Use of a taste-sensing system to discriminate Kasseki (Aluminum Silicate Hydrate with Silicon Dioxide) in The Japanese Pharmacopoeia from Huashi (Talc) in Pharmacopoeia of The People's Republic of China

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Abstract

‘Kasseki’ in Japanese or ‘Huashi’ in Chinese are highly similar crude mineral drugs. Though almost the same Chinese characters are used for both, the definition of the former in The Japanese Pharmacopoeia (JP) is different from that of the latter in Pharmacopoeia of The People’s Republic of China (CP). Namely, Kasseki is defined as “a mineral substance, mainly composed of aluminum silicate hydrate and silicon dioxide” in JP, while Huashi is defined as “mainly hydrated magnesium silicate” in CP. Since the Kasseki used in Japan is imported from China, discrimination of these two is important from the viewpoint of regulatory science. In this report we applied a taste-sensing system having artificial lipid membrane sensors to discriminate between Kasseki and Huashi.

First, seven types of sensors were tested on serial concentrations of water extracts of Kasseki and Huashi. The results suggested that the AC0 and AAE sensors were appropriate for our purpose when 1% (w/w) water extracts of samples were used. Next, we tested ten each of Kasseki and Huashi samples in this condition. For the Kasseki samples, both sensors showed specifically localized output values ranging from 0 to -5 mV. By contrast, for the Huashi samples, AC0 characteristically showed output values deviating from the range within ±5 mV and AAE showed a wide range of output values, from -22 to 1 mV. These data suggest that the taste-sensing system can discriminate Kasseki from Huashi when their 1% (w/w) water extracts are measured by AC0 and AAE sensors.

Key words Kasseki, Huashi, Pharmacopoeia, Discrimination, Taste-sensing system, Artificial lipid membrane sensors.

Introduction

‘Kasseki’ and ‘Huashi’ are crude mineral drugs. The former has been used as a component of some Kampo formulae such as bofutsushosan and choreito, and recently its consumption increases. Though almost the same Chinese characters are used for both, they have different definitions. In Japan, in 2011, Kasseki was newly adapted to The Japanese Pharmacopoeia Sixteenth Edition (JP16) with its English name of Aluminum Silicate Hydrate with Silicon Dioxide and defined as
“a mineral substance, mainly composed of aluminum silicate hydrate and silicon dioxide; it is not the same substance as mineralogical talc”. Although the Kaskei used in today’s Japanese markets is almost completely imported from China, it has been never found as Huashi in Chinese markets. Huashi, in the Pharmacopoeia of The People’s Republic of China (CP), is defined as “mainly hydrated magnesium silicate” with the Latin name of ‘TALCUM’ and the English name of ‘Talc’. Thus, Huashi as defined in CP is thought to be equivalent to the “mineralogical talc”, or magnesium salt, distinguished from Kesseki in JP16. In fact, JP16 describes a confirmation test which distinguishes Kaskei from talc by the identification of aluminum in Kaskei. It should be noted that this confirmation test focuses only on aluminum, but not on the whole of Kaskei, which consists of aluminum silicate hydrate and silicon dioxide. X-ray fluorescence analysis and X-ray diffraction are available in order to strictly analyze Kaskei. They are particularly effective methods for the analysis of minerals, but they utilize somewhat expensive instruments.

Meanwhile, we have used a taste-sensing system as one of the comprehensive and objective methods for quality evaluation of Kampo formulae, crude drugs and foods. The taste-sensing system is a tool for objective taste measurement, which is based on the concept of modeling the mechanism of human taste recognition. This system is composed of a sensor unit, consisting of various artificial lipid membrane sensor probes, and a personal computer. The taste data are obtained from the change of the electric potential of the artificial lipid membranes when the taste substances interact electrically with, or are adsorbed to, the membrane. In this study, we report the possibility of this system as a new method to discriminate Kaskei from Huashi.

Materials and Methods

Materials

Samples: Ten Kaskei samples were kindly supplied from Japan Medicinal Plant Federation (Osaka, Japan). Ten Huashi samples were obtained from eight drug stores, a pharmaceutical factory and a drug market in China. The main component of Huashi samples was preliminarily identified as hydrated magnesium silicate by the X-ray diffraction method. In addition, a talc which is sold as a commercial reagent (average particle size 7 ~ 12 μm) and an equivalent to Huashi was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Information about the sources of Kaskei and Huashi samples is summarized in Table 1. Every sample was investigated the X-ray diffraction method in order to identify the origin of minerals (data not shown). The brick-type samples were pulverized using an electric mill and passed through a No. 100 sieve (150 μm). All samples are stored at Museum of Materia Medica, Research Center for Ethnomedicine, Institute of Natural Medicine, University of Toyama, Japan.

Chemicals and reagents: A solution consisting of 30 mM potassium chloride in 0.3 mM tartaric acid was employed as the reference solution in the measurement by the taste-sensing system. As the washing solution for negatively charged artificial lipid membrane sensor probes, 30% ethanol aqueous solution containing 100 mM hydrochloric acid was used. As the washing solution for positively charged artificial lipid membrane sensor probes, 30% ethanol aqueous solution containing 100 mM potassium chloride and 10 mM potassium hydroxide was used. Potassium chloride, tartaric acid, ethanol (99.5 v/v%) and potassium hydroxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hydrochloric acid (35 ~ 37%) was purchased from Nakalai Tesque, Inc. (Kyoto, Japan). Ultra pure water was obtained by an EQS-10L system (Nihon Millipore K.K., Tokyo, Japan). The internal solution for the artificial lipid membrane sensor probes containing 3.3 M potassium chloride in saturated silver chloride aqueous solution was obtained from Intelligent Sensor Technology, Inc. (Atsugi, Japan).

Sample preparation

A pulverized and sieved Kaskei (TMPW 25309 in Table 1) and the reagent talc were weighed and suspended in ultra pure water at serial concentrations (Kaskei: 0.01 ~ 10% (w/w), reagent talc: 0.01 ~ 1% (w/w)), and then extracted by sonication for 10 minutes at 25°C. After centrifuging at 1,710 × g for 10 minutes at 25°C, the supernatant was filtered with No. 2 filter paper (JIS P3801). Potassium chloride and tartaric acid

Taste sensors sort Kaskei from Huashi
Table 1: Collection data of Kaskei (Aluminum Silicate Hydrate with Silicon Dioxide) in Japanese markets and Huashi (Talc) in Chinese markets

<table>
<thead>
<tr>
<th>Drug Name in Pharmacopoeia</th>
<th>No.</th>
<th>TMPW No.</th>
<th>Market</th>
<th>Origin</th>
<th>Date</th>
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<tbody>
<tr>
<td>J-1</td>
<td>26809</td>
<td>Japan Medicinal Plant Federation, Osaka, Japan (日本生薬連合会, 大阪)</td>
<td>Fujian Prov.</td>
<td>Oct., 2006</td>
<td></td>
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<td>J-2</td>
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<td>26831</td>
<td></td>
<td></td>
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<td>J-4</td>
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<td>J-5</td>
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TMPW No.: The specimen reference number of the Museum of Materia Medica, Research Center for Ethnomedicine, Institute of Natural Medicine, University of Toyama.

were added to the filtrate at concentrations of 10 mM and 0.1 mM, respectively.

The test solutions were initially applied to the taste-sensing system to determine the optimal concentration and sensor types. Next, ten Kaskei and ten Huashi samples were extracted to the optimal concentration in the same way as described above, and measured by the taste-sensing system. Aqueous solution containing 10 mM potassium chloride and 0.1 mM tartaric acid was used as a blank control.

Measurement
We measured sensor outputs of water extracts of Kaskei and Huashi samples under the following conditions, which were chosen as described in our previous report.²³

Instrument: Taste-sensing system SA402B (Intelligent Sensor Technology, Inc.). Artificial lipid membrane sensor probes: AAE, CT0, CA0, C00, AE1, AC0 and AN0 (Intelligent Sensor Technology, Inc.). In this study, we used only the sensor output values called relative
potentials, that is, the electrical potential changes between the sample solution and the reference solution.

Results

First, graduated concentration solutions of Kasseki and reagent talc were measured by the Taste-sensing system SA402B with seven types of artificial lipid membrane sensor probes. As the result, the AC0 and AAE sensors showed slightly negative output values for Kasseki water extracts, with little concentration-dependent sensor output changes. For the reagent talc, by contrast, two sensors’ output values changed depending on sample concentrations. The output values of the AC0 sensor to 1% (w/w) of Kasseki and the reagent talc solutions were -3.5 mV and -15.7 mV (Fig. 1-a), while those of the AAE sensor were -1.5 mV and -9.3 mV (Fig. 1-b), respectively. These two sensors’ output values were stable and their measurement deviations were small. Thus, we judged that the AC0 and AAE sensors were sufficient to discriminate Kasseki from Huashi at the extract condition of 1% (w/w). There were several reasons that the other five sensors were considered inappropriate. The CT0 and AE1 sensors did not respond to any of the sample solutions and the CO0 sensor showed no constant concentration-dependent sensor output changes for either sample type (data not shown). The AN0 and CA0 sensors showed similar output tendencies to those of AC0 and AAE sensors, respectively; however, these two sensors were less durable.

Then, each of the ten samples of both Kasseki and Huashi were extracted at the condition of 1% (w/w) and their sensor output values were measured by the AC0 and AAE sensors. As a result, both sensors showed specifically localized output values ranging from 0 to -5 mV for all ten Kasseki sample solutions (Fig. 2) while, for the Huashi samples, AC0 characteristically showed output values deviating from the range within ± 5 mV (actual figure ranges were from -21 to -9 mV and from +9 to +15 mV) and AAE showed a wide range of output values, from -22 to 1 mV (Fig. 2).

![Graphs](image)

Fig. 1 Concentration dependences of electrical output changes on artificial lipid membrane sensors (AC0 and AAE) to Kasseki (Aluminum Silicate Hydrate with Silicon Dioxide) and reagent talc (Wako Pure Chemical Industries, Ltd.). AC0 (a) and AAE (b). To avoid damage to the sensor, higher concentrations than 1% (w/w) were not tested for the reagent talc. Each value represents the mean ± standard deviation of three measurements.
Discussion

In this study, we tried to discriminate Kasseei from Huashi by a taste-sensing system. In the measurements, both AC0 and AAE sensors showed slight responsiveness to Kasseei samples, ranging from 0 to -5 mV, whereas, for the Huashi samples, a wide variety responsiveness deviating from the output value range from 0 to -5 mV. These data clearly suggest that the taste-sensing system can discriminate Kasseei from Huashi when their 1% (w/w) water extracts are measured by AC0 and AAE sensors. In other words, under the conditions of this study, when the tested sample showed an output value deviating from the range from 0 mV to -5mV, the sample is not referable to Kasseei and may be to Huashi.

Although AC0 and AAE sensors used in this study are normally utilized for detecting the taste factors of cationic bitterness and umami respectively, none of the human subjects in the gustatory test noted a specific taste for either Kasseei or Huashi water extracts. Thus, it is unlikely that sensor output values to these extracts result from the sensor responsiveness to taste substances of cationic bitterness and umami. The Kasseei and Huashi samples tested in this study originate mainly from “aluminum silicate hydrate and silicon dioxide” and “hydrated magnesium silicate”, respectively. It is assumed that the differences in the sensor output values obtained by the taste-sensing system with AC0 and AAE sensors reflect the differences of kinds and amounts of minerals eluted from Kasseei or Huashi to water. Therefore, the data in this study may offer a new application of the taste-sensing system in the field of mineralogy in addition to drug regulatory science. In this study, we employed not AN0 sensor but AC0 sensor for discriminating Kasseei from Huashi because of the difference in their endurance. Both sensors are usually applied to evaluate a taste value of cationic bitterness and the difference of their endurance is likely to be due to the compositional differences of the corresponding artificial lipid membranes.

All of the Kasseei samples showed similar sensor output values. In contrast, Huashi showed wide-ranging values (Fig. 2). This result seems to denote a rich diversity of the Huashi in Chinese markets. It is of interest that the AC0 sensor showed both positive and negative values for Huashi samples. These phenomena may be due to compositional differences of the Huashi samples. Huashi as defined in CP is though to be equivalent to the JP Talc. According to the JP16, Talc may contain
related mineral substances consisting chiefly of chlorite, magnesite, calcite and dolomite.\textsuperscript{15}) The existence of these related substances may affect sensor responses, although detailed analyses of Huashi samples by other analytical instruments would be needed to clarify such variations.

Since ancient times, most of the Kasseki used in Japan has been imported from China. Masutomi mentioned that the origin of Kasseki housed at Shosoin as early as the eight century was mainly hydrated halloysite, namely aluminum silicate hydrate.\textsuperscript{16}) In addition, it is known that all of Kasseki products in the Japanese market at the present day are imports from Fujian Province, China. On the basis of the survey analyses of Kasseki used in Japan, JP16 defined Kasseki as “a mineral substance, mainly composed of aluminum silicate hydrate and silicon dioxide”. While in China, Huashi has been defined as “mainly hydrated magnesium silicate” in CP. Several papers have reported that Huashi having different combinations of minerals from that defined in CP are sometimes sold in Chinese markets.\textsuperscript{5,6) These facts suggest that mineral crude drugs other than Kasseki may be mistakenly imported as Kasseki from the Chinese market to Japan in the future. Thus, it is important to continually monitor the mineral crude drugs imported from China. Since the measurement of mineral extracts by the taste-sensing system is not time-consuming and does not require expertise in pharmacognosy, this method may be suitable for the monitoring analysis.

**Conclusion**

This study showed that some artificial lipid membrane sensors of Taste-sensing system SA402B were able to discern the difference between Kasseki and Huashi. When a 1% (w/w) water extract of tested samples is measured by AC0 and AAE sensors and these sensors show their output values from 0 to -5 mV, the tested sample is identifiable as Kasseki.

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