

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

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Abbreviations: P-gp, P-glycoprotein; CYP3A, cytochrome P450 3A; SD, Sprague-Dawley; *ABCB1*, ATP-binding cassette protein B1 gene; AUC, area under the concentration curve; C_{\max} , maximum plasma concentration; CL_{oral} , apparent oral clearance; t_{\max} , time of maximum plasma concentration; HPLC, high performance liquid chromatography; ADME, absorption, distribution, metabolism and excretion.

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ABSTRACT

The aim of this study was to evaluate whether curcumin could modulate P-gp and CYP3A expression, and in turn modify the pharmacokinetic profiles of P-gp and CYP3A substrates in the male SD rats. Intragastric gavage of the rats with 60 mg/kg of 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. The regular curcumin consumption also caused the C_{\max} and area under the concentration-time curve (AUC_{0-8} and total AUC) of peroral celiprolol (a P-gp substrate with negligible CYP 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.

INTRODUCTION

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 (Johne 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*) (Johne 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-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 UK (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, since 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 which are P-gp substrates. Neither has any study correlates curcumin consumption to tissue P-gp expression. Moreover, the effects of curcumin on the CYP3A enzymes remain unknown. Given the co-localization and overlapping substrate specificities of the P-gp and CYP3A (Wacher et al., 1995), it will not

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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 CYP3A substrate that is not a substrate of the P-gp (Kim et al., 1999; Galetin et al., 2005), were used as model drugs.

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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 12-h dark-light cycle. All 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 4-5 rats in each group. Rats in the treated group were gavaged (16 gauge gavage needle, Kent Scientific, Torrington, CT, USA) once daily with curcumin (Sigma-Aldrich, St. Louis, USA) 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 scarification 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, USA), then solubilized in lysis buffer for 20 min. The lysis buffer consisted of phosphate-buffered saline (PBS) with 1% of Triton X-100 and 1 mM each of PMSF, DTT and protease inhibitor (Roche Diagnosis, Basel, Switzerland). The lysates were centrifuged at 10,000 x g for 20 min at 4°C (MIKRO 22R, Andreas Hettich GmbH & Co KG, Tuttlingen, Germany) and the supernatant was collected and stored at -80°C till use.

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Protein in the supernatant was quantified by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA) and known quantities of albumin, similarly processed, were used as reference standards. Protein samples equivalent to 40 μ g of protein were size-fractionated by electrophoresis on a 7.5% SDS-polyacrylamide gel (PAGE) (Mini-PROTEAN 3 System, Bio-Rad Laboratories, Hercules, CA, USA) at 150 V for 1 h, and transferred to PVDF membranes, which were blocked by overnight incubation in a buffer containing 5% of nonfat milk, 200 mM of NaCl, 50 mM of Tris and 0.1% of Tween 20 (TBST). The membranes were then probed with either the monoclonal anti-P-gp antibody, C219 (1:1000, Signet Laboratories, Dedham, MA, USA), anti-CYP3A2 polyclonal antibody (1:1000, BD Gentest, San Jose, CA, USA), or anti- β -actin antibody as control (1:5000, Sigma, St. Louis, MO, USA) for 2 h. After washing thrice with TBST, each washing lasting 10 min, the membranes were incubated with secondary antibody labeled with horseradish peroxidase (1:10,000) for P-gp, β -actin (Amersham Biosciences, Piscataway, NJ, USA), and CYP3A (Pierce, Rockford, IL, USA), respectively, for 1 h at room temperature. The P-gp and CYP3A proteins were detected with the Supersignal west femto chemiluminescence system (Pierce, Rockford, IL, USA) while the β -actin protein was detected with the Supersignal west pico chemiluminescence system (Pierce, Rockford, IL, USA). Bands were visualized in X-ray film, and the band density was scanned and quantified using Quantity One 4.5.2 image analysis software (Bio-Rad, Hercules, CA, USA). P-gp and CYP3A protein band intensities were normalized to the intensities of the β -actin protein band. Since C219 is known to cross-react with the gene product of *mdr2* and this protein is highly expressed in the rat liver, the hepatic P-gp Western blot was also probed with another P-gp antibody, the C494 (1:1000, Signet Laboratories, Dedham, MA, USA), which is not known to cross-react with this protein (Warrington et al., 2004). Findings were similar using this antibody.

Pharmacokinetic studies

Rats were randomly divided into three groups, with 5 rats in each group. Rats assigned to the treatment group were gavaged (16 gauge gavage needle, Kent Scientific, Torrington, CT, USA) with 60 mg/kg/day (1% Tween 80 as vehicle) of curcumin for 4 consecutive days. Tween 80 was added to the vehicle to ensure curcumin dissolution and accurate dosing. Rats in the co-administered and control groups were similarly administered with equivalent volume (about 1 ml) of the 1% Tween 80 vehicle, taking into consideration reports that suggest Tween 80 to have inhibitory effects on the function of P-gp and CYP3A (Mountfield et al., 2000; Zhang et al., 2003). All rats were allowed free access to food and water. On day 5, rats in the control group continued to receive only the vehicle but those in the co-administered and treatment groups were given 60 mg/kg of curcumin. Half an hour later, rats in all 3 groups were gavaged with 30 mg/kg of celiprolol (Nycomed Pharma, Vienna, Austria) dissolved at 5 mg/ml in distilled water. At 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min following celiprolol administration, blood samples of about 120 μ l were drawn from the saphenous vein and collected into heparinized microcuvettes (CB300, Sarstedt AG & Co., Nümbrecht, Germany). Blood samples were centrifuged for 10 min at 10,000 x g at 4°C to obtain plasma samples, which were frozen at -80°C until analysis.

The experimental protocols for the pharmacokinetic study of midazolam were similar to those described for celiprolol, except that the rats were dosed with midazolam. Dormicum®, an injection containing 5 mg/ml of midazolam (Roche Diagnosis, Basel, Switherland), was administered to the rats by gavage at 20 mg/kg. Blood (~120 μ l) was sampled from the saphenous vein at 0, 5, 10, 20, 30, 45, 60, 90, 120, and 240min after administration, and the plasma samples generated from the blood samples were stored at -80°C until analysis.

Doses of 30 mg/kg for celiprolol and 20 mg/kg for midazolam were chosen based on literature values. The celiprolol dose emulated the dose used in another pharmacokinetic study in SD rats to evaluate its role as a P-gp substrate (Cornaire et al., 2004). For midazolam, oral doses in the range of 15 to 20 mg/kg were often used to examine its role as a CYP3A substrate in the SD rat model (Kotegawa et al., 2002; Kanazu et al., 2005). The upper dose limit of 20 mg/kg was applied for this study.

HPLC analysis of plasma celiprolol and midazolam

Methods for the assay of plasma celiprolol and midazolam concentrations were adapted from those described by Caudron et al. (Caudron et al., 2004) and ter Horst et al. (ter Horst, et al., 2003), respectively. Propranolol and norclomipramine (both from Sigma) were the respective internal standards. Briefly, the plasma sample (50 μ l) was mixed with 0.5 μ l of internal standard solution (100 μ g/ml in methanol) before the addition of 50 μ l of acetonitrile to precipitate the plasma proteins. After vortex mixing, the mixture was centrifuged at 10,000 \times g at 4°C for 10 min, and 20 μ l of the supernatant was subject to HPLC analysis. The HPLC system consisted of an Agilent 1100 system (Agilent Technologies, Palo Alto, CA, USA) with multiple-wavelength detector and a C₁₈ column (200 mm \times 4.6 mm, 5 μ m) (Waters, Milford, MA, USA) preceded by a C₁₈ guard column (Waters). Isocratic elution was performed for both drugs at a flow rate of 1.0 ml/min. Mobile phase for celiprolol analysis consisted of 27% of acetonitrile and 73% of buffer solution (20 mM KH₂PO₄, pH 3.8) while that for midazolam analysis consisted of 36% of acetonitrile and 64% of buffer. The detection wavelengths of 231 and 250 nm were employed for celiprolol and midazolam, respectively. The HPLC peaks were recorded and integrated using the Agilent data analysis software.

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The HPLC was calibrated with celiprolol and midazolam in the concentration range of 0.05-3.2 $\mu\text{g/ml}$. The standard solutions were prepared by diluting stock solutions (drug and internal standard dissolved in methanol at 1 mg/ml) with blank plasma. The intraday and interday coefficients of variation for celiprolol analysis were less than 9% and the lower limit of quantification was 50 ng/ml. The average recovery for celiprolol was 106% over the concentration range. Typical retention times recorded for celiprolol and propranolol were 5.5 and 12.5 min, respectively. The intraday and interday coefficients of variation for midazolam analysis were less than 10% and the lower limit of quantification was 50 ng/ml. The average recovery for midazolam was 103% over the concentration range. Typical retention times recorded for midazolam and norclomipramine were 11.9 and 18.5 min, respectively.

Pharmacokinetic analysis

Celiprolol and midazolam pharmacokinetics data were determined using the standard noncompartmental methods (*Win-Nonlin 1.1*, Pharsight, Mountain View, CA, USA). Area under the plasma concentration-time curves of celiprolol from 0 to 8 h (AUC_{0-8}) and midazolam from 0 to 4 h (AUC_{0-4}) were determined by use of the trapezoidal rule. The terminal elimination rate constant (k_e) was calculated using the slope of the log-linear regression of the terminal elimination phase. Area under the plasma concentration versus time curve from zero to infinity ($\text{AUC}_{0-\infty}$) was calculated using the trapezoidal rule up to the last measured time concentration (C_{last}) with extrapolation to infinity using C_{last}/k_e . The apparent oral clearance (CL_{oral}) was calculated as $\text{dose}/\text{AUC}_{0-\infty}$. Peak plasma drug concentrations (C_{max}) and the times when they occurred (t_{max}) were derived directly from the plasma concentration versus time curves.

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Statistical analysis

Statistical analysis of protein expression data was performed using the Student's *t*-test, while the analysis of pharmacokinetic parameters was conducted using One-way ANOVA, with the Tukey's test applied for comparison of means (SPSS 10.0, SPSS Inc., Chicago, IL). A *p* value ≤ 0.05 was considered statistically significant.

RESULTS

Effects of curcumin on P-gp and CYP 3A protein levels in rodent tissues

In the present study, we demonstrated 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 β -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 of curcumin showed a 49% decrease in P-gp protein level in the small intestine after 4 days ($p = 0.02$), while 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 of curcumin (FIG. 2, $p = 0.04$), while 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 oral administration of 30 mg/kg of celiprolol to rats in the control, co-administered 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 prior to drug administration showed significantly higher C_{\max} and AUC values. The C_{\max} , AUC_{0-8} 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. CL_{oral} value for celiprolol in the treatment group was, however, 22% lower than the CL_{oral} value for the control group ($p = 0.01$). By contrast, rats administered with curcumin 30 min prior to celiprolol treatment (co-administered group) did not have significantly different C_{\max} ($p = 0.95$), AUC_{0-8} ($p = 0.83$), total AUC ($p = 0.19$) and CL_{oral} ($p = 0.06$) values compared to rats in the control group. Significant differences in C_{\max} ($p = 0.01$) and AUC_{0-8} ($p = 0.04$) were observed between rats in the co-administered and treatment groups, but there were no significant differences in total AUC ($p = 0.32$) and CL_{oral} ($p = 0.48$) between these two groups. The t_{\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 oral administration of 20 mg/kg of midazolam to rats in the control, co-administered and treatment groups are shown in FIG. 4 and the pharmacokinetic parameters are summarized in TABLE 2. The t_{\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, and lower CL_{oral} (75% lower, $p = 0.02$) values, compared to control. C_{\max} of midazolam was not significantly different ($p = 0.10$) between the treatment and control groups. In comparison, rats in the co-administered group did not show significantly different C_{\max} ($p = 0.17$), AUC_{0-4} ($p = 0.29$), total AUC ($p = 0.79$) and CL_{oral} values ($p = 0.15$) from rats in the control group. Neither were there significant

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differences in the C_{\max} ($p = 0.94$), AUC_{0-4} ($p = 0.38$), total AUC ($p = 0.10$) and CL_{oral} values ($p = 0.33$) between rats in the co-administered and treatment groups.

DISCUSSION

The overlapping tissue distribution of CYP3A and P-gp, and the broad spectrum of drugs that interact with both proteins have presented significant challenges to drug absorption and delivery to the systemic circulation (Yasuda et al., 2002). Moreover, the interaction of co-administered 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 co-administered 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 orally administered curcumin was capable of not only changing the P-gp and CYP3A protein levels in the rodent intestine, it also modified the P-gp and CYP3A levels in the rodent liver and kidney. Curcumin administered by the oral route is known to be absorbed into the systemic circulation in rats (Ravindranath and Chandrasekhara, 1980) and human (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. (Lown et al., 1994), who showed that the hepatic CYP3A4 activity in human 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). Since 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 of curcumin for 4 days compared to 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 prior to 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_{\max} , AUC_{0-8} and total AUC of celiprolol, a P-gp substrate, were raised while 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_{\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_{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_{\max} and AUC of celiprolol, and its decreased systemic clearance via biliary elimination (Hartmann et al., 2005). It may therefore 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 demonstrated by Greiner et al. for digoxin (Greiner et al., 1999), and by Lown et al. for cyclosporine (Lown et al., 1997).

Curcumin consumed over 4 consecutive days also caused the AUC of midazolam to increase, while the CL_{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 since it is not a substrate of P-gp (Kim et al., 1999), its pharmacokinetic profile would not be affected by the co-regulation 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 (Chaobal and Kharasch, 2005). Although CYP3A in the kidney might 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_{oral} of midazolam. Despite the plethora of ADME 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

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evaluation of curcumin-drug interactions in human subjects in order 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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Footnotes

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LEGENDS FOR FIGURES

FIG. 1. Effects of curcumin treatment on the expression of intestinal, hepatic, and renal P-gp.

A: Data analysis of Western blot results; B: Image of Western blot results. Rats ($n = 4-5$) were gavaged once daily with 1% of Tween 80 (control) or 60 mg/kg/day of curcumin (treated) for 4 consecutive days. Intestine, liver and kidney were collected on day 5. P-gp levels were measured using the C219 antibody as described under *Methods*. β -actin was used as control. Bars represent mean values \pm SEM of band densities of P-gp normalized to β -actin (*, $p < 0.05$).

FIG. 2. Effects of curcumin treatment on the expression of intestinal, hepatic, and renal CYP3A. A: Data analysis of Western blot results; B: Image of Western blot results. Rats ($n = 4-5$) were gavaged once daily with 1% of Tween 80 (control) or 60 mg/kg/day of curcumin (treated) for 4 consecutive days. Intestine, liver and kidney were collected on day 5. CYP3A levels were measured using the anti-rat CYP3A2 antibody as described under *Methods*. β -actin was used as control. Bars represent mean values \pm SEM of band densities of CYP3A normalized to β -actin (*, $p < 0.05$).

FIG. 3. Time course of the plasma concentrations of celiprolol in rats assigned to the control, co-administered and treatment groups. Rats ($n = 5$) were gavaged once daily with 1% of Tween 80 (control, diamond symbols; co-administered group, triangle symbols) or 60 mg/kg/day of curcumin (treatment, square symbols) for 4 consecutive days. On day 5, rats were gavaged with vehicle (control) or 60 mg/kg of curcumin (co-administered and treatment groups) 30 min before they were gavaged with 30 mg/kg of celiprolol. Data points represent mean \pm SEM.

FIG. 4. Time course of the plasma concentrations of midazolam in rats assigned to the control, co-administered and treatment groups. Rats ($n = 5$) were gavaged once daily with 1% Tween 80 (control, diamond symbols; co-administered group, triangle symbols) or 60 mg/kg/day of

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curcumin (treatment, square symbols) for 4 consecutive days. On day 5, rats were gavaged with vehicle (control) or 60 mg/kg of curcumin (co-administered and treatment group) 30 min before they were gavaged with 20 mg/kg of midazolam. Data points represent mean \pm SEM.

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TABLE 1

Pharmacokinetic parameters of celiprolol in rat plasma in control, co-administered and treatment groups following an oral dose of 30 mg/kg

| Parameter | Control | Co-administered | Treatment |
|--------------------------------|------------------|------------------|-------------------|
| C_{\max} (ng/ml) | 492.65 ± 33.21 | 458.21 ± 78.05 | 960.14 ± 93.39** |
| t_{\max} (min) | 120.0 ± 0.0 | 108.0 ± 6.6 | 120.0 ± 0.0 |
| AUC ₀₋₈ (ng/ml × h) | 2140.04 ± 187.87 | 2347.63 ± 287.83 | 3440.26 ± 210.45* |
| Total AUC (ng/ml × h) | 3570.91 ± 106.26 | 4114.26 ± 103.22 | 4582.18 ± 382.65* |
| CL _{oral} (ml/h) | 2.36 ± 0.07 | 2.02 ± 0.06 | 1.85 ± 0.15** |

Total AUC: Area under concentration curve extrapolated to infinity. Data are presented as Mean ± SEM ($n = 5$). * $p \leq 0.05$ significant difference compare to control. ** $p \leq 0.01$ significant difference compare to control.

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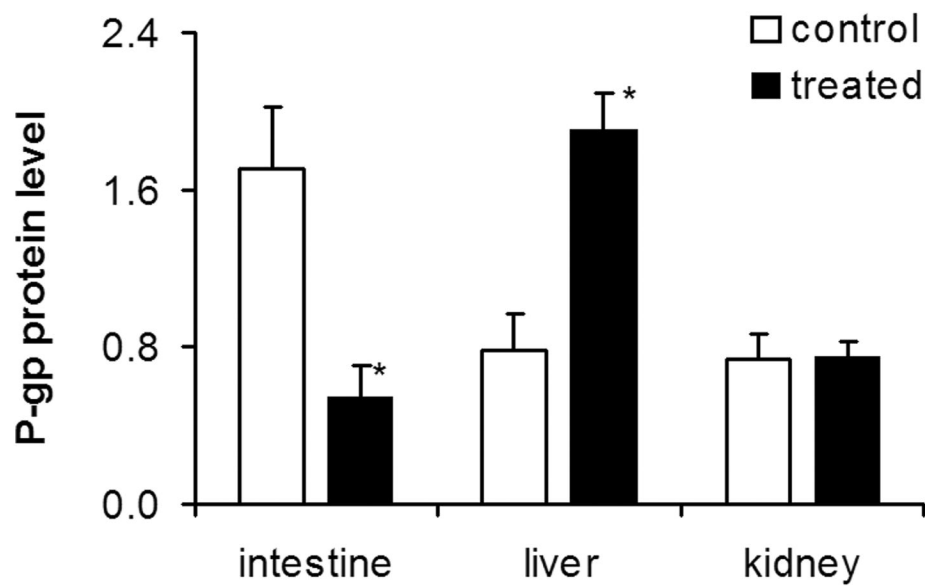
TABLE 2

Pharmacokinetic parameters of midazolam in rat plasma in control, co-administered and treatment groups following an oral dose of 20 mg/kg

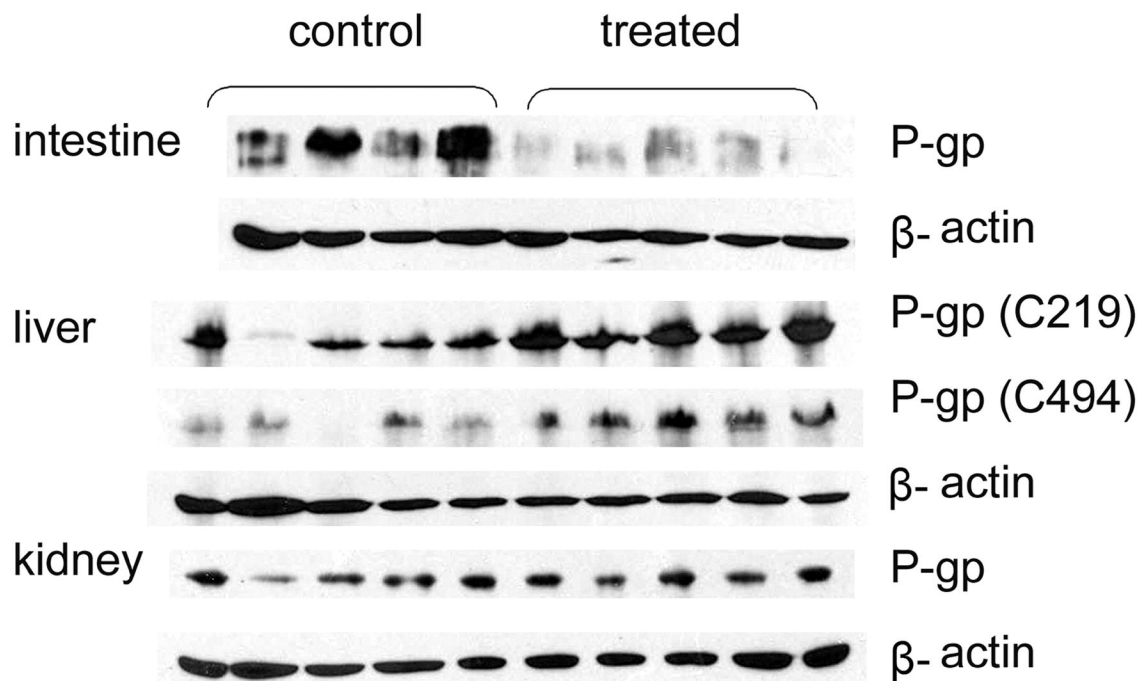
| Parameter | Control | Co-administered | Treatment |
|---------------------------------------|---------------------|--------------------|-----------------------|
| C_{\max} (ng/ml) | 123.21 \pm 14.82 | 239.71 \pm 46.82 | 259.20 \pm 38.71 |
| t_{\max} (min) | 28.8 \pm 5.9 | 28.8 \pm 4.7 | 32.5 \pm 5.8 |
| AUC ₀₋₄ (ng/ml \times h) | 255.06 \pm 27.40 | 470.01 \pm 88.36 | 657.02 \pm 135.59* |
| Total AUC (ng/ml \times h) | 476.88 \pm 109.50 | 776.18 \pm 94.58 | 1835.40 \pm 422.13* |
| CL _{oral} (ml/h) | 13.40 \pm 3.21 | 7.59 \pm 0.76 | 3.33 \pm 0.66* |

Total AUC: Area under concentration curve extrapolated to infinity. Data are presented as Mean \pm SEM ($n = 5$). * $p \leq 0.05$ significant difference compare to control.

FIG. 1

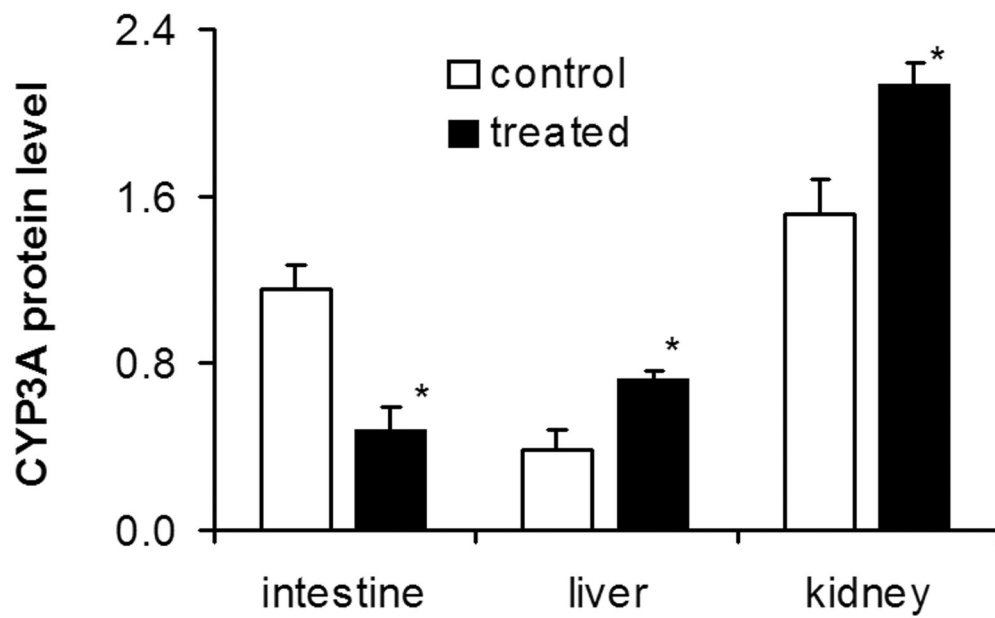


A

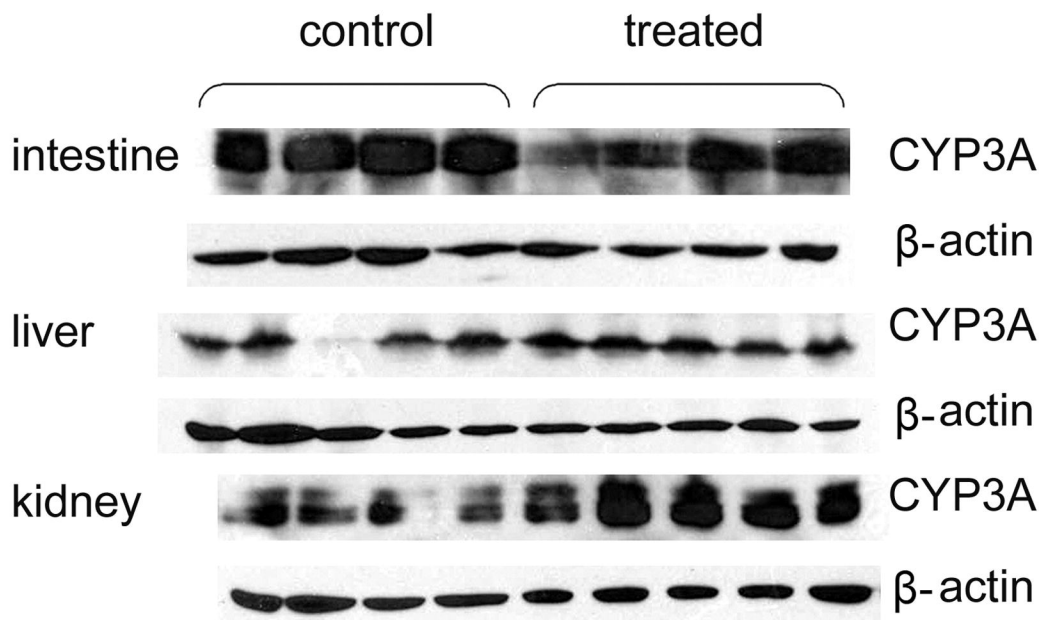


B

FIG. 2



A



B

FIG. 3

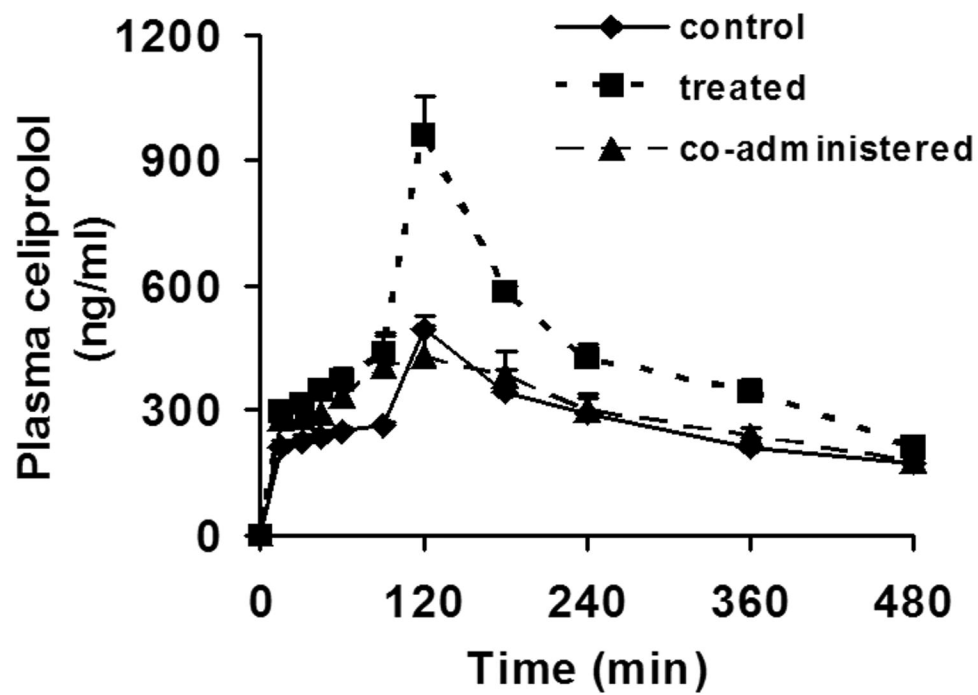


FIG. 4

