Effect of Clarithromycin on the Bioavailability of Cyclosporin in Rats

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This study was conducted to determine the effect of clarithromycin (CAM) on the bioavailability of cyclosporin (CYA) in rats, and to compare its effect with that of erythromycin (EM). The area under the blood CYA concentration–time curve (AUC) values after intravenous administration of CYA (2 mg/kg) in combination with CAM or EM (100 mg/kg, p.o.) were significantly increased compared with those of CYA alone, suggesting that there was metabolic inhibition of CYA in the liver by CAM or EM. The time to reach the peak concentration after oral administration of CYA (10 mg/kg) tended to be longer with increasing doses of both CAM and EM (10 and 100 mg/kg, p.o.). Each AUC value for the CAM or EM coadministration group, except the EM (100 mg/kg) coadministration group (about 77% increase), was comparable to that for the CYA alone group. Both CAM and EM (10 and 100 mg/kg, p.o.) were shown to delay gastric emptying in a dose-dependent manner. The gastric emptying in the group treated with CAM (100 mg/kg) was significantly lower than that with EM (100 mg/kg). It is suggested that CAM as well as EM might affect the oral bioavailability of CYA by inhibiting its metabolism and simultaneously by changing the gastrointestinal motility in rats. Thus, caution is recommended when administering CYA concomitantly with CAM to humans.

Key words cyclosporin; clarithromycin; bioavailability; pharmacokinetic interaction; erythromycin; rat

Cyclosporin (CYA), a strong immunosuppressive drug, is widely used for the prevention of graft rejection in organ transplantation.1) However, CYA has not only a narrow therapeutic range, but also serious side effects such as nephrotoxicity and hepatotoxicity.2−5) Its bioavailability is very low and variable because it is metabolized by the cytochrome P450IIA (CYP3A) in the stomach,6−7) the wall of the small intestine,8,9) and the liver.10,11) CYP3A-inducing drugs (such as rifampicin12,13) and CYP3A-inhibiting drugs (such as ketoconazole14,15) have a considerable effect on CYA metabolism. In addition, its absorption is also affected by food intake16) and gastrointestinal motility. Gastric emptying rate (GER)-enhancing drugs (metoclopramide,16) cisapride,17) etc.) and GER-suppressing drugs (atropine,18) etc.) show a potent influence on the absorption of CYA.

On the other hand, macrolide antibiotics are clinically used in combination with CYA when organ transplant patients have some bacterial infection. It has been reported that erythromycin (EM)18,19) and josamycin18,20) increase the blood CYA concentration, whereas spiramycin18,21) and roxithromycin18,22) have no effect on the blood CYA concentration in humans. However, the effect of clarithromycin (CAM), a relatively new macrolide,23,24) on the bioavailability of CYA remains unknown.

This study was conducted to investigate the effect of CAM on the bioavailability of CYA in rats, and to compare its effect with that of EM.

MATERIALS AND METHODS

Materials CYA injection and oral solutions (50 and 100 mg/ml, respectively) were purchased from Sandoz, Ltd. (Tokyo, Japan). CAM powder (Lot No. OITX05) was generously supplied by Taisho Pharmaceutical, Ltd. (Tokyo, Japan), and EM of biochemical grade was obtained commercially from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All other chemicals used were of analytical grade.

Animals Male Wistar rats (Clea, Tokyo, Japan), weighing 206−293 g, were used throughout this study, and were fasted but allowed free access to water overnight before all experiments. The left carotid artery was cannulated with polyethylene tubing (PE-50; Clay Adams, Dickinson & Co., Parsippany, NJ) under pentobarbital anesthesia (50 mg/kg, intraperitoneally) the day before the bioavailability experiment.

Drug Administration and Sampling For single intravenous (i.v.) administration, the CYA injection solution was used and injected via the tail vein to unanesthetized rats as a bolus (2 mg/kg). The CYA oral solution was administered orally without anesthesia. Blood samples (0.25 ml) were collected through the cannula into heparinized plastic microcentrifuge tubes (1.5 ml) before administration, and 0.083, 0.25, 0.5, 1, 2, 4, 6, 10, 24 and 48 h (i.v.), and 0.5, 1, 2, 3, 4, 6, 8, 10, 24 and 48 h (oral) after administration, and then frozen at −20°C until assayed. The assays were performed within 3 d of collection.

CAM or EM (10 or 100 mg/kg) suspended in a 5% (w/v) arabic gum solution was administered orally 1 h before i.v. or oral administration of CYA.

Measurement of Blood CYA Concentrations Blood CYA concentrations were measured by means of a monochromatic fluorescence polarization immunonassay specific for CYA, with an automated TDX analyzer (Dainabot, Tokyo, Japan).25,26) Within-run and between-run variabilities for rat blood samples containing various amounts of CYA were within 3% and 7% (coefficient of variation), respectively. The limit of quantification was about 25 ng/ml.

Measurement of Gastric Emptying Gastric emptying was measured according to the method reported by

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Scarpignato et al.\textsuperscript{27} with several modifications. Briefly, a 5% (w/v) arabic gum suspension (vehicle), or CAM or EM (10 or 100 mg/kg) suspended in a constant volume (1 ml/kg) of the vehicle, was administered orally to unanesthetized rats 1 h before the oral administration of 1.5 ml of the pre-warmed meal (35°C) containing both 2.5% (w/v) phenol red and 2% (w/v) aqueous methylcellulose (400 cps). The rats were killed by cervical dislocation immediately or 40 min after administration of the meal, and then a standard stomach (100% phenol red in the stomach) or test stomach was exposed by laparotomy, quickly ligated at the pylorus and cardia, and then removed. The stomach was homogenized with a Polytron\textsuperscript{®} homogenizer (Model PT10TSKR; Kinematica AG) in 100 ml of 0.1 N NaOH. Five ml of the solution was added to 0.5 ml of a 20% (w/v) trichloroacetic acid solution, followed by vortexing for 1 min and then centrifugation for 20 min (2500 \times g). The supernatant (5 ml) was added to 4 ml of 0.5 N NaOH and then vortexed for 1 min. The absorption of the solution was then measured at 560 nm with a spectrophotometer (Model 200-20; Hitachi). Gastric emptying for each rat was calculated according to the following equation:

\[
\text{gastric emptying} = \frac{\text{amount of phenol red recovered from the test stomach}}{\text{average amount of phenol red recovered from a standard stomach}} \times 100
\]

Pharmacokinetic Analysis The peak concentration \((C_{\text{max}})\) and time to reach \(C_{\text{max}} (T_{\text{max}})\) were determined directly from the concentration–time profile. The area under the blood concentration–time curve after single bolus i.v. administration \((AUC_{i.v.})\) was calculated by the trapezoidal method from time zero to the final sampling time, and extrapolation of this time to infinity with the terminal elimination rate constant, which was determined by log-linear regression analysis of the last two or three concentration points. \(AUC_{p.o.}\) was calculated according to the trapezoidal rule from time zero to 48 h after oral administration of CYA. Total body clearance \((CL_t)\) was determined as the administered dose \((D)\) divided by \(AUC_{i.v.}\). The volume of distribution at steady-state \((V_{\text{ss}})\) was calculated as \(D \times AUMC_{i.v.}/(AUC_{i.v.})^2\). AUMC meaning the area under the first moment curve. The absolute bioavailability after oral administration \((F_{p.o.})\) was estimated as follows: \((AUC_{p.o.} \times D_{i.v.})/(AUC_{i.v.} \times (\text{CYA alone}) \times D_{p.o.}) \times 100\).

Statistical Analysis All data are expressed as means \(\pm\) S.E. Statistical analysis was performed by analysis of variance (ANOVA) with StatView J4.02 for Macintosh (Abacus Concepts Inc., Berkeley, CA), and differences were considered statistically significant at \(p \leq 0.05\).

RESULTS

Effect of CAM or EM on the Bioavailability of CYA after Single Bolus i.v. Administration Blood CYA concentration–time curves after single bolus i.v. administration of CYA alone (2 mg/kg), and in combination with CAM or EM (100 mg/kg) in rats are shown in Fig. 1. CYA disappeared from the systemic circulation with a three-exponential decay. The elimination of CYA from the blood decreased with the coadministration of CAM or EM. The pharmacokinetic parameters are listed in Table 1. \(CL_t\) (0.136 l/h/kg) and \(V_{\text{ss}}\) (1.741 l/kg) for the CYA alone group were similar to the values previously reported by Bernareggi and Rowland \((CL_t = 0.161 l/h/kg; V_{\text{ss}} = 2.671 l/kg)\)\textsuperscript{25} and Quijano et al. \((CL_t = 0.124 l/h/kg; V_{\text{ss}} = 2.13 l/kg)\).\textsuperscript{6} The \(AUC_{i.v.}\) and \(CL_t\) values for the CAM coadministration group were significantly increased and decreased, respectively, compared with those for the CYA alone group, but the \(V_{\text{ss}}\) value did not change significantly. In contrast, the coadministration of EM significantly increased the \(AUC_{i.v.}\) value and decreased the \(CL_t\) value. There was a significant difference in the \(V_{\text{ss}}\) value between the CYA alone and EM coadministration groups, while the \(AUC_{i.v.}\), \(CL_t\) and \(V_{\text{ss}}\) values were not significantly different between these two groups.

![Fig. 1. Blood CYA Concentration–Time Curves after Single Bolus i.v. Administration of CYA (2 mg/kg) Alone and in Combination with CAM or EM in Rats](image)

Each point represents the mean \(\pm\) S.E. \((n = 4–5)\). CAM or EM was administered orally 1 h before administration of CYA. ○, CYA alone; ▲, CYA + CAM (100 mg/kg); □, CYA + EM (100 mg/kg).

Table 1. Effects of CAM or EM (100 mg/kg) on Pharmacokinetic Parameters of CYA after Single Bolus i.v. Administration of CYA (2 mg/kg) in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(n)</th>
<th>(AUC_{i.v.}) ((\mu g\cdot h/ml))</th>
<th>(AUC_{i.v.}) ratio (%)</th>
<th>(CL_t) (l/h/kg)</th>
<th>(V_{\text{ss}}) (l/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYA alone</td>
<td>5</td>
<td>15.2 ± 1.4</td>
<td>100.0</td>
<td>0.136 ± 0.014</td>
<td>1.74 ± 0.19</td>
</tr>
<tr>
<td>CYA + CAM</td>
<td>4</td>
<td>26.4 ± 3.3\textsuperscript{b}</td>
<td>173.7</td>
<td>0.080 ± 0.010\textsuperscript{a}</td>
<td>1.33 ± 0.17</td>
</tr>
<tr>
<td>CYA + EM</td>
<td>4</td>
<td>30.3 ± 0.7\textsuperscript{b}</td>
<td>199.3</td>
<td>0.066 ± 0.002\textsuperscript{b}</td>
<td>1.08 ± 0.05\textsuperscript{a}</td>
</tr>
</tbody>
</table>

CAM or EM was administered orally 1 h before administration of CYA. Each value represents the mean \(\pm\) S.E. a) \(p < 0.05\), b) \(p < 0.01\) vs. CYA alone group (ANOVA).
Table 2. Effect of CAM or EM (10 or 100 mg/kg) on Pharmacokinetic Parameters of CYA after Single Oral Administration of CYA (10 mg/kg) in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of CAM or EM (mg/kg)</th>
<th>n</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$\text{AUC}_{p.o.}$ (μg h/ml)</th>
<th>$F_{p.o.}$ (%)</th>
<th>$F_{p.o.}$ ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYA alone</td>
<td>—</td>
<td>8</td>
<td>1.42 ± 0.08</td>
<td>3.6 ± 1.1</td>
<td>19.3 ± 1.0</td>
<td>25.4</td>
<td>100.0</td>
</tr>
<tr>
<td>CYA + CAM</td>
<td>10</td>
<td>4</td>
<td>1.09 ± 0.08</td>
<td>4.8 ± 1.4</td>
<td>17.0 ± 0.4</td>
<td>22.4</td>
<td>88.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9</td>
<td>0.82 ± 0.19</td>
<td>9.6 ± 3.0</td>
<td>18.8 ± 3.2</td>
<td>24.7</td>
<td>97.2</td>
</tr>
<tr>
<td>CYA + EM</td>
<td>10</td>
<td>3</td>
<td>1.43 ± 0.25</td>
<td>4.7 ± 1.8</td>
<td>24.6 ± 2.9</td>
<td>32.4</td>
<td>127.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>1.47 ± 0.10</td>
<td>9.0 ± 0.6</td>
<td>34.2 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.0</td>
<td>177.2</td>
</tr>
</tbody>
</table>

CAM or EM was administered orally 1 h before administration of CYA. Each value represents the mean ± S.E. *a* $p<0.05$ vs. CYA alone group (ANOVA).

Effect of CAM or EM on the Bioavailability of CYA after Single Oral Administration

Figure 2 shows mean blood CYA concentration–time curves after single oral administration of CYA alone (10 mg/kg), and in combination with CAM or EM (10 or 100 mg/kg) in rats. The range of blood CYA concentrations and the slope of the curve corresponding to the elimination phase after oral administration of CYA alone were similar to those after i.v. administration. Table 2 summarizes the pharmacokinetic parameters. The $C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_{p.o.}$, and $F_{p.o.}$ values after single oral administration of CYA alone were 1.42 μg/ml, 3.6 h, 19.3 μg·h/ml and 25.4%. The $F_{p.o.}$ value was almost the same as the values reported by Bernareggi and Rowland (24%)<sup>28</sup> and Quijano et al. (27%).<sup>6</sup> The $C_{\text{max}}$ and $T_{\text{max}}$ values tended to be lower and higher with increasing doses of CAM, respectively, although there were no significant differences. The $\text{AUC}_{p.o.}$ values for the CAM coadministration groups were comparable to that for the CYA alone group. In the case of coadministration of EM, the $C_{\text{max}}$ and $T_{\text{max}}$ values did not significantly change with increasing EM doses, but the latter value tended to be higher as in the case of CAM. The $\text{AUC}_{p.o.}$ value after coadministration of EM (100 mg/kg) was about twice larger than that after administration of CYA alone ($p<0.05$).

Effect of CAM or EM on Gastric Emptying

The effect of CAM or EM on gastric emptying in rats is shown in Fig. 3. The gastric emptying in the vehicle group was 80.3%. That in the groups treated with 10 and 100 mg/kg of CAM was 53.3 and 16.6%, respectively, and each value for these groups was significantly lower than that for the vehicle only group. Furthermore, EM also decreased gastric emptying, which was 65.5 and 40.9%, respectively, when rats were treated with 10 and 100 mg/kg of EM. Both CAM and EM were shown to delay gastric emptying in a dose-dependent manner. The gastric emptying in the group treated with CAM (100 mg/kg) was significantly lower than that with EM (100 mg/kg).

DISCUSSION

The elimination after i.v. administration of CYA (2 mg/kg) was lowered by EM (100 mg/kg, p.o.) coadministration (Fig. 1), and the $\text{AUC}_{i.v.}$ and $CL_i$ values for the EM coadministration group were significantly increased and decreased, respectively, compared with those for the CYA alone group (Table 1). Hedayat and Rowland<sup>29</sup> reported that EM extensively decreased the $CL_i$ value for CYA after i.v. administration of CYA in rats; our results agree with theirs.

Renal excretion is a minor pathway of elimination of CYA in humans and rats.<sup>40</sup> Only 2% of the i.v. dose is excreted as unchanged CYA in the urine in rats.<sup>30</sup> Therefore, the renal excretion pathway of CYA is thought to be little affected by other drugs such as CAM or EM. The major pathway of elimination of CYA after i.v. administration appears to be metabolism in the liver. CYA as a parent drug excreted in the bile is less than 1%, although the biliary excretion of radioactivity accounts...
for approximately 59% of the intravenously administered
$^3$H-labeled CYA.\(^{30}\) Furthermore, the biotransformation
of CYA has been demonstrated to be inhibited by EM
using an isolated perfused rat liver model,\(^{31}\) or rabbit
hepatocytes and microsomal fractions.\(^{32}\) It has been
shown that EM, like CYA, is a good substrate for
CYP3A.\(^{33}\) Accordingly, the significant decrease in the
$CL_t$ value was confirmed to be due to the inhibition of
CYA metabolism in the liver by EM. However, there was
a significant difference in the $V_{dss}$ value between the CYA
alone and EM coadministration groups. It has been
reported by Aoki et al.\(^{34}\) that the $V_{dss}$ value for CYA was
reduced during EM therapy in humans. The reason for
this decrease is unclear, although CYA is distributed
extensively in peripheral tissues.\(^{40}\) Further detailed study
should be undertaken to clarify this mechanism.

CAM (100 mg/kg, p.o.) also decreased the $CL_t$ value for
CYA administered intravenously (Table 1). It has been
reported that CAM, like EM, inhibits the metabolism of
terfenadine, which is a substrate of CYP3A, in human
liver in vitro, indicating that CAM may be one of
many CYP3A substrates.\(^{35}\) In addition, CAM has been
shown to form an inactive P450-metabolite complex in
glucocorticoid-pretreated rats.\(^{36}\) Based on these facts, it
is suggested that the inhibition of CYA metabolism in the
liver by CAM may be responsible for the significantly
decreased $CL_t$ value. The $AUC_{t-infty}$, $CL_t$ and $V_{dss}$ values
were not significantly different between the CAM and EM
coadministration groups, suggesting that the extent of
metabolic inhibition by CAM was nearly equal to that by
EM.

Next, we investigated the effect of CAM or EM (10 or
100 mg/kg) on the bioavailability of CYA after oral
administration of CYA (10 mg/kg) to rats (Fig. 2 and
Table 2). Each $AUC_{p.o.}$ value for the CAM and EM
coadministration groups, except the EM (100 mg/kg)
coadministration group (about 77% increase, $p<0.05$),
was comparable to that for the CYA alone group.
Furthermore, the $F_{p.o.}$ ratios (97.2 and 177.2%) for
CAM and EM coadministration (100 mg/kg) were about
one-half and nine-tenths larger than the $AUC_{t-infty}$ ratios
(173.7 and 199.3%, Table 1), respectively. These results
mean that CAM and EM have some prehepatic mechanism
of action by which the bioavailability of CYA after oral
administration is reduced. GER-suppressing drugs such
as atropine decrease its absorption.\(^{63}\) This is believed
due to the fact that CYA is degraded in the stomach or
greatly metabolized by CYP3A in the wall of the small
intestine.\(^{6-90}\)

We therefore determined whether or not CAM or EM
(10 or 100 mg/kg) decreased gastrointestinal motility by
measuring the gastric emptying of the standard meal
containing phenol red in rats. As shown in Fig. 3, both
drugs administered orally delayed gastric emptying in a
dose-dependent manner. The gastric emptying in the group
receiving CAM (100 mg/kg) was significantly lower than
that with EM (100 mg/kg), indicating that CAM reduced
the gastrointestinal motility and thus might increase the
contribution of the prehepatic first pass metabolism more
potently than EM in rats. For this reason, the $F_{p.o.}$ value
for CAM coadministration is speculated to be almost the
same as the value for the CYA alone group and to be
smaller than that for EM coadministration, which is
significantly greater than that for CYA alone group.
It is generally known that EM, which acts as a motilin
receptor agonist,\(^{37}\) stimulates the gastrointestinal motor
activity in dogs\(^{38}\) and humans.\(^{39}\) However, Minocha and
Galligan\(^{40}\) indicated that EM at high doses can have an
inhibitory action on intestinal motility in the guinea pig,
which is not mediated at motilin receptors. In addition,
oral administration of CAM (300 mg/kg) induced a
decrease in gastrointestinal motility in mice, although the
spontaneous motility of the stomach in anesthetized
rabbits was enhanced by CAM (1 mg/kg, i.v.).\(^{41}\) It was
speculated that both EM and CAM might show dual
actions on the gastrointestinal motility at different doses
or in different species.

In conclusion, it was suggested that CAM as well as
EM might affect the bioavailability of CYA by inhibiting
the metabolism of CYA, and simultaneously by changing
the gastrointestinal motility in rats. Thus, caution is
recommended when administering CAM concomitantly
with CAM to humans.

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REFERENCES AND NOTES

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Higashinada-ku, Kobe 658, Japan.

2) The Canadian Multicentre Transplant Study Group, N. Engl. J.


Pharmacokin., 11, 107 (1986).


6) Quijano R. F., Onishi N., Umeda K., Komada F., Iwakawa S.,

7) Kolars J. C., Schmiedlin-Ren P., Dobbins III W. O., Schuetz J.,


9) Kolars J. C., Stetsen P. L., Rush B. D., Ruvirt W. M.,
Schmiedlin-Ren P., Duell E. A., Voorhees J. J., Watkins P. B.,

650 (1988).

11) Combaltbert J., Fabre I., Fabre G., Dalet I., Derancourt J., Cano


14) First M. R., Schroeder T. J., Alexander J. W., Stephens G. W.,
Wakszticiel P., Myre S. A., Pease A. J., Transplant., 51, 365

15) Ohnishi N., Iwakawa S., Okumura K., Ota K., Kitagawa K.,
Hachiwaka H., Yoshikawa N., Nakamura H., Miyazaki J., Yasuno
H., Matsumoto O., Kaminodono S., Jpn. J. Ther. Drug Monit., 9,

16) Wadzwa N. K., Schroeder T. J., O’Flaherty E., Pesce A. J., Myre

17) Finet L., Westeel P. F., Hary L., Maurel G.,Andrejka M., Dupas