Influence of Swallowing Aids on the Adsorption and Palatability of Kremezin®

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The purpose of this study was to evaluate the effect of three swallowing aids on the adsorbent properties and palatability of Kremezin®, a mixture of the oral charcoal adsorbent, Kremezin®. None of the swallowing aids had any effect on the adsorption of indole by Kremezin®, either in vitro and in vivo. In gustatory sensation tests of the palatability of the swallowing aids with Kremezin®, 14 items were evaluated according to the semantic differential (SD) method. Factor analysis of the results identified two main factors ‘Remaining after removing from mouth’ and ‘Sense of holding in mouth’ as predominantly determining the palatability. The swallowing aids with the highest viscosity allowed the best dispersion of Kremezin®, and also improved the palatability of Kremezin® the most.

Key words Kremezin®; swallowing aid; adsorption; indole; palatability

Accumulation of some of these uremic retention solutes in the body has been reported to impose various impairment to different organs. When toxicity is proven, they are called uremic toxins. Indoxyl sulfate is one of the uremic toxins. Tryptophan contained in food is transformed to indole by the intestinal bacteria, and the indole absorbed from the intestine is transformed in the liver into indoxyl and then to Indoxyl sulfate. Since 90% of Indoxyl sulfate in blood binds with albumin, it is excreted through the kidney mainly from the proximal renal tubules into urine. In chronic renal failure, the blood concentration of uremic toxins increases markedly due to lowered renal clearance. Compared with healthy persons, the blood Indoxyl sulfate concentration is approximately 30 times higher in pre-dialysis patients with chronic renal failure and 80 times higher in patients before initiation of dialysis. Indoxyl sulfate is known to accelerate the progression of renal failure, and is a surrogate parameter of renal function. Patients who accumulate larger amounts of Indoxyl sulfate tend to show a higher speed of progression of renal failure. Administration of Indoxyl sulfate or its precursor, indole, to rats with renal failure results in lowered renal function. Circulating uremic toxins are thought to be one of the factors accelerating the progression of chronic renal failure (CRF). There have been several reports that administration of an oral adsorbent (Kremezin®, Kureha Corporation, Tokyo, Japan) may retard the progression of CRF in both uremic rats and undialysed uremic patients by adsorbing hydrophilic water-soluble, ionic uremic substances in the gastrointestinal tract, which are then excreted in faeces. Kremezin® consists of fine spherical particles approximately 0.2–0.4 mm in diameter, composed of porous microcrystalline carbon with an oxygen complex including a surface oxide. Kremezin® is insoluble in water and organic solvents. It is not decomposed by digestive enzymes or intestinal bacteria, and does not adsorb electrolytes such as sodium, potassium, calcium or phosphate. At present, Kremezin® is widely used as an approved drug in Japan for the treatment of approximately 50000 undialysed uremic patients to delay the progression of CRF. However, the single dose is quite large (2 g), which frequently gives rise to noncompliance. A device is therefore needed to improve compliance for Kremezin®.

Recently, a number of swallowing aids have been developed that aim to improve compliance by easing difficulties in swallowing. There are, however, few reports in which the effects of jellies have been evaluated as swallowing aids for Kremezin®.

In this study, we investigated the effects of jellies and pastes as swallowing aids on the adsorption capacity of Kremezin®. Secondly, we evaluated the improvement of palatability of Kremezin® with the addition of these swallowing aids, using human gustatory sensation testing. Palatability scores were evaluated by the semantic differential (SD) method. A factor analysis (rotated with the varimax method) was performed on the data, and ‘Remaining after removing from mouth’ and ‘Sense of holding in mouth’ were identified as the two main factors determining palatability. Finally, we investigated the viscosity and dispersion of Kremezin® with each swallowing aid.

Experimental

Materials Kremezin® was purchased from the market circulation. The following three swallowing aids were used in this study: Okusurinometane (jelly product, taste peach), Pestojoynoooburato (paste product, taste plain) and TROMELIN®GRA. (a semi-paste product). Okusurinometane was a gift from Ryukakusan Co., Ltd., Tokyo, Japan. TROMELIN®GRA. and Pesutojyonoooburato were purchased from Sanwa Kagaku Kenkusho Co., Ltd., Nagoya, Japan.

Adsorption of Indole by Kremezin® (in Vitro) Kremezin® (200 mg) and indole (initial concentration: 1 g/l) was suspended in 0.75 g of water (control), Okusurinometane, Pestojoynoooburato, or 3% TROMELIN®GRA. solution. Each sample was added to 200 ml of 50 mM phosphate-buffered saline (PBS) at pH 7.4. After shaking for 3 h at 37 °C, samples were centrifuged at 15000 rpm for 10 min. The concentration of indole in the supernatant was measured by high performance liquid chromatography (HPLC). The ratio of adsorption to elimination of indole was calculated using the following formula:

$$\text{ratio of adsorption to elimination} = \frac{\text{initial concentration} - \text{free concentration in the supernatant}}{\text{initial concentration}}$$

Adsorption of Indole by Kremezin® (in Vitro) Male Sprague-Dawley strain rats (11 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan). They were housed in a light-controlled room (lights on from 7:00 a.m. to 7:00 p.m.) at room temperature (24±2 °C) and humidity 55±10% with food and water ad libitum. The rats were allowed to adapt to these conditions for 10 days before the start of the experiments. The indole solution was administered in 0.5% methylcellulose solution containing 0.1%
Twen 20. Firstly, groups of rats each were given single per os (p.o.) dose of indole at 25 mg/kg, 50 mg/kg, 100 mg/kg and blood samples were collected at 0, 0.5, 1, 3, 6, 10 h after indole p.o. injection for dose finding and time course study. Serum samples were obtained after centrifugation at 3000 rpm for 30 min. The serum levels of indoxyl sulfate (a hepatic metabolite of indole) were determined by HPLC. The serum levels of indoxyl sulfate were shown in dose dependent manner. At 6 h after indole p.o. injection, the serum levels of indoxyl sulfate of all groups were showed peak concentration. Moreover, the serum levels of indoxyl sulfate of group at 6 h after 50 mg/kg indole p.o. injection had the smallest interindividual difference in all groups. The serum levels of indoxyl sulfate of group at 6 h after 50 mg/kg indole p.o. injection were accepted as a proper dose and blood sampling time in our experiment (data not shown).

The rats were divided into five groups (n = 3). Group 1 (control) received only indole (50 mg/kg, p.o.). Group 2 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g of 1% methylcellulose solution. Group 3 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g of 3% TROMELIN®Gra. solution. Group 4 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g of Pesutojyonooburato (plain). Group 5 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g of Pesutojyonooburato.

All animal experiments were approved by the Animal Care and Use Committee of Mukogawa Women’s University.

Determination of Serum Indoxyl Sulfate Level by HPLC Indoxyl sulfate was used as an internal standard. The HPLC system consisted of an LC-10Advp pump (Shimadzu, Kyoto, Japan), a Shimadzu SPD-10Avp UV–vis detector, a Shimadzu SCL-10Avp auto injector, and a Shimadzu SCL-10Avp system controller. The system was equipped with a CAPCELL-PAK C18 UG120 column (5 μm, 4.6 × 250 mm; SHISEIDO). The mobile phase consisted of water : acetonitrile (5 : 5, v/v) and was delivered at a flow rate of 1.0 ml/min at 40 °C. Detection was monitored at an UV wavelength of 238 nm. Under these conditions, the coefficients of the intra- and inter-day variations were below 5%.

Gustatory Sensation Tests For human gustatory sensation tests, 10 ml of Okusurinometane, Pesutojyonooburato or 3% TROMELIN®Gra. solution (or water as a control) was uniformly mixed with 2 g of Kremezin® for 10 s using a spoon.

Samples of Kremezin® mixed with water (control), Okusurinometane (peach), Pesutojyonooburato (plain) or TROMELIN®Gra., with or without Kremezin®, were used for gustatory sensation testing in eight well-trained volunteers. Each volunteer provided informed consent for the procedures, which were approved by ethical committees of the Mukogawa Women’s University.

The sample size was 10 ml, and all samples were kept in the mouth for 10 s. After testing, subjects gargled well before tasting the next sample. Various palatability scores were evaluated using the semantic differential (SD) method as follows16): the subjects were asked to score the samples on the following items: 1) Bad/Good odour (orthonasal) in mixing, the scores of Kremezin® with Okusurinometane (peach) were much higher than control. In item 4) Taste bad/Taste good, all the scores were much higher than control, especially that of Okusurinometane (peach). In items 9) Taste remaining/Not remaining after spitting out, and 10) Rough feeling/No rough feeling after spitting out, all the scores were higher than control, especially those of Okusurinometane (peach) and Pesutojyonooburato.

A Factor Analysis of Palatability of Kremezin® with Swallowing Aids as Determined by Gustatory Sensation Tests A factor analysis (rotated using the varimax method) was performed on the data obtained by the SD method. As a result, two factors with values greater than 1.0 were identified. A factor analysis was performed according to method of Results and Discussion

The Adsorption of Indole by Kremezin® (in Vitro) Figure 1 shows the ratio of adsorption to elimination of indole by Kremezin® with various swallowing aids compared with control (Kremezin® plus water) in vitro. The ratio of adsorption to elimination in the presence of any of the swallowing aids was almost the same as with the control. This suggests that the swallowing aids had no effect on the adsorption of indole by Kremezin® in vitro.

The Adsorption of Indole by Kremezin® (in Vivo) Figure 2 shows the serum indoxyl sulfate levels in groups 2, 3, 4 and 5 after administration of indole and Kremezin® with each of the three swallowing aids or with solvent alone, compared with group 1. The serum levels of indoxyl sulfate in groups 2, 3, 4 and 5 were similar to each other and significantly lower than those in group 1. The serum indoxyl sulfate levels showed no significant difference between groups 2, 3, 4 and 5 after administration of indole and Kremezin® with solvent alone or with each of the three swallowing aids. This suggests that the swallowing aids had no effect on the adsorption of indole by Kremezin® in vivo.

The Palatability of Kremezin® Plus Swallowing Aids Evaluated by the SD Method Figure 3 shows the palatability scores for Kremezin® plus each of the swallowing aids compared with control, evaluated by the SD method. In item 1) Bad/Good odour (orthonasal) in mixing, the scores of Kremezin® with Okusurinometane (peach) in particular, were higher than control. In item 4) Taste bad/Taste good, all the scores were higher than control, especially that of Okusurinometane (peach). In items 9) Taste remaining/Not remaining after spitting out, and 10) Rough feeling/No rough feeling after spitting out, all the scores were higher than control, especially those of Okusurinometane (peach) and Pesutojyonooburato.

Each value was mean ± S.E.M. of three samples.
The contributions of these factors (factors I and II) were 36.4% and 22.4%, respectively. Among the 14 palatability items, two items, item 12) Remaining/Not remaining after gargling five times and 13) Rough feeling/No rough feeling after gargling five times, showed high factor loadings of factor I. ‘Remaining after removing from mouth’ was adopted as the composite factor for these items. Items 3) Easy/Difficult to keep in mouth, 9) Taste remaining/Not remaining after spitting out and 10) Rough feeling/No rough feeling after spitting out, showed high factor loadings of factor II. ‘Sense of holding in mouth’ was adopted as the composite factor for these items.

The factor score shows relation between each group (each of the swallowing aids and water) and each factor. A scatterplot of Factor scores of Factor I and II for Kremezin® plus each of the swallowing aids compared with control was shown in Fig. 4. The high score in Factor scores of Factor I means less of ‘Remaining after removing from mouth’ and the low score in Factor scores of Factor I means much of ‘Remaining after removing from mouth.’ The high score in Factor scores of Factor II means less of ‘Sense of holding in mouth’ and the low score in Factor scores of Factor II means much of ‘Sense of holding in mouth.’ The palatabilities of Kremezin® with the various swallowing aids or water (control) were divided into two groups. Okusurinometane (peach) and Pesutojyonooburato were showed high score in Factor score of Factor I and Factor II. These two products were suggested to be products which are less of ‘Remaining after removing from mouth’ and less of ‘Sense of holding in mouth.’ While in the case of 3% TROMELIN®Gra. and control, both of Factor scores of Factor I and Factor II were low. 3% TROMELIN®Gra. and water (control) were suggested to be products which are much of ‘Remaining after removing from mouth’ and much of ‘Sense of holding in mouth.’ Consequently, the palatability of Kremezin® with Okusurinometane (peach) and Pesutojyonooburato (plain) were suggested to be improved compared with control when analysed on the basis of the two factors arising from a factor analysis.

Viscosity of Water and Swallowing Aids before and after Addition of Kremezin® (2 g/10 g)

The solvent for the indole solution was a 0.5% methylcellulose solution containing 0.1% Tween 20. Group 1 rats received indole (50 mg/kg, p.o.), group 2 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g 1% methylcellulose solution, group 3 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g 3% TROMELIN®Gra. solution, group 4 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g Okusurinometane, and group 5 received indole (50 mg/kg, p.o.) plus Kremezin® (1 g/kg, p.o.) suspended in 10 g Pesutojyonooburato. Each value is mean±SEM of three rats. **p<0.01; compared with Group 1 (control) using the Dunnet test.

Fig. 2. Serum Indoxyl Sulfate Levels in Groups 2, 3, 4 and 5 (after Administration of Indole Plus Kremezin® in the Presence of Swallowing Aids or Solvent Only) Compared with Group 1 (Control)

The data represent the mean of six values. **p<0.01; compared with water (control) using the Dunnet test.

Fig. 4. A Scatterplot of Factor Scores of Factor I and II in the Palatability of Kremezin® Plus Each of the Swallowing Aids

Each value is mean of six subjects.
properties, while adhesion of micro particles to the tongue is prevented, and bitterness perception thereby decreased. Fukui et al. has reported on the physicochemical characteristics of jellies (viscosity, strength, loss of water content), and their effects on swallowing. In this study, both the jelly product and a paste product had higher viscosities than TROMELIN®Gra. and water (control). However Kremezin® was completely precipitated in control or was slightly precipitated in TROMELIN®Gra., Kremezin®, Kremezin® were uniformly dispersed in both Okusurinometane (peach) and Pesutojyonooburato (plain). Both Okusurinometane (peach) and Pesutojyonooburato (plain) were suggested to be products which is less of ‘Remaining after removing from mouth’ and less of ‘Sense of holding in mouth’ in factor analysis. A fine dispersion of products made swallowing easier due to prevention of sticking Kremezin® in the mouth and improved palatability.

**Conclusion**

In this study, three swallowing aids showed no effects on the ability of Kremezin® to adsorb the uremic substance, indole, either *in vitro* or *in vivo*. Among three swallowing aids, the jelly and the paste product which had a fine dispersion of Kremezin® with high viscosity comparatively were suggested to improve the palatability of Kremezin® considerably.

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**References**