Factors Affecting the Bitterness Intensities of Ten Commercial Formulations of Ambroxol

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The bitterness of 10 different products with ambroxol as active ingredient, the original and nine generics, were evaluated by human gustatory sensation tests in which the tablets were kept in the mouth, with water, at 20 and 37°C. The products all showed different bitterness intensities. The original and some of the generic products had comparatively low bitterness intensities but some of the generic products had comparatively high bitterness intensities. The bitterness intensities of these 10 was found to be significantly correlated with both the disintegration time, as evaluated using the ODT-101 (a recently developed apparatus), and the drug concentration in dissolved medium, as measured in a conventional dissolution test. The bitterness threshold of ambroxol solution was found to increase when the temperature of the water with which the tablets were taken, was raised from 20 to 37°C. The equation was calculated to predict the bitterness intensity of ambroxol, a function based on temperature and the ambroxol concentration using data from a standard ambroxol solution at 4, 20 and 37°C. The bitterness intensities obtained for the 10 ambroxol formulations with water at 20 and 37°C, coincided with the bitterness values predicted by the equation.

Key words ambroxol tablet; bitterness threshold; prediction; temperature

The treatment of respiratory diseases, among others, is greatly affected by compliance issues, with patients often failing to take medications with an unpleasant taste, thus reducing their therapeutic effects. Bitterness is thought to be one of the main reasons for this.1–4 For orally disintegrating tablets especially, the bitterness of the formulation seems to be the critical factor determining palatability. There are large differences between products with respect to bitterness intensity,5–7 and even conventional tablets that disintegrate or dissolve in saliva comparatively slowly, carry a risk that patients will be put off from swallowing them if they taste bitter.

Ambroxol formulations, including a dry syrup, are widely used in respiratory medicine, but in some cases, the unpleasant taste and bitterness of the product have been claimed to limit clinical usage.8–10 Patients have even been heard to complain about the bitterness of ambroxol commercial tablets. In spite of this, there have not been any reports in which bitterness intensity has been evaluated quantitatively.

In the present study, we focused on the bitterness of 10 commercial ambroxol tablets, the original product and nine generic copies. Firstly, the bitterness of the tablets was evaluated in human gustatory sensation tests, in which the temperature of the water with which the tablets were taken varied between 20 and 37°C. Secondly, the critical factor affecting bitterness intensity was determined by establishing the correlation between the bitterness score measured in human gustatory sensation testing, the disintegration time evaluated using the ODT-101 (a recently developed apparatus), and the drug concentration in the solute (water) measured in a conventional brief dissolution test.

The relationship between the bitterness threshold of the ambroxol solution and the temperature of the water with which the tablets were taken was also investigated, using temperatures of 20 and 37°C. The equation was calculated to predict the bitterness intensity of ambroxol, a function based on the temperature and the ambroxol concentration, using data from a standard ambroxol solution at 20 and 37°C. When the temperature and the ambroxol concentration (obtained using a conventional brief dissolution test), were put into the equation, the bitterness of each of the 10 tablets could be predicted. Thus, we propose a method of predicting the bitterness intensity of various tablets without using gustatory sensation testing, based on data from standard solutions of the active ingredient and a conventional brief dissolution test, assuming at least partial dissolution of the active ingredient in the mouth.

The protocol was approved in advance by ethical committees of Mukogawa Women’s University.

Experimental

Materials Ten different formulations of 15-mg ambroxol tablets were used in the present study; the original product, Mucosalvan® tablets 15mg (Teijin Pharma Ltd., Japan), and the following nine generic products: Ponopen® tablets 15mg (Aska Pharmaceutical Co., Ltd., Japan), Coughnol (Nichii-Iko Pharmaceutical Co., Ltd., Japan), Nontas® tablet 15mg (Ono Pharmaceutical Co., Ltd., Japan), Pulsmarin® A tablets 15mg (Takata Seiyaku Co., Ltd., Japan), Fuzuleban (Tatsumi Kagaku Co., Ltd., Japan), Grinkool® (Nippon Chemiphar Co., Ltd., Japan), ambroxol hydrochloride tablets 15mg [Ze] (Zen-sei Pharmaceutical Industries Co., Ltd., Japan), ambroxol hydrochloride tablets 15mg [Sawai] (Sawai Pharmaceutical Co., Ltd., Japan), and ambroxol hydrochloride tablets 15mg [Ch] (Mylan Seiyaku Ltd., Japan). The nine generic products were randomly assigned letters A to I, and the original product was assigned J. Quinine hydrochloride (bitterness standard) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and ambroxol hydrochloride from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were high grade.

Gustatory Sensation Tests in Human Volunteers All subjects were volunteers and gave their informed consent. The protocol was approved in advance by ethical committees of Mukogawa Women’s University.

Gustatory Sensation Testing of Standard Solutions of Quinine Chloride and Ambroxol The standard ambroxol hydrochloride and quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30, and 1.00 mm. These solutions were
maintained at 4, 20 or 37°C. After tasting each sample, subjects \(n=14\) gargled well and waited at least 20 min before testing the next sample.

**Gustatory Sensation Testing of 10 Ambroxol Formulations** Gustatory sensation testing was performed according to a previously described method.\(^{11}\) The standard quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30, and 1.0 m\(\text{M}\) and the corresponding bitterness intensity scores were defined as 0, 1, 2, 3, and 4, respectively. Before testing well-trained healthy adult volunteers \(n=14\) were asked to keep 2 mL of the standard solutions in their mouths for 2 min each, and told their concentrations and bitterness intensity scores. In evaluation of the samples, 25 mL of water, maintained at 20 or 37°C, was kept in the mouth, together with the tablet. Test samples were removed from the mouth (2 mL) and bitterness intensity scores recorded after 10, 20 and 30 s.

**Evaluation of Disintegration Time of 10 Commercial Ambroxol Tablets** In order to evaluate the disintegration times of 10 commercial ambroxol tablets, the disintegration apparatus described in the Japanese Pharmacopoeia (JP-XVI, and a newly developed apparatus called the ODT-101\(^{12}\) (Toyama Sangyo Co., Ltd., Osaka, Japan) were both employed. For the disintegration apparatus described in JP-XVI, tablets were placed in each of the six tubes of basket and the disks were added. A device for raising and lowering the basket in the water as the immersion fluid maintained at 37°C was kept in the mouth, with a stainless-steel basket (diameter and depth is 20 mm and 30 mm, respectively) with tablet was placed in 25 mL of purified water in the beaker on the water bath and shaken at 150 rpm, through a distance of about 30 mm. The medium temperature was set to 20, 37°C. When the tablet was shaken for 10, 20 and 30 s, stainless-steel basket with tablet was removed from the beaker. Medium in the beaker was filtrated, the concentration of drug in the medium was determined by HPLC, as described above.

**Statistical Analysis** All data were expressed as mean±S.E. Dunnett’s test was also used for post-hoc analysis. Two-way repeated measures analysis of variance (ANOVA) was used for statistical comparisons. Prediction formula in bitterness of ambroxol was calculated based on multiple regression analysis. Correlation between the actual measurement value in bitterness of ambroxol and value forecast in bitterness of ambroxol and was examined using Spearman test. Values of \(p<0.05\) were considered significant.

**Results and Discussion** The Bitterness Intensities of 10 Commercial Ambroxol Tablets by Human Gustatory Sensation Testing at 20°C (A) and 37°C (B)

Fig. 1. The Bitterness Intensities of 10 Commercial Ambroxol Tablets by Human Gustatory Sensation Testing at 20°C (A) and 37°C (B)

Data are presented as mean values±S.E. \((n=12)\). Product J is the original drug and the other nine products are generic versions. \(*p<0.05, \#p<0.01\) compared with product J at 10 s; \(\dagger\) \(p<0.01\) at 20 s; and \(\ddagger\) \(p<0.05, \#\#p<0.01\) at 30 s (Dunnett’s test).
Formulations by Human Gustatory Sensation Test  
Gustatory sensation tests were performed to estimate the bitterness intensities of 10 commercial ambroxol formulations when tablets were kept in the mouth for up to 30 s each, together with 25 mL of water maintained at 20 or 37°C.

As shown in Figs. 1A (20°C) and B (37°C), the bitterness intensities of all products increased as time increased from 10 to 30 s. The bitterness intensities at 37°C were greater than the corresponding values at 20°C for all products.

The bitterness intensities of tablets A, E and J (original), were under 2.0 when the tablets were held in the mouth with water maintained at 20°C for 30 s, indicating low bitterness (Fig. 1A), while the bitterness intensities of tablets C, F, and I were over 3.0 when the tablets were held in the mouth with water maintained at 37°C for 30 s, indicating strong bitterness (Fig. 1B), significantly higher than that of tablet J (the original) (\(F_{[9,110]} = 7.164, p < 0.01\)).

The Relationship between Disintegration Time and Bitterness Intensity as Measured by Gustatory Sensation Testing of 10 Commercial Ambroxol Formulations  
As mentioned in the previous paragraph, the bitterness intensities of the 10 commercial ambroxol products were quite different. In order to try to find the underlying cause for this, two factors affecting bitterness intensity (disintegration time and dissolved drug concentrations in oral cavity) were examined for the 10 commercial ambroxol formulations.

Firstly, the disintegration times of the tablets were examined using two different methods: the disintegration test described in the JPXVI, and a recently developed alternative method using the ODT-101. \(^{12}\) The results are presented in Figs. 2A and B, respectively.

Using the conventional JPXVI method, the disintegration times of products A and E were over 5 min, showing that these formulations do not disintegrate easily. All the other tablets disintegrated within 90 s. As the bitterness intensities of products A and E are also lower than those of the other products, these results suggest that bitterness may be related to their limited disintegration. Although the relationship between bitterness intensity and the logarithm of disintegration time seemed to be negatively linear, a statistically significant relationship was not confirmed (\(r_s = 0.63; \) Fig. 2A).

When the relationship between bitterness intensity and the logarithm of disintegration time was evaluated using the ODT-101, however, it was found to be significantly negative (\(r_s = 0.69 (p < 0.05), \) Fig. 2B). There were no significant differences in correlation between conventional JPXVI method and ODT-101. However, the disintegration times obtained using the ODT-101 was smaller than those obtained with the JP method. This could be due to the fact that, with the ODT-101, the shearing stress is applied to the surface of the tablet via the anchor, and may reflect more accurately the conditions in the human oral cavity.

The Effect of the Dissolved Drug Concentration on the Bitterness Intensity Measured by Gustatory Sensation Testing of 10 Commercial Ambroxol Formulations  
As well as the relationship between bitterness intensity and disintegration time, discussed above, the concentration of drug dissolved may also be a critical factor in determining the bitterness intensity demonstrated in human gustatory sensation tests.

In the next experiment, therefore, the effect of the dissolved drug concentration on the bitterness intensities of the 10 commercial ambroxol formulations was examined. The dissolution media were maintained at 20 or 37°C, to mimic the tablets being kept in the mouth with water maintained at 20 or 37°C.

The relationship between the bitterness intensity measured by gustatory testing at 20 and 37°C, and the dissolved drug concentration in the medium, as measured by both the JP method and the brief conventional dissolution test, are shown in Figs. 3A and B, respectively.

No relationship was detected between the bitterness intensity measured by human gustatory testing at 20 and 37°C, and the dissolved drug concentration at 30 s measured using the JP method (\(r_s = 0.68, r_s = 0.33\), respectively, Fig. 3A). However, when the drug concentration was measured using the brief conventional dissolution test, a relationship was found between the dissolved drug concentration at 30 s and the bitterness intensity measured by human gustatory testing at 20 and 37°C (\(r_s = 0.80 (p < 0.05), r_s = 0.91 (p < 0.01)\), respectively; Fig. 3B). The drug concentrations in the dissolved medium at 37°C

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**Fig. 2. Correlation between Bitterness Intensities and Disintegration Times as Evaluated Using the JP Method (A) or the ODT-101 (B)**

Data are presented as mean values±S.E. (\(n = 6\)). The equation was obtained by single regression. *\(p < 0.05\) is regarded as significant (Spearman’s test).

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\[ y = -0.40x + 4.51 \]

\[ r^2 = 0.72 \]

\[ y = -0.45x + 4.53 \]

\[ r^2 = 0.63^* \]

\(\text{Bitterness intensity score (F)}\)

\(\text{Disintegration time (sec)}\)

\(\text{Fig. 3A} \) (20°C) and \(\text{Fig. 3B} \) (37°C), the bitterness intensities of tablets A, E and J (original), were under 2.0 when the tablets were held in the mouth with water maintained at 20°C for 30 s, indicating low bitterness (Fig. 1A), while the bitterness intensities of tablets C, F, and I were over 3.0 when the tablets were held in the mouth with water maintained at 37°C for 30 s, indicating strong bitterness (Fig. 1B), significantly higher than that of tablet J (the original) (\(F_{[9,110]} = 7.164, p < 0.01\)).
were greater than those at 20°C.

The relationship between the bitterness intensity in humans (y value as τ), and the dissolved drug concentration (mM) at 30 s, measured using the brief conventional dissolution test (x value) at 20°C, could be represented as follows: $y = 0.91x + 1.61$ ($r^2 = 0.82, \quad rs = 0.80$). The corresponding equation at 37°C was: $y = 1.14x + 1.63$ ($r^2 = 0.90, \quad rs = 0.91$). The difference in the slope of the equation, indicates the possibility of a change of threshold depending on the temperature. When human take the ambroxol tablets with hot water, the threshold of bitterness was decreased.

The Evaluation of Bitterness of 10 Commercial Ambroxol Formulations in Human Gustatory Sensation Tests: Effect of Temperature

Standard Solutions of Quinine Hydrochloride and Ambroxol Hydrochloride: The relation between the bitterness intensity and the dissolved standard ambroxol hydrochloride or quinine hydrochloride concentrations (0.01, 0.03, 0.10, 0.30, 1.0 mM) was studied at 4, 20 and 37°C. The regression data for quinine hydrochloride (control) and ambroxol hydrochloride are shown in Figs. 4A and B, respectively. With quinine, the bitterness intensity at 4°C was a little less than at 20 and 37°C (Fig. 4A), but there was no difference between the bitterness intensities at 20 and 37°C. The obtained bitterness intensity of 0.1 mM standard quinine hydrochloride at 20°C was 1.29±0.48 as shown in Fig. 4A, even though the bitterness intensity score of 0.1 mM standard quinine hydrochloride was defined to be 2.0. The inconsistent between theoretical and obtained score seemed to be caused by variation among panels.

In general, lower temperatures tend to be associated with slightly higher bitterness thresholds. However, at concentrations over 1.0 mM, the bitterness intensities of ambroxol hydrochloride solution increased with increasing temperature (Fig. 4B). When calculated by single regression analysis using ambroxol hydrochloride solution data at three different temperatures (4, 20, 37°C), the slopes were 1.39, 2.16, and 2.52, respectively. Therefore, the bitterness intensity of ambroxol hydrochloride solution increases as the temperature
rises, unlike that of quinine hydrochloride. Interactions of concentration and temperature in quinine solution or ambroxol solution were determined by two tailed two-way repeated measures analysis of variance (ANOVA). The significant interaction of concentration and temperature in quinine solution was not obtained ($p=0.963>0.05$). While significant interaction of concentration and temperature in ambroxol solution was obtained ($p<0.01$). However, the reason for the differences between quinine and ambroxol solution in interaction of concentration and temperature was not clear. The additional study will need to clarify this issue.

Effect of Temperature on Prediction of Bitterness Intensity Scores of 10 Ambroxol Tablets: Prediction formula in bitterness intensity of ambroxol was derived from multiple regression analysis using three items, namely, concentration, temperature, and their interaction. To avoid multicollinearity due to correlation between main effect items (concentration and temperature) and their interaction, explanatory variables were converted to centration value (average in variables in each item was converted to be 0). The equation was calculated as follows: $Y$ (as bitterness intensity ($r$)) = $2.1629 + 1.4022 \times ($centration value of ambroxol concentration (mM)) + 0.01154 \times ($centration value of temperature (K)) $- 0.0005 \times ($centration value of ambroxol concentration (mM)) \times ($centration value of temperature (K)) $r^2=0.8393, F_{[3,50]}=97.5272, p<0.01$).

Partial regression coefficient of centration value of ambroxol concentration (mM) was 1.4022 ($n=60$, average=0, S.D.=0.436, 95% confidence interval: 1.2260–1.5783, $p<0.01$). Partial regression coefficient of centration value of temperature (K) was 0.01154 ($n=60$, average=0, S.D.=8.572, 95% confidence interval: 0.0025–0.0204, $p<0.05$). Partial regression coefficient of centration value of ambroxol concentration (mM) $\times$ (centration value of temperature (K)) was $-0.0005$ ($n=60$, average=0.801, S.D.=3.613, 95% confidence interval: $-0.0210–0.0202$, $p=0.9619>0.05$). From these results, ambroxol concentration and temperature were suggested to affect strongly the bitterness intensity of ambroxol.

In Fig. 5, the bitterness values obtained in human gustatory testing of the 10 tablets at 20 and 37°C were plotted on the y-axis while the corresponding values predicted from

The probability surface as a function of temperature and the ambroxol concentration obtained using the brief conventional dissolution test, were plotted on the x-axis. The bitterness intensities obtained for the ambroxol tablets on human testing were in good agreement with the predicted values taken from the prediction formula as a function of temperature and the ambroxol concentration obtained using the brief conventional dissolution test at 20 and 37°C.

The data for most of the tablets were located near the $y=x$ line ($y=x-10^{-14}, r^2=0.84, rs=0.97, q^2=0.84, p<0.01$), but for tablets A and E, there were comparatively large discrepancies between the values of x and y. This may be due to differences between the concentrations of ambroxol dissolved in conventional dissolution medium and in saliva.

In conclusion, we were able to predict the bitterness intensities of 10 ambroxol tablets in water at 20 and 37°C, by putting the temperature and ambroxol concentration obtained in a conventional dissolution test, into an equation prediction formula derived from multiple regression analysis. Slow disintegration seems to be the main explanation for formulations with low bitterness scores (products A and E), although product J, the original formulation, was found to disintegrate relatively quickly while having a low bitterness score.

**Conclusion**

1. The bitterness intensities of 10 commercial ambroxol formulations in human volunteers varied between products; the temperature of the water with which the tablets were taken was also a critical factor.

2. The bitterness scores of the 10 products in human gustatory sensation testing were significantly correlated with the disintegration time, evaluated using the ODT-101, a recently developed apparatus, and the drug concentration in dissolved medium, evaluated using a conventional brief dissolution test.

3. The bitterness threshold of ambroxol solutions increased when the temperature of the water with which the tablets were taken was increased from 20 to 37°C.

4. Finally, we were able to predict the bitterness of each tablet formulation by putting the temperature and the ambroxol concentration obtained using a conventional brief dissolution test, into a predictive formula derived in this study.

This method may be capable of predicting the bitterness of many commercial tablets which disintegrate in the oral cavity and thereby give rise to bitterness. We will be evaluating the palatability of different products in a forthcoming study.

**References**


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