The Quantitative Prediction of Bitterness-Suppressing Effect of Sweeteners on the Bitterness of Famotidine by Sweetness-Responsive Sensor

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The purpose of the present study was the quantitative prediction of the bitterness-suppressing effect of sweeteners (sucrose or sugar alcohols) on the bitterness of famotidine (or quinine sulfate as control) solutions using an artificial taste sensor. Firstly, we examined the response characteristics of the sensor response to sweetness. The sensor membrane is charged negatively in the presence of sweeteners, which tend to receive protons from one of the components of the sensor membrane. The magnitude of the sensor response was shown to increase in direct proportion to the concentration of the sweetener. Secondly, we used direct or indirect methods to evaluate and predict the bitterness-suppressing effect of sweeteners on 1 mg/ml famotidine and 81.4 μM quinine sulfate solutions. In direct method, a regression between the sensor output of the sweetness-responsive sensor and the bitterness intensity obtained in human gustatory tests of famotidine solutions containing sweeteners at various concentrations, was performed. As a result, we were able to predict directly the bitterness intensity of the mixed solution. Finally, we also evaluated the bitterness intensity of the dissolution media of commercially available, orally disintegrating tablets containing famotidine by the combined usage of bitterness- and sweetness-responsive sensor. We found that the sugar alcohols in the tablet seem to be effective in the bitterness-suppression of famotidine from these tablets, especially in the initial phase (within 30 s) of the disintegration process.

Key words taste sensor; bitterness-suppression; sweetness; famotidine; sweeteners; orally disintegrating tablet

Famotidine, a histamine-2 receptor antagonist, is widely available as a treatment for gastric ulcers. The bitterness of famotidine is the main obstacle to good compliance for this medicine; the drug is frequently administered as an orally disintegrating tablet so that high concentrations of famotidine are delivered directly into the mouth. Therefore the quantitative evaluation of the effect of various sweeteners such as sugar alcohols, which are included in the formulation, on the bitterness-suppression of famotidine seems so important. Hashimoto *et al.* provided in Japanese Patent No. 2705787[1] that sugar alcohols suppress famotidine's bitterness. But it's not desirable to examine a bitterness-suppression by human repeatedly. If an alternative method is found, without exposures of famotidine or any medicine, more bitterness-suppression formulations are able to be produced easily and safely.

Some articles reported on the quantitative evaluation of the bitterness of medicines, amino acids and elemental diets using the artificial taste sensor.[2—4] The sensor was also successful in evaluating or predicting the bitterness-suppression of quinine hydrochloride by phospholipids,[5—8] which suppress bitterness by selectively competing at the bitterness receptor, or by adsorbing and coating the bitter substances. This result demonstrated that it is possible to predict the bitterness-suppression of human medicines at peripheral site (receptor sites in human taste cells) using the sensor's response to bitterness. However, it has been more difficult for the sensors to predict bitterness-suppression by sweet substances,[6] since bitterness-suppression seems to occur during the process of neurotransmission, which means the central bitterness-suppression. It also has been reported that the receptors for bitterness and for sweetness are different.[9—15] Therefore a sensor which responds to bitterness would be unable to evaluate bitterness-suppression by sweet substances.

The purpose of the present study was to develop a quantitative prediction method for the bitterness-suppressing effect of sweeteners (sucrose and several sugar alcohols) on famotidine solutions, and also to examine the bitterness intensity of commercially available, orally disintegrating tablets containing famotidine, using the artificial taste sensor.

Firstly, we characterized this newly developed sweetness-responsive sensor[16] for sweet-tasting substances such as sucrose and sugar alcohol solutions. We found that the negative output values of the various sweeteners became larger as the sweetener concentrations increased.

Secondly, we used two different methods (indirect and direct) for the quantitative evaluation of bitterness-suppression, and demonstrated the usefulness of the sweetness-responsive sensor in this evaluation. In both methods, we tried to evaluate bitterness-suppression of famotidine or quinine sulfate solution by using obtained sensor output of unknown concentrated sucrose or sugar alcohols. We fixed the concentrations of famotidine (1 mg/ml) or quinine sulfate solutions (81.4 μM: quinine sulfate was used as a standard for bitterness) and examined the bitterness-suppressing effects of added sweeteners using the sweetness-responsive sensor.

In Method 1 (indirect method) a regression equation was calculated for the various sweeteners using the output values of the sensor responding to sweetness and the sweetness intensity obtained in human gustatory sensation tests. The sensor output value of an unknown sweetener was then substituted into the above regression equation and its sweetness in-

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Table 1. The Concentration of Sweetener Solutions Used in the Present Study

Standard sweetness intensity score	Sweetener	Sucrose	Sorbitol	Erythritol	Xylitol	Mannitol	Lactitol	Maltitol
	Sweetness ratio	1	1.43	1.25	1	2	2.5	1.25
	Sweetener concentration (mM) which shows correponding standard sweetness intensity							
1		29.2	41.8	36.6	29.2	58.5	73.1	36.6
2		87.7	125.3	109.7	87.7	175.4	219.3	109.7
3		187.1	267.3	233.9	187.1	374.3	467.8	233.9
4		409.3	584.8	511.7	409.3	818.7	1023.4	511.7
5		994.1	1420.2	1242.7	994.1	—	—	—

Solutions of mannitol, lactitol and maltitol of sweetness intensity 5 could not be prepared due to solubility problems.

tensity predicted. And next, using the relationship between gustatory sweetness intensities of solutions of various sweeteners and that of quinine sulfate or famotidine solutions containing sweeteners, we are thus able to predict the bitterness-suppression effect of unknown sweetener solution.

In Method 2 (direct method), the regression between the sweetness intensity of the sweeteners and the bitterness intensity of 1 mg/ml famotidine or 81.4 μM quinine sulfate solution containing the sweeteners at different concentrations, was used to determine the bitterness-suppressing effect of the various sweeteners directly.

Finally, we tried to evaluate bitterness-suppression of dissolution media for commercially available, orally disintegrating tablets by combined use of bitterness- and sweetness-responsive sensor channels.

Experimental

Materials The famotidine powder and orally disintegrating tablets (Gaster®D) were gifts from Astellas Pharma Inc. (Tokyo, Japan). Quinine sulfate, sucrose and six sugar alcohols (sorbitol, erythritol, xylitol, mannitol, lactitol and maltitol) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were of special reagent grade.

Preparation of Standard Solutions with Sweeteners (Sucrose and Sugar Alcohols) for Response Characteristic of Sensor Responsive to Sweeteners For standard sucrose solutions, concentrations of 29.2, 87.7, 187.1, 409.4, and 994.1 mM were used, corresponding sweetness intensity scores were defined as 1, 2, 3, 4, and 5, respectively as shown in Table 1 according to previous articles.[17,18] The concentration and corresponding sweet scores for other sweet substances were simultaneously determined according to previous articles.[17,18] Their concentrations and scores were essentially defined by Fechner's law.[19] The human sweetness threshold of sucrose, sorbitol, erythritol, xylitol, mannitol, lactitol and maltitol were 10.0,[20,21] 14.3, 12.5, 10.0, 20.0, 25.0 and 12.5 mM, respectively, and sweet intensity for various concentrated solutions were all defined as the sweetness ratio as shown in Table 1.[20,21] The sweetness ratio means the ratio of the sugar alcohols' sweetness compared with the sweetness of sucrose as 1 in Table 1.[22] The human sweetness thresholds of sugar alcohols are believed same as the sweetness threshold of sucrose, therefore they are derived with 10.0 mM by the sweetness ratio.

Preparation of 1 mg/ml Famotidine or 81.4 μM Quinine Sulfate Solutions Consisting of Various Concentrated Sweetener Solutions Famotidine or quinine sulfate powder was diluted using various concentrated sweetener solutions with defined sweetness intensities of 3, 4, and 5 (except that solutions of mannitol, lactitol and maltitol of sweetness intensity 5 could not be prepared due to solubility problems), and drug concentration was finally adjusted to 1 mg/ml (famotidine) or 81.4 μM (quinine sulfate).

Preparation of Dissolution Media of Orally Disintegrating Tablet The dissolution media of orally disintegrating tablets were prepared as follows: five tablets (corresponding to 50 mg famotidine) were placed in a 100-ml beaker containing 50 ml of pH 7.4 phosphatase buffer containing 10 mM KCl. After 1, 5, 10, 30 and 60 s, the suspensions were decanted, the obtained supernatant filtered through a 0.45 μm-millipore filter and the famotidine concentration of the filtered solution determined by HPLC as follows: 50 μl of the filtered sample was injected into a chromatograph (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10AV), an integrator (Shimadzu C-R4A), and a reversed-phase column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai Tesque Co., Ltd., Kyoto, Japan). The following mobile phase systems were used: A, 0.01 mM heptane sulfonate; B, acetonitrile; C, methanol; (A : B : C, 25 : 6 : 1). The pH of the mobile phase was finally adjusted to pH 3.0 with diluted perchloric acid. The flow rate was 1.0 ml·min^{-1} and the wavelength was set at 254 nm. In the HPLC study, the recovery of famotidine was over 99.5%, and its related standard deviation was within 1.0% in the range of the measured concentration. The detection limit was 0.02 μg·ml^{-1} and much lower than the sample concentration. The filtered solution from dissolution media was also used for the above gustatory sensation test.

Gustatory Sensation Tests The gustatory sensation tests were performed with nine well-trained healthy human volunteers. The tests were performed using a quantitative 5-point scale as described in previous papers.[2,17,18]

Famotidine or quinine sulfate powder was diluted using various concentrated sweetener solutions with defined sweetness intensities of 3, 4, and 5 (except that solutions of mannitol, lactitol and maltitol of sweetness intensity 5 could not be prepared due to solubility problems), and drug concentration was finally adjusted to 1 mg/ml (famotidine) or 81.4 μM (quinine sulfate). For quinine solution, this concentration (81.4 μM) and corresponding bitterness intensity score of 4 was adopted from the previous article.[5] In the case of famotidine, the concentration of 1 mg/ml famotidine is defined by the clinical dose of the famotidine liquid formulation as shown in the previous article.[23] The 1 mg/ml famotidine solution represents averaged bitterness intensity of 4.5 obtained by another gustatory sensation test that was essentially same as following study using 9 volunteers. For 1 mg/ml famotidine or 81.4 μM quinine sulfate solutions consisting of various concentrated sweetener solutions, the bitterness suppression effect of sweetener for each drug was evaluated.

Before testing, the volunteers (n=9) were asked to keep the standard solutions in their mouths for 15 s, and were told their concentrations and their bitterness intensities. All unknown samples were randomly and blindly supplied to each volunteer. They were then asked to taste 2.5 ml aliquots of the sample solutions and to give each sample bitterness and/or sweetness intensity. All samples were kept in the mouth for 15 s. After tasting the sample, subjects gargled well and waited for at least 20 min before tasting the next sample.

Sensor Measurement The taste-responding system SA402B of Intelligent Sensor Technology, Ltd., Japan, was used to measure the electric potential of solutions of famotidine, sucrose, and six sugar alcohols. The electrode set is attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes made of lipid/polymer membranes (channels).

The lipid components of the sensors used in the present study are shown in Table 2. Each lipid was mixed in a test tube containing poly(vinylchloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran in a test tube, and dried on a glass plate at a temperature of 30 °C to form a transparent thin film, almost 200 μm thick. Each electrode was composed of a silver wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3.3 M KCl solution. The difference between the electric potential of the working electrode and the reference electrode was measured by means of a high input impedance amplifier connected to a computer.

We searched sensors which were responded to sweetness of sucrose and sugar alcohols. The sensor was sensor channel 8 consists of dioctyl phenyl phosphonate, phenylphosphoric acid mono-octyl ester, and tetradodecyl am-

monium bromide. The hydrophobic groups of these lipids are long and do not usually dissociate easily. Since the sweeteners tend to receive a proton from the sensor molecule, the surface of the sensor membrane is easily charged negatively, as illustrated in Fig. 1.[23] The negative output values caused by the sweetener increased as the concentration of the sweetener increases. On the other hand, in sensor channel 3, bitter substances such as famotidine donate protons to the sensor, such that the sensor is charged positively.

Samples consisting of solutions of various concentrations of famotidine, sucrose and sugar alcohols in 10 mM KCl solution were used in the study. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference sample and also to rinse the electrodes after every measurement according to the previous articles.[2]

The following method was used to measure the sensitivity and the selectivity of adsorption of the samples. The electrode is first dipped into the reference solution (Vr) and then into the sample solution (Vs). The sensor output is taken as the difference (Vs−Vr) between the potentials of the sample and the reference solution. Each measurement interval was set at 30 s, and electrodes were thoroughly rinsed after each measurement. The concentrations of all samples were adjusted with 10 mM KCl, which has no taste, to improve conductibility.

S-PLUS 2000J (Mathematical Systems, Inc. (Tokyo, Japan) was used as calculation software for regression analysis.

Analysis of Bitterness-Suppression for Dissolved Famotidine/Standard Quinine Sulfate Solutions by Indirect or Direct Method Method 1 (indirect method): The following four steps were performed:

(1) A correlation equation was calculated for the sensor output values obtained in human gustatory tests and the output of the sensor response for the sweeteners tested (see Figs. 4A, 5A).

(2) Obtained sensor output for an unknown concentrated sucrose or sugar alcohols solution was substituted into the above equation obtained in (1). (see Figs. 4A, 5A). Then we obtained sweetness intensity score of unknown concentrated sucrose or sugar alcohols.

(3) A correlation equation was obtained for the sweetness intensities of the sweetener solutions and the bitterness intensity of 1 mg/ml famotidine or 81.4 μM quinine sulfate solutions containing different concentrations of the sweetener obtained from gustatory sensation test results (see Figs. 4B, 5B).

(4) The sweetness intensity value predicted from (2) above was further substituted into the correlation equation determined in (3), to predict the bitterness-suppression effect of the sweeteners.

Using the relationship between the sweetness of solutions of sweeteners and that of quinine sulfate or famotidine solutions containing sweeteners, we are thus able to predict the bitterness-suppression effect of the sweeteners.

Method 2 (direct method): In this method, a regression equation was calculated for the relation between the sensor output of channel 8 and the bitterness intensity obtained in human gustatory tests of famotidine solutions containing sweeteners. Using this regression analysis using channels 8, we were able to predict directly the bitterness intensity of the mixed solution from the sensor output (see Figs. 6A, B). Method 2 was more convenient than method 1.

Using the above two schemes, and with quinine sulfate as a standard for bitterness, data were obtained for six sugar alcohols (sorbitol, erythritol, xylitol, mannitol, lactitol and maltitol). Sorbitol, mannitol, and erythritol were chosen as sweeteners in evaluation of bitterness-suppression of famotidine because they were actually included in the commercial famotidine formulation and were expected to be effective in bitterness-suppression.

Analysis of Bitterness-Suppression of Famotidine Released from Orally Disintegrating Tablet by Direct Method For the analysis of data from the dissolution media of orally disintegrating tablets, the bitterness intensity was evaluated using data from channel 3, which is responsive to famotidine, and channel 8, responsive to sugar alcohols and famotidine.

Table 2. Lipids Used for the Membranes

Channel	Lipid component
1	Dioctyl phenyl-phosphonate Phosphoric acid di-*n*-decyl ester
2	Phosphoric acid di-*n*-decyl ester 2-Nitrophenyl octyl ether
3	Dioctyl phenyl-phosphonate Hexadecanoic acid
4	Dioctyl phenyl-phosphonate
5	Tetradodecyl ammonium bromide Dioctyl phenyl-phosphonate
6	Tetradodecyl ammonium bromide 2-Nitrophenyl octyl ether
7	Tetradodecyl ammonium bromide Dioctyl phenyl phosphonate 2-Nitrophenyl octyl ether
8	Tetradodecyl ammonium bromide Dioctyl phenyl-phosphonate Phenylphospholic acid monooctyl ester

Results and Discussion

Response Characteristic of the Sensor Response to Sweetness Firstly, we tried to characterize the newly developed sweetness-responsive sensors. Figure 2 shows the response electric potential patterns for single-component solutions of 1 mg/ml famotidine, solutions of sucrose and six sugar alcohols corresponding to a sweetness intensity of 4 (409.3 mM sucrose, 584.8 mM sorbitol, 511.7 mM erythritol, 409.3 mM xylitol, 818.7 mM mannitol, 1023.4 mM lactitol, and

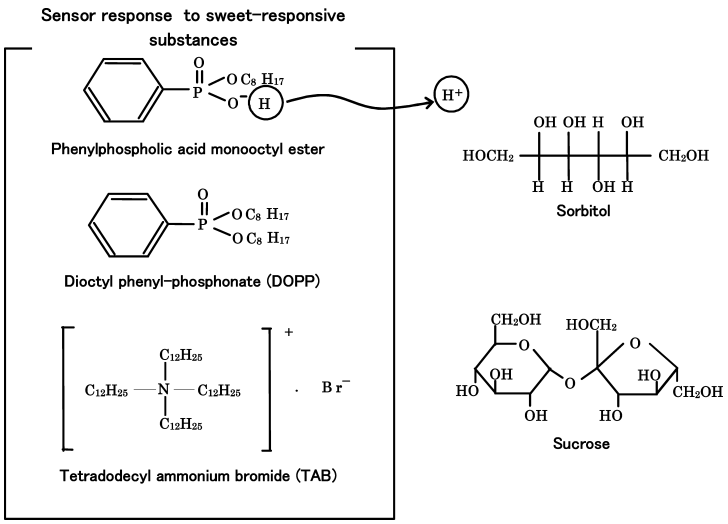

Fig. 1. Molecules Comprising the Sweetness-Responsive Sensor and Proposed Mechanism of Action

The proton seems to be released from a membrane component and donated to the sucrose or sweetener molecule.

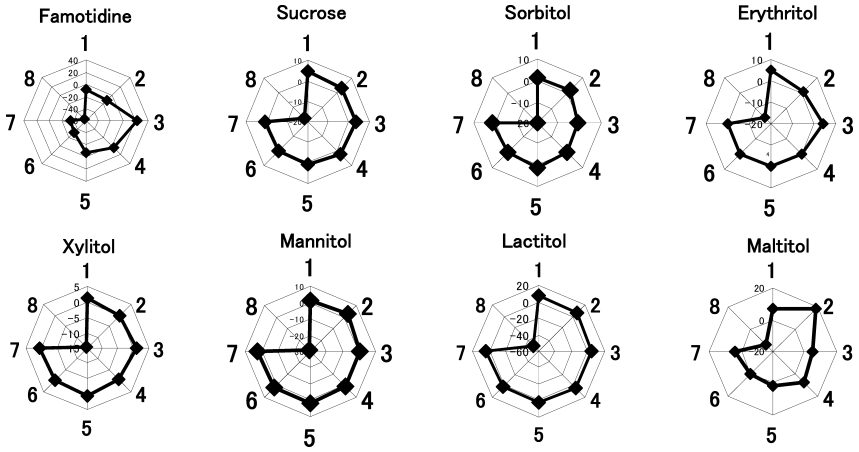

Fig. 2. Sensor Response Output Electric Potential Patterns of the Sweetness-Responsive Sensor to Famotidine, Sucrose and Six Sugar Alcohols
The concentration of the sweetener sample solution corresponded to a taste intensity of 4.

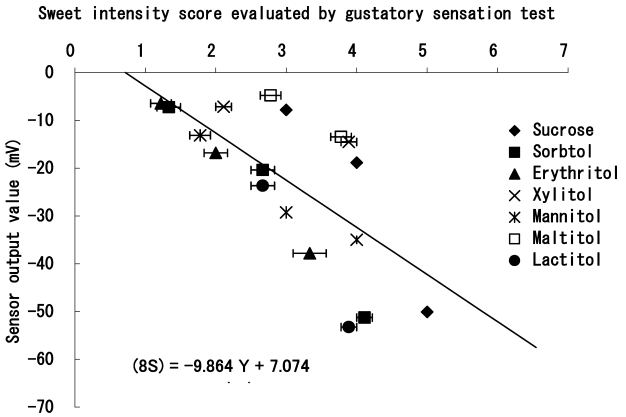

Fig. 3. The Relationship between Obtained Sensor Output of Channel 8 and Sweet Intensity Score of Different Concentrations of Sucrose (Standard for Sweetness) and Six Sugar Alcohol Solutions, Evaluated by Gustatory Sensation

511.7 mM maltitol).

Apart from channel 8, the magnitude of the sensor output to sweeteners was very low. The sweetness-responsive sensor also responds to the famotidine molecule.

Figure 3 shows the relationship between obtained sensor output of channel 8 and sweet intensity score of different concentrations of sucrose (standard for sweetness) and six sugar alcohol solutions, evaluated by gustatory sensation. The magnitude of the negative sensor output increased as the sweet intensities increased. Since sweeteners tended to receive a proton from the component molecule of the sensor membrane, the sensor membrane is charged negatively in the presence of sweeteners, in direct proportion to the concentration of the sweetener.

The response electric potentials caused by famotidine were comparatively large in channels 1—4 (which have a negative charge), with the largest sensor output being observed in channel 3. There was a good correlation between sensor output and the concentration of famotidine in the range 0.1 to 3.0 mM ($r=0.999$) (detailed data not shown). There was no response to famotidine in channels 5, 6, or 7 (which have a positive charge), as famotidine is a basic substance. Therefore, it was decided to use data from channels 3 and 8 in the data analysis.

Method 1 for the Evaluation of Bitterness-Suppression Firstly we assumed the bitterness-suppression by sweeteners (unknown concentrated sucrose or sugar alcohols) using a 81.4 μM quinine sulfate solution with a bitterness intensity of 4 as a standard bitter solution. As shown in step (1) in method section, we have to prepare the correlationship between sweetness intensities obtained in human gustatory sensation test and output of sensor responsive for known sucrose and sugar alcohols solutions in advance.

The derived regression equation was

$$(8S) = -9.864Y + 7.074 \quad (r = 0.725, p < 0.01) \tag{1}$$

Where Y represents the predicted sweetness intensity and 8S represents the output values of the sensor response (channel 8) to sweetness. A comparatively good correlation was observed between the sweetness intensity evaluated by human gustatory tests and the sensor output value calculated using the above equation (Fig. 4A).

As second step as show in step (2) in method section, the predicted sensor output for an unknown sugar alcohol (sweetener) was substituted into the above Eq. 1. For example, if we obtain −32 mV as sensor output value for an unknown concentrated sucrose or sugar alcohol solutions, we obtained 4.0 for the sweetness intensity calculated using Eq. 1.

Thirdly, as shown in step (3) in method section, a correlation between the sweetness intensity of the sweetener and the bitterness intensity of the 81.4 μM quinine sulfate solution containing various concentrations of the same sweetener was demonstrated (correlation with gustatory sensation results).

The bitterness intensity of quinine sulfate was reduced by addition of the sweeteners; the results are shown in Fig. 4B. The relation between the bitterness intensities obtained from the human gustatory sensation tests for 81.4 μM quinine sulfate solutions containing various concentrations of sweetener solutions (y-axis) and the sweetness intensities of single-component solutions of the sweeteners obtained in human gustatory sensation tests (x-axis) is represented by the straight line:

$$y = -0.633x + 4 \quad (r = -0.964, p < 0.001) \tag{2}$$

A high correlation was obtained between y and x values.

Finally, as shown in step (4) in method section, a predicted

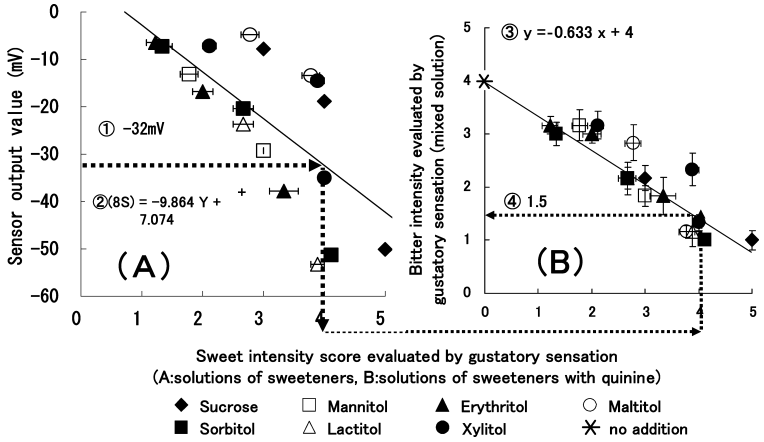

Fig. 4. The Relationship between Obtained Sweetness Intensity Scores of Sweetener Solutions in Human Gustatory Tests (x Axis) and the Taste Sensor Output Values (8S: Output of Sensor (Channel 8)) of Single-Component Solutions of the Sweeteners (y Axis) (A) and the Relationship between Obtained Bitterness Intensity in Human Gustatory Tests, of Quinine Sulfate Solutions to Which Sweeteners Had Been Added and Predicted Sweetness Intensity (B)

In both figs. 1, 2, 3, 4, and 5 in x axis means the standard sweetness intensities. Error bar represents S.E. (n=9).

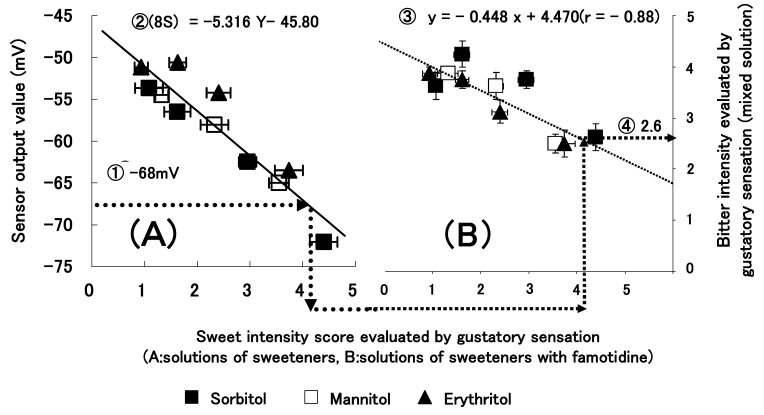

Fig. 5. The Relationship between Obtained Sweetness Intensity Scores of Sweetener Solutions in Human Gustatory Tests (x Axis) and the Taste Sensor Output Values (8S: Output of Sensor (Channel 8)) of Single-Component Solutions of the Sweeteners (y Axis) (A) and the Relationship between Obtained Bitterness Intensity in Human Gustatory Tests, of Famotidine to Which Sweeteners Had Been Added and Predicted Sweetness Intensity (B)

In both figs., 1, 2, 3, 4, and 5 in x axis means the standard sweetness intensities. Error bar represents S.E. (n=9).

sweetness intensity of 4.0 for an unknown sugar alcohol sample was substituted into Eq. 2; this resulted in a predicted bitterness intensity of 1.5 for a mixed solution of 81.4 μM quinine sulfate solution and the unknown sugar alcohol. In other words, the bitterness intensity (4.0) of a 81.4 μM quinine sulfate solution is predicted to be reduced to 1.5 by the addition of an unknown concentrated sucrose or sugar alcohol solutions.

In the above method, we fixed the concentration of the quinine sulfate solution, a model bitter substance, and examined bitterness-suppression by sucrose or sugar alcohols. To evaluate bitterness-suppression of drugs, we first evaluate the drug's bitterness intensity using the bitterness-responsive sensor and then we evaluate the effect of bitterness-suppression using method 1.

Whereas in the case of famotidine, bitterness-suppression by sorbitol, mannitol, and erythritol were examined, as these sweeteners are contained in commercially available orally disintegrating tablets and have been reported to be effective in bitterness-suppression. According to the first step as shown in method section, the sweetness intensities of the solutions of these sweeteners obtained from human gustatory sensation tests and from the sensor output values were used in the regression analysis. The derived regression equation was

$$(8S) = -5.316Y - 45.80 \quad (r = 0.9308, p < 0.01) \tag{3}$$

Where Y represents the predicted the sweetness intensity and 8S represents the output values of sensor channel 8 to sweetness. A comparatively good correlation was observed between the sensor output and the value evaluated by human gustatory tests using the above equation (Fig. 5A).

As second step as shown in method section, the predicted sensor output of an unknown sugar alcohol (sweetener) was substituted into the above Eq. 3. For example, if we obtain −68 mV as sensor output value for an unknown sugar alcohol, we obtain 4.2 for the sweetness intensity calculated using Eq. 3.

Thirdly as shown in method section, the correlation between the sweetness intensity of the sweeteners and the bitterness intensity of the 1 mg/ml famotidine solution containing various concentrations of the same sweeteners (sorbitol, mannitol and erythritol) from gustatory sensation tests was examined.

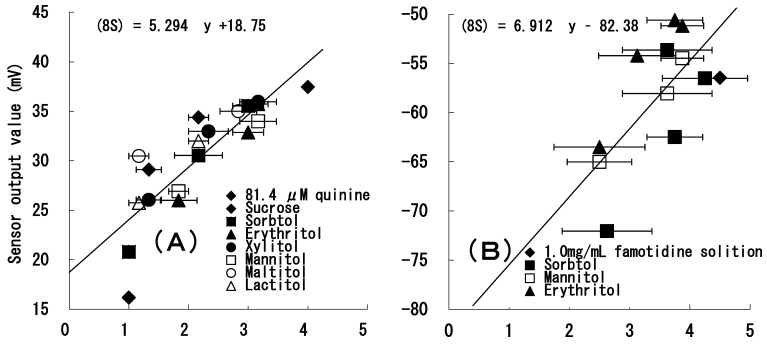

Fig. 6. Regression Analysis of the Sensor Output Values of Quinine Sulfate (A) and Famotidine (B) Solutions Plus Sweeteners Obtained Using the Taste Sensor (8S: Output of Sensor (Channel 8)), and Their Bitterness Intensities Obtained in Human Gustatory Tests

Error bar represents S.E. ($n=9$).

The bitterness intensity of famotidine was reduced by addition of the sweeteners; the results are shown in Fig. 5B. The relation between the bitterness intensities obtained from the human gustatory sensation tests for 1 mg/ml famotidine solutions containing various concentrations of sweetener solutions (y-axis) and the sweetness intensities of single-component solutions of the sweeteners obtained in human gustatory sensation tests (x-axis) is represented by the straight line:

$$y=-0.448x+4.47 \quad (r=-0.882, p<0.001) \tag{4}$$

A high correlation was obtained between y and x values.

Finally, a predicted sweetness intensity of 4.2 for an unknown sugar alcohol sample was substituted into Eq. 4; this resulted in a predicted bitterness intensity of 2.6 for a mixed solution of 1 mg/ml famotidine solution and the unknown sugar alcohol. In other words, the bitterness intensity (4.5) of a 1 mg/ml famotidine solution is predicted to be reduced to 2.6 by the addition of the sugar alcohol solution.

In the above method, we fixed the concentration of famotidine solution as a model substance and evaluated the effect of bitterness-suppression by sorbitol, mannitol and erythritol. It is expected that other sugar alcohols' bitterness-suppressions are examined.

Method 2 for the Evaluation of Bitterness-Suppression The sensor output value decreases as the amount of sweetener increases, because the sweeteners absorb the protons that quinine sulfate or famotidine donate to the sensor. If sweetener is added to bitterness solution such as quinine sulfate or famotidine, sensor output of drug in channel 8 (8S) must be decrease as increased concentration of added sweeteners.

As a result, the bitterness-suppression of quinine sulfate or famotidine by addition of sweeteners could be predicted directly by regression analysis of sensor output and the bitterness intensity of the mixed solutions obtained in human gustatory sensation tests (see Figs. 6A, B). The derived regression equations for quinine sulfate and famotidine can be written as shown in Eqs. 5 and 6, respectively.

$$(8S)=5.294y+18.75 \quad (r=0.8463, p<0.01) \tag{5}$$

$$(8S)=6.912y-82.38 \quad (r=0.7045, p<0.01) \tag{6}$$

Where y represents the predicted bitterness intensity and (8S) represents the output values of channel 8 of the sensor. Good correlations were obtained between the bitterness intensities derived from human gustatory tests (x axis values in both figs.) and the sensor output values (y axis values in both figs.) calculated using the above equations. This result demonstrates that the bitterness-suppressing effects of the sweeteners on quinine sulfate or famotidine solutions could be predicted with good accuracy using the taste sensor.

Comparison of the Two Methods Two methods are proposed for the evaluation of bitterness-suppression. Deviations of predicted bitterness intensity from obtained bitterness by gustatory sensation for the two methods were found to be significantly different (detailed data not shown) for the two drugs. Predictability of two methods does not seem so different.

Method 2 is the more convenient, and is also useful when only the mixed solution is available (for example, when evaluating a solution of a medicine containing both a bitter drug and a sweetener). If we are able to obtain the dissolution media for the drug product, we can predict the bitterness-suppression using the sweetness-responsive sensor and the bitterness of the bitter substance in the mixture using the bitterness-responsive sensor, whose outputs for sweetness is relatively low. Since the taste-responding system SA402 can utilize maximally eight sensors simultaneously, we can evaluate the effect of bitterness and the scope for bitterness-suppression of a medicine at the same time. In this way the taste sensor may be useful when designing drug products.

In Method 1, the characteristics of sweeteners (*i.e.*, relative magnitude of sweetening effect) could be easily established, although additionally gustatory sensation tests are required. The method is based on the mechanism of bitterness-suppression in humans. Method 2 is more direct and convenient on the basis of assumption that the magnitude of the negative sensor output reflects an inhibitory ability of sweeteners.

Evaluation of Taste-Masking Effect in Commercial Orally Disintegrating Tablets There has been developed taste masked formulations such as chewable tablets.[24,25] The commercially available, orally disintegrating tablet, Gaster®D is also developed and easily taken without water and is therefore suitable for elderly patients with diminished swallowing ability. As mentioned in the previous section, the channel 8 sensor is responsive to sweeteners, whereas famotidine elicits a high response from channel 3, which is responsive to bitterness.

Figure 7 shows the bitterness intensities of dissolution

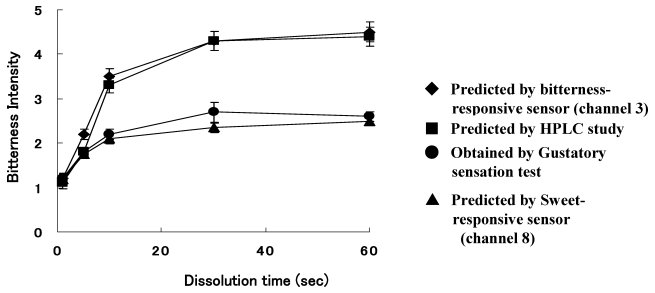

Fig. 7. Time-Course of the of Bitterness Intensity in Dissolution Media of Orally Disintegrating Tablets Containing Famotidine

The bitterness was evaluated by four kind of method. Closed diamonds show the simulated bitterness intensity of famotidine itself if we assume there are not any additives such as sugar alcohols and only famotidine is dissolved in the media. Closed squares represent the calculated bitterness intensity based on the famotidine concentration determined by HPLC method. Closed circles show the bitterness intensity determined by human gustatory sensation test. Closed triangles show the predicted bitterness intensity using sweetness- and bitterness-responsive sensors. Error bar represents S.E. (n=9).

media from orally disintegrating tablets after 1, 5, 10, 30, and 60 s dissolution. The bitterness was evaluated by four kinds of methods. Closed diamond symbols represent the simulated bitterness intensity of famotidine if we assume there are not any additives such as sugar alcohols and only famotidine is completely dissolved in the media. The bitterness intensities were predicted using only data from channel 3. The bitterness intensity increased dramatically as time passed, with an intensity of nearly 4 at 30 s. We also confirmed the immediate release of famotidine from orally disintegrating tablets by HPLC method, and dissolved famotidine concentration transferred to bitterness intensity and plotted as closed square. Closed diamond symbols data was very similar to the simulated bitterness intensity (closed square) derived from HPLC data.

The closed circle data shows the bitterness intensity evaluated by human gustatory sensation test. Closed triangles show the predicted bitterness intensity by data from channel 8 using Method 2. The two sets of data correlate well, although small discrepancies were observed at some points. The bitterness of dissolved famotidine seems to be effectively masked by the additives (such as sugar alcohols) in the tablet, as both the observed and predicted bitterness intensities (shown in closed circles and triangles, respectively) were much lower than the simulated data for similar tablets without sugar alcohols or additives.

Conclusion

The sensor output values of the sweeteners (sucrose and sugar alcohols) on the sweetness-responsive sensor were negative and their absolute values increased in proportion to the concentration of the sweeteners.

We propose two prediction methods for bitterness-suppression, one indirect and another direct. Using these methods, we were able to predict successfully the bitterness-suppressing ability of sugar alcohols in famotidine solutions. The direct method was more convenient than the indirect.

When we the bitterness intensity of the dissolution media of commercially available, orally disintegrating tablets containing famotidine using data from both bitterness- and sweetness-responsive sensors, we found that the sugar alcohols in the tablet play effectively in the bitterness-suppression of famotidine, especially in the initial phase (within 30 s) of the disintegration process.

In the present study we used sugar alcohols as model sweeteners, and it has shown that sweetness and bitterness are mutually suppressive, and the suppression occurs central rather than peripheral sites of the taste system. The artificial taste sensor should in theory have good predictive power to assess such bitterness suppression and actually showed the effect even though complicated taste–taste interactions were reported recently.[26] Many different mechanisms may exist for bitterness-suppression by different sweeteners. For example, aspartame and cyclodextrin differ in their mechanism of bitterness-suppression. An attempt will be made to elucidate these mechanisms further in a future study.

Several models of sweetness chemoreception has been developed such as receptor binding theories.[27—29] If such binding study using model sensors sensitive to bitter or sweetness is available, we might evaluate the characteristics of the drug binding to receptor more detail and it must contribute for taste masking of target drug.

James *et al.*[30] evaluate that the difference of temperature caused a different displacement of L-alanine from L-[3*H*] alanine already bind with the fraction made by taste buds of the catfish. It might be one of the studies of the absorption rate of the drug. Anyway, we try to improve not only specificity or sensitivity of sensor but also but also the value and usefulness of sensor as new analytical tool for distinguishing or characterization of various kinds of bitter or sweetness.

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