and B revealed slightly similar inhibitory activities on α-amylase of 29.8% and 36.0%, respectively, with significant difference ($p<0.02$). Each final concentration of the test sample in the reaction solution was calculated as: (yield of one-day dose supernatant extract×1/5)/300 ml.

### Table 1 Yield of Decoction Extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>g/one-day dose</th>
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</thead>
<tbody>
<tr>
<td>Decoction A</td>
<td>2.380 ± 0.006*</td>
</tr>
<tr>
<td>Decoction B</td>
<td>2.197 ± 0.016*</td>
</tr>
<tr>
<td>Decoction C</td>
<td>1.454 ± 0.027</td>
</tr>
<tr>
<td>Ephedra Herb</td>
<td>1.053 ± 0.010</td>
</tr>
</tbody>
</table>

All values represent the mean±S.E. (n=3). a) General Maoto decoction, b) Maoto decoction indicated by Shokan-ron, c) Maoto lacking Ephedra herb decoction. * Significantly different between decoctions A and B, $p<0.001$.

**Fig.1** Change in Inhibitory Effect on Mouse Plasma Amylase Activity by Addition of Various Decoction Extracts

Each value represents the mean of 9 samples per group. Horizontal lines show the standard error of the mean. Concentration of test samples in reaction solution, (yield of one-day dose extract×1/10)/300 ml. a) General Maoto decoction, b) Maoto decoction indicated by Shokan-ron, c) Maoto removed Ephedra herb decoction. * Significantly different between decoctions A and B, $p<0.01$.

### Table 2 Yields of Polysaccharides

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yields (g/one-day dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction A</td>
<td>2.380 ± 0.006*</td>
</tr>
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<td>Decoction B</td>
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All values represent the mean±S.E. (n=3). a) General Maoto decoction, b) Maoto decoction indicated by Shokan-ron, c) Maoto lacking Ephedra herb decoction. * Significantly different between decoctions A and B, $p<0.001$.

IV. Discussion

The decoction of Ephedra herb in advance has been carried out for many Kampo prescriptions containing Ephedra herb following the descriptions in Shokan-ron. Although some studies on the reasonability of these descriptions exist, they have been reported from the standpoint of the elution of ephedrine. We already reported the intense inhibitory
activity of Ephedra herb and Maoto on mouse plasma α-amylase activity. Consequently, differences in this activity between extracts derived from two decoction procedures: that described in Shokan-ron and that carried out generally in the present medical field, were examined in this study.

Commonly, the yield of Kampo formula extracts is not simply equal to sum of yields of extracts of each blended crude drug. The efficiency of extraction is influenced by various factors, and complex interactions exist among blended crude drugs. The shorter decoction time using the 3 crude drugs, disturbance of extraction of the 3 crude drugs by Ephedra herb which is decocted in advance, and so on are considered as reasons for the lower yield of extract of decoction B than A. From the standpoint of amylase inhibition, a biologically qualitative difference was apparently found between decoction B, following Shokan-ron, and decoction A, as shown in Fig.1. Ephedrains have been reported as polysaccharides in Ephedra herb showing hypoglycemic effects in alloxan-induced hyperglycemic mice by intraperitoneal administration. However, the relation with amylase is uncertain. Owing to insufficient purification, the polysaccharide fraction we obtained from Ephedra herb exhibited a far lower purity. Regardless, it exhibited a strong inhibitory action against mouse α-amylase activity, but no significant difference was found between polysaccharide fractions from decoctions A and B. Difference was also not found in activity between supernatant extracts of decoctions A and B. The value of B appears similar to the sum of activities of supernatant extracts of C and Ephedra herb.

These facts described above suggest that the difference in the inhibitory action of decoction extracts of A and B seems to be the result of the total effects caused by polysaccharides, tannins and all of other unknown active components.

As for the relationship between stress and increases in the amylase activity, it has been reported that pancreatic amylase secretion increases with the stimulation of amylase protein synthesis due to elevated glucocorticoid secretion by the pancreas caused by hyperactivity of the pituitary-adrenal system or with increased dopamine secretion under stress, but that this action is mediated by adrenaline β-receptors rather than dopamine D1-receptors. Since ephedrine contained in Ephedra herb shows a strong, direct β-stimulator action, it may cause excess increases in amylase activity due to the sympathetic stimulant activities of alkaloids contained in Ephedra herb or Maoto.

It is known that starch in Pueraria root contributes to further elution and a greater remnant of ephedrine in the decoction of Kakkonto, one of the prescriptions containing Ephedra herb, through the decoction procedure directed by Shokan-ron (Ephedra herb and Pueraria root are infused together before other crude drugs are added and infused).

### Table 2 Yield of Crude Polysaccharide Fraction

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg/one-day dose of decoction extract</th>
<th>Total sugar content (as Glu., %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction A</td>
<td>309.11 ± 3.17*a</td>
<td>31.0</td>
</tr>
<tr>
<td>Decoction B</td>
<td>318.57 ± 5.61*a</td>
<td>31.0</td>
</tr>
<tr>
<td>Decoction C</td>
<td>179.71 ± 2.87</td>
<td>35.6</td>
</tr>
<tr>
<td>Ephedra herb</td>
<td>208.49 ± 3.05</td>
<td>25.9</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. (n=3). a) Determined by the phenol-sulfuric acid method, b) general Maoto decoction, c) Maoto decoction indicated by Shokan-ron, d) Maoto without Ephedra herb decoction. * Significantly different between decoctions A and B, p < 0.01.
increases in the ephedrine content in the decoction procedure of Maoto through the direction of Shokan-ron were also reported, at 12% and 8%, but these were not significant. Such quantitative physico-chemical analyses have previously been conducted, but no biological evaluation such as presented in this paper has been reported.

V. Conclusion

In this study, we employed the inhibitory effect on amylase activity as a characteristic and found that the activity of decoction extract was apparently promoted in decoction B (manner directed by Shokan-ron) compared with decoction A (general manner used today). Hence, the decoction method described in Shokan-ron is presumed to have an aim for reducing the stress to the body caused by Maoto and mitigation of side effect by Ephedra herb. That is to say, obvious qualitative change in bioactivity on performed decoction (medicine) was demonstrated by comparing the two decoction procedures.

The enzyme inhibitory activity measured in this experiment suggested that the order of decoction of Maoto described in Shokan-ron has some basis. To discuss the adequacy of descriptions concerning decoction methods, not only physicochemical analysis represented by component determination but also investigation of biological qualitative changes during the decoction process is necessary. Understanding of the whole drug by investigation of its biological activity is superior to measurement of a portion of the active components with regard to the evaluation of crude drugs and Kampo formulae. Scientific elucidation of the basis and usefulness of decoction and ingestion methods described in Shokan-ron and Kinki-yoryaku is beneficial for consideration of their uses and instruction in clinical practice, and may increase their therapeutic effects in clinical field.

Table 3  Yield of Supernatant Extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>g/one-day dose of decoction extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction A</td>
<td>1.574 ± 0.025</td>
</tr>
<tr>
<td>Decoction B</td>
<td>1.494 ± 0.009</td>
</tr>
<tr>
<td>Decoction C</td>
<td>1.135 ± 0.031</td>
</tr>
<tr>
<td>Ephedra herb</td>
<td>0.613 ± 0.031</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. (n=3). a) general Maoto decoction, b) Maoto decoction indicated by Shokan-ron, c) Maoto without Ephedra herb decoction.

Fig.3  Variation in Inhibitory Activities of Different Supernatant Extracts on Amylase Activity in Mouse Plasma

All values represent the mean±S.E. (n=9). Horizontal lines show the standard error of the mean. Concentration of test samples in reaction solution, (yield of one-day dose extract×1/5)/300 ml. a) General Maoto decoction, b) Maoto decoction indicated by Shokan-ron, c) Maoto without Ephedra herb decoction. * Significantly different between decoctions A and B, p<0.02.

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