Impact of Curcumin-Induced Changes in P-Glycoprotein and CYP3A Expression on the Pharmacokinetics of Peroral Celiprolol and Midazolam in Rats

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ABSTRACT:

The aim of this study was to evaluate whether curcumin could modulate P-glycoprotein (P-gp) and CYP3A expression, and in turn modify the pharmacokinetic profiles of P-gp and CYP3A substrates in male Sprague-Dawley rats. Intragastric gavage of the rats with 60 mg/kg curcumin for 4 consecutive days led to a down-regulation of the intestinal P-gp level. There was a concomitant up-regulation of hepatic P-gp level, but the renal P-gp level was unaffected. Curcumin also attenuated the CYP3A level in the small intestine but induced CYP3A expression in the liver and kidney. Regular curcumin consumption also caused the C_{max} and area under the concentration-time curve (AUC_{0-4} and total AUC) of peroral celiprolol (a P-gp substrate with negligible cytochrome P450 metabolism) at 30 mg/kg to increase, but the apparent oral clearance (CL_{oral}) of the drug was reduced. Similarly, rats treated with curcumin for 4 consecutive days showed higher AUC (AUC_{0-4} and total AUC) and lower CL_{oral} for peroral midazolam (a CYP3A substrate that does not interact with the P-gp) at 20 mg/kg in comparison with vehicle-treated rats. In contrast, curcumin administered 30 min before the respective drug treatments did not significantly modify the pharmacokinetic parameters of the drugs. Analysis of the data suggests that the changes in the pharmacokinetic profiles of peroral celiprolol and midazolam in the rat model were contributed mainly by the curcumin-mediated down-regulation of intestinal P-gp and CYP3A protein levels, respectively.

Food-drug interactions, including those between herbs and drugs, have become a major concern in recent years. This may be attributed to a greater awareness as more research and therefore publicity is generated in this area (John et al., 1999; Piscitelli et al., 2002), as well as the growing popularity of herbs as complementary medicines (Tindle et al., 2005). Drug interactions have been reported with some herbal medicines and supplements, such as St. John’s wort (Hypericum perforatum) (John et al., 1999) and garlic (Allium sativum) (Piscitelli et al., 2002). St. John’s wort, in particular, has been relatively well researched, with the chronic intake of St. John’s wort shown to reduce the oral bioavailability of drugs through an up-regulation of the intestinal CYP3A4 and P-glycoprotein (P-gp) levels (Dürr et al., 2000; Mai et al., 2004).

Curcumin is a natural phenolic coloring component in Curcuma longa, usually called turmeric, which has long been widely used as a spice and medicinal agent in traditional Chinese and Indian medicines (Ammon and Wahl, 1991). In recent years, curcumin has attracted the attention of Western medical practitioners because of its potential application as a relatively safe therapeutic adjuvant for cancer patients (Sharma et al., 2001; Cruz-Correa et al., 2006). On this basis, Phase I clinical trials of curcumin in cancer patients have been initiated in the United Kingdom (Sharma et al., 2004) and Taiwan (Cheng et al., 2001). Given its regular consumption in certain populations, curcumin could also give rise to drug interactions because it has been reported to inhibit both the function and expression of the P-gp in human KB cells (Anuchapreeda et al., 2002). To date, however, there has been no report on whether curcumin could modulate the pharmacokinetics of drugs that are P-gp substrates, nor has any study correlated curcumin consumption to tissue P-gp expression. Moreover, the effects of curcumin on the CYP3A enzymes remain unknown. Given the colocalization and overlapping substrate specificities of the P-gp and CYP3A (Wacher et al., 1995), it will not be surprising for curcumin to also modulate the activity and expression of the CYP3A enzymes.

The objective of this study was to evaluate whether curcumin, when consumed on a regular basis, was capable of influencing the pharmacokinetic parameters of peroral P-gp and CYP3A4 substrates. To examine the basis for the interactions, we measured the P-gp and CYP3A proteins in the small intestine, liver, and kidney of male Sprague-Dawley (SD) rats following curcumin consumption over 4 days. Celiprolol, a P-gp substrate that is negligibly metabolized by CYP3A (Karlsson et al., 1993; Lilja et al., 2003), and midazolam, a

ABBREVIATIONS: P-gp, P-glycoprotein; SD, Sprague-Dawley; HPLC, high-performance liquid chromatography; AUC, area under the concentration curve; PXR, pregnane X receptor.
CYP3A substrate that is not a substrate of the P-gp (Kim et al., 1999; Galetin et al., 2005), were used as model drugs.

Materials and Methods

Animals. Healthy male SD rats (220–250 g) were purchased from the Laboratory Animals Centre, National University of Singapore. Rats were housed in cages at the animal holding unit under controlled temperature (23–24°C) and a 12-h dark/light cycle. All the animal procedures were approved by the Animal Ethics Committee of the National University of Singapore.

Analysis of P-gp and CYP3A Protein Expression. Rats were randomly divided into two groups with four or five rats in each group. Rats in the treated group were gavaged (16-gauge gavage needle, Kent Scientific, Torrington, CT) once daily with curcumin (Sigma-Aldrich, St. Louis, MO) at 60 mg/kg/day (1% Tween 80 as vehicle) for 4 consecutive days. Rats in the control group were similarly gavaged with the equivalent volume (about 1 ml) of vehicle. Animals were allowed free access to food and water but were fasted overnight before sacrificing to reduce the intestinal content. On day 5, rats were euthanized by i.p. injection with 30 mg/kg pentobarbital, and the small intestine, liver, and kidney were excised, snap-frozen in liquid nitrogen, and stored at −80°C until analysis.

Samples for protein analysis were homogenized (50 mg of small intestine, 30 mg of liver, or 30 mg of kidney) in freshly prepared ice-cold lysis buffer using a pellet pestle (Sigma, St. Louis, MO) and then solubilized in lysis buffer consisting of phosphate-buffered saline with 1% Triton X-100 and 1 mM each of phenylmethylsulfonyl fluoride, dithiothreitol, and protease inhibitor (Roche Diagnose, Basel, Switzerland). The lysates were centrifuged at 10,000 × g for 20 min at 4°C (MIKRO 22R, Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany), and the supernatant was collected and stored at −80°C until use.

Protein in the supernatant was subjected to the Bio-Rad (Hercules, CA) protein assay, and known quantities of albumin, similarly processed, were used as reference standards. Protein samples equivalent to 40 μg of protein were size-fractionated by electrophoresis on a 7.5% SDS-polyacrylamide gel (Mini-PROTEAN 3 System, Bio-Rad) at 150 V for 1 h and transferred to polyvinylidene difluoride membranes, which were blocked by overnight incubation in a buffer containing 5% nonfat milk, 200 mM NaCl, 50 mM Tris, and 0.1% Tween 20. The membranes were then probed with either the monoclonal anti-P-gp antibody C219 (1:1000, Signet Laboratories, Dedham, MA), anti-CYP3A2 polyclonal antibody (1:1000, BD Gentest, San Jose, CA), or anti-β-actin antibody as control (1:5000, Sigma) for 2 h. After washing thrice with Tris-buffered saline/Tween 20, each washing lasting 10 min, the membranes were incubated with secondary antibody labeled with horseradish peroxidase (Mini-PROTEAN 3 System). Western blot was also probed with another P-gp antibody, the C494 (1:1000, Rockford, IL), whereas the P-gp and CYP3A proteins were detected with the Supersignal West Femto CYP3A (Pierce, Rockford, IL), respectively, for 1 h at room temperature. The Western blots were incubated with secondary antibody labeled with horseradish peroxidase (Mini-PROTEAN 3 System). Western blot was also probed with another P-gp antibody, the C494 (1:1000, Signet Laboratories), which is not known to cross-react with this protein (Galetin et al., 2005), were used as model drugs.

Statistical Analysis. Statistical analysis of protein expression data was performed using the Student’s t-test, whereas the analysis of pharmacokinetic data were determined using the standard noncompartmental methods (WinNonlin 1.1, Pharsight, Mountain View, CA).
Parameters was conducted using one-way analysis of variance, with the Tukey test applied for comparison of means (SPSS 10.0, SPSS Inc., Chicago, IL). A $p \leq 0.05$ was considered statistically significant.

Results

Effects of Curcumin on P-gp and CYP3A Protein Levels in Rodent Tissues. In the present study, we showed that curcumin consumption could cause the P-gp and CYP3A to be differentially regulated in the small intestine, liver, and kidney of male SD rats. After 4 days of treatment, there were no significant differences in mean body weight between the vehicle- and curcumin-treated rats (data not shown). P-gp protein levels in the intestinal, hepatic, and renal crude membranes isolated from the rats were determined by Western blot analysis using the C219 and C494 antibodies, with $\beta$-actin as control. The C219 anti-P-gp antibody recognized a protein band of about 170 kDa, consistent with the known molecular mass of P-gp. Compared with vehicle-treated rats, rats dosed with 60 mg/kg/day curcumin showed a 49% decrease in P-gp protein level in the small intestine after 4 days ($p = 0.02$), whereas the hepatic P-gp level was increased by 144% ($p = 0.01$). There was no change in the renal P-gp level ($p = 1.00$). Probing the hepatic P-gp protein with C494 antibody yielded comparable results as the C219 antibody (Fig. 1).

CYP3A protein level in the rodent tissues was determined by Western blot analysis using the polyclonal anti-rat CYP3A2 antibody. This antibody recognized one band of about 55 kDa in the liver tissues, but more than one band was detected for the small intestine and kidney tissues. Intestinal CYP3A protein level was observed to decrease by 42% after 4 days of treatment with 60 mg/kg/day curcumin (Fig. 2, $p = 0.04$), whereas the hepatic CYP3A protein level was increased by 91% ($p = 0.05$). Unlike the renal P-gp, the renal CYP3A protein level was enhanced by 41% after the curcumin treatment (Fig. 2, $p = 0.02$).

Effects of Curcumin on Celiprolol Pharmacokinetics in Rats. Plasma celiprolol concentration-time profiles obtained after p.o. administration of 30 mg/kg celiprolol to rats in the control, coadministered, and treatment groups are shown in Fig. 3. The corresponding
pharmacokinetic parameters are summarized in Table 1. Compared with rats in the control group, rats treated with curcumin for 4 days before drug administration showed significantly higher \( C_{\text{max}} \) and AUC values. The \( C_{\text{max}} \), \( AUC_{0-\text{t}} \), and total AUC of celiprolol were, respectively, 1.9- (\( p = 0.01 \)), 1.6- (\( p = 0.01 \)), and 1.3-fold (\( p = 0.02 \)) greater for rats in the treated group. However, \( CL_{\text{oral}} \) value for celiprolol in the treatment group was 22% lower than the \( CL_{\text{oral}} \) value for the control group (\( p = 0.01 \)). By contrast, rats administered with curcumin 30 min before celiprolol treatment (coadministered group) did not have significantly different \( C_{\text{max}} \) (\( p = 0.95 \)), \( AUC_{0-\text{t}} \) (\( p = 0.83 \)), total AUC (\( p = 0.19 \)), and \( CL_{\text{oral}} \) (\( p = 0.06 \)) values compared with rats in the control group. Significant differences in \( C_{\text{max}} \) (\( p = 0.01 \)) and \( AUC_{0-\text{t}} \) (\( p = 0.04 \)) were observed between rats in the coadministered and treatment groups, but there were no significant differences in total AUC (\( p = 0.32 \)) and \( CL_{\text{oral}} \) (\( p = 0.48 \)) between these two groups. The \( t_{\text{max}} \) value was not significantly different among the three groups (Table 1, \( p > 0.05 \)).

Effects of Curcumin on Midazolam Pharmacokinetics in Rats.

Plasma midazolam concentration-time profiles obtained after p.o. administration of 20 mg/kg midazolam to rats in the control, coadministered, and treatment groups are shown in Fig. 4, and the pharmacokinetic parameters are summarized in Table 2. The \( t_{\text{max}} \) value of midazolam was not significantly different among the rats in the three groups (\( p > 0.05 \)). However, rats in the treatment group showed higher AUC\(_{0-4} \) (2.6-fold, \( p = 0.04 \)) and total AUC (3.8-fold, \( p = 0.03 \)) values, as well as lower \( CL_{\text{oral}} \) (75% lower, \( p = 0.02 \)) values, compared with control. \( C_{\text{max}} \) of midazolam was not significantly different (\( p = 0.10 \)) between the treatment and control groups. In comparison, rats in the coadministered group did not show significantly different \( C_{\text{max}} \) (\( p = 0.17 \)), \( AUC_{0-4} \) (\( p = 0.29 \)), total AUC (\( p = 0.79 \)), and \( CL_{\text{oral}} \) values (\( p = 0.15 \)) from rats in the control group, nor were there significant differences in the \( t_{\text{max}} \) (\( p = 0.94 \)), \( AUC_{0-4} \) (\( p = 0.38 \)), total AUC (\( p = 0.10 \)), and \( CL_{\text{oral}} \) Values (\( p = 0.33 \)) between rats in the coadministered and treatment groups.

Discussion

The overlapping tissue distribution of CYP3A and P-gp, as well as the broad spectrum of drugs that interact with both proteins, has presented significant challenges to drug absorption and delivery to the systemic circulation (Yasuda et al., 2002). Moreover, the interaction of coadministered drugs with CYP3A and P-gp in the gut can lead to major drug-drug interactions (Yasuda et al., 2002). Given the substantial overlaps in substrate, inducer, and inhibitor specificities between the P-gp and CYP3A, it is important to understand the relative contribution of CYP3A and P-gp to specific drug interactions (Wacher et al., 1995; Yasuda et al., 2002). Inhibition and induction of the CYP3A have been shown to, respectively, reduce and enhance the metabolism of drugs that are CYP3A substrates, and to consequently alter their pharmacokinetic profiles (Luo et al., 2003; Chaobal and Kharasch, 2005). Conversely, the inhibition and induction of P-gp would have opposite effects on the fate of coadministered drugs that are P-gp substrates (Luo et al., 2003; Gurley et al., 2006).

Our results show that curcumin was a modulator of P-gp and CYP3A expression in the rat. Significantly, the curcumin administered p.o. was capable of not only changing the P-gp and CYP3A protein levels in the rodent intestine, but also it modified the P-gp and CYP3A levels in the rodent liver and kidney. Curcumin administered p.o. is known to be absorbed into the systemic circulation in rats.

### Table 1

**Pharmacokinetic parameters of celiprolol in rat plasma in control, coadministered, and treatment groups following a p.o. dose of 30 mg/kg**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Coadministered</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>492.65 ± 33.21</td>
<td>458.21 ± 78.05</td>
<td>960.14 ± 93.39*</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (min)</td>
<td>120.0 ± 0.0</td>
<td>108.0 ± 6.6</td>
<td>120.0 ± 0.0</td>
</tr>
<tr>
<td>( AUC_{0-\text{t}} ) (ng/ml × h)</td>
<td>2140.04 ± 187.87</td>
<td>2347.63 ± 298.83</td>
<td>3440.26 ± 210.45*</td>
</tr>
<tr>
<td>Total AUC(^*) (ng/ml × h)</td>
<td>3570.91 ± 106.26</td>
<td>4114.26 ± 103.22</td>
<td>4582.18 ± 382.65*</td>
</tr>
<tr>
<td>( CL_{\text{oral}} ) (ml/h)</td>
<td>2.36 ± 0.07</td>
<td>2.02 ± 0.06</td>
<td>1.85 ± 0.15**</td>
</tr>
</tbody>
</table>

* \( p \leq 0.05 \) significant difference compared with control.

* Total AUC, area under concentration curve extrapolated to infinity.

### Table 2

**Pharmacokinetic parameters of midazolam in rat plasma in control, coadministered, and treatment groups following a p.o. dose of 20 mg/kg**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Coadministered</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>123.21 ± 14.82</td>
<td>239.71 ± 46.82</td>
<td>259.20 ± 38.71</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (min)</td>
<td>28.8 ± 5.9</td>
<td>28.8 ± 4.7</td>
<td>32.5 ± 5.8</td>
</tr>
<tr>
<td>( AUC_{0-\text{t}} ) (ng/ml × h)</td>
<td>255.06 ± 27.40</td>
<td>470.01 ± 88.36</td>
<td>657.02 ± 135.59*</td>
</tr>
<tr>
<td>Total AUC(^*) (ng/ml × h)</td>
<td>476.88 ± 109.50</td>
<td>776.18 ± 94.58</td>
<td>1835.40 ± 422.13*</td>
</tr>
<tr>
<td>( CL_{\text{oral}} ) (ml/h)</td>
<td>13.40 ± 3.21</td>
<td>7.59 ± 0.76</td>
<td>3.33 ± 0.66*</td>
</tr>
</tbody>
</table>

* \( p \leq 0.05 \) significant difference compared with control.

* Total AUC, area under concentration curve extrapolated to infinity.
(Ravindranath and Chandrasekhara, 1980) and humans (Sharma et al., 2001). However, it is reported to have a relatively low oral bioavailability of about 60% (Ravindranath and Chandrasekhara, 1980) because of extensive metabolism by human phenol sulfotransferase isoenzymes (SULT1A1 and SULT1A3) and equine alcohol dehydrogenase (Ireson et al., 2002). Nevertheless, peroral curcumin has been shown in this study to exert both local and systemic effects on protein expression when it was administered to the rats at a dose of 60 mg/kg/day for 4 days. The cumulative absorption of curcumin into the general circulation was adequate to modify the expression of P-gp and CYP3A in the hepatic and renal tissues.

Data in this study also implicate the presence of differential regulatory mechanisms for protein expression in the rodent organs. Curcumin concomitantly suppressed the P-gp and CYP3A protein levels in the rodent intestine yet up-regulated the expression of these proteins in the rodent liver. Moreover, the P-gp and CYP3A levels in the liver and intestine were not coordinately regulated, which is consistent with the findings of Lown et al. (1994), who showed that the hepatic CYP3A4 activity in humans did not correlate with the intestinal CYP3A4 activity and protein content. Such tissue-specific response of P-gp and/or CYP3A expression to treatment has been reported by other researchers (Hartmann et al., 2005) and is thought to facilitate the removal of potentially toxic substances in abnormal states where the excretory capacity of a specific organ is diminished. There is evidence that curcumin may modulate the transcription of CYP3A through the pregnane X receptor (PXR) (Liu et al., 2006). Because the activation of PXR is known also to influence the expression of the P-gp protein (Synold et al., 2001), the regulating effect of curcumin on P-gp expression may well occur through its regulation of the PXR.

Pharmacokinetic profiles of celiprolol and midazolam were different in rats fed with 60 mg/kg/day curcumin for 4 days compared with control rats. Such pharmacokinetic modulations might be attributed to changes in the function and/or expression of proteins that participate in the transport and metabolism of the drugs. Indeed, curcumin has been shown to inhibit P-gp efflux activity in vitro (Anuchapreeda et al., 2002), and studies in our laboratory have indicated that it also attenuates the activity of CYP3A enzymes in liver microsomes. However, the administration of a single dose of curcumin 30 min before drug treatment did not modify the pharmacokinetic profiles of either celiprolol or midazolam, indicating that the pharmacokinetic changes were unlikely to be contributed by modifications in protein function. Rather, they were caused by the curcumin-mediated changes in tissue P-gp and CYP3A levels.

C\text{max}, AUC\text{0–8}, and total AUC of celiprolol, a P-gp substrate, were increased, whereas its systemic clearance was reduced in rats fed with curcumin for 4 days. These findings were in agreement with another study, in which the inhibition of intestinal P-gp by clarithromycin in humans caused the C\text{max} and AUC of digoxin, another P-gp substrate, to increase (Gurley et al., 2006). The inhibition of renal P-gp by clarithromycin also reduced the CL\text{oral} of digoxin (Gurley et al., 2006), which is largely eliminated by renal excretion (Doherty et al., 1977). Celiprolol, on the other hand, is excreted equally into the urine and the bile (Pruss et al., 1988). Its clearance via the kidney was unlikely to be significantly affected by curcumin, which did not alter the renal P-gp protein level. The hepatic P-gp also appeared to play a minor role in influencing the pharmacokinetics of celiprolol. This is because curcumin mediated an up-regulation of the hepatic P-gp level, whereas a down-regulation of hepatic P-gp level would be more in line with the observed increased C\text{max} and AUC of celiprolol and its decreased systemic clearance via biliary elimination (Hartmann et al., 2005). Therefore, it may be concluded that the down-regulation of intestinal P-gp level by curcumin was influential in increasing the oral bioavailability of celiprolol. By its effect on the enterohepatic circulation of the drug, the lower P-gp level could also reduce the systemic clearance of celiprolol. The importance of the intestinal P-gp, relative to those in the liver and kidney, in influencing drug bioavailability is not unusual and has been shown by Greiner et al. (1999) for digoxin and by Lown et al. (1997) for cyclosporine.

Curcumin consumed over 4 consecutive days also caused the AUC of midazolam to increase, whereas the CL\text{oral} of midazolam was reduced. These results are consistent with the changes in CYP3A level in the rodent tissues. Midazolam is almost entirely eliminated by CYP3A-mediated metabolism (Kronbach et al., 1989), and because it is not a substrate of P-gp (Kim et al., 1999), its pharmacokinetic profile would not be affected by the coregulation of P-gp by curcumin. Pharmacokinetic studies have consistently associated an up-regulation of hepatic and/or intestinal CYP3A level with decreased oral bioavailability and increased systemic clearance of CYP3A substrates (Dürr et al., 2000; Dresser et al., 2003; Mai et al., 2004). Conversely, the down-regulation of CYP3A in either tissue would have opposite consequences. Clinical studies have further shown midazolam to have lower AUC following intestinal and hepatic CYP3A induction by rifampin and higher AUC after intestinal and hepatic CYP3A inhibition by troleandomycin (Chaubal and Karhas, 2005). Although CYP3A in the kidney may also contribute to drug metabolism, the up-regulation of renal CYP3A expression by curcumin is not expected to play an important role in the CL\text{oral} of midazolam. Despite the plethora of absorption, distribution, metabolism, and excretion studies on midazolam, none to date has implicated renal metabolism as a significant route for the systemic clearance of midazolam. In the present study, curcumin caused a 91% up-regulation of hepatic CYP3A protein level compared with a 41% elevation of renal CYP3A. This makes it even less likely for renal metabolism to exert a predominant influence on the systemic clearance of midazolam following spice administration. Taken together, the data in this study suggest that the curcumin-mediated down-regulation of the intestinal CYP3A protein level was the main contributing factor for the increased oral bioavailability and decreased systemic clearance of midazolam.

In summary, curcumin-induced regulation of P-gp and CYP3A expression in the rat tissues can cause changes to the pharmacokinetic profiles of celiprolol, a P-gp substrate, and midazolam, a CYP3A substrate. The implication is that the regular consumption of curcumin by certain populations could probably influence the pharmacokinetics of drugs that are P-gp and/or CYP3A substrates. Our data suggest that it is imperative to conduct quantitative evaluation of curcumin-drug interactions in human subjects to provide a better understanding of the effects of curcumin on population pharmacokinetics. It will also allow for predictive clinical intervention to be made so that undesirable curcumin-drug interactions can be avoided in susceptible populations.

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References


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