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

Miyako Yoshida, Mai Hazekawa, Tamami Haraguchi, and Takahiro Uchida*

School of Pharmaceutical Science, Mukogawa Women's University; 11–68 Koshien 9-Bancho, Nishinomiya 663–8179, Japan. Received August 2, 2010; accepted January 5, 2011; published online January 12, 2011

The purpose of this study was to evaluate the effect of three swallowing aids on the adsorbent properties and palatability of 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 aid 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.[1] 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.[2] 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.[3] Administration of Indoxyl sulfate or its precursor, indole, to rats with renal failure results in lowered renal function.[4,5] Circulatimg 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[6—8] and undialysed uremic patients,[9—11] by adsorbing hydrophilic water-soluble, ionic uremic substancesin 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.[12—15] 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), Pestojyonooburato (paste product, taste plain) and TROMELIN®Gra. (a semi-paste product). Okusurinometane was a gift from Ryukakusan Co., Ltd., Tokyo, Japan. TROMELIN®Gra. and Pesutojyonooburato were purchased from Sanwa Kagaku Kenkyusho 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, Pesutojyonooburato, 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 Vivo)* 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 d before the start of the experiments. The indole solution was administered in 0.5% methylcellulose solution containing 0.1%

* To whom correspondence should be addressed. e-mail: takahiro@mukogawa-u.ac.jp

© 2011 Pharmaceutical Society of Japan

Tween 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 3 min. The serum levels of indoxyl sulfate (a hepatic metabolite of indole) were determined by HPLC. The serum levels of indoxyl sulfate were showed 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 Okusurinometane. 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 SIL-10ADvp 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. 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 follows[16]: the subjects were asked to score the samples on the basis of eight items, expressed as symmetrical terms representing both extremities, as follows: 1) Bad/Good odour (orthonasal) of mixture, 2) Not uniformly mixed/Uniformly mixed, 3) Easy/Difficult to keep in mouth, 4) Bad/Good taste, 5) Bad/Good odour (retronasal), 6) Feeling/Not feeling rough, 7) Bad/Good sensation in mouth, 8) Sandy/Not sandy, 9) Taste remaining/Not remaining after spitting out, 10) Rough feeling/No rough feeling after spitting out, 11) Aftertase/No aftertase after spitting out, 12) Taste remaining/Not remaining after gargling five times, 13) Rough feeling/No rough feeling after gargling five times, 14) Aftertaste/No aftertaste after gargling five times. Each item was scored using the following rating scale: 1, extremely; 2, slightly; 3, neither; 4, slightly; 5, extremely. No adverse effect was observed during the testing.

Viscosity Test The viscosities of control, Okusurinometane (peach), Pesutojyonooburato (plain) and TROMELIN®Gra., with or without Kremezin®, were determined using a viscotester (VT-03F, VT-04F, RION Co., Ltd., Tokyo, Japan).

Optical Determination of Dispersion of Kremezin® with Each Swallowing Aid Samples (10 ml) of Okusurinometane, Pesutojyonooburato, 3% TROMELIN®Gra. solution, or water (control) were uniformly mixed with 2 g Kremezin® for 10 s using a spoon. After 5 min, the degree of dispersion of Kremezin® with each swallowing aid was determined with the naked eye.

Data Analysis S-PLUS 2000J (Mathematical Systems, Inc., Tokyo, Japan) was used for factor analysis. The Dunnet test was used for multiple comparisons. The 5% level of probability was considered significant.

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 (plain).

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

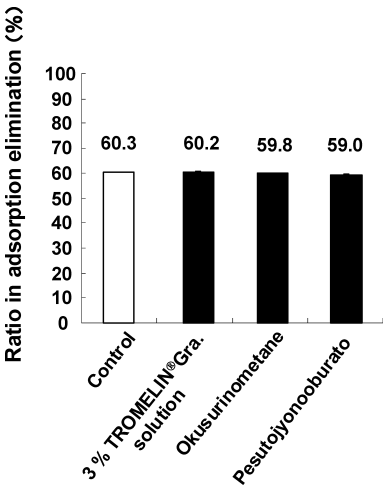

Fig. 1. The Ratio of Adsorption : Elimination for Kremezin® with Swallowing Aids Compared with Control (Kremezin® with Water) in the Adsorption of Kremezin® to Indole *in Vitro*

Each value was mean±S.E.M. of three samples.

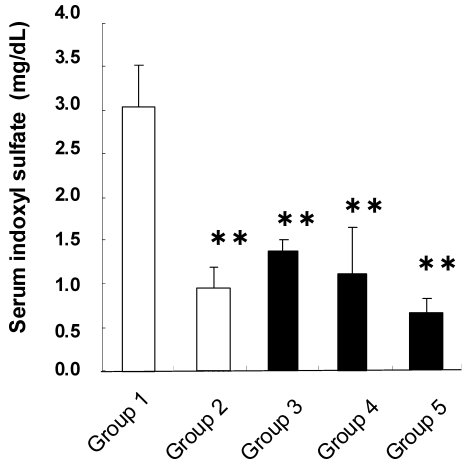

Fig. 2. Serum Indoxyl Sulfate Levels in Groups 2, 3, 4 and 5 (after Administration of Indole Plus Kremezin® in the Presence of Swallowing Aids or Solvent Only) Compared with Group 1 (Control)

The 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.

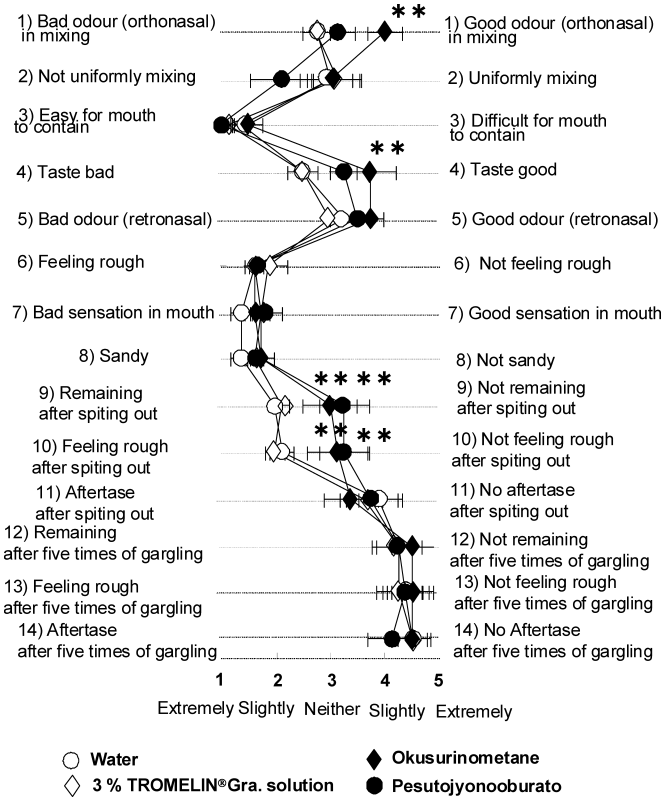

Fig. 3. Palatability Scores of Kremezin® Plus Each of the Swallowing Aids Using the SD Method

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

Mukai *et al.*[17] 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.

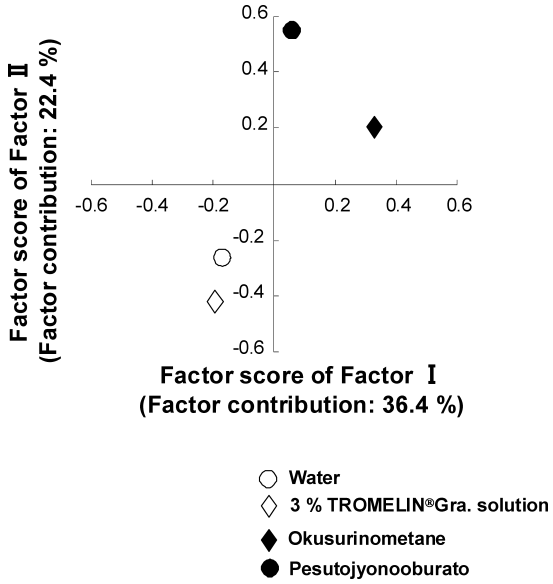

Fig. 4. A Scatterplot of Factor Scores of Factor I and II in the Palatability of Kremezin® Plus Each of the Swallowing Aids

Each value is mean of six subjects.

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 (plain) 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 viscosities of water, Okusurinometane (peach), Pesutojyonooburato (plain)

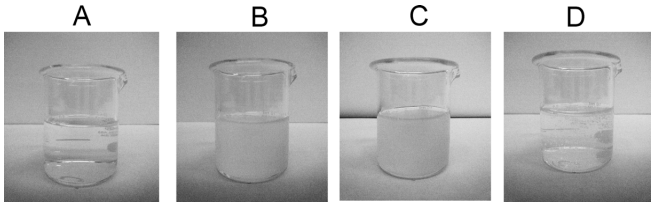

Fig. 5. The Dispersion of Kremezin® with Each Swallowing Aid 5 min after Mixing, as Determined by the Naked Eye before Adding Kremezin®

(A) Water, (B) 3% TROMELIN®Gra. solution, (C) Okusurinometane, (D) Pesutojyonooburato.

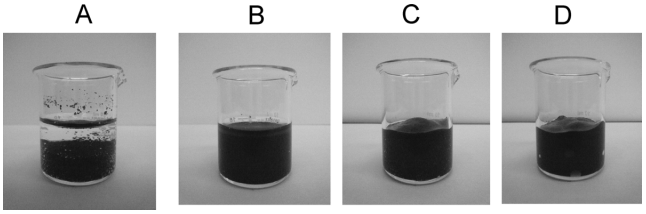

Fig. 6. The Dispersion of Kremezin® with Each Swallowing Aid 5 min after Mixing, as Determined by the Naked Eye, after Adding 2 g/10 g of Kremezin®

(A) Water, (B) 3% TROMELIN®Gra. solution, (C) Okusurinometane, (D) Pesutojyonooburato.

or TROMELIN®Gra. solution before adding Kremezin® were 0.0154±0.0000 dPa·s, 1.56±0.05 dPa·s, 4.18±0.04 dPa·s and 80.80±0.49 dPa·s, respectively, compared with 25.80±0.58 dPa·s, 48.40±0.60 dPa·s and 109.00±1.00 dPa·s, respectively, after adding Kremezin® (2 g/10 g). The viscosity of water plus Kremezin® (2 g/10 g) was not determined because Kremezin® completely precipitated in the water. Each value is the mean±S.E.M. of six samples.

Optical Determination of Dispersion of Kremezin® with Each Swallowing Aid The dispersion of Kremezin® with each swallowing aid 5 min after mixing was determined with the naked eye as shown in Figs. 5 and 6. In the control, Kremezin® was completely precipitated. In TROMELIN®Gra., Kremezin® was slightly precipitated. However the viscosity of TROMELIN®Gra. solution after adding Kremezin® was higher than before adding, the viscosity of TROMELIN®Gra. solution after adding Kremezin® was too low to obtain fine dispersions of Kremezin®. Fine dispersions of Kremezin® were observed with Okusurinometane (peach) and Pesutojyonooburato (plain). The viscosities of Okusurinometane (peach) after adding Kremezin® and Pesutojyonooburato (plain) after adding Kremezin® were higher than that of TROMELIN®Gra. solution after adding Kremezin®. These results suggest that the viscosity of the swallowing aids is related to dispersion of Kremezin® with each swallowing aid. Moreover, fine dispersion of Kremezin® with each swallowing aid was suggested to prevent sticking in the mouth and to improve the palatability of Kremezin® having specific material character consisted of spherical shape and micro particle. It is beneficial for chronic kidney disease (CKD) patients especially, elderly patients to utilize swallowing aids because they lack salivary secretion.

When swallowing aids are added to drugs, swallowing is made easier due to their moderate adhesion and liquidity 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.[18] 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.

Acknowledgements This work was supported by Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science 20590166 (to T.U.).

References

1) Niwa T., Yazawa T., Ise M., Sugano M., Kodama T., Uehara Y., Maeda K., *Nephron*, **57**, 84—88 (1991).
2) Niwa T., "Textbook of Nephrology," 4th ed., Lippincott, Williams and Wilkins, Baltimore, 2000, pp. 1269—1272.
3) Niwa T., Aoyama I., Takayama F., Niwa T., Aoyama I., Takayama F., Tsukushi S., Miyazaki T., Owada A., Shiigai T., *Miner. Electrolyte. Metab.*, **25**, 118—122 (1999).
4) Niwa T., Ise M., Miyazaki T., *Am. J. Nephrol.*, **14**, 207—212 (1994).
5) Niwa T., Ise M., *J. Lab. Clin. Med.*, **124**, 96—104 (1994).
6) Motojima M., Nishijima F., Ikoma M., Kawamura T., Yoshioka T., Fogo A. B., Sakai T., Ichikawa I., *Kidney Int.*, **40**, 461—469 (1991).
7) Yoshida Y., Sakai T., Ise M., *Nephron.*, **62**, 305—314 (1992).
8) Niwa T., Miyazaki T., Hashimoto N., Hayashi H., Ise M., Uehara Y., Maeda K., *Am. J. Nephrol.*, **12**, 201—206 (1992).
9) Sanaka T., Sugino N., Teraoka S., Ota K., *Am. J. Kidney Dis.*, **12**, 97—103 (1988).
10) Owada A., Nakao M., Koike J., Ujiie K., Tomita K., Shiigai T., *Kidney Int.*, **63** (Suppl.), S188—S190 (1997).
11) Niwa T., Nomura T., Sugiyama S., Miyazaki T., Tsukushi S., Tsutsui S., *Kidney Int.*, **62** (Suppl.), S23—S28 (1997).
12) Hanawa T., Watanabe A., Tsuchiya T., Ikoma R., Hidaka M., Sugihara M. *Chem. Pharm. Bull.*, **43**, 872—876 (1995).
13) Kaneuchi M., Kohri N., Senbongi K., Sakurai H., Iseki K., *Yakugaku Zasshi*, **125**, 187—196 (2005).
14) Ando M., Kouzai T., Higuchi F., Ichihashi T., Takeda M., Mizutani Y., Uesaka H., *Yakuzaigaku*, **60**, 261—270 (2000).
15) Fukui A., Fujii R., Yonezawa Y., Sunada H., *Chem. Pharm. Bull.*, **52**, 298—302 (2004).
16) Osgood E. C., Suci G. J., Tannenbaum P. H., "The Measurement of Meaning," University of Illinois Press, Urbana, 1957.
17) Mukai J., Miyanaga Y., Ishizaka T., Asaka K., Nakai Y., Tsuji E., Uchida T., *Chem. Pharm. Bull.*, **52**, 1416—1421 (2004).
18) Fukui A., Fujii R., Sunada H., *Yakuzaigaku*, **60**, 62—70 (2000).