Influence of Food on Rifampicin Pharmacokinetics in Rats

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**Regular Article**

Rifampicin (RFP; 30mg/kg) was orally administered to fasted or fed rats using ultrapure water as the vehicle, and the influence of food on its pharmacokinetics was investigated. To examine the influence of intragastric pH and RFP solubility, similar experiments were performed using 0.1s HCl (pH 1.0), 0.1s phosphate buffer (pH 6.8), or 10% Tween 80 vehicles. Plasma RFP concentrations were measured by HPLC-UV for 24h. The administration of RFP to fed rats in ultrapure water (10% dissolved) resulted in a significant 40% reduction in the maximum plasma drug concentration (C_{max}) and area under the concentration–time curve (AUC_{0-24}), as compared with fasted rats (p<0.05). RFP administration in 0.1s phosphate buffer (10% dissolved) produced approximately 25% lower C_{max} and AUC_{0-24} values, as compared with those achieved by RFP in ultrapure water in fasted rats. The administration of RFP in 0.1s HCl (100% dissolved) to fasted rats increased the AUC_{0-24} by approximately 1.8-fold, compared with ultrapure water, suggesting that increasing RFP solubility increased its absorption. The 10% Tween 80 vehicles (60% dissolved) enhanced the absorption of RFP to a similar level as observed when using 0.1s HCl solution, suggesting that both the improvement in solubility and P-glycoprotein inhibition by Tween 80 increased the absorption. This study suggested that RFP solubility in gastrointestinal fluid may be an important determinant of absorption and that it would be beneficial to change the timing of RFP administration to patients with insufficient clinical outcomes by administration after a meal.

**Key words** rifampicin; pH; solubility; plasma concentration; food; pharmacokinetics

The standard treatment for tuberculosis consists of rifampicin (RFP), isoniazid, and pyrazinamide, plus either ethambutol or streptomycin. RFP is one of the first-line drugs for tuberculosis, and administration is recommended before breakfast because the absorption of RFP is decreased by food. Low serum RFP concentration may cause therapy failure in tuberculosis patients. In patients with insufficient clinical outcomes, its effects were reported to improve when the dose was increased, because this increased the serum RFP concentration. Therefore, administration of RFP after a meal can reduce its serum concentration and compromise its efficacy. However, there is an opposite view that maintains that administration of RFP after a meal does not influence efficacy because it does not significantly reduce the maximum plasma drug concentration (C_{max}) even though the time to C_{max} (T_{max}) is delayed. In practice, anti-tuberculosis drugs are generally administered together after a meal in order to prevent gastrointestinal dysfunction, to facilitate Directly Observed Treatment-Short course, and to optimize patient adherence. Although previous studies have indicated that an elevated gastric pH or an increase in gastric emptying time after a meal can influence RFP absorption, the details are unclear.

In the present study, we orally administered RFP suspensions to rats under fasting or fed conditions, and investigated the influence of food on RFP pharmacokinetics by measuring plasma RFP concentrations for 24h. We also examined the effect of gastric pH by administering RFP in 0.1M HCl solution (pH 1.0) or 0.1M phosphate buffer (pH 6.8). Furthermore, we used 10% Tween 80 to improve the solubility of RFP.

**MATERIALS AND METHODS**

**Chemicals** RFP (for biochemistry, lot no. DCJ4885) and 25-desacetyl RFP (DR; lot no. 3-SBT-85-2), a major active metabolite of RFP, were purchased from Wako Pure Chemical Industries, Ltd., while flufenamic acid (lot no. STBC740V), which served as an internal standard for analysis, was purchased from Sigma-Aldrich. All other chemicals were of special grade. RACOL®-NF Liquid for Enteral Use (RACOL®; 200mL), for the diet of rats, and EASYGEL®, the food for making various enteral nutrients into jelly, were purchased from Otsuka Pharmaceutical Co., Ltd.

**Animals** Male Wistar rats (age, 11 weeks; weight, 230–260g) were purchased from Sankyo Labo Service Co. They were housed under standard conditions (23±1°C, 55±5% relative humidity) and maintained under a 12-h light/dark cycle with free access to diet and water for at least 5d before use. This study was conducted in compliance with the ethical guidelines for animal studies devised by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Study protocols were approved by the Institutional Animal Care and Use Committee of Tokyo University of Science (approval nos. Y12001, Y13009 and Y14001).

**Administration of RFP and Blood Collection**

In Vivo Oral RFP Administration to Fasting and Fed Rats

All rats were fasted overnight and had an indwelling polyethylene cannula (SP31, inner diameter 0.5mm, outer diameter 0.8mm; Natsume Seisakusho Co., Ltd.) implanted in the femoral artery for blood sampling. Implantation surgery was performed under isoflurane anesthesia. After the effects of the anesthesia had disappeared, the rats in the fasting condition group (n=3) were orally administered RFP (30mg/2mL/kg; 15mg RFP/mL) in ultrapure water. Approximately 0.3mL of blood was collected into a heparinized tube from the indwelling cannula prior to RFP administration (0h), and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24h after administration. Plasma samples (100µL) were obtained from the upper layer after centrifuging blood at 1800×g at 4°C for 10min, 10µL of 5% ascorbic
acid solution was added as an antioxidant, and the samples were stored at ~30°C until analysis. For rats in the fed condition group (n=3), a meal was prepared using EASYGEL® and RACOL® liquid (100 mL) was mixed with 22 g EASYGEL® liquid 1 for 20–30 s; 5 g of liquid 2 was added and mixed again for 20–30 s, and allowed to gel at room temperature for 30 min. The gel was administered to rats that had been fasted for 24 h (2.5 mL/kg). Ten minutes later, the rats were orally administered RFP (30 mg/kg) and blood samples were collected as described above.

In Vivo Oral RFP Administration Using Different Solvents

To examine the influence of solvent pH on RFP pharmacokinetics in fasted rats, 0.1 M HCl solution (pH 1.0) and 0.1 M phosphate buffer (pH 6.8) were prepared. To improve RFP solubility, 10% Tween 80 solution was used, and the pharmacokinetics of RFP were compared in fasting and fed rats. RFP is difficult to dissolve in water and it was therefore either dissolved or homogeneously suspended by sonication on ice in each solvent immediately prior to oral administration. The doses of RFP employed and the experimental methods were as described above. After measuring the pH, each prepared solution or suspension was centrifuged, the RFP level in the supernatant was measured, and this was used to determine the amount (%) of dissolved RFP in each solution or suspension.

Measurements of the Plasma RFP and DR Concentration

The plasma concentrations of RFP and DR were measured using the HPLC-UV method described by Shibata et al. and Ishii and Ogata, with modifications. Plasma samples (100 µL) were mixed with 200 µL of 10 µg/mL flufenamic acid solution in methanol (internal standard); 50 µL of methanol was added to precipitate protein. After centrifugation at 19000×g for 3 min at 4°C, the supernatants were added to 0.5 M phosphate buffer, adjusted to pH 7.2, and extracted with 1 mL ethyl acetate. The organic solvent was evaporated and the residue was reconstituted in 50 µL HPLC mobile phase. A 20-µL aliquot of this solution was injected into the HPLC. The HPLC system (HITACHI Ltd.) consisted of an L-2130 pump and L-2455 detector. The separations were performed on a reverse phase column, PEGASIL ODS SP100 (5 µm, 4.6×250 mm), monitored by UV absorbance at 340 nm, and operated at 1.0 mL/min using 40:60% acetonitrile:10 mM phosphate buffer (pH 7.2) as the mobile phase. The column temperature was maintained at 30°C. Five standards containing known amounts of RFP (final concentration: 2, 5, 10, 20 and 50 µg/mL) and DR (final concentration: 1, 2, 5 and 10 µg/mL) were added to 100 µL blank plasma. These standards were then carried through the sample preparation procedure described above and injected into the HPLC. RFP was also determined in each prepared solution or suspension using the same HPLC conditions employed for plasma RFP measurements. The calibration graphs were obtained by linear fitting of the peak-area ratios of RFP and DR to internal standard versus the concentrations of RFP and DR, T<sub>max</sub>, C<sub>max</sub> and the area under the curve from 0 to 24 h (AUC<sub>0–24</sub>) were obtained from concentration–time profile of plasma RFP. The absorption rate constant (k<sub>a</sub>), elimination rate constant (k<sub>e</sub>), and T<sub>max</sub> were used to compare RFP pharmacokinetics in fasting and fed rats.

Statistical Analysis

Each pharmacokinetics parameter was presented as the mean±standard deviation (S.D.). Student’s t-test was used to compare study groups. A value of p<0.05 was considered to be statistically significant.

RESULTS

Pharmacokinetics of Oral RFP in Rats

Pharmacokinetics of RFP in Ultrapure Water in Fasting and Fed Rats

Following administration to fasted rats in ultrapure water, the mean plasma concentration of RFP increased immediately, reached a C<sub>max</sub> of 24.5±3.9 µg/mL at 1.2 h, and the AUC<sub>0–24</sub> was 406.8±82.7 µg·h/mL (n=3). When administered to fed rats, the T<sub>max</sub> was 2.2±1.8 h, slightly delayed as compared with the fasted rats. Administration of RFP to fed rats resulted in significant decreases in the C<sub>max</sub> and AUC<sub>0–24</sub> to approximately 60% of the levels observed in fasted rats (p<0.05); C<sub>max</sub> was 14.9±4.4 µg/mL and the AUC<sub>0–24</sub> was 230.0±85.1 µg·h/mL. k<sub>a</sub>, k<sub>e</sub>, and the elimination half-life (T<sub>1/2</sub>) were not significantly different in the fed and fasted rats (Fig. 1, Table 1). The plasma concentrations of DR were below the detection limit (1 µg/mL) in all animals.

Pharmacokinetics of RFP in Different Solvents in Fasted Rats

Figure 2 shows the plasma concentration–time profiles for
24 h after oral RFP administration to fasted rats in different solvents. The pharmacokinetics parameters of RFP are summarized in Table 2. The administration of RFP using 0.1 M HCl solution (pH 1.5 after RFP addition) resulted in a slight increase in $C_{\text{max}}$ and significant increase in $AUC_{0-24}$ to approximately 1.2 times and 1.8 times, respectively, the values observed following RFP administration in ultrapure water ($p<0.05$); the mean values were 29.1±µg/mL for $C_{\text{max}}$ and 712.6±µg·h/mL for $AUC_{0-24}$. When administered using 0.1 M phosphate buffer (pH 6.8), the mean $C_{\text{max}}$ and $AUC_{0-24}$ values were 18.3±1.6µg/mL and 313.3±61.0µg·h/mL, respectively. Although these $C_{\text{max}}$ and $AUC_{0-24}$ values were approximately 1.2 times and 1.8 times, respectively, compared to the values observed in the RFP administration in ultrapure water, there was no significant difference in the value of $AUC_{0-24}$ ($p<0.05$). When administered using 10% Tween 80 solution (pH 3.5–4.0 after RFP addition), the mean $C_{\text{max}}$ (28.7±µg/mL) and $AUC_{0-24}$ (686.1±µg·h/mL) were similar to those achieved by administration using 0.1 M HCl solution. The plasma concentrations of DR were below the detection limit (1µg/mL) in all rats. The administration of RFP using 0.1 M HCl solution and 10% Tween 80 solution resulted in a significant decrease in $k_e$ compared to the value observed in the RFP administration in ultrapure water ($p<0.05$), whereas $k_e$ was not significantly different. The values of $T_{1/2}$ observed in the RFP administration in 0.1 M HCl solution and 10% Tween 80 were prolonged to 4.4 times and 3.2 times, respectively, compared to the values observed in the RFP administration in ultrapure water.

Pharmacokinetics of RFP Administered to Fed Rats in 10% Tween 80

When administered to fed rats using 10% Tween 80 solution, the mean RFP $C_{\text{max}}$ and $AUC_{0-24}$ were 36.8±µg/mL and 600±µg·h/mL, respectively. As compared to RFP administration to fasted rats in 10% Tween 80 (see above), there was no significant difference in the $AUC_{0-24}$, whereas the $C_{\text{max}}$ was significantly increased by approximately 1.3-fold ($p<0.05$). $k_e$, $k_i$, and $T_{1/2}$ were not significantly different in the fed and fasted rats (Table 2). The plasma concentrations of DR were below the detection limit (1µg/mL) in all rats.

The Amount (%) of Dissolved RFP in Each Solution or Suspension RFP concentrations in the supernatants of each solution or suspension, which were all prepared using 15 mg RFP/mL, were measured by HPLC-UV (Table 2). The amount (%) of dissolved RFP was approximately 100% in 0.1 M HCl and 60% in 10% Tween 80, but only 10% in ultrapure water and 0.1 M phosphate buffer (pH 6.8).

DISCUSSION

This study examined the usefulness of oral RFP administration before a meal and investigated the influence of gastric pH and dissolution state on RFP pharmacokinetics in rats.

First, we compared the pharmacokinetics of RFP under fasting conditions with those under fed conditions in rats. The administration of RFP after a meal resulted in significantly lower $C_{\text{max}}$ and $AUC_{0-24}$ values, which were approximately 60% of the levels observed in fasted rats (Fig. 1, Table 1). These results were consistent with many previous clinical studies of healthy adults or tuberculosis patients, which compared the pharmacokinetics of RFP after oral administration under fasting and fed conditions. Therefore, our results appeared relevant to clinical practice. The plasma concentration of DR, a major active metabolite of RFP, was lower than that of RFP (below the detection limit of 1µg/mL) in both fasted and fed rats. Thus, we could not clarify the time-course of the plasma concentration of DR.

Generally, drug solubility in solvents and the membrane permeability at absorption sites are factors considered to influence the absorption of drugs. The pH of vehicle and the excipients in formulations are important to the dissolution of the drugs. The lipophilicity of drugs and the expression of efflux transporter such as P-glycoprotein (Pgp), which hamper the uptake of drugs through the intestinal epithelial, play an important role in the membrane permeability. RFP is a good substrate for Pgp and permeates to a higher extent through the proximal part of small intestine in comparison with the

![Fig. 2. Time–Course of Plasma Rifampicin (RFP) Concentration in Fasted Rats Administered RFP in Ultrapure Water (○), 0.1 M HCl (■), 10% Tween 80 (△) or 0.1 M Phosphate Buffer (▲) (n=3, Mean±S.D.)](Image 55x231 to 283x407)

Table 2. Summary of pH after Preparation, Amount (%) of Dissolved RFP in the Solvent and Pharmacokinetic Parameters among Each Treatment

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Diet</th>
<th>pH after preparation</th>
<th>Amount (%) of dissolved RFP</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>$AUC_{0-24}$ (µg·h/mL)</th>
<th>$k_e$ (h⁻¹)</th>
<th>$k_i$ (h⁻¹)</th>
<th>$T_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrapure water</td>
<td>Fasting</td>
<td>5.0–6.0</td>
<td>9.5</td>
<td>1.2±0.8</td>
<td>24.5±3.9</td>
<td>406.8±82.7</td>
<td>0.709±0.007</td>
<td>0.070±0.019</td>
<td>10.3±2.5</td>
</tr>
<tr>
<td>0.1 M HCl</td>
<td>Fasting</td>
<td>1.5</td>
<td>100.2</td>
<td>8.0±4.0*</td>
<td>29.1±2.1</td>
<td>712.6±66.5</td>
<td>0.719±0.008</td>
<td>0.019±0.011*</td>
<td>45.6±26.5*</td>
</tr>
<tr>
<td>0.1 M phosphate buffer</td>
<td>Fasting</td>
<td>6.8</td>
<td>101.2</td>
<td>2.0±0.0</td>
<td>18.3±1.6*</td>
<td>313.4±61.0</td>
<td>0.710±0.003</td>
<td>0.044±0.020</td>
<td>18.3±9.2</td>
</tr>
<tr>
<td>10% Tween 80</td>
<td>Fasting</td>
<td>3.5–4.0</td>
<td>61.2</td>
<td>5.7±4.0</td>
<td>28.7±3.7</td>
<td>686.1±45.2</td>
<td>0.716±0.007</td>
<td>0.025±0.011*</td>
<td>32.7±18.6</td>
</tr>
<tr>
<td>10% Tween 80</td>
<td>Fed</td>
<td>3.5–4.0</td>
<td>61.2</td>
<td>3.0±1.7</td>
<td>36.8±5.2</td>
<td>600.8±79.3</td>
<td>0.737±0.033</td>
<td>0.050±0.043</td>
<td>58.2±83.0</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters are given as the mean±S.D., n=3; *p<0.05.
distal part.\textsuperscript{17} Although the expression level of Pgp is low in the proximal part of small intestine,\textsuperscript{28,29} the absorption of RFP may be influenced by Pgp to some extent. On the other hand, it is known that an organic anion transporter is also involved in the transportation of RFP,\textsuperscript{20} but the details concerning the contribution to the RFP absorption from gastrointestinal tract are unclear.

In this study, the meal-related decreases in $C_{\text{max}}$ and $AUC_{0-24}$ appeared to reflect decreased absorption of RFP from the gastrointestinal tract and we therefore conducted an \textit{in vivo} study of oral RFP administration using various kinds of solvents. The administration of RFP using 0.1 M HCl solution (pH 1.5 after RFP addition) resulted in remarkably higher $C_{\text{max}}$ and $AUC_{0-24}$ values, as compared with ultrapure water, whereas the use of 0.1 M phosphate buffer (pH 6.8) decreased these values (Fig. 2, Table 2). The solubility of RFP in water has been reported to increase in low pH areas and decrease in neutral pH areas.\textsuperscript{\textsuperscript{21}} In the present study, approximately 100% of RFP dissolved in 0.1 M HCl (pH 1.5 after RFP addition), but only 10% dissolved in ultrapure water or 0.1 M phosphate buffer (pH 6.8). Therefore, these results suggest that the reduced solubility of RFP caused by a meal-induced shift of gastrointestinal tract pH from acidic to neutral reduces the plasma RFP concentration. Although RFP did not completely dissolve (approximately 60% dissolved) in 10% Tween 80 solution (pH 3.5–4.0 after RFP addition), the resulting $C_{\text{max}}$ and $AUC_{0-24}$ were increased, as compared with ultrapure water, and were similar to those observed using 0.1 M HCl solution (with almost 100% RFP solubility). Recently, it was reported that the excipients in formulations such as Tween 80 improved the absorption of drugs by inhibiting an efflux transporter.\textsuperscript{22,23} Therefore, this effect of Tween 80 might have some influence on our results. Based on these findings, we infer that both the improvement in solubility and Pgp inhibition by Tween 80 increased the absorption of RFP to a similar level observed using 0.1 M HCl solution (with almost 100% RFP solubility).

However, the amount (% of dissolved RFP in ultrapure water and 0.1 M phosphate buffer were 10%, whereas the values in 10% Tween 80 and 0.1 M HCl solution were more than 60%. In contrast, the value of $AUC_{0-24}$ after oral administration by 10% Tween 80 and 0.1 M HCl solution increased only at most two folds, compared with those by ultrapure water and 0.1 M phosphate buffer. Furthermore, the values of $C_{\text{max}}$ in this study were higher than average clinical RFP concentration (8–20 μg/mL).\textsuperscript{\textsuperscript{24}} A previous study showed that the proximal region of small intestine was higher in the permeability of RFP than the distal region, and that the permeation through the proximal region was saturable.\textsuperscript{\textsuperscript{17}} Accordingly, it seems likely that the absorption of RFP from the gastrointestinal tract in this study reached saturation. The mean values of $k_{\text{a}}$ were almost the same levels among each treatment, whereas the decrease in $k_{\text{a}}$ and the prolongation in $T_{1/2}$ were observed in the groups which produced sufficient absorption compared to the group using ultrapure water. The terminal slopes of the time–concentration profile after RFP oral administration representing the $k_{\text{a}}$ values were altered remarkably with regard to administration forms, whereas the $k_{\text{a}}$ values remained almost constant irrespective of administration forms. This may be indication for the occurrence of a flip-flop kinetic phenomenon, therefore the rate constant in the terminal phase might reflect the $k_{\text{a}}$ values. Many factors are known to contribute to the flip-flop kinetic phenomenon including limited drug solubility, sustained release formulations, or transport-mediated absorption.\textsuperscript{\textsuperscript{25}} A recent report suggested that the time–concentration profile of oral RFP administration was involved in this phenomenon because of its poor aqueous solubility and transport-mediated absorption.\textsuperscript{\textsuperscript{26}} Therefore, a large amount of dissolved RFP remained in the gastrointestinal tract, and was absorbed slowly and continuously for a long term when administered with 10% Tween 80 and 0.1 M HCl solution. Conceivably, the delay of the $T_{\text{max}}$ and the increase of the $AUC_{0-24}$ attributes to this flip-flop kinetic phenomenon. Concerning the reduction in the the $k_{\text{a}}$ values of these groups, the rate constant might be estimated to be small in calculation, because the absorption of RFP reached saturation. Even if a large amount of RFP remained in the gastrointestinal tract, there is a limit of the absorption, and this might decrease the $k_{\text{a}}$ values.

We also examined the influence of diet on the pharmacokinetics of RFP, administered in 10% Tween 80 solution. The $C_{\text{max}}$ of RFP in fed rats was significantly higher than that observed in fasted rats, whereas the $AUC_{0-24}$ was unchanged (Table 2). These results may indicate that the diet-induced rise in gastrointestinal pH has little influence on RFP absorption if RFP is dissolved in the solvent by using Tween 80 which enhances the solubility (and also inhibits Pgp).

The $n$-octanol/water distribution coefficient of RFP shows a maximum at pH 6–7, and the lipophilicity of RFP is known to increase within this pH range.\textsuperscript{27} According to pH–partition theory, the unionized drugs which have high lipophilicity are an important factor to determine gastrointestinal absorption of drugs.\textsuperscript{28} If absorption of RFP operates in accordance with this theory, it appears that RFP can easily penetrate the lipid membrane in a neutral pH range, and the absorption from the gastrointestinal tract increases. However, when suspended RFP was administered using ultrapure water, it seems likely that the diet-induced rise in gastric pH reduced RFP solubility further. Because the pH–partition theory applies when RFP is dissolved, we infer that the absorption of RFP in this study decreased. In the present study, when RFP was suspended (not dissolved) in solvents, or when we raised gastric pH by administering 0.1 M phosphate buffer (pH 6.8) or a meal, the $AUC_{0-24}$ value was reduced. Therefore, we infer that RFP solubility was a more important determinant of RFP pharmacokinetics than the pH-induced rise in proportion of the unionized drugs.

In conclusion, RFP solubility in the gastrointestinal fluid depended on pH and affected the absorption of RFP. It was suggested that the low solubility of RFP in the presence of an elevated gastric pH reduced the $C_{\text{max}}$ and $AUC_{0-24}$ of RFP when administered after a meal in clinical practice. Based on these findings, it would be beneficial to change the timing of RFP administration to patients, because clinical outcomes could be compromised by administration after a meal. Moreover, it was observed that the absorption of RFP was not affected by a meal when RFP was dissolved using 10% Tween 80. Accordingly, these results could inform the development of oral RFP formulations that improve availability and efficacy by optimizing RFP solubility. We also expect further improvement in availability by adding an appropriate excipient which enhances the solubility of RFP in formulations and inhibits Pgp in the gastrointestinal tract.
Conflict of Interest  The authors declare no conflict of interest.

REFERENCES


3) Mehta JB, Shantaveerapa H, Byrd RP Jr, Morton SE, Fountain F, Roy TM. Utility of rifampin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest, 120, 1520–1524 (2001).


