
Regular Article

**Effect of Clarithromycin on the Pharmacokinetics of Pranlukast in Healthy Volunteers**

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**Summary:** Pranlukast is a cysteinyl leukotriene receptor antagonist that has been used to treat bronchial asthma and allergic rhinitis. In vitro data suggest that pranlukast is a substrate of CYP3A4. Thus, the effect of clarithromycin, a potent CYP3A4 inhibitor, on the pharmacokinetics of pranlukast was examined in an open-label, randomized, two-way crossover study in 16 healthy male volunteers. In treatment A, volunteers received a single, 225 mg dose of pranlukast. In treatment B, 200 mg of clarithromycin was administered twice daily for 7 days and a single, 225 mg dose of pranlukast was coadministered on day 7. Blood samples were collected up to 24 hours after treatment, and pranlukast concentrations in the plasma were measured. The geometric mean ratios [GMR] (90% confidence intervals [CIs]) for pranlukast AUC0- and Cmax (with/without clarithromycin) were 1.06 (0.91, 1.24) and 1.17 (0.95, 1.45), respectively. In conclusion, clarithromycin and pranlukast could be coadministered without dose adjustment because clarithromycin minimally affected the pharmacokinetics of pranlukast.

**Keywords:** clarithromycin; CYP3A4; drug-drug interaction; pranlukast; pharmacokinetics

**Introduction**

Cysteinyl leukotrienes (cysLTs) are 5-lipoxygenase pathway products of arachidonate that induce bronchoconstriction, vascular hyperpermeability, mucosal edema accumulation, and mucus secretion.1–3) Pranlukast is a selective cysLTs receptor antagonist, and a 225 mg twice-daily dose has been used to treat bronchial asthma and allergic rhinitis in Japan.

The absorption ratio of pranlukast is estimated to be approximately 20% based on excretion ratios in the feces following oral administration (the absolute bioavailability has not been reported). Plasma concentrations peak approximately 3 hours after oral administration with an apparent lag-time of at least 1–2 hours under fasting conditions.4) Pranlukast absorption is delayed by concurrent food ingestion.5) The terminal elimination half-life of pranlukast in the plasma is approximately 2 hours. In vitro studies show that pranlukast is predominantly metabolized by CYP3A4 and CYP2C8.6) Pranlukast is minimally excreted in the urine. The major metabolic pathway of pranlukast is shown in Figure 1. The plasma binding ratio of pranlukast is more than 99%, and the major binding protein is albumin.

Some 14-membered macrolide antibiotics such as clarithromycin and erythromycin are irreversible inhibitors of CYP3A4 and CYP2C8.7,8) Thus, the present study was designed to evaluate the effect of clarithromycin on pranlukast pharmacokinetics. Clarithromycin was chosen because it is the moderate to strong CYP3A4 inhibitor.9,10)

**Methods**

**Subjects:** Sixteen healthy male subjects, aged 20–31 years with a body weight range of 51.7–72.3 kg,
were eligible to participate in this study. The subjects were considered healthy based on a medical interview, physical examination, vital signs, clinical laboratory tests, and an electrocardiogram. Written informed consent was obtained from each subject upon entering the study. Subjects were required to abstain from taking any medication without prior consent of the investigator, drinking alcohol, smoking, and consuming food or beverages containing grapefruit, St John’s wort, and caffeine.

**Study design:** The study, conducted in accordance with good clinical practice (GCP), was a single center (Kan-nondai Clinic, Ibaraki, Japan), open-label, randomized, two-way crossover study. The study protocol, informed consent documents, and other documents were approved by the institutional review board of Kan-nondai Clinic.

Subjects received two treatment regimens in random order, with at least a 7-day washout period between treatments. In treatment A, subjects received a single 225 mg dose of pranlukast after a standard breakfast on day 1. This treatment regime was designed to evaluate the pharmacokinetics of pranlukast without clarithromycin. In treatment B, 200 mg of clarithromycin (Clarith tab.200, Taisho Toyama Pharmaceutical Co., Ltd., Tokyo, Japan) was administered twice daily in the morning and evening for 7 days. On day 7, clarithromycin and pranlukast were coadministered in the morning after a standard breakfast. The pharmacokinetics of pranlukast were evaluated on day 7, while clarithromycin pharmacokinetics were evaluated on days 5 and 7. The pranlukast capsule was manufactured in accordance with Good Manufacturing Practice. The capsules, each containing 112.5 mg of pranlukast, were manufactured by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). The dose of clarithromycin used in this study was the maximum dose for sinusitis patients according to the Japanese label.

**Pharmacokinetic sampling:** On the day of pharmacokinetic evaluation, blood samples (2 mL) were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours post-pranlukast administration in order to measure plasma concentrations of pranlukast and its metabolites (HM-1, HM-2, M-1, and M-1-S). Blood samples (1 mL) used to measure clarithromycin plasma concentrations were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours post treatment. In addition, for clarithromycin, blood samples were collected before drug administration on days 1 through 7. Samples were collected into tubes containing sodium heparin, centrifuged, and plasma was harvested and stored below −20°C until analysis.

**Assays for pranlukast and its four metabolites in plasma:** Plasma concentrations of pranlukast and its metabolites except M-1-S were measured simultaneously using a validated LC/MS/MS analytical procedure. Briefly, 0.1 mL of plasma was transferred into glass tubes, and 0.01 mL of internal standard (IS) solution and 0.5 mL of 0.3 mol/L potassium dihydrogen phosphate were added.
The analytes were extracted with 2 mL of ethyl acetate. After centrifugation (1000 g, 5 min), the organic layer was transferred to a new glass tube and evaporated until dry at 40°C under a gentle nitrogen stream. The solution was reconstituted with 1 mL of 10 mmol/L ammonium acetate/acetonitrile (11:9, v/v), filtered through a 0.45 μm membrane (type W-MR, Kurabo, Osaka, Japan), and a 5 μL aliquot was injected into the LC/MS/MS. The LC/MS/MS system consisted of a 2795 separation module (Waters, Milford, USA) and API4000 (Applied Biosystems, Foster, USA). Discovery RP-Amide C16, 5 μm, 2.1 × 150 mm (Supelco, Bellefonte, USA) was used for separation and kept at 30°C. The flow rate was 0.25 mL/min and acetonitrile and 10 mmol/L ammonium acetate were used as mobile phase A and B, respectively, for step gradient separation. The ratio of mobile phase A was as follows: 0–4.5 min 45%, 4.5–5.5 min 70% and 5.5–10 min 45%. A negative MRM detection mode was used and the MRM transitional pairs were as follows: pranlukast (480–424), M-1 (496–292), HM-1 (496–292), HM-2 (494–292) and IS (494–438). The calibration curves for pranlukast and the metabolites were linear over a concentration range of 2 to 200 ng/mL. Intra- and inter-day precision (CV) for each analyte were within 14.9%, and accuracy (RE) were within ±8.6%.

To examine M-1-S, 0.1 mL of plasma was incubated with sulfatase (Type H-1 Helix pomstia, Sigma) at 37°C for 10 min to hydrolyze M-1-S to M-1. After hydrolysis, the M-1 concentration was measured as described above. The plasma levels of M-1-S were calculated by subtracting the M-1 concentration obtained without hydrolysis from that after hydrolysis.

Assays for clarithromycin in plasma: A validated LC/MS/MS method was used to examine clarithromycin in the plasma. Briefly, 0.02 mL of plasma, 0.02 mL of IS (roxithromycin) solution, and 0.2 mL of methanol/acetonitrile (1:2, v/v) were mixed. After centrifugation, 0.1 mL of the supernatant was transferred to a polypropylene tube containing 0.1 mL of 0.1 vol% acetic acid. A 5 μL aliquot was injected into an LC/MS/MS consisting of an autosampler (SIL-HTc, Shimadzu, Kyoto, Japan), pump (LC-10ADvp, Shimadzu), column oven (CTO-10Asvp, Shimadzu), and API4000. ZORBAX Extend-C18, 3 μm, 2.1 × 50 mm (Agilent technologies, Palo Alto, USA) column was used and kept at 40°C. 0.1 vol% acetic acid and acetonitrile/methanol (1:1, v/v) were used as mobile phase A and B, respectively, for step gradient separation. The ratio of mobile phase A was as follows: 0–1 min 80%, 1–4 min 10% and 4–7 min 80%. The flow rate was set at 0.25 mL/min from 0 min to 1 min, 0.40 mL/min from 1 to 5 min and 0.25 mL/min from 5 to 7 min. A positive MRM detection mode was used and the MRM transitional pairs were as follows: clarithromycin (748–158), roxithromycin (838–158). The calibration curves for clarithromycin were linear over a concentration range of 5 to 5000 ng/mL. Intra- and inter-day precision (CV) were within 8.6% and accuracy (RE) were within ±8.4%.

Pharmacokinetic analysis: The area under the plasma concentration versus time curve from 0 to 12 hours or infinity (AUC0–12 for clarithromycin or AUC0–∞ for pranlukast) was estimated using the linear trapezoidal rule. The peak plasma concentration (Cmax) values and the time associated with the maximal concentration (tmax) were obtained from the data. The elimination rate constant (kz) was calculated from the slope of the linear regression of the log-transformed concentration values versus time in the terminal phase. The elimination half-life (t1/2) was calculated as 0.693/kz. These parameters were calculated using WinNonlin Professional Ver. 4.0.1 software (Pharsight, Mountain View, USA).

Statistical methods: A power analysis was performed to calculate the number of subjects that were necessary to show a 50% change in the AUC0–∞ or Cmax of pranlukast with 80% power and a significance level of p<0.05. The reference and test treatments were pranlukast alone and pranlukast with clarithromycin, respectively. An ANOVA was performed on pranlukast log-transformed AUC0–∞ and Cmax with treatment as a fixed effect and subject as a random effect. The results are expressed as the test/reference ratio of the geometric means and 90% confidential interval (CI). All statistical analyses were performed using the SAS software program version 8.2 (SAS Institute Japan, Tokyo, Japan).

Safety and tolerability: The following safety assessments were performed prior to dosing and at regular intervals post treatment: adverse event reports, clinical laboratory tests (hematology, blood chemistry and urinalysis), vital signs (blood pressure, heart rate, respiration rate, and body temperature), physical examination (body weight), 12-Lead ECG, and continuous Lead-II ECG monitoring.

Results

Fifteen of the 16 subjects completed the study. One subject was withdrawn from the study due to discontinued participation. Therefore, data from 15 subjects were included in the pharmacokinetic analysis.

Pranlukast pharmacokinetics: Table 1 summarizes the pharmacokinetic parameters and the statistical analysis of the AUC0–∞ and Cmax of pranlukast. The mean concentration-time profiles of pranlukast were shown in Figure 2. When pranlukast was administered with or without clarithromycin, the mean plasma pranlukast concentrations, AUC0–∞, Cmax, and t1/2 were similar, and the median tmax value was identical. The geometric mean ratios (pranlukast with clarithromycin/pranlukast alone) of...
Table 1. Pharmacokinetic parameters of pranlukast after single administration of 225 mg pranlukast alone or coadministration with 200 mg BID clarithromycin in healthy male subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pranlukast alone</th>
<th>pranlukast with clarithromycin</th>
<th>Ratio of geometric means</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>937 ± 408</td>
<td>1090 ± 500</td>
<td>1.17</td>
<td>0.95–1.45</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>5.0 (4.0–12)</td>
<td>5.0 (4.0–6.0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–12&lt;/sub&gt; (ng h/mL)</td>
<td>2870 ± 1060</td>
<td>3060 ± 1070</td>
<td>1.06</td>
<td>0.91–1.24</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>5.0 ± 1.2</td>
<td>5.0 ± 0.6</td>
<td>–</td>
<td>–</td>
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</table>

Values are means ± standard deviation except for t<sub>max</sub>, which is median (range), n = 15, CI: confidence interval

Pranlukast metabolites pharmacokinetics: The mean concentration-time profiles of the three pranlukast metabolites (M1-S, HM-1, and HM-2) are shown in Figure 2. Although coadministration of clarithromycin slightly elevated the plasma concentrations of all metabolites, their plasma concentration-time profiles resembled that of pranlukast alone. M-1 was hardly detected either pranlukast alone or in combination with clarithromycin.

Clarithromycin pharmacokinetics: The effect of pranlukast on clarithromycin pharmacokinetics was a secondary parameter of this trial. The trough plasma clarithromycin concentrations showed that steady state was almost achieved by day 5. The plasma clarithromycin concentration on day 7 were higher than those on day 5, and the geometric mean AUC<sub>0–12</sub> and C<sub>max</sub> of clarithromycin were 13% and 20% higher, respectively.

Clinical safety: Eight of the 15 subjects reported a total of 11 clinical adverse events, including mushy stool (n = 6), increased total bilirubin (n = 2), increased alanine aminotransferase (n = 2), and increased triglycerides (n = 1). ECGs and vital signs showed no clinically significant changes. All of the adverse events were mild and resolved without medication.
Discussion

The major metabolic pathway of pranlukast is the hydroxylation of its terminal benzene ring to form M-1. M-1 is further sulfated and exists as M-1-S in the plasma. Other metabolites include HM-1, generated by hydroxylation at the benzylic position of the terminal benzene ring. HM-1 is further oxidized to the ketone body, HM-2. The involvement of CYP3A4 and CYP2C8 in each hydroxylation process remains unclear. Of these metabolites, only HM-1 shows in vitro cysteine receptor antagonist activity of about one-half of pranlukast.

Clarithromycin 250 mg BID (200 mg BID was used in this study) causes a 3.6- to 7.0-fold increase in the AUC of midazolam, a typical CYP3A4 and CYP3A5 substrate, mainly by mechanism-based inhibition. In the present study, clarithromycin 200 mg BID minimally affected the pharmacokinetics of pranlukast and its metabolites. According to our in-house data, in vitro studies using human liver microsomes have demonstrated that both ketoconazole and anti-CYP3A4 antibody inhibited approximately 50% of the pranlukast metabolism. However, macrolide antibiotics such as clarithromycin and erythromycin hardly inhibited the pranlukast metabolism in in vitro studies using human liver microsomes pre-incubated with those macrolide antibiotics for 30 minutes. Therefore, the kinetic parameters for enzyme inactivation, the apparent dissociation constant and the maximum inactivation rate constant, could not be calculated. In these in vitro studies, pranlukast concentration was 1 and 5 μmol/L (pranlukast C_max is approximately 2 μmol/L), whereas clarithromycin and erythromycin concentrations ranged from 5 to 100 μmol/L (macrolides C_max is less than 10 μmol/L). The test system used in this study was confirmed to be adequate since the effects of macrolides on the metabolism of midazolam, which were evaluated as control, were in good accordance with the previous report. Although the cause of the inconsistency between the competitive inhibition and the mechanism-based inhibition remains unclear, these findings suggest that the involvement of CYP3A4 in pranlukast in vitro metabolism is limited and that CYP2C8 may functionally compensate for CYP3A4.

On the other hand, the major metabolic pathways of clarithromycin are oxidation to the active metabolite, 14-hydroxy clarithromycin, and demethylation to the inactive metabolite, N-desmethylclarithromycin. Both processes are catalyzed by CYP3A4 isozymes. Pranlukast inhibits CYP3A4 with a Ki value of 4.1 μmol/L (in-house data). However, this study indicated that steady-state clarithromycin pharmacokinetics are unlikely to be affected by coadministration of pranlukast, which is consistent with the 1000-fold difference between the Ki value and free C_max (4.4 nmol/L) following administration of 225 mg of pranlukast, a clinical dosage. Clarithromycin is a drug with a wide safety margin. In multiple dose-ranging studies, clarithromycin was well tolerated when 250 to 2000 mg was administered orally twice daily.14-16 The design of the present study is insufficient to evaluate the effects of pranlukast on clarithromycin pharmacokinetics. However, the results of this study and the above information suggest that pranlukast is unlikely to induce a clinically significant drug-drug interaction with clarithromycin.

No serious adverse events were reported during the study, and all adverse events were mild and resolved without medication. Thus, coadministration of pranlukast and clarithromycin was well tolerated in healthy volunteers.

In conclusion, clarithromycin and pranlukast could be coadministered without dose adjustment because clarithromycin minimally affected the pharmacokinetics of pranlukast.

References


