In Vitro Dissolution Tests Corresponding to the in Vivo Dissolution of Clarithromycin Tablets in the Stomach and Intestine

Kuniaki Ishi, Yoko Katayama, Shigeru Itai, Yuji Ito, and Hidehumi Hayashi

Department of Pharmaceutics, Research Center, Taisho Pharmaceutical Co., Ltd., No. 403, Yoshino-cho 1-chome, Ohmiya-shi, Saitama 330, Japan. Received March 15, 1995; accepted July 21, 1995

The correlation between in vivo and in vitro dissolution of clarithromycin (CAM) tablets was examined. In vitro dissolution rate constants in the stomach and the intestine were obtained from analysis of the urinary excretion data of CAM following oral administration to humans in the fasting or postprandial state using a pharmacokinetic model including gastrointestinal transit.

In the present study, the flow-through cell method with moderate agitation was used, as the in vitro dissolution test related to the in vivo dissolution rate constants. Both the effects of pH of the dissolution medium and the volumetric solvent flow rate on the dissolution rate in the flow-through cell method were examined. The pH of the dissolution medium and the flow rate were related to the in vitro dissolution rate. Therefore, the conditions of the flow-through cell method in correlation with the in vitro dissolution rates in the stomach and intestine were determined by controlling the flow rate at pH 3.0 and 6.8 dissolution medium. The urinary excretion of CAM, simulated by substituting the in vitro dissolution rate constants into the equation, were consistent with the in vivo data. The in vitro tests corresponding to the in vivo dissolution in the stomach and intestine following a single oral administration in the fasting or postprandial state for a CAM tablet were established.

Key words in vitro— in vivo correlation; flow-through cell method; dissolution rate; clarithromycin tablet; pharmacokinetic model

In the development of oral solid dosage forms, if the rate of dissolution of a drug formulation is the limiting factor in drug absorption, dissolution testing is useful for predicting drug absorption. Therefore, numerous attempts have been made to determine the correlation between in vitro release and in vivo performance. Four levels of in vitro— in vivo correlation have been defined by the USP subcommittee on biopharmaceutics. The highest level of correlation is a 1:1 relationship between in vitro and in vivo dissolution. In vitro dissolution profiles can be obtained by the Wagner—Nelson method or the Roo—Rieglman method (model dependent method) and by the direct mathematical deconvolution method (model independent method).

In general, however, it is not easy to obtain in vitro dissolution profiles correlated with in vivo dissolution profiles, since physiological conditions such as gastric emptying and the pH profile in the gastrointestinal tract are individually specific. In particular, drug release from formulations containing drugs with pH-dependent solubility is significantly affected by pH in the gastrointestinal tract. Therefore, in order to adapt dissolution test parameters to physiological conditions, methods of dissolution with changes in pH during testing have been developed.

However, these dissolution methods are more complicated than conventional methods such as the paddle method. Moreover, in these dissolution methods, the effect of gastrointestinal transit rate on absorption cannot be taken into consideration.

In the present study, the in vivo dissolution rate constants in the stomach and intestine were obtained from the urinary excretion of clarithromycin (CAM) following oral administration to humans in the fasting or postprandial state using the pharmacokinetic model (Chart 1) described in our previous paper. We then attempted to estimate the in vivo behavior of drugs, including gastrointestinal transit, using these parameters.

Furthermore, we examined the in vitro dissolution tests corresponding to the in vivo dissolution rates in the stomach or intestine separately. When the dissolution test for CAM tablets using the paddle method with conventional conditions was carried out, the in vitro dissolution rate was found to be higher than the in vivo dissolution rate. In the present study, therefore, the flow-through cell method with moderate agitation was used as the in vitro dissolution test.

Materials and Methods

Materials CAM and tablets containing 200 mg (potency of CAM were supplied by Taisho Pharmaceutical Co., Ltd. All other chemicals were used of reagent grade.

In Vivo Data and Pharmacokinetic Analysis The urinary excretion data for CAM after a single oral administration to humans reported by Sowa et al. were used.

The equation for cumulative urinary excretion was derived from the pharmacokinetic model and was assumed to proceed with first-order kinetics for the entire process.

Cumulative urinary excretion (E) can be expressed by the following equation in terms of time (t):

\[ E = \int_0^t K_t \cdot F_t \left( \frac{a - e^{-K_t \cdot t} + \beta \cdot K_t \cdot e^{-K_t \cdot t} - \gamma \cdot K_t \cdot e^{-K_t \cdot t} + K_1 \cdot K_2 \cdot K_4 \cdot e^{-K_t \cdot t}}{K_1 \cdot K_2} \right) \]

where

\[ a = \frac{k_{g2} \cdot k_8 + k_{g1} \cdot (K_1 - k_0)}{(K_1 - k_0) \cdot (K_2 - k_0)} \]

\[ \beta = \frac{k_{g2} \cdot k_8 - k_{g2} \cdot (K_2 - k_1)}{(K_2 - k_1) \cdot (K_2 - k_0)} \]

\[ \gamma = \frac{k_{g2} \cdot k_8}{(K_2 - k_1) \cdot (K_1 - k_0)} \]

A_0 and F_t represent the dose and fraction of drug absorbed, respectively. k_2, k_21, k_2, and k_20 represent the rate constants for

© 1995 Pharmaceutical Society of Japan

* To whom correspondence should be addressed.
gastrointestinal transit rate, dissolution in stomach, dissolution in intestine, exclusion, and elimination, respectively, and \( K_1 \) and \( K_2 \) are defined as follows:

\[
K_1 = (k_{d1} + k_a)
\]

\[
K_2 = (k_{d2} + k_g)
\]

Pharmacokinetic parameters of Eq. 1 were calculated by simultaneously fitting the individual urinary excretion data for CAM after oral administration in the fasting and postprandial states with MULTIT10 using nonlinear least-squares regression analysis.

**In Vitro Dissolution Test** The dissolution test was carried out using Dissoltest-100 (Toyamasaengyo, Osaka, Japan) as the flow-through cell apparatus. The flow-through cell method was used in accordance with Supplement 1 in the Pharmacopeia of Japan, Twelfth Edition. Into a 22.6 mm inner diameter cell, one was placed glass bead 5 mm in diameter and 1 g of glass beads 1 mm in diameter, along with one tablet on a holder. After an assembly of two filters was attached, the dissolution medium, warmed at \( 37^\circ \text{C} \), was introduced through the bottom of the cell by using a piston pump. Britton-Robinson buffer solution with a pH range of 3.0 to 7.8 was used as the dissolution medium. At each dissolution test, there were used five tablets for each of the dissolution medium. A single dissolution medium was prepared in each experiment using dissolution media of pH 3.0, 5.0, 6.0, 6.5, 6.8, 7.2 and 7.8 at a 10 ml/min flow rate for studying the effect of pH on the dissolution. The change of flow rates on dissolution was tested with the flow rates of 0.5, 1.5, 3 and 5 ml/min at pH 3.0 of the dissolution medium, and with the flow rates of 1.5, 5, 10, and 15 ml/min at pH 6.8 of the dissolution medium. During each experiment, the dissolution medium was pumped through a cell, and the eluate was collected in separate fractions during different time periods, i.e., 0–5, 5–10, 10–15, 15–20, 20–30, 30–45, 45–60, 60–90, and 90–120 min. The eluate was filtered through a membrane filter with a 0.45 μm pore size.

Then, the amount of CAM released into the dissolution medium was quantitatively determined by HPLC with the following operating conditions: ultraviolet absorption photometer detector: wavelength, 210 nm; column: 4.6 mm i.d. × 15 cm stainless-steel column packed with ODS-80TM (Tosoh): column temperature, \( 50^\circ \text{C} \); mobile phase: a mixture of 1/15 M monobasic potassium phosphate and acetonitrile (13:7); and a flow rate of 1 ml/min.

**Determination of in Vitro Dissolution Rate Constant** The in vitro dissolution rate constants with first-order kinetics were calculated by fitting each dissolution value for a CAM tablet using MULTIT10, the nonlinear least-squares regression analysis.

**Results and Discussion**

Figure 1 shows the urinary excretion data for CAM obtained after a single oral administration of 200 mg CAM tablets to healthy volunteers in the fasting or postprandial state, as reported by Suwa et al.9) The fitted lines were obtained by nonlinear least-squares regression analysis. The pharmacokinetic parameters obtained are listed in Table 1. The parameters obtained from the serum concentration data were nearly equal to that obtained from the urinary excretion data.

For a single oral administration in the fasting state, the in vivo dissolution rate of CAM in the stomach was higher than that in the intestine. Therefore, we attempted to characterize the flow-through cell methods corresponding to the stomach and intestine separately.

On the other hand, following postprandial administration, the in vivo dissolution rate in the stomach was nearly equal to that in the intestine. Consequently, for postprandial administration, we used the same condition for flow-through cell method corresponding to the stomach and the intestine. For CAM tablets, the dissolution rate was determined by the rate-determining step, since the rate of disintegration is much larger than that of dissolution. Therefore, although in the pharmacokinetic model (Chart 1) solid drug in the intestine compartment is assumed to be dissolved from granules gastric-emptied following first order kinetic, the dissolution test for the intestine was carried out by the whole tablet. In order to characterize the flow-through cell method, the effects of the pH of the dissolution medium and the volumetric solvent flow rate on dissolution rate for CAM were examined. These correlations may be useful for adapting the in vitro dissolution rate to the in vivo dissolution rate.

**Relationship between pH of Dissolution Medium and in Vitro Dissolution Rate Constant (Kd) 10** To clarify the effect of pH of the dissolution medium on the in vitro dissolution rate in the flow-through cell method for CAM, we tested the pH range of 3.0 to 7.8 at 10 ml/min flow rate. As the pH value increased, the in vitro dissolution rate decreased, as shown in Fig. 2.

The in vitro dissolution rate constants with first-order kinetics, \( K_{d,\text{in}} \), were calculated by fitting the in vitro dissolution profiles for each dissolution medium pH. As can be seen in Fig. 3, the plot of \( \log(K_{d,\text{in}}) \) against each pH of dissolution medium yielded a straight line with the following equation:

\[
\log(Y) = -0.588 \cdot X + 3.918 \quad r = 0.996
\]

Fig. 1. Cumulative Urinary Excretion of CAM Following Oral Administration to Healthy Volunteers in Fasting or Postprandial State

Values are expressed as means ± S.E. for 8 volunteers. The solid lines were obtained by non-linear least-squares regression analysis.

**Table 1. Effect of Food on Pharmacokinetic Parameters for a Single Oral Administration of CAM (200 mg) to Healthy Volunteers**

<table>
<thead>
<tr>
<th>State</th>
<th>( k_{d1} ) (h⁻¹)</th>
<th>( k_{d2} ) (h⁻¹)</th>
<th>( k_{g} ) (h⁻¹)</th>
<th>( k_{a0} ) (h⁻¹)</th>
<th>( t_{d} ) (h)</th>
<th>( F ) (%)</th>
<th>( k_{11} ) (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>1.873 ± 1.412</td>
<td>0.254 ± 0.208</td>
<td>4.499 ± 6.476</td>
<td>0.047 ± 0.081</td>
<td>0.260 ± 0.492</td>
<td>40.2 ± 8.9</td>
<td>0.305 ± 0.070</td>
</tr>
<tr>
<td>Postprandial</td>
<td>0.407 ± 0.482</td>
<td>0.464 ± 0.503</td>
<td>0.449 ± 0.534</td>
<td>0.089 ± 0.124</td>
<td>0.799 ± 0.641</td>
<td>36.2 ± 5.9</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. of 8 volunteers.
where $C$ is the concentration of solute at time $t$, $C_s$ is the equilibrium solubility of the solute at the experimental temperature, $K$ is a constant with dimension length/time, $S$ is the surface area of solute available for dissolution, and $V$ is the volume of the dissolution medium. In general, since sink conditions are maintained in the flow-through cell method, the dissolution rate can be considered to depend on drug solubility. Thus, the following correlation can be obtained:

$$K_d_{vitra} \propto C_s$$  \hspace{1cm} (9)

And the correlation can be rewritten as follows:

$$\log(K_d_{vitra}) \propto \log(C_s)$$  \hspace{1cm} (10)

In addition, the relationship between pH and solubility of a weak base drug can be explained using Henderson-Hasselbach's equation.

$$\text{pH} = pK_a + \log\left(\frac{C_i}{C_b}\right)$$  \hspace{1cm} (11)

where $pK_a$ is the dissociation constant, $C_i$ is the solubility of the dissociated molecule, and $C_b$ is the solubility of the undissociated molecule. In the case of CAM, in which a weak base drug which exhibits $pK_a 8.76$, $C_s$ is nearly equal to $C_b$, since $C_s$ is much larger than $C_b$ below pH 7.8. Therefore, Eq. 11 is given by:

$$\log(C_i) = -pH + pK_a + \log(C_b)$$  \hspace{1cm} (12)

The following correlation can be obtained:

$$\log(C_i) \propto pH$$  \hspace{1cm} (13)

These correlations suggest that $\log(K_d_{vitra})$ may be proportional to pH.

**Relationship between $K_d_{vitra}$ and $C_s$** The solubilities of CAM at each pH were calculated by using the $pK_a$ and $C_b$ of CAM reported by Nakagawa et al.\textsuperscript{31} An approximately linear relationship was found between the logarithms of the solubility for CAM calculated and the logarithms of the in vitro dissolution rate constant as shown in Fig. 4. The linear regression of these data produces Eq. 14.

$$\log(Y) = 1.533 \cdot \log(X) + 2.890 \hspace{1cm} r = 0.995$$  \hspace{1cm} (14)

![Chart 1. A Simplified Version of the Pharmacokinetic Model Including in Vitro Dissolution and Gastrointestinal Transit Parameters](chart)

$A$, and $B$ represent the amounts of solid drug in the stomach and undissolved drug in the intestine. $C_1$, $C_2$, and $C$ represent the amounts of drug dissolved in the stomach and absorbed in the intestine, drug dissolved in the intestine and absorbed in the intestine, and the total amount of drug absorbed in the gastrointestinal compartment, respectively. $D$ and $E$ represent the amounts of undissolved drug and of urinary excretion of drug.

![Fig. 2. The Effect of pH of Dissolution Medium on the in Vitro Dissolution Rate](graph1)

**Key:** ¥, pH 3.0; ●, pH 5.0; ▲, pH 6.0; ■, pH 6.5; ○, pH 6.8 △, pH 7.2; □, pH 7.8.

![Fig. 3. Relationship between pH of Dissolution Medium and in Vitro Dissolution Rate Constant (Kd vitra)](graph2)

This finding indicates that logism of the in vitro dissolution rate is proportional to the dissolution medium pH. This appears to be due to the pH-dependent solubility of CAM.

Wagner\textsuperscript{11} reported that under sink conditions, the dissolution rate could be represented as

$$\frac{dC}{dt} = \frac{K S}{V} C_s$$  \hspace{1cm} (8)

![Fig. 4. Relationship between Solubility and Kd vitra](graph3)
From Eq. 10, derived from Eq. 8 reported by Wagner, Eq. 14 can be explained.

Relationship between $K_d$ and Flow Rate  To confirm the effect of volumetric solvent flow rate on the dissolution rate in the flow-through cell method, we changed the flow rate from 0.5 to 5 ml/min at pH 3.0 and from 1.5 to 15 ml/min at pH 6.8. As the flow rate increased, the in vitro dissolution rate also increased, as shown in Fig. 5. $K_d$ was calculated by fitting the in vitro dissolution profiles at each flow rate. As can be seen in Fig. 6, the plot of $K_d$ against the flow rate yielded a straight line with the following equations:

$$Y = 2.2292X + 0.0217 \quad r = 0.995 \quad \text{at pH 3.0} \quad (15)$$

$$Y = 0.0917X - 0.0087 \quad r = 1.000 \quad \text{at pH 6.8} \quad (16)$$

These findings indicate that the in vitro dissolution rate is proportional to the flow rate.

In the flow-through cell method, the dissolution rate can be determined using the following equation $^{13}$:

$$\frac{dm}{dt} = C_e Q \quad (17)$$

where $m$ is the amount of drug dissolved, $C_e$ is the concentration during time $t$, and $Q$ is the flow rate of the dissolution medium. This relationship also shows that the in vitro dissolution rate is proportional to flow rate.

Determination of Conditions for the Flow-Through Cell Method Corresponding to in Vivo Data  The above findings proved that the in vitro dissolution rate is related to both the pH of dissolution medium and flow rate. Therefore, the in vitro dissolution rate can be controlled by the pH of the dissolution medium and flow rate. In the present study, the conditions under which the flow-through cell method would correlate with the in vivo dissolution rate were determined by controlling the flow rate. In the fasting state, the dissolution medium of pH 3.0 for the stomach and pH 6.8 for the intestine were used. The gastric pH of human is 1 to 3.5. $^{14}$ Though macrolide antibiotics were inactivated by gastric acid, CAM is relatively stable in acidic solution. $^{12}$ In particular, CAM is stable above pH 3. Moreover, the 5-O-desosaminyl-6-O-methlyerythronolide, the degradation product by acid, was only slightly detected in the urinary excretion of CAM following oral administration. $^{9}$ Therefore, the pH of the stomach can be estimated to be about pH 3. And in general, since the
Fig. 7. Relationship between in Vivo Dissolution and in Vitro Dissolution for Oral Administration in Fasting State

Key: a, simulated in vivo dissolution in stomach; b, simulated in vivo dissolution in intestine; ■, in vitro dissolution data for stomach; ○, in vitro dissolution data for intestine.

Fig. 8. Correlation between Fitting Curve Obtained from in Vivo Data and Predicted Curve Obtained from in Vitro Dissolution for Cumulative Urinary Excretion of CAM Following Oral Administration in Fasting State

The dotted line was obtained by fitting the cumulative urinary excretion data of CAM following oral administration in fasting state. The solid line was predicted from in vitro dissolution profiles for the stomach and intestine.

Fig. 9. Relationship between in Vivo Dissolution and in Vitro Dissolution after a Meal

Key: a, simulated in vivo dissolution in stomach; b, simulated in vivo dissolution in intestine; ■, in vitro dissolution data in stomach and intestine.

Fig. 10. Correlation between Fitting Curve Obtained from in Vivo Data and Predicted Curve Obtained from in Vitro Dissolution for Cumulative Urinary Excretion of CAM Following Oral Administration after a Meal

The dotted line was obtained by fitting the cumulative urinary excretion data of CAM following oral administration after a meal. The solid line was predicted from the in vitro dissolution profile.

pH in the intestine of human is 5 to 7.146 a pH of 6.8 for the intestine was selected. In contrast, the same dissolution medium of pH 6.8 for the stomach and the intestine in the postprandial state were used, since the pH in the stomach may be increased by food.155

Since the in vivo dissolution rate constant in the stomach in the fasting state was 1.873 h⁻¹, as shown in Table 1, the flow rate at pH 3.0 of the dissolution medium was 0.8 ml/min, from Eq. 15. It seems reasonable to assume that the flow rate is 0.8 ml/min, since the gastric secretion rate in the fasting state is 0.08—3 ml/min.146 Similarly, since the in vivo dissolution rate in the intestine in the fasting state was 0.254 h⁻¹, the flow rate at pH 6.8 of the dissolution medium was 2.9 ml/min, from Eq. 16. For a single postprandial oral administration, since the in vivo dissolution rate in the stomach is nearly equal to that in the intestine, the average of the values of the in vivo dissolution in the stomach and intestine was used, and the flow rate at pH 6.8 of dissolution medium was found to be 4.9 ml/min using Eq. 16.

Also, it can be assumed that these flow rates in the test are reasonable, because the flow rates of the intestinal contents in the fasting and fed state are 0.33—1.8 ml/min, 3.0—8.3 ml/min, respectively.177

In Vitro—in Vivo Correlation for Dissolution of a CAM Tablet In order to correspond to the in vivo dissolution of a CAM tablet in the stomach in the fasting state, the flow-through cell method was used with a flow rate of 0.8 ml/min at pH 3.0 of the dissolution medium. The in vitro dissolution data were consistent with the in vivo dissolution profile, as shown in Fig. 7.

Similarly, the conditions for the intestine in the fasting state were used with a flow rate of 2.9 ml/min and a dissolution medium of pH 6.8. The in vitro dissolution data were also consistent with the in vivo dissolution profile, as again shown in Fig. 7.

Figure 8 shows the urinary excretion of CAM after a single oral administration in the fasting state simulated by substituting the in vitro dissolution rate constants
obtained into the equation for cumulative urinary excretion.

For postprandial administration, the same conditions of the flow-through cell method were used with dissolution medium of pH 6.8 at a flow rate of 4.9 ml/min. The dissolution data obtained were consistent with the \textit{in vivo} dissolution profiles, as shown in Fig. 9. Figure 10 shows the urinary excretion of CAM following a single postprandial oral administration simulated by substituting the \textit{in vitro} dissolution rate constants into Eq. 1. In the case of a single postprandial oral administration, the line obtained was also consistent with the \textit{in vivo} data.

\textbf{Conclusion}

\textit{In vitro} dissolution tests corresponding to \textit{in vivo} dissolution in the stomach and intestine following a single oral administration in the fasting or postprandial state for CAM tablet were established.

Little difference was found between \textit{in vitro} and \textit{in vivo} dissolution, and a good \textit{in vitro–in vivo} correlation was obtained. The values of urinary excretion of CAM predicted by substituting the \textit{in vitro} dissolution rate constants and the \textit{in vivo} gastrointestinal transit rate into the pharmacokinetic model fit the actual experimental data well. In the case of different subjects, the prediction obtained from the \textit{in vitro} dissolution may deviate a little from the \textit{in vivo} urinary excretion. However, this method is useful for the study of formulation, quality control and minor changes in formulation, since the \textit{in vitro} dissolution corresponding to the urinary excretion in the biobatch can be obtained. And, for oral solid dosage forms containing CAM, this method is useful for predicting \textit{in vivo} performance.

\textbf{References}


