Bitterness Evaluation of Medicines for Pediatric Use by a Taste Sensor

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The purpose of this study was to evaluate the bitterness of 18 different antibiotic and antiviral drug formulations, widely used to treat infectious diseases in children and infants, in human gustatory sensation tests and using an artificial taste sensor. Seven of the formulations were found to have a bitterness intensity exceeding 1.0 in gustatory sensation tests (evaluated against quinine as a standard) and were therefore assumed to have an unpleasant taste to children. The bitterness intensity scores of the medicines were examined using suspensions in water or an acidic sports drink. In the case of three macrolide antibiotic formulations containing erythromycin (ERYTHROCIN® dry syrup), clarithromycin (CLARITH® dry syrup for pediatric), and azithromycin (ZITHROMAC® fine granules for pediatric use), the bitterness intensities of suspensions in acidic sports drinks were dramatically enhanced compared with the corresponding scores of suspensions in water. This enhancement could be predicted using the taste sensor. On the other hand, a reduction of bitterness intensity was observed for an acidic sports drink suspension of an amantadine product (SYMMETREL® fine granules) compared with an aqueous suspension. This reduction in bitterness could also be predicted using the taste sensor output value. Thus, the taste sensor could predict whether or not suspension in an acidic sports drink would enhance or reduce the bitterness intensity of pediatric drug formulations, compared with suspensions in water.

Key words pediatric medicine; sports drink; taste sensor; antibiotic; macrolide; bitterness

The treatment of infectious diseases in pediatric patients is greatly affected by compliance issues, as the unpleasant taste of many antibiotic or antiviral drug formulations often gives rise to a refusal to take the medication and thus reduces therapeutic effect. The bitterness of these medicines is thought to be one of the main reasons for this. For example, in a study of the bitterness of clarithromycin, there is evidence that compliance improves when the antibiotic is taken with sweet foods such as chocolate milk, ice creams, and soft adzuki-bean jellies.[1] Various additives have been used to improve the bitterness of the drug formulation.[2—7] In interview panels involving these commercial medicines, the drugs themselves are frequently described as tasting "bitter," while their formulations have been described as tasting "sweet." This indicates that the formulation of these drugs have involved some modifications, such as coating *etc.*, which has affected their taste. The strength of bitterness may vary greatly between different formulations of the same drug, however, presumably due to difference in the success of the taste-masking attempts.[8—10] Therefore, it is not possible to predict the bitterness strength of a formulation on the basis of data from interview panels.

We have previously reported the quantitative evaluation of bitterness of various drug formulations, such as antibiotics and amino-acid preparations, *etc.* using a taste sensor.[11—14] In these studies we have demonstrated that the sensor has good reproducibility and sensitivity in the evaluation of bitterness and shown that it may be useful in predicting the bitterness of medicines. In the present study, we examined the bitterness of 18 antibiotic and antiviral drug formulations marketed for pediatric use in Japan, in human gustatory sensation tests and using the taste sensor. Children sometimes take drug formulations suspended in acidic sports drinks or together with acidic foods in order to reduce their bitterness. However, in some cases, this may make the drugs taste more bitter than when they are taken with water.[15] This phenomena is caused by the acidity of the food or sports drink, as basic drugs dissolve more readily in acidic conditions, and the large amount of released bitter drug obtained cannot be masked by the sweetness of the food or drink.

The goal of the present study, therefore, was to examine whether or not enhancement of the bitterness of pediatric drug formulations by acidic sports drinks could be predicted by the taste sensor.

Experimental

Materials The following 18 pediatric drug formulations widely used to treat infectious diseases were used in the study: eight different β-lactam antibiotics (amipicillin: VICCILLIN® dry syrup (Meiji Seika Co., Ltd., Tokyo, Japan); amoxicillin: SAWACILLIN® fine granules (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan); cefaclor: KEFRAL® (Shionogi Co., Ltd., Osaka, Japan); cefdinir: CEFZON® fine granules for pediatric (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan); cefcapene: FLOMOX® (Shionogi Co., Ltd., Osaka, Japan); cefteram: TOMIRON® (Toyama Chemical Co., Ltd., Tokyo, Japan): faropenem: FAROM® dry syrup for pediatric (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan); cefditoren: MEIACT® fine granules (Meiji Seika Co., Ltd., Tokyo, Japan)), four different macrolide antibiotics (azithromycin: ZITHROMAC® fine granules for pediatric use (Pfizer Pharmaceutical Co., Ltd., Tokyo, Japan); clarithromycin: CLARITH® dry syrup for pediatric (Taisho Toyama Co., Ltd., Tokyo, Japan); erythromycin: ERYTHROCIN® (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan); midecamycin: MIOKAMYCIN® dry syrup (Meiji Seika Co., Ltd., Tokyo, Japan)), and formulations of six other drugs (fosfomycin: FOSMICIN® dry syrup (Meiji Seika Co., Ltd., Tokyo, Japan); norfloxacin: BACCIDAL® tablet for children (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan); sulfamethoxazole/trimethoprim: BAKTAR® (Shionogi Co., Ltd., Osaka, Japan); aciclovir: ZOVIRAX® granules (GlaxoSmithKline Co., Ltd., Tokyo, Japan); oseltamivir: TAMIFLU® dry syrup (Roche Co., Ltd., Tokyo, Japan); and amantadine: SYMMETREL® (Novartis Pharma Co., Ltd., Tokyo, Japan)). The acidic sports drink, POCARI SWEAT® (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was used in the study as diluent.

Gustatory Sensation The samples used in the gustatory sensation test were prepared on the basis of a single dose for a 15-kg child. The weighed

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samples were suspended in 10 ml of water (pH 6.6) or acidic sports drink (pH 3.5), and stirred for 1 h using an agitator. This 1 h seems enough time for drug release from each formulations. Gustatory sensation tests were done using the equivalent density examination method of Katsuragi *et al.*[16] 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 ($n=7$) were asked to keep the above standard quinine solutions in their mouths, and were told the concentrations and bitterness scores for each solution. After tasting 2 ml of a test drug formulation suspension in water, they were asked to give the sample a bitterness score. All samples were kept in the mouth for 15 s. After testing the sample, the volunteers gargled their mouth well and waited for at least 20 min before tasting the next sample. For any formulations in which the bitterness intensity exceeded 1.0 when suspended with water, a second series of gustatory sensation tests was carried out with the formulations suspended both in water and in an acidic sports drink. In this case, the concentration of the medicine was diluted to 25% of that used in the first series of tests (corresponding to a dose for a child of 3.75 kg) because an initial indication of the increased bitterness of the suspension in the acidic sports drink (over 4), suggested that too high a concentration might lead to saturation of receptor sites.

Sensor Measurement and Data Analysis The taste sensor system and the lipid components of the sensor used in the present study are essentially same as those described in a previous paper.[17—22] The taste sensor system, SA402B of Intelligent Sensor Technology Co., Ltd., Atsugi, Japan, was used to measure the electric potential of the drug suspensions. In this sensor, the electrode set is attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes composed of lipid/polymer membranes. 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 consist of an Ag wire whose surface is plated with Ag/AgCl, with an internal cavity filled with 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 of the pediatric drug formulations, suspended in water or acidic sports drink for one hour, were evaluated in the following manner. 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 method used to measure the sensitivity and the selectivity of adsorption of the samples is summarized in Chart 1. The electrode is first dipped into the reference solution (Vr) and then into the sample solution or suspension (Vs). The relative sensor output is represented as the difference (Vs−Vr) between the potentials of the sample and the reference solution. When the electrode is 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. In the present study, relative sensor output values (R), and CPA values were used to predict the bitterness of the pediatric drug formulations.

Statistical Analysis The difference between the bitterness intensity of water suspensions and that of acidic sports drink suspensions was analyzed using the Student's unpaired *t*-test. A value of $p<0.05$ or $p<0.005$ was accepted as indicating a significant differences between values. S-PLUS 2000J (Mathematical Systems, Inc.,Tokyo, Japan) was used for regression analysis.

Results and Discussion

Gustatory Sensation Test Results for Drug Formulations Suspended in Water Figure 1 shows the result of the gustatory sensation tests for the 18 pediatric drug formulations suspended in water. The *Y* axis value represents the bitterness intensity score. The drug used as a standard for bitterness was quinine hydrochloride. From results obtained in previous studies in our laboratory, we know that a bitterness score of 1.0 (corresponding to the bitterness of a 0.03 mM quinine hydrochloride solution) represents the threshold at which the unpleasantness of the solution starts to increase appreciably with increasing quinine hydrochloride concentration. As shown in Fig. 1, there were seven drug formulations whose bitterness score exceeded 1 in the gustatory sensation tests: one β-lactam antibiotic (the cefcapene product FLOMOX®), three of the macrolide antibiotic formulations (the azithromycin, clarithromycin, and erythromycin products, ZITHROMAC®, CLARITH®, and ERYTHROCIN®, respectively), and three of the other formulations (the norfloxacin, sulfamethoxazole/trimethoprim, and amantadine products, BACCIDAL®, BAKTAR®, and SYMMETREL®, respectively).

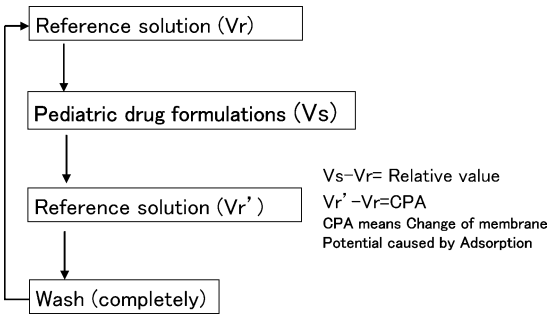

Chart 1. Measuring Procedure in This Study

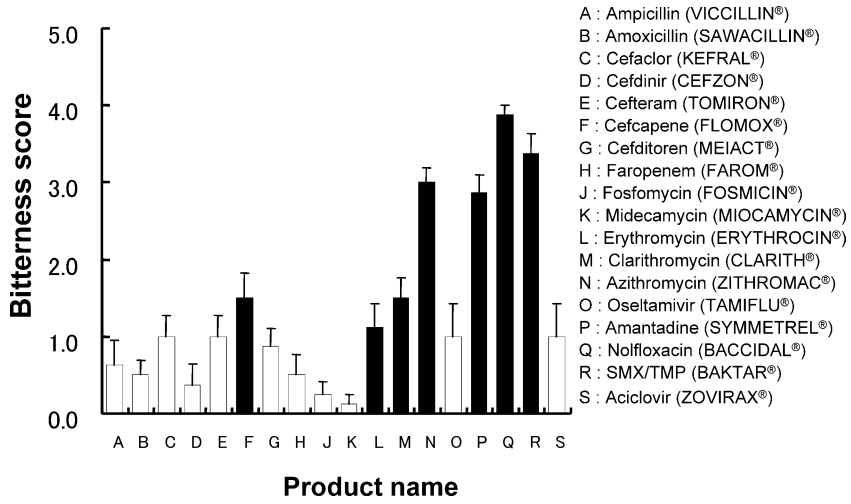

Fig. 1. Bitterness Intensity Scores Obtained in Gustatory Sensation Tests for Various Concentrations of Drug Formulations in 10 ml of Water

Table 1. Physicochemical Properties for Pediatric Drug Formulations Used in This Study

Drug (formulation)	Molecular weight[a)]	pK_a[a)]	pH of water suspension[b)]	pH of acidic sports drink suspension[b)]
Cefcapene (FLOMOX®)	622.11	3.7	5.07	3.42
Erythromycin (ERYTHROCIN®)	862.05	7.1	6.98	4.86
Clarithromycin (CLARITH®)	747.95	8.48	10.70	8.08
Azithromycin (ZITHROMAC®)	785.03	8.1, 8.8	9.26	7.16
Amantadine (SYMMETREL®)	187.71	10.3	4.21	3.42
Norfloxacin (BACCIDAL®)	319.33	6.34, 8.75	7.01	4.38
SMZ/TMP (BAKTAR®)	253.28 290.32	SMZ 5.94 TMP 7.11	5.65	4.46

a) Values for each drug. *b*) Values for each product.

ZITHROMAC®, BACCIDAL®, BAKTAR®, and SYMMETREL® were found to be particularly bitter, with bitterness scores of over 3, corresponding with the bitterness of 0.3 mM quinine hydrochloride.

These seven formulations were studied further (see 2. below).

Gustatory Sensation Test Results for Seven Bitter Drug Formulations Suspended in Water or Acidic Sports Drink In general, children can swallow formulations whose bitterness score is under 1 relatively easily, but tend to take more bitter medicines with syrup or with acidic sports drinks which contain sweeteners. In the recent article,[15)] however, we reported that drug formulations suspended in acidic sports drinks had an enhanced bitterness compared with their bitterness in water, since basic drug formulations dissolved more easily in acidic conditions, and the increased amount of bitter drug released could not be masked by the sweetness of the drink. Therefore, for the seven formulations whose bitterness scores exceeded 1, we performed further gustatory sensation tests with lower concentrations of the formulations suspended both in water and in an acidic sports drink. Some physicochemical properties for seven formulations and involved drugs were also summarized in Table 1.

A comparison of the result of gustatory sensation tests of the seven drug formulations suspended in water or acidic sports drink is shown in Fig. 2. All the formulations except the amantadine product (SYMMETREL®) showed an enhancement of bitterness when suspended in acidic sports drink. In particular, the bitterness of the three macrolide antibiotics products was significantly increased when the drug was suspended in the acidic sports drink ($p<0.05$).

As shown in Table 1, three macrolide formulations, involved drugs are basic drugs and have pK_a values 7—9. Therefore, in acidic condition, the release rate from each macrolide formulation is expected to increase as decrease pH value of formulation suspensions. When suspension medium was changed from purified water (pH 6.6) to acidic sports drink (pH 3.5), pH of dry syrup suspensions were dramatically changed from 6.98 to 4.86 in erythromycin product (ERYTHROCIN®), 10.7 to 8.08 in the clarithromycin product (CLARITH®), and 9.26 to 7.16 in azithromycin product (ZITHROMAC®), respectively. This pH jump seems the critical factor for enhancing the bitterness that caused by increase of released amount of each macrolide drug. In clarithromycin formulation, pH of clarithromycin product suspension in acidic sports drink is 8.08, and still basic. Even though the released amount of clarithromycin is not likely to be so large but the released drug might be enough for giving severe bitterness. Because the clarithromycin shows very low solubility in water and has hydrophobic characteristics as shown in its interviewform.[23)] As mentioned in previous articles, hydrophobicity seems the key for the bitterness.[24—27)] In the case of azithromycin product, involved azithromycin has a comparatively large solubility in water as mentioned in the interviewform.[28)] Therefore, even in the case of water suspensions, the comparatively severe bitterness for water suspension seems to be due to a comparatively large solubility in water.

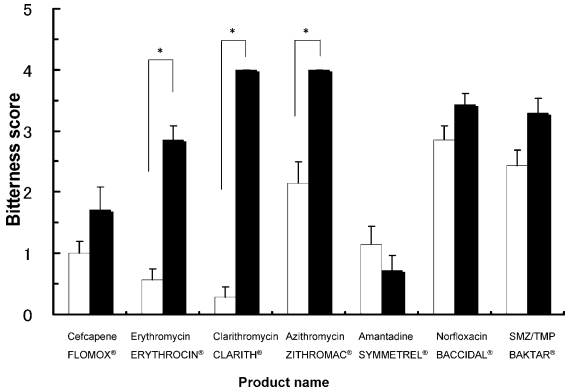

Fig. 2. The Result of Gustatory Sensation Tests of the Seven Most Bitter Drug Formulations Suspended in Water (□) or Acidic Sports Drink (■)

The data represents the mean±S.E. $*p<0.05$ compared with water suspension ($n=7$).

Whereas in the case of SYMMETREL®, the bitterness intensity was reduced when the product was suspended in acidic sports drink (0.71), compared with suspension in water (1.14). Involved amantadine is also basic drug, but its pK_a value was 10.3. In this case, pH of the dry syrup suspension was slightly changed from 4.21 (water) to 3.42 (sports drink). In this pH region, involved amantadine seems to give enough solubility in water. Therefore it was expected that

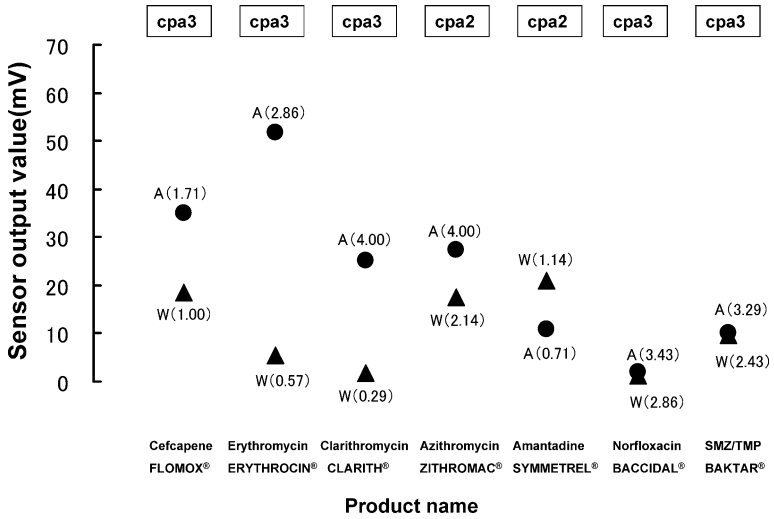

Fig. 3. CPA Data from Channels 2 or 3 of the Taste Sensor for the Seven Most Bitter Drug Formulations Suspended in Water (▲) or Acidic Sports Drink (●)

Values in parentheses are averages of the bitterness scores obtained in human gustatory sensation tests.

amantadine have moderate bitterness and it could be masked by sweetners involved in acidic sports drink.

Relationship between Bitterness Intensities Evaluated by Gustatory Sensation Tests and by the Taste Sensor for Seven Bitter Drug Formulations Suspended in Water or Acidic Sports Drink The seven formulations with bitterness scores over 1 were also tested using the taste sensor. Figure 3 shows the CPA data from the taste sensor which are considered to reflect the bitterness intensity of these formulations. The values in parentheses are the average bitterness scores obtained in human volunteers. For example, in the case of azithromycin, W(2.14) or A(4.00) means obtained bitterness score for water suspensions or acidic sports drink suspensions was 17.58, or 27.42 mV, respectively. We adopted sensory CPA data from channels 2 or 3, which was represented as CPA2 or CPA3, respectively. The components of channel 2 or 3, were; phosphroc acid di-*n*-decyl ester/2-nitrophenyl octyl ester, or hexadecanoic acid/dioctylphenyl phosphonate, respectively. Their components were different, and by binding of positively charged drug to the surface of membrane, the positive charge was given to negatively charged membranes.

The increase in CPA2 or CPA3 is due to the addition of the charge of the basic drug to the surface of the sensor membrane which was negatively charged due to the presence of a large number of phosphoric groups. The larger CPA2 or CPA3 values, the greater the bitterness intensity, even though its absolute values were different. Six of the formulations showed an increase in both sensor output values and gustatory sensation test scores when suspended in acidic sport drink; only SYMMETREL® showed a decrease using both methods. The output value of the taste sensor could therefore be used to predict the gustatory sensation data, and thus to predict the effect of suspension in an acidic sports drink on bitterness.

A calibration curve was made for each drug formulation individually, to investigate whether it would be possible to predict precisely the gustatory sensation bitterness score of a suspension of the drug in acidic sport drink on the basis of gustatory sensation data from a water suspension and taste sensor data from formulations suspended in water and the acidic sports drink. The results are summarized in Fig. 4. The bitterness strength of acidic sports drink suspensions predicted by the taste sensor was very close to the scores obtained in gustatory sensation tests. For example, with the cefcapene product FLOMOX® (top left in Fig. 4), the closed triangle (▲) shows the bitterness score of the water suspension (1.00), and the closed circle (●) shows the bitterness score of an acidic sports drink suspension (1.89) derived from gustatory sensation tests. The open circle (○) shows the bitterness score of an acidic sport drink suspension as predicted by the taste sensor (1.79). This is very close to the value actually obtained in human gustatory tests (1.71).

It can be concluded that the gustatory bitterness score of an acidic sports drink suspension can be predicted by the taste sensor using the sensor output value and gustatory bitterness scores for a water suspension, and the sensor output value for the acidic sport drink suspension.

It was necessary to select the most bitterness-specific sensor data for the calibration curve. In some cases, the CPA2 best reflected the bitterness of the drug formulation, but in other cases channel 3 was more specific. For the three macrolides, a high correlation was obtained between the CPA2 and the obtained bitterness intensity using a six-point data set ($r=0.90$) (detailed data not shown).

We then examined the relationship between the gustatory bitterness scores and the data predicted by the taste sensor for both water and acidic sport drink suspensions for all seven drugs. The results are shown in Fig. 5.

Originally, we had thought that there was a correlation between the large output of CPA2 or CPA3 and the bitterness score of an individual medicine, as mentioned above. For example, CPA3 values for clarithromycin suspensions in water or acidic sports drink was 1.82 or 25.10 mV, respectively. Whereas as CPA2 value, 1.55 or 21.47 mV, were obtained for output for suspension in water or acidic sports drink, respectively. However, when all seven medicines were examined, it was not possible to predict individual bitterness scores on the

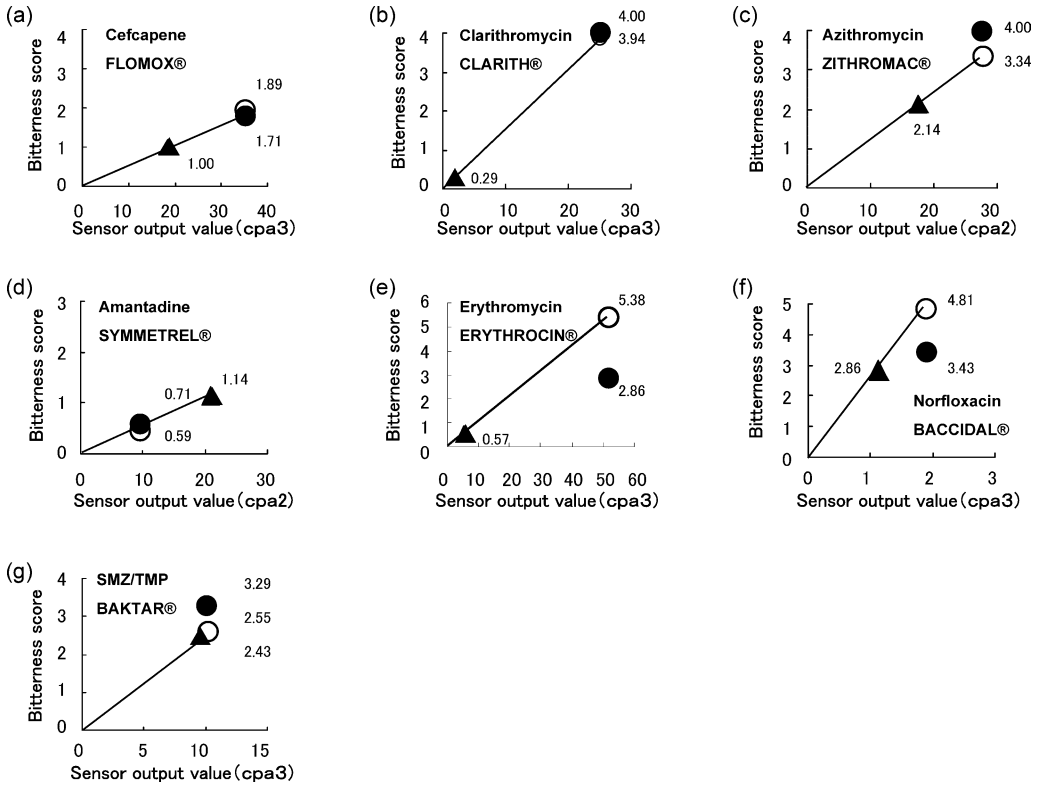

Fig. 4. Predicted and Obtained Bitterness Scores for Acidic Sports Drink Suspensions

The calibration curves were made for each individual drug formulation. ▲; the bitterness scores of a water suspension. ●; the bitterness scores of an acidic sports drink suspension. ○; bitterness scores of an acidic sport drink suspension as predicted by the taste sensor.

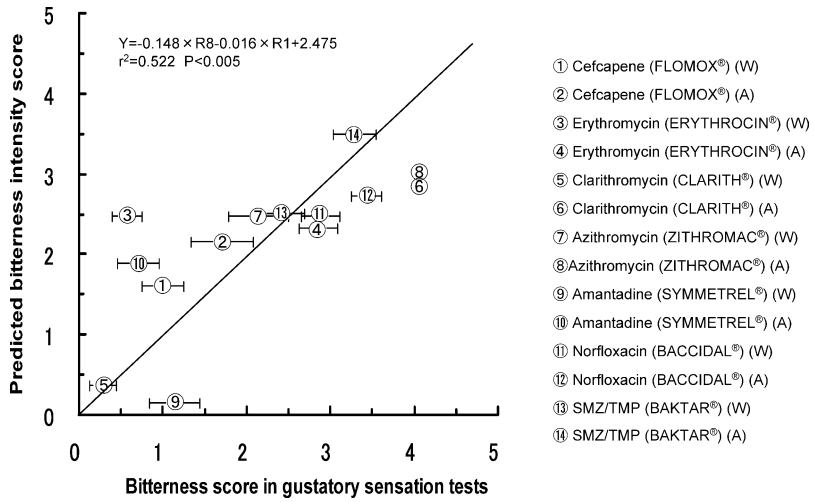

Fig. 5. The Relationship between the Bitterness Intensity Scores Obtained from Human Volunteers and the Predicted Values Calculated from the Equation Derived from Multiple Regression Analysis Using Data from Channels 1 and 8 of the Taste Sensor

R1 or R8 represents the relative values for channels 1 or 8, respectively. The data represents the mean±S.E. (n=7). In figure, cefcapene (FLOMOX®) (W) and cefcapene (FLOMOX®) (A) represent suspension in water, and acidic sports drink suspension respectively.

basis of the data from whichever of these two channels had the larger individual output value.

Therefore, the most optimized choice of sensors was examined to minimize residues between obtained and predicted bitterness scores by multicollinearity and stepwise test using S-PLUS. As a result, a comparatively good correlation could be obtained for all drugs. When the relative output values of channels 1 and 8, defined as R1 or R8, respectively, were used, the following multiple regression equation could be calculated:

$$Y = -0.148 \times R8 - 0.016 \times R1 + 2.475 \quad (r^2 = 0.522)$$

As shown in Fig. 5, use of these data enabled a good correlation to be obtained. Therefore, using the above-mentioned regression equation, the bitterness score of a water suspension or acidic sports drink suspension is quantitatively

predictable, to some degree, on the basis of their sensor output values. In this regression equation, the prediction of erythromycin product (③) or amantadine product (⑩) seems impossible.

It is expected that systematic optimization of the sensor, to improve sensor sensitivity and variety, may be possible in the future.

Quantitative bitterness prediction might also be possible using HPLC. However, if one needs to evaluate the quantitative bitterness of a considerable number of different medicines, the taste sensor has several advantages, as the methodology is well-established and the procedure is comparatively inexpensive and easy to conduct. Taste sensors would also be useful in screening for bitterness during the development of new drug formulations, and would reduce dependence on gustatory sensation data.

Whereas we have a task to be dissolved in our taste sensor system: increasing sensitivity or specificity for bitterness. We have to improve this issue by using novel type taste sensor, for example, that attached surface modified membrane. The taste sensor has an obvious potential in the evaluation of bitterness or other tastes in medicines.

Conclusions

1. It is possible to measure the bitterness of several different types of antibiotic and antiviral formulations using the taste sensor.
2. The bitterness of these formulations was frequently enhanced when the product was taken with an acidic sport drink; the taste sensor was able to predict this enhancement effect using data derived from human gustatory tests with the product suspended in water.
3. The taste sensor may offer an alternative methodology to gustatory sensation tests in the assessment of bitterness.

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