Quantitative Prediction of the Bitterness Suppression of Elemental Diets by Various Flavors Using a Taste Sensor

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Purpose. The purpose of the study was to develop a method for the quantitative prediction of the bitterness suppression of elemental diets by various flavors and to predict the optimum composition of such elemental diets for oral administration using a multichannel taste sensor.

Methods. We examined the effects of varying the volume of water used for dilution and of adding varying quantities of five flavors (pineapple, apple, milky coffee, powdered green tea, and banana) on the bitterness of the elemental diet, Aminoreban EN®. Gustatory sensation tests with human volunteers (n = 9) and measurements using the artificial taste sensor were performed on 50 g Aminoreban EN® dissolved in various volumes (140, 180, 220, 260, 300, 420, 660, 1140, and 2100 ml) of water, and on 50 g Aminoreban EN® dissolved in 180 ml of water with the addition of 3–9 g of various flavors for taste masking.

Results. In gustatory sensation tests, the relationship between the logarithmic values of the volumes of water used for dilution and the bitterness intensity scores awarded by the volunteers proved to be linear. The addition of flavors also reduced the bitterness of elemental diets in gustatory sensation tests; the magnitude of this effect was, in decreasing order, apple, pineapple, milky coffee, powdered green tea, and banana. With the artificial taste sensor, large changes of membrane potential in channel 1, caused by adsorption (CPA values, corresponding to a bitter aftertaste), were observed for Aminoreban EN® but not for any of the flavors. There was a good correlation between the CPA values in channel 1 and the results of the human gustatory tests, indicating that the taste sensor is capable of evaluating not only the bitterness of Aminoreban EN® itself but also the bitterness-suppressing effect of the five flavors, which contained many elements such as organic acids and flavor components, and the effect of dilution (by water) on this bitterness. Using regression analysis of data derived from the taste sensor and from human gustatory data for four representative points, we were able to predict the bitterness of 50 g Aminoreban EN® solutions diluted with various volumes of water (140–300 ml), with or without the addition of a selected flavor.

Conclusions. Even though this prediction method does not offer perfect simulation of human taste sensations, the artificial taste sensor may be useful for predicting the bitterness intensity of elemental diets containing various flavors in the absence of results from full gustatory sensation tests.

KEY WORDS: taste sensor; bitter taste; human gustatory sensation; elemental diet; Aminoreban EN®; flavors.

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INTRODUCTION

Many human pharmaceutical medicines have a bitter taste, which makes them difficult or unpleasant to take, and which may give rise to noncompliance and thus decrease therapeutic efficacy. The evaluation of the bitterness of medicines is, therefore, an important factor in drug design. The multichannel taste sensor, originally developed by Toko, can be regarded as an electric "tongue" with global selectivity. It comprises several kinds of lipid/polymer membranes that transform information about substances producing taste into electrical signals that are then analyzed by the computer (1–3). The sensor output has been shown to produce different patterns for groups of chemical substances with similar tastes, such that the tastes of various foodstuffs such as beer (4), coffee (5), sake (6), and tea can be expressed quantitatively using the sensor.

We have previously evaluated the bitterness of various medicines and suggested that the taste sensor could be used to obtain quantitative predictive data on the bitterness of commercial medicines (7–10). In the present study, we investigated the bitterness of an elemental diet that is used in the treatment of hepatic diseases. In order to improve Fischer's rate (11,12), these elemental diets contain high concentrations of branched-chained amino acids such as isoleucine, leucine, and valine, which have a bitter taste. Patients suffering from severe hepatic diseases usually drink 50 g Aminoreban EN®, dissolved in 180 ml water and mixed with 6 g flavor, three times a day, as instructed in the package insert of the product. This diet must often be maintained for a long period, and its bitterness is not only unpleasant but may also decrease compliance or intake.

In the present study, our goal was, first, to develop a method for the quantitative prediction of the bitterness suppression of elemental diets by various flavors using the taste sensor, and second, to use the taste sensor to predict the optimal composition (with respect to volume of diluent and addition of flavors) of such elemental diets in order to increase their palatability to patients.

The package insert of Aminoreban EN®, a commercial elemental diet, states that 50 g of Aminorelan EN® should be taken in 180 ml of water. The addition of 6.0 g of one of five flavors is also recommended, per 180 ml Aminoreban EN® solution (one dosage), to improve palatability. We examined the effects of varying the volume of water taken with Aminoreban EN® and of adding varying amounts of one of five different flavors on the bitterness of the diet, with the aim of determining the optimal composition of the diet (in terms of volume of water and concentration/choice of flavor) for palatibility.

METHODS

Materials

The Aminoreban EN® elemental diet and the flavors were gifts from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). There were five flavors: apple, banana, pineapple, powdered green tea, and milky coffee which contained about 10.0%, 3.0%, 12.0%, 2.0%, 2.5% of citric acid, respectively. And apple and pineapple flavors also contained about 0.1%,

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and 0.05% of malic acid, respectively. Additionally, all flavors contained flavor components and sweeteners. These communications in relation to five kinds of flavors were privately obtained from Otsuka Pharmaceutical Co., Ltd. Solutions containing 50 g Aminoreban EN® dissolved in 140, 180, 220, 260, 300, 420, 660, 1140, and 2100 ml water were prepared. For each dilution, a number of samples were prepared containing each of the five flavors at concentrations of 0, 3, 6, or 9 g/sample, depending on the particular experiment (see below). The bitterness of these samples was evaluated using the taste sensor and/or in human gustatory sensation tests. For the taste sensor tests, the samples were diluted with 10 mM KCl to improve conductibility. The diluents for sample preparations in the taste sensor tests was 10 mM KCl solution, which has no taste.

Gustatory Sensation Test

The gustatory sensation tests were performed with nine healthy human volunteers, which were well trained, according to a previously described method (13–15). The volunteers were claimed to focus on the bitterness and not to receive the influence of smell in this examination. The standard quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30, and 1.00 mM, and the corresponding bitterness scores were defined as 0, 1, 2, 3, and 4, respectively. Before testing, the volunteers were asked to keep the above standard quinine solutions in their mouths for 10 s and were told their bitterness scores. The volunteers were then asked to give bitterness intensity scores to solutions of Aminoreban EN® dissolved in various volumes of water (140, 180, 300, 420, 660, 1140, and 2100 ml), in the presence or absence of flavor. The five flavors (6 g of each) were added to 50 g Aminoreban EN® dissolved in 180 ml water. The sample size was 10 ml, and all samples were kept in the mouth for 10 s. After tasting, subjects gargled well before tasting the next sample.

Taste-Sensor Measurements and Data Analysis

The taste-sensing system SA402 of Intelligent Sensor Technology Co., Ltd., (Atsugi, Kanagawa, Japan) was used to measure the electric potential of various sample solutions (Fig. 1). The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes, each composed of a lipid/polymer membrane. The lipid components of the sensor used in the present study are the same as those described in a previous paper (16). Each lipid was mixed in a test tube containing poly(vinylchloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran, and dried on a glass plate at 30°C to form a transparent thin film, almost 200 μm thick. The electrodes consisted of an Ag 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.

Samples consisting of various concentrations of Aminoreban EN® 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 (Vr) and also to rinse the electrodes after every measurement.

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 relative sensor output is taken as the difference (Vs – Vr) between the potentials of the sample and the reference solution. When the electrode is

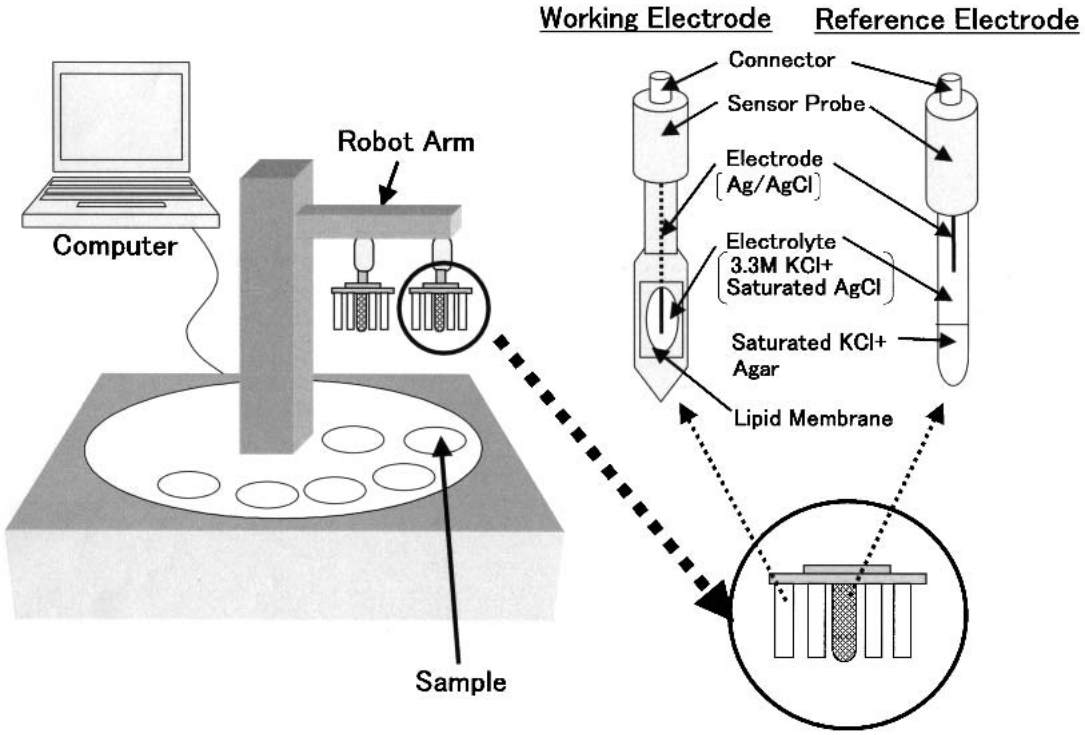

Fig. 1. The Multichannel Taste-Sensing System (SA402) used in the present study.

dipped into the reference solution again, the new potential of the reference solution is defined as Vr′. The difference (Vr′ – Vr) between the potentials of the reference solution before and after sample measurement is defined as CPA (change of membrane potential caused by adsorption) and corresponds to aftertaste. Each measuring time was set at 30 s, and the electrodes were rinsed after each measurement. S-PLUS 2000J (Mathematical Systems, Inc., Tokyo, Japan) was used for regression analysis.

RESULTS AND DISCUSSION

Human Gustatory Sensation Tests

The bitterness intensity scores for solutions of Aminoreban EN® dissolved in 140, 180, 300, 420, 660, 1140, or 2100 ml water and for Aminoreban EN® dissolved in 180 ml water inthe presence of 6 g of each of the five flavors are shown in Fig. 2.

For Aminoreban EN® solution dissolved in various volumes of water in the absence of flavor, the relationship between the logarithmic value of the volume of water and the bitterness intensity score was shown to be linear, as represented by the straight line:

$$Y = -2.207X + 7.391 \ (r^2 = 0.968, p < 0.001) \qquad (1)$$

where the Y axis value represents the bitterness intensity score as obtained from the human gustatory sensation test and the X axis value represents the logarithmic value of the volume of water added to the 50 g Aminoreban EN®.

As shown in Fig. 2, the bitterness intensity of the Aminoreban EN® was reduced to a varying extent by the addition of 6 g flavor. The bitterness-suppressing effect of the different flavors on Aminoreban EN® was, in descending order of magnitude, apple, pineapple, milky coffee, powdered green tea, and banana (see Fig. 2 and Table I). This correlates well

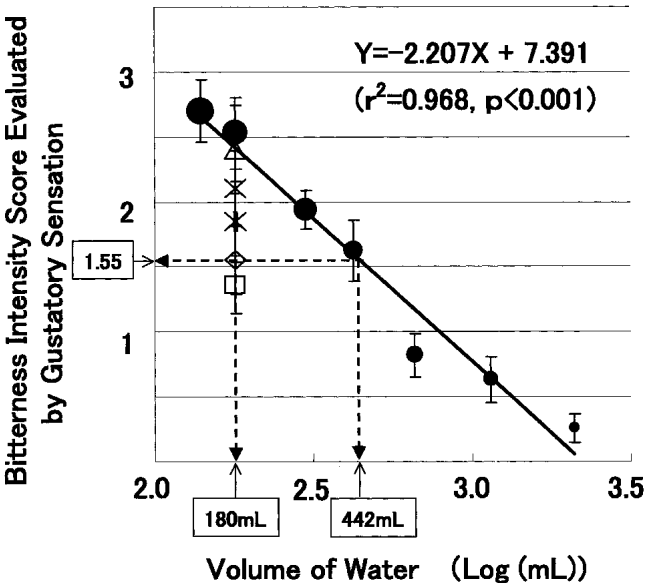

Fig. 2. Human bitterness intensity scores for Aminoreban EN® dissolved in various volumes of water (140, 180, 300, 420, 660, 1140, 2100 ml) in the presence or absence of flavor. (Each *bar* represents mean ± SE.)

Table I. Effect of Five Kinds of Flavors on Bitterness Suppression Evaluated by Gustatory Sensation Tests

Flavor	(1)	(2)	(3)	(4)
None	2.52	100		
Pineapple	1.55	33	38	442
Apple	1.36	26	24	542
Milk Coffee	1.85	46	19	326
Powdered Green Tea	2.10	62	10	249
Banana	2.40	87	9	183

(1) Bitterness intensity scores.
(2) Bitterness intensity scores expressed as equivalent quinine concentration ratio (%).
(3) Actual clinical usage (%).
(4) Volume of water that would be required to suppress the bitterness to the same degree as the added flavor (ml).

with the relative actual usages of these five flavors in clinical practice (pineapple 38%, apple 24%, milky coffee 19%, powdered green tea 10%, and banana 9%; private communication, Otsuka Pharmaceutical Co.). From Table I it can be seen that 50 g Aminoreban EN® dissolved in 180 ml water in the presence of 6 g pineapple flavor (the most popular flavor in clinical practice) has a bitterness intensity score of 1.55, and the value is significantly smaller than the bitterness intensity score of 2.52 with 50 g Aminoreban EN® dissolved in 180 ml of water in the absence of the flavor. In previous papers (17,18) that compared bitterness of two kinds of substances, obtained bitterness intensities could be converted to quinine concentration. We named this converted concentration "equivalent quinine concentration," and the ratio of this equivalent quinine concentration has been used for evaluation of the taste-masking effect in our previous papers (8,9). Simultaneously, in this case, the values for bitterness intensity scores in column (1) in Table I were converted to equivalent quinine concentration ratio as shown in column (2) in Table I. For example, the equivalent quinine concentrations corresponding to 2.52 (no flavor as negative control) and 1.55 (with pineapple flavor) were calculated to be 0.179 mM and 0.059 mM, respectively. Thus, the ratio of these concentrations was calculated to be 0.33 or 33% as percentage.

In other words, as shown in Fig. 2, in order to reduce the bitterness intensity of 50 g Aminoreban EN® to 1.55 in the absence of flavors, 442 ml of water must be used as diluent, as calculated on the basis of eq. (1) above.

Thus, if flavors are not added to Aminoreban EN®, the volume of water used to dissolve the Aminoreban EN® powder must be increased significantly to achieve an equivalent reduction in bitterness.

Taste-Sensor Studies

The Response Electric Potential Patterns for Elemental Diet With and Without Flavors

The data of the sensor output and CPA values for channels 1–8 obtained were summarized as described in the previous paper (8,10). Figure 3 shows the response electric potential patterns of relative output value (Fig. 3A) and CPA value (Fig. 3B) for 50 g Aminoreban EN® or 6 g of each of the five different flavors, dissolved in 180 ml water. The CPA,

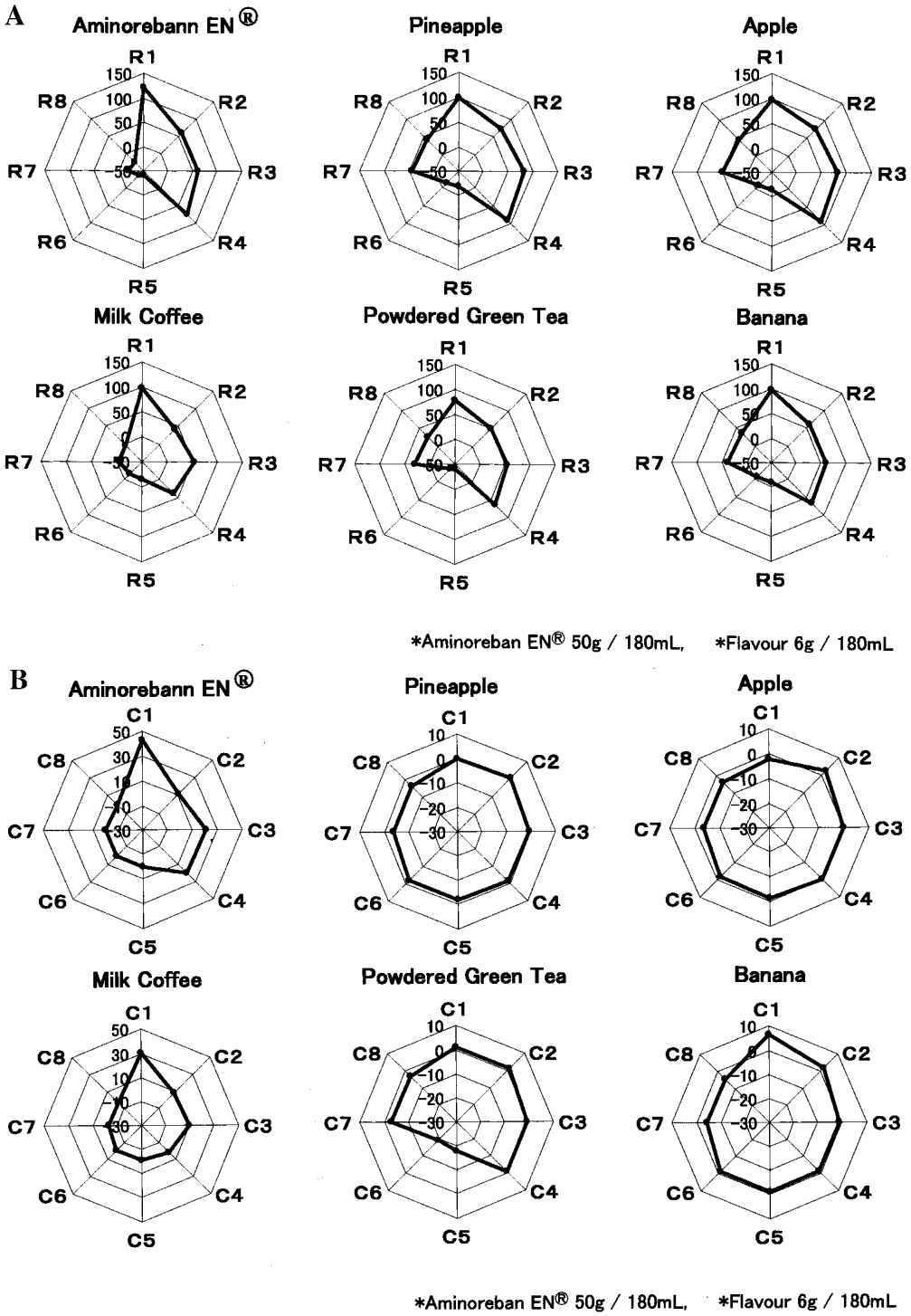

Fig. 3. Sensor response output electric potential patterns for Aminoreban EN® and five kinds of flavors. (A) Relative value. (B) CPA value. For detailed explanation of CPA, see text.

defined as the change of membrane potential caused by adsorption, corresponds to aftertaste (7).

First, as shown in Fig. 3A, the relative output values of the flavors themselves were so large that we could neither ignore them nor use their relative values. These large values might be caused by many elements because Aminoreban EN® contains not only branched-chain amino acids but also many other elements with various characteristics that may increase the relative value in sensor output.

The CPA values for Aminoreban EN® were comparatively large (almost 45 mV in channel 1), as shown in Fig. 3B. However, flavor-only solutions showed much smaller CPA values, although milky coffee did have CPA values in channel 1 of over 30 mV. Powdered green tea had a value of almost –30 mV in channels 5 and 6, which corresponds to astringency. This indicates that, in general, the adsorption of tastes other than bitterness to the sensor membrane surface is weak for these five flavors, as could be predicted on the basis of

their hydrophobicity, which is much less than that of Aminoreban EN®.

The fact that the flavors themselves have no bitterness (having small CPAs) is an advantage because there is no risk of the output value of the flavors interfering with the output value of the elemental diet. In the present study, therefore, the CPA value in channel 1 could be used to predict bitterness.

Effect of the Volume of Water and Choice of Flavor on the Prediction of Bitterness of Aminoreban EN® Solutions

The sensory CPA data from channel 1 of the taste sensor was used to predict the bitterness of various Aminoreban EN® solutions (Fig. 2). As in the human gustatory sensation tests, 12 samples were used for sensor measurement: 50 g Aminoreban EN® dissolved in 140, 180, 300, 420, 660, 1140, or 2100 ml water, 6 g of each of the five flavors mixed with 50 g Aminoreban EN® dissolved in 180 ml water, plus the 10 mM KCl control solution with which all samples were diluted. The CPA values and bitterness intensities obtained were used in the regression analysis. The results are shown in Fig. 4A. The derived regression equation was:

$$Y = 0.083 \times Cl - 0.954 \ (r = 0.960, p < 0.001) \qquad (2)$$

where Y represents the predicted bitterness intensity score, and C1 represents the CPA value observed in channel 1.

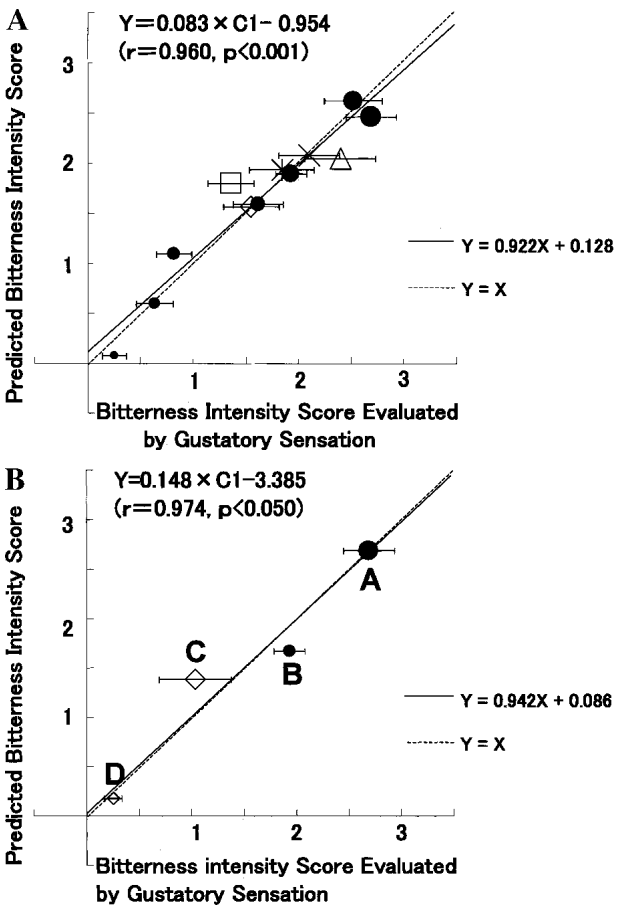

Fig. 4. Regression equation for predicting bitterness intensity score. (Each *bar* represents mean ± SE.)

The calculated regression equation represented as a solid line was:

$$Y = 0.922 X + 0.128 \ (r = 0.960, p < 0.001) \qquad (2')$$

where Y and X mean the predicted and observed bitterness scores, respectively. So, the observed gustatory bitterness and the predicted bitterness calculated by the above equation were almost located near the diagonal line (Y = X, represented as a dotted line in Fig. 4A). A good correlation was observed between bitterness intensity scores as evaluated by human gustatory tests and the predicted bitterness intensity score calculated using the above equation. Strictly speaking, the bitterness suppression caused by increasing the amount of water and that caused by adding flavors are separate effects because dilution of the solution and competing effect of organic acids in the flavors with amino acids on the surface of sensor membrane will be independent. The bitterness suppression caused by addition of the five flavors could be predicted by a different regression equation:

$$Y = 0.092 \times Cl - 1.287 \ (r = 0.837, p < 0.050) \qquad (3)$$

In this regression, we used data on five kinds of flavors (represented as open symbols in Fig. 4A) plus data in the absence of flavor (control).

Although the regression equation derived solely from the data on the five flavors was not exactly the same as that derived from the data using all 12 samples, there is a correlation between the bitterness intensity score determined by human gustatory sensation tests and the predicted bitterness intensity score calculated using Eq. (3), at least for the five flavors.

This bitterness suppression caused by flavors may result from organic acids such as citric or malic acid. The organic acids with negative charge are able to compete with the positive charge of branched-chained amino acids involved in the elemental diet. In fact, flavors with a comparatively large amount of organic acid. i.e., apple and pineapple flavors used in the present study were more effective at taste masking Aminoreban EN® than the other flavors.

As reported above, dilution with water and the addition of flavors were effective in bitterness suppression, as measured not only by the taste sensor but also in gustatory sensation tests. In general, lipophilic compounds such as phosphatidic acid, a representative bitterness-suppressing agent, compete with quinine for binding sites in human receptors (19–22). We and other researchers have demonstrated that the taste sensor output reflects this bitterness-suppressing effect (8,9,23,24). The application of sensor data to hydrophilic compounds is not so clear. Takagi *et al.* reported that the bitterness-depressing effect of sucrose on quinine was not observed on sensor output (25). In their article, these authors suggest that bitterness suppression by sucrose in human gustatory sensation tests occurs as a result of a central effect (i.e., in the brain) rather than a peripheral one (i.e., in the taste cells), which is why the taste-sensor data were unable to predict the bitterness-suppressing effect. Nevertheless, we have recently been successful in demonstrating a bitterness-suppressing effect of sodium chloride on quinine hydrochloride solutions using the sensor. Sodium chloride is known to be a bitterness-suppressing agent that acts both centrally and pe-

ripherally; our sensor output data could predict only its peripheral bitterness-suppressing effect (8,9).

Thus, it is possible to evaluate not only bitterness itself using the taste sensor but also bitterness suppression by various substances, even though these substances themselves may have strong tastes. To the best of our knowledge, this is the first trial to evaluate the bitterness-suppressing effect of flavors when added to elemental diets. We expect that some perfume components of flavors precisely inhibit bitterness of elemental diets. Flavors usually have characteristic smells that may interfere with the precise evaluation of bitterness suppression. In addition, it is difficult to take into account the individual taste preferences of human volunteers or patients. Evaluation of the bitterness-suppressing effects of flavors by the taste sensor is both reproducible and objective because it excludes the individual subjectivity and taste preferences of human volunteers.

Development of a Quick Method for Predicting the Bitterness of Elemental Diets Containing Various Amounts of Water and Flavors

If we were to develop fully the regression equation shown in Fig. 4A as a method of bitterness prediction, we would need to perform 12 gustatory sensation tests for each combination, which is impractical for our volunteers. We therefore sought to develop an abbreviated prediction method using more limited gustatory sensation data. We selected four samples for evaluation: 50 g Aminoreban EN® solution dissolved in 140 or 300 ml water in the absence or presence of 9 g pineapple flavor. Our reason for choosing these samples was as follows: 140–300 ml water seems to represent the acceptable range; more than 300 ml of water is commonly regarded as too much liquid, but the elemental diet could not be completely dissolved in less than 140 ml. As far as the amount of added flavor was concerned, 9 g was shown to have the greatest bitterness-suppressing effect (more than 10 g flavor was found to be too strong or even unpleasant in a pilot study; results not shown). We therefore selected conditions (140–300 ml water and 9 g flavor) that are likely to reflect the clinical situation. The result of this evaluation is shown in Fig. 4B. The derived regression equation was:

$$Y = 0.148 \times Cl - 3.385 \ (r = 0.974, p < 0.050) \qquad (4)$$

where Y represents the predicted bitterness intensity score, and C1 represents the CPA value observed in channel 1. The calculated regression equation represented as a solid line was:

$$Y = 0.942X + 0.086 \ (r = 0.974, p < 0.050) \qquad (4')$$

where Y and X mean the predicted and observed bitterness score, respectively. So, the observed gustatory bitterness and the predicted bitterness calculated by the above equation were located very close to the diagonal line (Y = X, represented as a dotted line in Fig 4B). A good correlation was observed between the bitterness intensity scores derived from gustatory sensation tests on these four solutions and their predicted bitterness intensity scores calculated according to Eq. (4).

The Optimal Composition for Administration of Aminoreban EN®

Using Eq. (4), described in Fig. 4B, we predicted the bitterness intensity scores of the following 20 samples: 50 g Aminoreban EN® dissolved in 140, 180, 220, 260, or 300 ml water containing 0, 3, 6, or 9 g pineapple flavor, the most popular flavor in clinical practice. The results are presented as a three-dimensional graph in Fig. 5. The numbers not in parentheses are bitterness intensity scores obtained from human gustatory sensation tests, and the numbers in parentheses are the values predicted on the basis of the abbreviated prediction method described above. As the volume of water and quantity of flavor increased, the bitterness intensity decreased proportionally. For example, the actual bitterness intensity of 50 g Aminoreban EN® when dissolved in 180 ml water was 2.52 (2.96); when dissolved in 180 ml water in the presence of 6 g pineapple flavor, it was 1.55 (1.09); and when dissolved in 260 ml in the presence of 6 g pineapple flavor, it was 0.52 (0.17), respectively.

These results show that the bitterness of Aminoreban EN® is considerably reduced when it is taken in over 260 ml of water and with more than 6 g pineapple flavor. Although there are some discrepancies between the two sets of values in Fig. 5, the predicted values calculated from the sensory CPA data on the basis of only four points of gustatory and sensor data (the values in parentheses) give a good indication of the bitterness intensity and would be useful in situations in which the results of full gustatory sensation tests are not available.

Although full data for the other flavors are not presented here, this method would also be useful for predicting the bitterness of elemental diet solutions containing other flavors. For example, for bitterness intensities for 50 g Aminoreban EN® dissolved in 260 ml water in the presence of 6 g apple flavor was 0.86 (0.69); dissolved in 140 ml water in the presence of 9 g milk coffee flavor, it was 1.90 (1.37); dissolved in 300 ml water in the presence of 9 g powdered green tea flavor, it was 1.07 (1.23), and dissolved in 260 ml water in the presence of 3 g banana flavor, it was 1.46 (1.72).

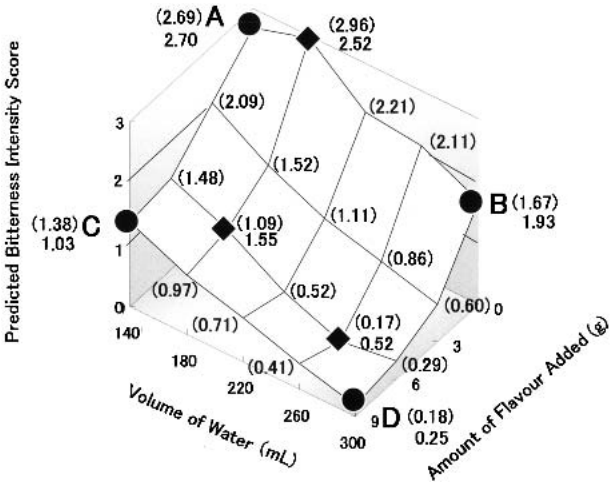

Fig. 5. The three-dimensional graph of predicted bitterness intensity scores of the following 20 samples: 50 g Aminoreban EN® dissolved in 140, 180, 220, 260, or 300 ml water containing 0, 3, 6, or 9 g pineapple flavor, the most popular flavor in clinical practice, using the equation described in Fig. 4B [Eq. (4)].

Even though this prediction method does not offer a perfect simulation of human taste sensations, the artificial taste sensor may be useful for predicting the bitterness intensity of elemental diets containing various flavors in the absence of results from full gustatory sensation tests.

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