Effect of Clarithromycin and Other Macrolides on the Sulfoxidation and 5-Hydroxylation of Lansoprazole in Dogs

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The purpose of this study was to evaluate a possible interaction between lansoprazole and clarithromycin as well as other macrolides in dogs. Lansoprazole (30 mg) was orally administered to male beagle dogs, with or without oral pretreatment with 200 mg clarithromycin twice a day for 5 d. The experiments had a randomized cross-over design with a two-week washout period between dosing regimens. Clarithromycin pretreatment for 5 d resulted in a significant increase in the area under the serum lansoprazole concentration-time curve (AUC), whereas the area for a lansoprazole metabolite, lansoprazole sulfone, was significantly reduced, as was the maximum serum concentration (Cmax) of lansoprazole sulfone. When the effects of clarithromycin on the metabolism of lansoprazole were studied using dog liver microsomes, it was found that clarithromycin significantly inhibited the formation of lansoprazole sulfone but not another metabolite, 5-hydroxylansoprazole. These results suggest that co-medication of lansoprazole with clarithromycin may produce a synergistic effect caused by the increased serum levels of lansoprazole of benefit in Helicobacter pylori eradication.

Key words lansoprazole; clarithromycin; sulfoxidation; metabolism; pharmacokinetics

Lansoprazole, a benzimidazole derivative, is a proton pump inhibitor that acts on the membrane H+/K+-adenosine triphosphatase in gastric parietal cells.1 This drug is being increasingly used in the treatment of duodenal ulcer, Zollinger-Ellison syndrome, and other related hyperacidic conditions.3 Lansoprazole is extensively metabolized following oral administration to sulfone and 5-hydroxylated metabolites by the cytochrome P450 (CYP) enzymes, CYP3A43 and CYP2C19,4 respectively.

The use of clarithromycin has been extended to the treatment of Helicobacter pylori infection which is a common cause of gastritis and gastroduodenal ulcers. Therefore, the drug is often used in combination with a proton pump inhibitor to inhibit acid secretion and eradicate Helicobacter pylori infection.5 Some macrolide antibiotics are also known to be potent inhibitors of CYP with CYP3A46 being most affected. A mechanism has been proposed whereby macrolide antibiotics are N-demethylated by CYP3A4 to nitrosourea, which combine with the haem of CYP to form stable complexes, thus rendering the enzyme inactive. The CYP3A4-mediated metabolism of drugs may, therefore, be inhibited by some macrolide antibiotics. The CYP3A subfamily is responsible for oxidation of a large number of compounds, such as steroids,7 human immunodeficiency virus (HIV) protease inhibitors,8 cyclosporine,9 anticancer drugs10 and macrolide antibiotics.11

The aim of the present study is to investigate a possible interaction between lansoprazole and clarithromycin in dogs in vivo and in vitro. We used beagle dogs in the present study since the metabolic pathways and major metabolites of lansoprazole are similar to those in humans.12

MATERIALS AND METHODS

Materials Lansoprazole and its metabolites: lansoprazole sulfone and 5-hydroxylansoprazole were generous gifts from Takeda Chemical Industries, Osaka. Clarithromycin was generously supplied by Taisho Pharmaceutical Co., Tokyo, Japan. Lansoprazole capsules (Takepran8, Takeda Chemical Industries) containing enteric-coated granules and clarithromycin tablets (Clarith, Taisho Pharmaceutical Co.) were purchased from a local wholesaler. All other chemicals used in this study were of analytical grade.

In Vivo Experiments In the control study, 6 male beagle dogs, 8—14 kg, were fasted overnight with free access to water. The next morning, a capsule containing 30 mg lansoprazole was administered orally. Blood samples (5 ml) were collected from the median antebrachial vein at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h after dosing. In the multiple dose study, a 200-mg clarithromycin tablet was administered orally to the dogs twice a day for 5 d and, after the last administration, the dogs were fasted overnight with free access to water. The next morning, a 200-mg clarithromycin tablet was administered orally to the dogs and, subsequently, a 30-mg lansoprazole capsule orally. Blood was collected as in the control study. These experiments were performed in a randomized cross-over design with a two-week washout period between regimens.

In Vitro Experiments Liver microsomes were prepared from male beagle dogs according to our previous report.13 All subsequent procedures were performed at 4°C or below. The amount of protein in the microsomal preparation was determined by the method of Lowry et al.14 The reaction mixture (500 µl), prepared from 100 µl microsomal suspension equivalent to 0.2 mg protein, 200 µl 0.3 M potassium phosphate buffer, pH 7.4, 100 µl 0.6 mM EDTA and lansoprazole (9—22.5 µM) with the macrolides (0—83.3 µM) at the final concentrations indicated, was preincubated at 37°C for 5 min. The reaction was started by addition of the NADPH-generating system (100 µl) containing 3 mM NADP, 2 mM glucose-6-phosphate, 6 units/ml glucose-6-phosphate dehydrogenase and 24 mM MgCl2. The reaction was terminated by addition of 3 ml diethyl ether–dichloromethane (7:3, v/v).

Analysis Lansoprazole, lansoprazole sulfone and 5-hydroxylansoprazole in serum and microsomal solution were determined using reversed phase high performance liquid
Fig. 1. Serum Concentration–Time Profiles of Lansoprazole (a), Lansoprazole Sulfone (b) and 5-Hydroxylansoprazole (c) after Oral Administration of 30 mg Lansoprazole to Dogs.

Each point represents the mean±S.E.M. of 6 dogs. Lansoprazole alone (○, △, □); lansoprazole with clarithromycin (●, ■, ■).}

chromatography (HPLC; model LC-6A, Shimadzu, Kyoto, Japan) as reported by Aoki et al.15) To a 10-ml centrifuge tube containing 1 mg internal standard (I.S.), isobutyl p-hydroxybenzoate, were added 0.5-ml serum or incubation sample and 3 ml diethyl ether–dichloromethane (7:3, v/v) mixture. The tube was shaken for 30 s and centrifuged at 3000 rpm for 10 min at 4°C. The extraction procedure was repeated twice. The combined organic layer was transferred to another test-tube and evaporated. The residue was dissolved in 200 µl mobile phase and agitated for 5 s. A 100-µl aliquot was injected into the HPLC column. Isolation was performed with a reversed phase column (Lichrospher 100 RP-18, 5 µm, 250 mm×4 mm i.d.). The mobile phase consisted of acetonitrile and water (34:66, pH adjusted by phosphoric acid to 7.0) containing 0.1% n-octylamine. The mobile phase was delivered at a flow rate of 1 ml/min at 40°C. The absorbance was measured at 285 nm.

Pharmacokinetic Analysis The maximum serum concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained graphically. The areas under the serum concentration–time curves (AUC_{0→∞}) were calculated using the trapezoidal rule and by extrapolating the time to infinity using the elimination rate constant (k_e) values. The terminal half-life (t_{1/2}) was calculated by dividing 0.693 by k_e. The apparent total body clearance (CL_{tot}/F) was calculated from CL_{tot}/F = dose/AUC_{0→∞}, F being the fraction of the dose absorbed. Enzyme kinetics were analysed by Lineweaver-Burk plots. Values of the Michaelis-Menten constant (K_m) and the maximum rate of metabolism (V_{max}) were assessed by least-squares linear regression. The inhibition constant (K_i) values were determined from linear regression analysis using the following equations: apparent K_m′ = K_m + K_i; I/K_i, where apparent K_m and K_m are the affinity constants in the presence and absence of inhibitor, respectively, and I is the inhibitor concentration.

Statistical Analysis Each result is expressed as a mean±S.E.M. Pharmacokinetic data were analyzed for statistical differences using Student’s t-test. Statistical significance is assumed when p<0.05.

RESULTS

In Vivo Study Figure 1 shows the serum concentration–time profiles of lansoprazole, lansoprazole sulfone and 5-hydroxylansoprazole after oral administration of 30-mg lansoprazole to beagle dogs, with or without pretreatment with clarithromycin, and Table 1 summarizes the pharmacokinetic parameters. Pretreatment with 200-mg clarithromycin twice a day for 5 days resulted in a significant increase in the AUC of lansoprazole compared with that of lansoprazole alone. The CL_{tot}/F values of lansoprazole were significantly reduced in the presence of clarithromycin. There was no significant dif-
Table 1. Pharmacokinetic Parameters of Lansoprazole, Lansoprazole Sulfone and 5-Hydroxylansoprazole after Oral Administration of 30 mg Lansoprazole, with or without Clarithromycin

<table>
<thead>
<tr>
<th></th>
<th>Lansoprazole</th>
<th>Lansoprazole sulfone</th>
<th>5-Hydroxylansoprazole</th>
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<tr>
<td></td>
<td>Without</td>
<td>With</td>
<td>Without</td>
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<tr>
<td>AUC_{0-∞} (µg·h/ml)</td>
<td>1.14±0.21</td>
<td>1.66±0.27*</td>
<td>2.03±0.44</td>
</tr>
<tr>
<td>C_{max} (µg/ml)</td>
<td>0.52±0.07</td>
<td>0.95±0.23</td>
<td>0.66±0.08</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>1.58±0.15</td>
<td>1.50±0.18</td>
<td>1.42±0.15</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>0.89±0.11</td>
<td>1.11±0.20</td>
<td>0.51±0.08</td>
</tr>
<tr>
<td>CL_{int}/F (ml/min/kg)</td>
<td>48.71±8.61</td>
<td>33.49±6.39*</td>
<td>1.60±0.33</td>
</tr>
</tbody>
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Each value represents the mean±S.E.M. of 6 dogs. * p<0.05 compared with control (lansoprazole alone).

Table 2. Metabolic Ratio of Lansoprazole Sulfone and 5-Hydroxylansoprazole after Oral Administration of 30 mg Lansoprazole, with or without Clarithromycin, in Dogs

<table>
<thead>
<tr>
<th></th>
<th>AUC_{lansoprazole}</th>
<th>AUC_{lansoprazole}</th>
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<tr>
<td></td>
<td></td>
<td>AUC_{sulfone}</td>
<td>AUC_{sulfone}</td>
<td>C_{max} sulfone</td>
<td>C_{max} sulfone</td>
<td>C_{max} sulfone</td>
</tr>
<tr>
<td>Lansoprazole alone</td>
<td>1.85±0.23</td>
<td>0.33±0.13</td>
<td>1.25±0.16</td>
<td>0.21±0.06</td>
<td></td>
<td></td>
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<tr>
<td>With clarithromycin</td>
<td>0.65±0.10**</td>
<td>0.17±0.03</td>
<td>0.57±0.18*</td>
<td>0.15±0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. of 6 dogs. * p<0.005 compared with control (lansoprazole alone). ** p<0.005 compared with control (lansoprazole alone).

Fig. 2. Lineweaver-Burk Plots for Inhibition of Sulfoxidation (a) and 5-Hydroxylation (b) of Lansoprazole by Clarithromycin in Dog Liver Microsomes

(a) Sulfoxidation

(b) Hydroxylation

In Vitro Study

To evaluate the inhibitory effect of macrolide antibiotics on lansoprazole metabolism, we performed an in vitro study using dog liver microsomes. Three types of 14-membered-ring macrolides, clarithromycin, erythromycin and roxithromycin, were used to compare the inhibitory effect. The Lineweaver-Burk plots for the inhibition of sulfoxidation and 5-hydroxylation of lansoprazole following addition of each macrolide are shown in Figs. 2–4. Addition of clarithromycin or erythromycin to microsomal incubates inhibited lansoprazole sulfoxidation in a concentration-dependent and competitive manner, whereas addition of roxithromycin had little effect on sulfoxidation. On the other hand, the three macrolides had hardly any inhibitory effect on lansoprazole 5-hydroxylation. Then, to compare the degree of inhibition of sulfoxidation, the Ki values were estimated from the K_{eq} values in the presence or absence of macrolide, and the I. The mean Ki values of clarithromycin, ery-
thromycin and roxithromycin were 54.4, 40.6 and 239.0 μM, respectively. Erythromycin had the smallest $K_i$ value of the three macrolides. These results suggest that clarithromycin and erythromycin significantly inhibit the sulfoxidation of lansoprazole.

**DISCUSSION**

We investigated the effect of clarithromycin on the pharmacokinetics of lansoprazole in dogs and also studied if three types of 14-membered-ring macrolides, clarithromycin, erythromycin and roxithromycin, could affect the sulfoxidation and/or 5-hydroxylation of lansoprazole using dog liver microsomes *in vitro*. In the *in vivo* study, a pretreatment with clarithromycin for 5 d resulted in a significantly increased $AUC_{D→∞}$ and reduced $CL_{int}/F$ of lansoprazole, and in a significantly reduced $AUC_{D→∞}$ and $C_{max}$ of lansoprazole sulfone (Fig. 1, Table 1). These results suggest that clarithromycin inhibited the sulfoxidation of lansoprazole, probably mediated by CYP3A, and thereby increased the serum levels of lansoprazole in dogs. Thus, the sulfoxidation of lansoprazole plays an important role in lansoprazole disposition.

In addition, pretreatment with clarithromycin tended to reduce the metabolic ratio of lansoprazole to 5-hydroxylansoprazole, although there was no significant difference between both groups (Table 2). This suggests that clarithromycin may also influence the hydroxylation of lansoprazole. Robin et al.\(^{16}\) reported that antibodies against rat CYP3A enzymes inhibited the rates of both 5-hydroxylation and sulfoxidation of lansoprazole and that the 5-hydroxylation of lansoprazole at pharmacologically relevant concentrations appeared to be catalyzed by CYP2C19, rather than by CYP3A. Their report supports the idea that clarithromycin reduces not only the sulfoxidation, but also the hydroxylation, of lansoprazole to a minor extent, probably mediated by CYP3A.

The $k_{cl}$ values of lansoprazole in the presence of clarithromycin were not significantly different from those in its absence in spite of the significantly reduced sulfoxidation...
(Tables 1 and 2). Recent studies have confirmed that CYP3A is present in the mucosa of the small intestine and orally administered drugs such as nifedipine, felodipine and cyclosporine undergo significant metabolism in the gut wall. The major site of metabolism of these drugs is considered to be the gut wall since the bioavailability is increased without changing the elimination half-life. Accordingly, these reports suggest that clarithromycin inhibits not only the hepatic, but also the intestinal metabolism of lansoprazole. This is further supported by the report that clarithromycin coadministration inhibited the CYP3A-mediated metabolism of cyclosporine not only in the liver but also in the intestine.

Lansoprazole is structurally related to omeprazole. It has been reported that lansoprazole and omeprazole are metabolized by CYP2C19 (5-hydroxylation) and by CYP3A4 (sulfoxidation) to a minor extent in humans and that the degree of involvement of the polymorphically expressed CYP2C19 in the metabolism of the two drugs is similar. Böttiger et al. also reported that the mean omeprazole AUC0→5 after administration of 20-mg omeprazole increased 1.4-fold (from 1660 to 2265 nmol·h/l) in extensive metabolizers and 2-fold (from 7715 to 15319 nmol·h/l) in poor metabolizers after simultaneous administration of 200-mg ketoconazole, a known CYP3A4 inhibitor. They confirmed that the formation of omeprazole sulfone is highly dependent on CYP3A4 and that this is the predominant metabolic pathway in poor metabolizers for CYP2C19. With this in mind, the present study suggests that clarithromycin may increase the serum levels of lansoprazole in patients taking lansoprazole, especially in the poor metabolizers for CYP2C19.

We have shown that both clarithromycin and erythromycin inhibited lansoprazole sulfoxidation in a concentration-dependent manner but did not significantly inhibit the 5-hydroxylation of lansoprazole over the range 8.3 to 83.3 μM in dog liver microsomes (Figs. 2 and 3). The in vitro data also suggest that the increased serum lansoprazole levels observed after pretreatment with clarithromycin may be attributed to inhibition of sulfoxidation by clarithromycin. On the other hand, roxithromycin had little effect on the sulfoxidation (Fig. 4). The Kᵢ value (54.4 μM) of clarithromycin estimated from the in vitro study was greater than the Cₘₐₓ value (about 3.0 μM) after a single oral dose of 200-mg in dogs (unpublished data). This may be attributed to the mechanism-based inhibition of CYP by the macrolides. It is known that some 14-membered-ring macrolides inhibit drug metabolism by formation of CYP–nitrosoalane complexes between their metabolites and CYP, leading to irreversible inactivation of the enzyme. In this study, we examined the inhibitory effects in dog liver microsomes without preincubating the macrolide antibiotics in the presence of NADPH. However, if dog liver microsomes were preincubated with inhibitor in the presence of NADPH, a greater inhibitory effect would be expected, probably by a mechanism-based inhibition, than that without preincubation. Further experiments are needed to study the mechanism-based inhibition of CYP by macrolide antibiotics. Periti et al. reported that erythromycin and troleandomycin interacted with the CYP-mediated metabolism of drugs to a high degree, whereas clarithromycin and roxithromycin did not. Billaud et al. also reported that roxithromycin seemed to interact with drugs only to a small extent. Their reports support our in vitro data that erythromycin showed the most potent inhibitory effect on sulfoxidation among the three macrolides and that roxithromycin exhibited very little effect on the sulfoxidation of lansoprazole in dog liver microsomes. There are many reports on interactions between clarithromycin and drugs such as carbamazepine, terfenadine, midazolam and cyclosporine, probably involving CYP3A4-mediated metabolism.

Thus, clarithromycin could be a potent inhibitor of lansoprazole sulfoxidation involving CYP3A4.

The macrolide antibiotics such as clarithromycin, erythromycin and roxithromycin have antimicrobial activity against Helicobacter pylori, which causes gastritis and peptic ulcers, and are able to eradicate this infection. Therefore, the macrolides are often used in combination with the proton pump inhibitor, lansoprazole, which inhibits gastric acid secretion and, additionally, has weak antimicrobial activity against the bacteria. Consequently, the present results provide additional information about the combination therapy of lansoprazole with clarithromycin for Helicobacter pylori eradication, namely that comedication may produce a synergistic effect, enhancing eradication, based on increased serum lansoprazole levels.

REFERENCES