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Evaluation of the bitterness of antibiotics using a taste sensor

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Abstract

The bitterness of nine commercial antibiotics (clarithromycin, erythromycin, cefdinil, doxycycline, vancomycin, tetracycline, minocycline, oxytetracycline and bacampicillin) was evaluated in human gustatory sensation tests with nine volunteers. The bitterness of 0.1–0.3 m_M solutions (or suspensions in the case of clarithromycin) of the antibiotics was then measured using an artificial multichannel taste sensor. In the sensor measurements, three variables were used to predict estimated bitterness in single and multiple regression analysis and principal component analysis: sensor output as relative value (R), the change of membrane potential caused by adsorption (C) and C/R. Particularly good correlation was obtained between obtained bitterness scores and predicted scores using C from channel 2 of the sensor ($r^2 = 0.870$, $P < 0.005$) and C/R values for channels 2 and 3 ($r^2 = 0.947$, $P < 0.005$). The taste sensor was also successful in assessing the bitterness intensity of clarithromycin powder suspensions of various concentrations. Clarithromycin has a low aqueous solubility but is the most bitter of the nine antibiotics. Sensory data from channel 3 of the sensor predicted the bitterness of clarithromycin powder suspensions and their filtered solutions well. Finally, the bitterness intensity of a commercial clarithromycin dry syrup product (Clarith dry syrup, Taisho Pharmaceutical Co. Ltd, Tokyo, Japan) was evaluated in gustatory sensation tests and using the taste sensor. In Clarith dry syrup the drug is coated with aminoalkyl methacrylate polymer using a spray congealing method. The taste sensor results confirmed that the polymer was successful in almost completely masking the bitter taste of the dry syrup product.

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Introduction

Antibiotics are widely used to treat various bacterial infections. Although they are sometimes administered parenterally, oral administration is more convenient and acceptable for patients. The oral administration of antibiotics, especially to children and elderly patients, is often hampered by their unpleasant bitter taste, leading to noncompliance and hindering therapeutic management. A method for the quantitative evaluation of the bitterness of antibiotics would be useful in the development and formulation of antibiotics. There have been many attempts to achieve taste masking physically, using techniques such as microsphere formation (Ueda et al 1993; Hashimoto et al 2002) and coating (Choi & Kim 2003). However, it would also be useful to be able to evaluate quantitatively the bitterness of the target drug itself, prior to formulation development and any decisions being made as to the appropriate taste-masking strategy.

We have recently reported a quantitative analytical method for the evaluation of the bitterness of medicines using a taste sensor (Uchida et al 2000, 2001). The taste sensor, an 'electric tongue' with global selectivity, was developed by Toko (1998a). It comprises several kinds of lipid/polymer membrane that are able to transform information about substances producing taste into electrical signals (Hayashi et al 1990; Fukunaga et al 1996; Iiyama et al 1996; Takagi et al 2001). The sensor output exhibits different patterns for chemical substances that have different taste qualities, such as saltiness, sourness, bitterness and umami-in (Japan), and exhibits similar patterns for chemical substances

with similar tastes. The selectivity of the sensor for different tastes, the sensor detection level and the reproducibility of measurements were also demonstrated by Toko (1998b).

Toko (1998b) reported that the sensor had detection errors (in units of logarithmic concentration) of 0.73% for saltiness, 0.65% for sourness and 2.4% for bitterness in aqueous solutions simultaneously containing different kinds of chemical substances producing different taste qualities. Humans cannot distinguish between two tastes with a concentration difference below 20% (Pfaffman 1959). Here, 20% means an error of 7.9% (= log1.2) therefore the detection ability of the taste sensor is superior to that of humans. Even in the evaluation of the taste of various foods and chemicals, the sensor can express the taste quantitatively.

We have previously evaluated the bitterness of various medicines and amino acids using the taste sensor and suggested that the sensor could be used to obtain quantitative predictive data on the bitterness of commercial medicines (Uchida et al 2001; Miyanaga 2002a, b). In the present study, our goal was to see whether or not the taste sensor could be used to predict quantitatively the bitterness of various antibiotic solutions. We also investigated whether or not the taste sensor is capable of measuring the bitterness of suspensions, and their filtered solutions, using the insoluble antibiotic clarithromycin, the most bitter of the antibiotics tested. Finally, we compared the bitterness of a commercially available clarithromycin dry syrup product, which contains aminoalkyl methacrylate polymer as a taste-masker, with clarithromycin powder suspensions, to evaluate the effectiveness of the coating in masking the bitterness.

Methods

Materials

Clarithromycin, clarithromycin dry syrup (Clarith dry syrup) and erythromycin were donated by Taisho Pharmaceutical Co. Ltd (Tokyo, Japan). Cefdinil was donated by Fujisawa Co. Ltd (Japan). Doxycycline, vancomycin, oxytetracycline, minocycline and tetracycline were from Yamanouchi Co. Ltd (Tokyo, Japan), and bacampicillin was from Nihonyakuhinnkougy o Co. Ltd (Toyama, Japan). The nine drugs were dissolved or diluted to form 0.1 to 0.3 mM solutions (or suspensions in the case of clarithromycin) with 10 mM KCl. Quinine hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO), dissolved and diluted to produce a 0.10 mM solution with 10 mM KCl. All other reagents were of special reagent grade.

Sensor measurement and data analysis

The taste-sensing system SA402B of Intelligent Sensor Technology Co. Ltd (Atsugi, Japan), which is commercially available, was used to measure the electric potential of various concentrations of antibiotics, as shown in Figure 1. The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes composed of lipid/polymer membranes. The lipid components of the sensor used in

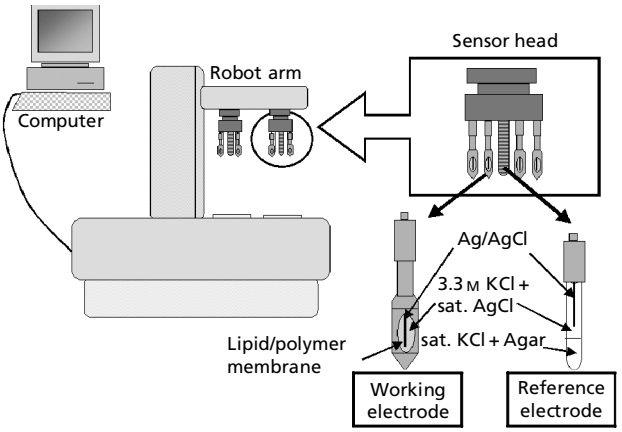

Figure 1 The multichannel taste sensor (SA402B).

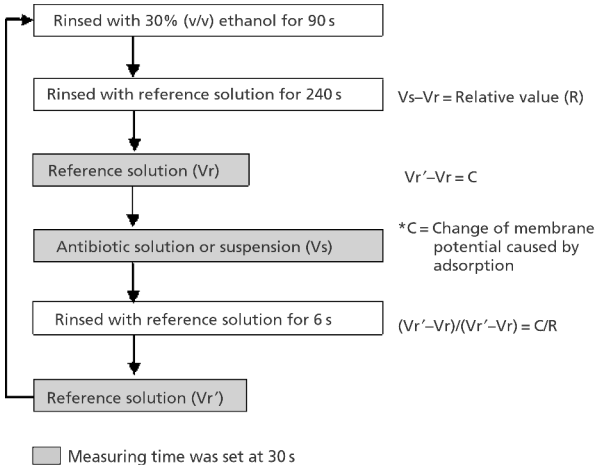

Figure 2 The measuring procedure in this study.

the present study were the same as those described previously (Uchida et al 2001; Miyanaga et al 2002b). Each lipid was mixed in a test-tube containing poly(vinylchloride) and dioctylphenylphospho nate 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 and an internal cavity filled with 3 mM 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 0.1–0.3 mM solutions (or suspensions in the case of clarithromycin) of the nine different antibiotics in 10 mM KCl were used in the study. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference solution (Vr) and also to rinse the electrodes after every measurement. The method used to measure the sensor output values produced by adsorption of the samples is summarized in Figure 2. The electrode was first dipped into the reference

solution and the electric potential (mV) obtained was defined as Vr. The electrode was then dipped into sample solution or suspension. The electric potential (mV) obtained was defined as Vs. The relative sensor output is represented by the difference (Vs–Vr) between the potentials of the sample and the reference solution. The electrode was then rinsed with fresh reference solution for 6 s. When the electrode was dipped into the reference solution again, the new potential of the reference solution was defined as Vr′. The difference (Vr′–Vr) between the potentials of the reference solution before and after sample measurement is the change of membrane potential caused by adsorption (C) and corresponds to aftertaste. In this experiment, each measuring time was set at 30 s. After measurement of a sample, the electrodes were rinsed with 30% (v/v) ethanol for 90 s, rinsed with fresh reference solution for 240 s and then the measurement of the electric potential (mV) of another sample was started. Thus enough rinsing was performed to exclude cross-contamination among samples. The continuous measurement set of different kinds of sample was repeated five times. The average values were used for the analysis. S-PLUS 2000J (Mathematical Systems, Inc., Tokyo, Japan) was used for regression analysis. In the present study, relative sensor output values (R), the change of membrane potential caused by adsorption (C) and C/R were used to predict the bitterness of nine antibiotics.

For the clarithromycin powder suspensions (concentrations 0.03, 0.1, 0.5, 1.0 and 3.0 mM), the samples were filtered through a 0.45-μm membrane filter. These filtered solutions and the corresponding unfiltered suspensions were compared in sensor measurement and gustatory sensation tests.

Gustatory sensation tests

The gustatory sensation tests were performed with nine well-trained and selected healthy human volunteers, according to a previously described method (Indow 1966; Katsuragi et al 1997). All volunteers could identify five tastes, especially bitterness. The subjects were asked to refrain from eating and drinking for at least 2 h prior to testing.

The standard quinine hydrochloride concentrations used were 0.003, 0.01, 0.03, 0.10, 0.3 and 1.00 mM and the corresponding bitterness scores were defined as −1, 0, 1, 2, 3 and 4, respectively. Before testing, the volunteers were asked to keep the above standard quinine solutions in their mouths for 15 s, and were told the concentrations and bitterness scores for each solution. After tasting the samples of antibiotic solution, they were asked to give them a bitterness score. 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.

In the case of clarithromycin powder and commercial dry syrup product, various quantities of powder or 1 g of dry syrup were adequately suspended in 50 mL of 10 mM KCl solution for 1 min.

Although Keast and Breslin (2002a) reported that cation or anion series of salts affect the bitterness of pharmaceutics, KCl, which was added to all the samples in the sensor study (for improving conductibility) and gustatory sensation tests, did not affect the bitterness intensity of clarithromycin in our pilot study (data not shown). Filtered solutions of the clarithromycin powder suspensions were also tested.

Determination of the solubility of clarithromycin

Clarithromycin powder suspensions of various concentrations (0.03, 0.1, 0.5, 1.0 and 3.0 mM) were filtered through a 0.45-μm membrane filter. The clarithromycin concentrations in the filtered solutions were determined using HPLC: 100 μL was injected onto a chromatograph (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10AV), an integrator (Shimadzu C-R6A) and a reversed-phase column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai Tesque Co., Ltd, Kyoto, Japan). The following mobile phase system was used: A, 1/15 M monobasic potassium phosphate; B, acetonitrile; (A:B = 13:7). The flow rate was adjusted so that the retention time for the clarithromycin peak was about 8 min. The wavelength was set at 210 nm. Samples for the standard curve were prepared by dissolving the clarithromycin powder in the above mobile phase. The slope and intercept of the standard curve was constant every time and reproducibility was confirmed. The clarithromycin concentration in the above filtered solution was determined using this standard curve.

Statistical analysis

The difference between the bitterness intensities of clarithromycin powder suspension and clarithromycin dry syrup was analysed using the Mann Whitney U-test, non-parametric method. The actual analysis was performed using software located in the website at http//aoki2.si.gunma-u.ac.jp/lecture/stats-by-excel/vba/html/two_sample.html. A value of $P < 0.005$ or $P < 0.001$ was accepted as indicating a significant difference between values.

Results and Discussion

Principal component analysis of sensor data

Principal component analysis was performed on the data obtained from the taste sensor for the nine antibiotics. Principal component analysis is a multivariate analytical method that reduces the dimensional space without losing any information. We used principal component analysis to estimate the largest and second largest relative contribution factors (PC1 and PC2) from all the sensor data. The results are shown in Figure 3. The relative contributions of PC1 and PC2 are 89 and 10%, respectively. We could not determine the precise meaning of the axes PC1 and PC2 from the data obtained. In the present study, we used only nine drugs, which were not representative of every type of antibiotic (two were macrolide antibiotics and four were from the tetracycline group). Principal component analysis may provide information on the similarity of a group of compounds in terms of overall taste.

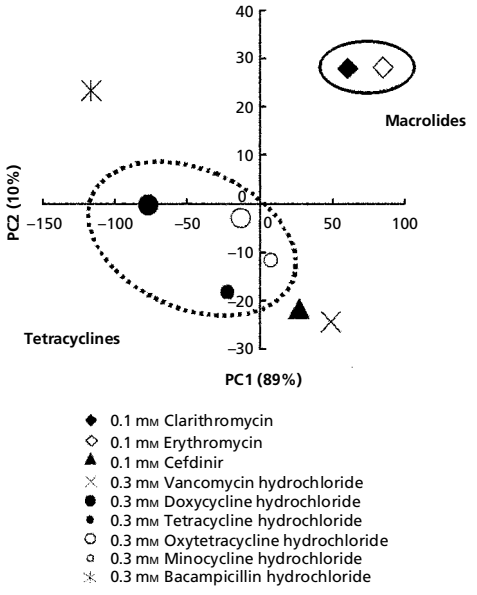

Figure 3 Principal component (PC) analysis of sensor output values for nine kinds of drug solutions (or suspensions in the case of clarithromycin). The relative contributions of PC1 and PC2 were calculated to be 89 and 10%, respectively. For further explanation see text.

Bitterness prediction of antibiotics

Three variables were used to predict the estimated bitterness of the nine antibiotics tested using single or multiple regression analysis: sensor output (R), the change of membrane potential caused by adsorption (C) and C/R. Good correlation was obtained between the bitterness scores obtained in the human gustatory sensation tests and scores derived from sensor data. The highest correlation between the human gustatory sensation data and the predicted bitterness scores obtained using the C value from channel 2 of the taste sensor (C2) is shown in Figure 4A. The derived equation by single regression was $y = 0.317 \times C2 + 0.380$ ($r^2 = 0.870$, $P < 0.0005$), where the y- and x-axis data represent the sensor-predicted and observed human bitterness scores, respectively. The observed gustatory bitterness and the predicted bitterness calculated by the above equation were located on or very close to the diagonal line in the graph. It was concluded that the bitterness of antibiotic drug solutions could be estimated with good accuracy using the C values of channel 2 of the taste sensor. Although the data are not shown, when other channels such as channel 3 were used for the analysis, a comparatively good correlation was obtained.

An even better correlation was obtained between the gustatory sensation data and predicted bitterness scores using the C/R of channels 2 and 3. The derived multiple regression equation was $y = 2.297 \times C2/R2 + 7.049 \times C3/R3 + 0.228$ ($r^2 = 0.947$, $P < 0.0005$), as shown in Figure 4B, where y represents the predicted bitterness scores. C2 and C3 represent the change of membrane potential caused by adsorption in channels 2 and 3, respectively, while R2 and R3 represent relative values for channels 2 and 3, respectively. Clarithromycin and bacampicillin were considerably more bitter than the other antibiotics. In this system, the bitterness of 0.1 mM and 1.0 mM quinine hydrochloride solutions would be 2.0 and 4.0, respectively. As the predicted bitterness of both the 0.1 mM clarithromycin suspension and the 0.3 mM bacampicillin solution was over 2.0, it was concluded that the bitterness of both solutions, and especially clarithromycin, was much greater than that of 0.1 mM quinine hydrochloride, the standard for bitterness.

Bitterness prediction of clarithromycin powder suspensions and their filtered solutions

As shown in Figures 4A and B, clarithromycin was the most bitter of the nine antibiotics in the present study. Clarithromycin has a low dissolution rate and poor aqueous solubility. In spite of considerable efforts to produce a solution of clarithromycin, such as heating to 50 °C, agitation and sonication (drug stability during the above treatments was confirmed; data not shown), we were unsuccessful and the clarithromycin samples used in this experiment were suspensions. We therefore determined whether or not the taste sensor was capable of predicting the bitterness of clarithromycin suspensions of various concentrations (0.03, 0.1, 0.5, 1.0 and 3.0 mM), as well as the solutions obtained after filtration of these suspensions through a 0.45-μm membrane filter.

Figures 5A and B show the relationship between the bitterness intensity scores obtained in human gustatory sensation tests and the predicted bitterness scores derived from sensor output using the C value of channel 3 for clarithromycin suspensions (Figure 5B) and their filtered solutions (Figure 5A). (In a previous paper (Uchida et al 2001) we demonstrated that C values are more specific to bitterness than R values.) In both figures, the y-axis represents the predicted bitterness intensity while the x-axis represents the bitterness intensity observed in human gustatory sensation tests. As can be seen in Figure 5A, at theoretical concentrations over 0.5 mM the filtered clarithromycin solutions were saturated, as the obtained and predicted bitterness intensities for filtered solutions of 0.5, 1.0 and 3.0 mM suspensions were essentially the same. In Figure 5B it can be seen that the obtained and predicted bitterness scores of the suspensions were almost same or a little higher than those of the corresponding filtered solutions. In addition, as shown in Figure 5B, at concentrations of 0.5 mM clarithromycin and above, the suspensions were probably saturated since both obtained and predicted bitterness scores were very similar. The solubility of clarithromycin was calculated to be 0.23 mM; HPLC analysis showed that the concentrations of the filtered samples from the 0.5–3 mM clarithromycin powder suspensions were very similar (almost 0.23 mM). The clarithromycin concentrations of the filtered solutions of 0.01, 0.03, 0.1 and 0.3 mM were 0.020, 0.037, 0.118 and 0.196 mM, respectively. Thus the concentration of the filtered solution increased as the concentration of the clarithromycin suspension increased. As mentioned above, the

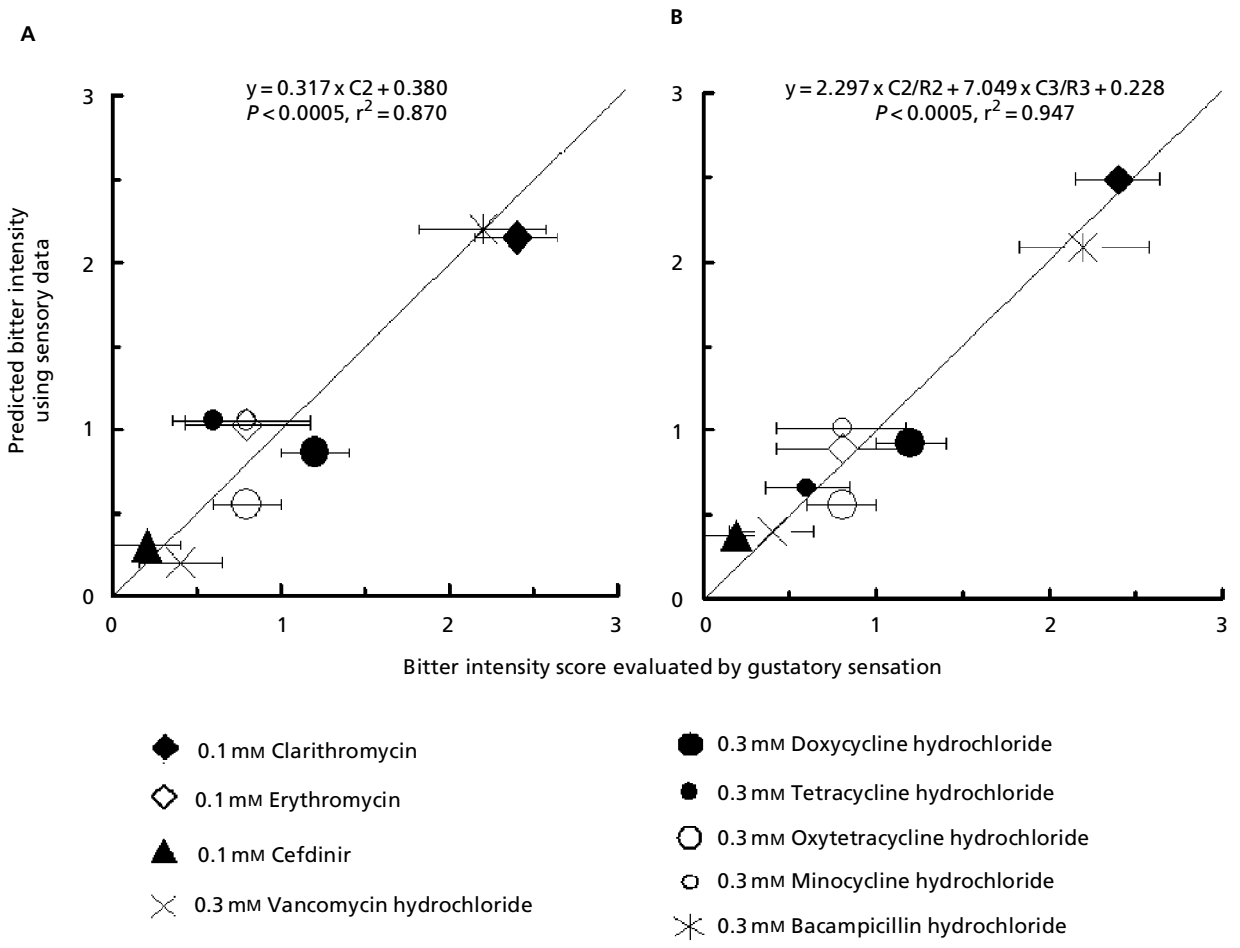

Figure 4 Single (A) and multiple (B) regression analysis results using relative sensor output values (R), the change of membrane potential caused by adsorption (C) and C/R values for channels 2 and 3. In single regression analysis the data are derived from the C value for channel 2 only. Multiple regression analysis data was calculated using C/R values for channels 2 and 3. For further explanation see text. Error bars represent the mean plus standard deviation (n = 9).

bitterness intensity scores obtained in human gustatory sensation tests could be predicted by sensor output. In the near future another target is to establish the correlation between drug concentration and predicted bitterness intensity scores by taste sensor in various kinds of drugs.

Bitterness evaluation of commercial clarithromycin dry syrup by taste sensor

Recently, Yajima et al (1999) developed a clarithromycin dry syrup (Clarith dry syrup, Taisho Co. Ltd, Tokyo, Japan) by a spray-congealing process and achieved good taste masking using aminoalkyl methacrylate polymer, which dissolves below pH 5. This commercially available syrup was used as a taste-masked formulation of clarithromycin.

Figure 6 shows that a good correlation was obtained between human gustatory sensation scores (x-axis) and the predicted bitterness intensity calculated from the C value in channel 3 (y-axis) for different concentrations of clarithromycin powder suspensions and dry syrup product. Comparatively large bitterness scores were obtained for the powder suspensions, while significantly lower values were obtained for 1.0 g of clarithromycin dry syrup (containing 100 mg of clarithromycin) suspended in 25 mL of 10 mM KCl solution: 0.111 (observed) and −0.025 (calculated), respectively. The bitterness of the dry syrup was significantly decreased compared to the powder suspensions.

The theoretical concentration of a clarithromycin drug suspension produced by suspending 1.0 g of clarithromycin powder directly in 25 mL of 10 mM KCl solution is 5.35 mM. This suspension would be expected to have a bitterness score of at least 4.0 (the bitterness score of a 3.0 mM clarithromycin suspension being about 4.0 in both human gustatory sensation tests and taste sensor tests). This bitterness score corresponds to that of a 1.0 mM quinine hydrochloride solution. However, the bitterness scores of dry syrup product obtained in a gustatory sensation test and predicted by the taste sensor were 0.111 and −0.025, respectively, equivalent to quinine concentrations of 0.01135 mM and 0.00943 mM, respectively. If the bitterness

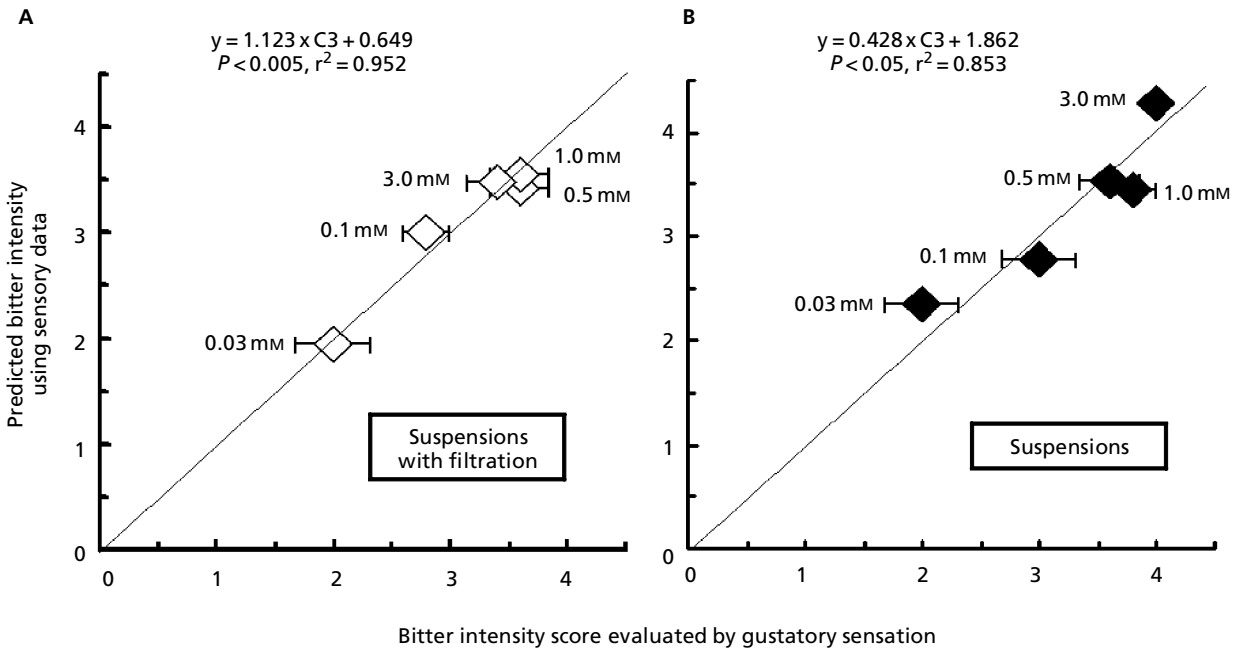

Figure 5 The relationship between bitterness intensity scores of clarithromycin obtained in human gustatory sensation tests and the predicted bitterness scores derived from the taste sensor output (C value of channel 3): (A) data from the filtered solutions; (B) data from the suspensions themselves. For further explanation see text. Error bars represent the mean plus standard deviation (n = 9).

scores of the clarithromycin powder suspension and dry syrup formulation are expressed in terms of equivalent quinine concentrations, the bitterness of clarithromycin dry syrup was reduced to about 1% of that of an equivalent powder suspension. In other words, almost 99% of the bitter taste was successfully masked in the dry syrup formulation, not only with respect to human gustatory sensation but also to taste sensor prediction.

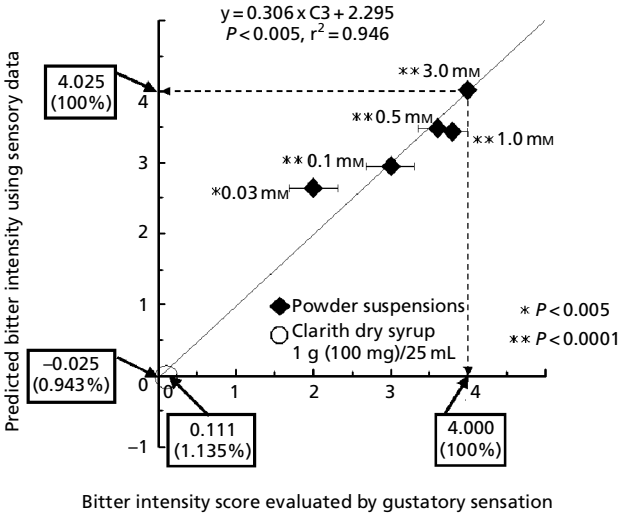

Figure 6 Relationship between bitterness scores obtained in human gustatory sensation tests and those predicted from taste sensor data (C3) using clarithromycin suspensions of different concentrations and the Clarith dry syrup. For further explanation see text. Error bars represent the mean plus standard deviation (n = 9). $^{*}P < 0.005$, $^{**}P < 0.001$, compared with control, the Clarith dry syrup.

In general, macrolide compounds are known to be strongly bitter and there have been many attempts to achieve effective taste masking of them. Lu et al (1991) proposed a polymer carrier system to reduce the bitterness of erythromycin and clarithromycin by absorption to Carbopol, a high molecular weight polyacrylic acid, and hoped thereby to remove the drug from the solution phase in an ion-free suspension. However, the taste suppression achieved was insufficient and the masking was therefore enhanced by encapsulating the adsorbate particles with polymer coatings using hydroxypropylmethylcellulose phthalate (HP-55). This method, which essentially requires two processes, is not cost effective. The simpler preparative method described by Yajima et al (1999) is essentially a spray-congealing method and it has already yielded products for the commercial Japanese market.

The Clarith dry syrup is bioequivalent to conventional dosage form (for example, tablet or powder suspension) since the drug is immediately released with dissolution of the polymer below pH 5 (in gastric conditions), whereas above pH 5 (oral cavity conditions) the drug cannot be released from the polymer matrix since the polymer cannot dissolve in that pH range.

These authors also demonstrated that release of clarithromycin in the mouth is initially very limited, and they were able to achieve adequate taste masking. The present taste sensor data obtained for the dry syrup supports these findings.

If it were possible to use the taste sensor to evaluate bitter-tasting drugs, the number of subjects required for

human gustatory sensation tests could be reduced. In addition, this method of quantitative bitterness evaluation might be useful in predicting the bitterness of other antibiotic solutions, suspensions and formulations.

Conclusions

The bitterness scores of nine commercial antibiotic solutions were predicted using an artificial multichannel taste sensor. The taste sensor was also capable of predicting the bitterness of clarithromycin powder suspensions with good accuracy. Finally, when the bitterness of clarithromycin powder suspensions was compared with that of a commercial clarithromycin dry syrup product that is taste masked with polymer (Clarith dry syrup), it was shown that, on the basis of both human gustatory sensation tests and taste-sensor data, almost 99% taste masking (expressed in terms of equivalent quinine concentrations) was achieved for the clarithromycin dry syrup product.

The mechanism of bitterness perception via taste receptors has been the subject of much recent discussion (Keast & Breslin 2002b; Nelson et al 2002), and several studies have shown that the action potential and Ca^{2+} levels in the taste cells play an important role in the perception of bitterness (Kashiwayanagi et al 1981; Kumazawa et al 1986). Recently, the cloning of a mammalian bitterness receptor has also been reported (Chandrashekar et al 2000). These results may allow us to produce a theoretical design of membrane components in the taste sensor.

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