Localization of $[^{14}C]$clarithromycin in rat gastric tissue when administered with lansoprazole and amoxicillin

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After oral and intravenous administration of $[^{14}C]$clarithromycin to rats, c. 60–70% of the radioactivity in the gastric tissue was found to be distributed in the mucosal layer. Co-administration of lansoprazole and amoxicillin had no apparent effect on this distribution pattern of $[^{14}C]$clarithromycin. The amount of unchanged $[^{14}C]$clarithromycin in gastric contents increased with co-administration of lansoprazole and amoxicillin. Microautoradiograms of the gastric mucosa showed that $[^{14}C]$clarithromycin was highly distributed in the mucous layer and in surface epithelial cells following oral administration. Homogeneous distribution of radioactivity was evident in the fundic gland. With iv administration, $[^{14}C]$clarithromycin seemed to be secreted by both secreting cells in the gland base and surface epithelial cells.

Introduction

Eradication of *Helicobacter pylori* is currently the most effective therapy for healing and prevention of recurrent gastritis and peptic ulcers. Triple therapy with lansoprazole, clarithromycin and amoxicillin can achieve high rates of eradication. Pharmacokinetic interactions between clarithromycin or amoxicillin and omeprazole in humans have been studied.1 Concentrations of antibiotics in gastric tissue or gastric juice are increased by the co-administration of omeprazole; however, the exact mechanism is not completely understood. To clarify the synergy affecting the eradication of *H. pylori*, the effects of lansoprazole and amoxicillin on the pharmacokinetics and distribution of $[^{14}C]$clarithromycin were investigated using rats.2 Uptake of $[^{14}C]$clarithromycin into gastric tissue was enhanced by co-administration of lansoprazole, and penetration of $[^{14}C]$clarithromycin in gastric tissue increased, dependent on the elevation of gastric pH by lansoprazole. In the present study, we investigated the localization of $[^{14}C]$clarithromycin in rat gastric mucosa, and attempted to clarify the exact distribution of $[^{14}C]$clarithromycin in gastric cells, using microautoradiography.

Materials and methods

Chemicals

$[6-O$-methyl-$^{14}C]$Clarithromycin, unlabelled clarithromycin, amoxicillin, lansoprazole and commercially available reagents were used, as described previously.2

Animals

All experiments were carried out in accordance with the Guidelines for Animal Experimentation and the Regulations for Animal Ethics in Taisho Pharmaceutical Co., Ltd. Male Wistar rats purchased from Nihon SLC Co., Ltd (Shizuoka, Japan) were acclimatized and 8-week-old rats, weighing 176–225 g, were used.2

Dosage and administration of drugs

Rats were given $[^{14}C]$clarithromycin 5 mg/kg body weight, lansoprazole and amoxicillin 10 mg/kg. These drugs were made up immediately before use, as described previously.2 Rats were allocated randomly into four groups and were given the drugs listed in Table 1.

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Measurement of radioactivity in gastric mucosal and muscular layers

At 15, 30, 60 and 240 min after oral (groups 1 and 2) and 15 and 60 min after intravenous (groups 3 and 4) administration of [14C]clarithromycin, the rats were exsanguinated following ether anaesthetization. The stomach was excised and subdivided into mucosal and muscular layers using the stripping method, as described previously.3 Radioactivity in each biological sample was measured using a liquid scintillation counter, as described previously.3 The distribution of radioactivity in mucosa/muscle was determined.

Measurement of the composition of unchanged [14C]clarithromycin in gastric contents

At 15, 30 and 60 min after oral administration of [14C]clarithromycin to rats (groups 1 and 2), gastric contents including mucus were collected, as described above. Radioactivity in the gastric contents was measured in the same manner as for gastric tissue. Part of the gastric contents was concentrated and filtered using Ultrafree-MC (0.45 µm; Millipore, Tokyo, Japan), and was then injected into the HPLC system. For HPLC, a TSK GEL ODS-120A column (4.6 mm inner diameter × 250 mm length; Tosoh, Tokyo, Japan) was used, the mobile phase was 80% methanol/water containing 0.04% ethanolamine, the column temperature was 40°C and the flow rate was 1 mL/min. The composition of unchanged [14C]clarithromycin was calculated on the basis of the peak area of radioactivity.

Microautoradiography of gastric mucosa

Sixty minutes after oral (groups 1 and 2) and 15 min after intravenous (groups 3 and 4) administration of [14C]clarithromycin, the rats were exsanguinated, following anaesthetization with ether. The stomach was excised and the frozen

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Table 1. Treatment regimens

<table>
<thead>
<tr>
<th>Route</th>
<th>Time (min)</th>
<th>Concentration (µg eq./g)</th>
<th>Mucosa/muscle distribution (%)</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>po</td>
<td>15</td>
<td>6.18 ± 1.06</td>
<td>64/36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9.34 ± 5.85</td>
<td>59/41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.06 ± 1.49</td>
<td>62/38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>2.65 ± 0.36</td>
<td>71/29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td>15</td>
<td>11.63 ± 1.33</td>
<td>73/27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12.11 ± 1.85</td>
<td>62/38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Concentration of radioactivity in the glandular stomach and distribution in mucosa/muscle after po or iv administration of [14C]clarithromycin to rats

Each value represents the mean ± S.D. of three or six (group 1, 15–60 min) animals. Significant difference (P < 0.01) of the level of radioactivity was observed between groups 1 and 2.

Measurement of radioactivity in gastric mucosal and muscular layers

At 15, 30, 60 and 240 min after oral (groups 1 and 2) and 15 and 60 min after intravenous (groups 3 and 4) administration of [14C]clarithromycin, the rats were exsanguinated following ether anaesthetization. The stomach was excised immediately and washed thoroughly in 20 mL of saline. The glandular stomach was separated and subdivided into mucosal and muscular layers using the stripping method, as described previously.3 Radioactivity in each biological sample was measured using a liquid scintillation counter, as described previously.3 The distribution of radioactivity in mucosa/muscle was determined.

Measurement of the composition of unchanged [14C]clarithromycin in gastric contents

At 15, 30 and 60 min after oral administration of [14C]clarithromycin to rats (groups 1 and 2), gastric contents including mucus were collected, as described above. Radioactivity in the gastric contents was measured in the same manner as for gastric tissue. Part of the gastric contents was concentrated and filtered using Ultrafree-MC (0.45 µm; Millipore, Tokyo, Japan), and was then injected into the HPLC system. For HPLC, a TSK GEL ODS-120A column (4.6 mm inner diameter × 250 mm length; Tosoh, Tokyo, Japan) was used, the mobile phase was 80% methanol/water containing 0.04% ethanolamine, the column temperature was 40°C and the flow rate was 1 mL/min. The composition of unchanged [14C]clarithromycin was calculated on the basis of the peak area of radioactivity.

Microautoradiography of gastric mucosa

Sixty minutes after oral (groups 1 and 2) and 15 min after intravenous (groups 3 and 4) administration of [14C]clarithromycin, the rats were exsanguinated, following anaesthetization with ether. The stomach was excised and the frozen

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sections of stomach corpus were cut, and slices were mounted on a glass slide dipped in nuclear emulsion, as described previously. After exposure at 4°C for 3 weeks, the glass slide was developed to observe the silver grains indicated by black coloration corresponding to the existence of radioactivity. The slide was stained with Methylene Blue–Fuchsin basic and observed under a light microscope.

Statistics

Results are expressed as mean ± S.D. The significance of differences was evaluated by variance analysis, using the SAS/STAT package. A significance level of 0.01 was used for all tests.

Results and discussion

*H. pylori* colonizes both the apical surface of surface epithelial cells and the surface mucous gel layer. There is thought to be an intracellular sanctuary site in which *H. pylori* evades the effects of antimicrobial therapy.

The concentration of radioactivity in the glandular stomach and the gastric mucosa/muscle distribution of radioactivity after administration of [14C]clarithromycin are shown in Table 2. After oral administration of [14C]clarithromycin, the concentration of radioactivity was several times higher when it was administered together with lansoprazole and amoxicillin (group 2) than when it was administered alone (group 1). After intravenous administration of [14C]clarithromycin (groups 3 and 4), there was no effect of co-administration, with regard to the concentration of radioactivity. With both oral and intravenous administration, c. 60–70% of the radioactivity, which contains both unchanged [14C]clarithromycin and metabolites, was distributed in the mucosal layer. There was no change in the distribution pattern of radioactivity at any time. Thus, clarithromycin would play an important role in eradication of *H. pylori* at the target site. No synergic effects on the mucosa/muscle distribution were detected when [14C]clarithromycin was co-administered with lansoprazole and amoxicillin.

The concentration of the active form of clarithromycin in the stomach is relevant to the eradication of *H. pylori*. When [14C]clarithromycin was administered orally alone, unchanged [14C]clarithromycin accounted for c. 64% of recovered radioactivity in gastric contents at 60 min after administration. On the other hand, when [14C]clarithromycin was co-administered with lansoprazole and amoxicillin, 94% of radioactivity was unchanged drug, which means that the stability of [14C]clarithromycin in gastric contents increased, depending on the lansoprazole-induced elevation of gastric pH. Thus, active [14C]clarithromycin can penetrate the gastric mucosa. The bioavailability of clarithromycin was high even after administration of clarithromycin alone. The stability of clarithromycin in the stomach after oral administration of lansoprazole seems to have little effect on the bioavailability of clarithromycin. The enhanced stability of clarithromycin with co-administration of lansoprazole would influence eradication of *H. pylori*, but the effect would only be at the target site.

The visual localization of [14C]clarithromycin in gastric mucosa was investigated microautoradiographically. Sixty minutes after oral administration of [14C]clarithromycin (group 2), silver grains indicating the existence of radioactivity were extensive in both the mucous layer and in surface epithelial cells in the corpus of the stomach (Figure 1a).

Figure 1. Microautoradiograms of the stomach corpus showing the distribution of radioactivity at 60 min after oral (a) or at 15 min after intravenous (b and c) administration of [14C]clarithromycin to rats (groups 2 and 4). Methylene Blue–Fuchsin basic stain: (a) and (b) magnification ×200, (c) magnification ×400.
Homogeneous radioactivity was observed in the fundic glands. After oral administration of $[^{14}\text{C}]$clarithromycin, it was incorporated into the mucous layer and distributed to surface epithelial cells, then it could penetrate the mucosal layer of the stomach. Fifteen minutes after intravenous administration (group 4), silver grains were observed in all of the fundic glands, particularly in the gland base (Figure 1b). A magnified microautoradiogram ($\times400$) of the gland base of Figure 1(b) is shown in Figure 1(c). Silver grains were present both in secreting cells that constituted the gland base and in the gland lumen. The gland base of the corpus has mainly zymogenic cells, which produce pepsinogen and parietal cells, which secrete acid. $[^{14}\text{C}]$Clarithromycin secreted by these cells may be transported through the gland lumen to the mucous gel layer. Many silver grains were also detected in surface epithelial cells, which indicates that $[^{14}\text{C}]$clarithromycin may be secreted by surface epithelial cells.

Consequently, $[^{14}\text{C}]$clarithromycin administered orally was widely distributed in both surface epithelial cells and the mucous layer by way of two routes: penetration from the gastric lumen and secretion through the blood circulation. Therefore, favourable behaviour of clarithromycin is likely to contribute to the eradication of \textit{H. pylori}. We reported previously that lansoprazole enhanced the affinity of $[^{14}\text{C}]$clarithromycin for the target site.\textsuperscript{2} In the present study in rats, we found that synergic effects consisted mainly of ‘quantitative modification’ of the penetration of $[^{14}\text{C}]$clarithromycin and did not influence the distribution pattern of $[^{14}\text{C}]$clarithromycin in gastric tissue. In addition, $[^{14}\text{C}]$clarithromycin was distributed not only in the apical surface of the surface epithelial cells and in the surface mucous layer but also in intracellular compartments that might be one sanctuary site of \textit{H. pylori}.

References