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Inhibition of human recombinant cytochrome P450s by curcumin and curcumin decomposition products

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Abstract

Curcumin (diferuloylmethane) is a major yellow pigment and dietary component derived from *Curcuma longa*. It has potent anti-inflammatory, anticarcinogenic, antioxidant and chemoprotective activities among others. We studied the interactions of curcumin, a mixture of its decomposition products, and four of its individually identified decomposition products (vanillin, vanillic acid, ferulic aldehyde and ferulic acid) on five major human drug-metabolizing cytochrome P450s (CYPs). Curcumin inhibited CYP1A2 (IC_{50}, 40.0 μM), CYP3A4 (IC_{50}, 16.3 μM), CYP2D6 (IC_{50}, 50.3 μM), CYP2C9 (IC_{50}, 4.3 μM) and CYP2B6 (IC_{50}, 24.5 μM). Curcumin showed a competitive type of inhibition towards CYP1A2, CYP3A4 and CYP2B6, whereas a non-competitive type of inhibition was observed with respect to CYP2D6 and CYP2C9. The inhibitory activity towards CYP3A4, shown by curcumin may have implications for drug–drug interactions in the intestines, in case of high exposure of the intestines to curcumin upon oral administration. In spite of the significant inhibitory activities shown towards the major CYPs in vitro, it remains to be established, whether curcumin will cause significant drug–drug interactions in the liver, given the reported low systemic exposure of the liver to curcumin. The decomposition products of curcumin showed no significant inhibitory activities towards the CYPs investigated, and therefore, are not likely to cause drug–drug interactions at the level of CYPs.

Keywords: Cytochrome P450; Curcumin; Drug–food interactions; Enzyme inhibition

1. Introduction

Multiple drug therapy is a common therapeutic practice especially in patients with multiple complications (Nadler et al., 2003; Hemaiswarya and Doble, 2006). If two or more drugs with affinity for the same cytochrome P450 (CYP) enzyme are co-administered, their biotransformation may be compromised, leading to undesirable accumulation of the drugs with toxic side effects as possible consequence. Drug–drug interactions involving CYPs have been identified as an important cause of adverse drug reactions and therapeutic failure (Honig

Abbreviations: CYP, cytochrome P450; HPLC, high performance liquid chromatography; BROD, benzyloxyresorufin O-debenzylase; MROD, methoxyresorufin O-demethylase; DBF, dibenzylfluorescein; BQ, 7-benzyloxyquinoline; BFC, 7-benzyloxy-4-trifluoromethyl-couma-rin; GSH, reduced glutathione; NAC, *N*-acetyl L-cysteine

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et al., 1993; Pea and Furlanut, 2001). Drug–drug interactions may be due to enzyme induction or inhibition, the latter being more common (Hemaiswarya and Doble, 2006; Zafar and Sharif, 2003). Next to drugs, several natural compounds have also been shown to cause significant interactions at the level of drug-metabolizing enzymes (Ioannides, 2002; Obach, 2000).

Curcumin, a polyphenolic component of turmeric (*Curcuma longa*), is a yellow pigment widely used for coloring of foods. It has been shown previously to exhibit anticancer, antioxidant, anti-inflammatory, antiparasitic and anti-HIV properties (Leu and Maa, 2002; Cole et al., 2004; Vajragupta et al., 2005; Reddy et al., 2005). Curcumin has also been shown to have chemoprotective, chemopreventive and immuno-modulating properties (Cole et al., 2004; Donatus et al., 1990; Cheng et al., 2001). Curcumin can be considered as a safe compound, because oral doses as high as 8 g/day administered to humans did not result in overt side effects (Cheng et al., 2001). Clinical trials for the use of curcumin as an anticancer agent are currently ongoing (Sharma et al., 2004).

Because relatively high doses of curcumin are evaluated in human studies, it might be anticipated that curcumin might cause drug–drug interactions at the level of intestinal and/or liver drug metabolism. Several in vivo and in vitro animal studies have shown that curcumin can significantly modulate the activity of several drug-metabolizing enzymes by down-regulation, induction or by direct inhibition. Oetari et al. (1996) and Thapliyal and Maru (2001) reported potent inhibition of rat liver microsomal CYP1A1, CYP1A2 and CYP2B1 enzymes by curcumin. In in vivo studies repetitive administration of curcumin to rats resulted in down-regulation of intestinal CYP3A-enzymes, whereas hepatic and renal CYP3A-levels were significantly induced (Zhang et al., 2006). Also, down-regulation of esophagal CYP2B1 and CYP2E1 was reported after intragastric treatment of rats, which might partially explain the chemopreventive activity of curcumin against carcinogenic N-nitrosamines (Mori et al., 2006).

As yet the effects of curcumin on the major human drug-metabolizing CYPs have not been studied. Due to the large species differences in the properties of metabolic enzymes and metabolic profiles of drugs, the animal studies described above are poorly predictive for the human situation (Eagling et al., 1998; Guengerich, 1997). The present in vitro investigation therefore was designed to assess the potential of curcumin to cause drug–drug interactions via inhibition of the five most important human drug-metabolizing CYPs. Major human CYP isoforms responsible for the metabolism and disposition of about 90% of the therapeutic drugs on the market include CYP1A2, CYP2D6, CYP2B6, CYP2E1, CYP2C9 and CYP3A4 (Shimada et al., 1994). Because curcumin has been shown to be chemically unstable under neutral and alkaline conditions (Wang et al., 1997), the inhibitory properties of these decomposition products were also studied as mixture and individually, if available. Degradation products which have been identified include *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, and minor products being vanillin, vanillic acid, ferulic aldehyde and ferulic acid (Fig. 1) (Wang et al., 1997). Apart from reversible inhibition, mechanism-based inhibition was also taken into consideration.

2. Materials and methods

2.1. Materials

Methoxyresorufin and benzyloxyresorufin were synthesized by the method of Burke et al. (1985) and the purity was determined by high performance liquid chromatography (HPLC), mass spectrometry and ^{1}H NMR. The plasmid, pSP19T7LT_2D6 containing human CYP2D6 bicistronically co-expressed with human cytochrome P450 NADPH reductase was kindly provided by Prof. M. Ingelman-Sundberg (Stockholm, Sweden). The plasmids, BMX100/h1A2 and pCWh3A4 with human cytochrome P450 NADPH reductase were kindly donated by Dr. M. Kranendonk (Lisbon, Portugal). Expression plasmids, pCWh2B6hNPR and pCWh2C9hNPR with human cytochrome P450 NADPH reductase were kindly provided by Prof. F.P. Guengerich (Nashville, TX, USA). All other chemicals were of analytical grade and obtained from standard suppliers.

2.2. CYP expression and membrane isolation

The plasmids containing cDNA of five human CYPs were transformed into *Escherichia coli* strain JM109. Expression of the CYPs was carried out in 3-L flasks containing 300 mL terrific broth (TB) with 1 mM δ-aminolevulinic acid, 0.5 mM thiamine, 400 μL/L trace elements, 100 μg/mL ampicillin, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), 0.5 mM $FeCl_3$ (for CYP2D6 and CYP3A4 only) and 30 μg/mL kanamycin (for CYP3A4 only). The culture media were inoculated with 3 mL overnight cultures of bacteria containing plasmids for the various CYPs. The cell cultures were incubated for about 40 h at 28 °C and 125 rpm and CYP contents were determined using the carbon monoxide (CO) difference spectra as described by Omura and Sato (1964). Cells were pelleted by centrifugation (4000 × *g*, 4 °C, 15 min) and resuspended in 30 mL Tris–sucrose–EDTA (TSE) buffer (50 mM Tris–acetate buffer, pH 7.6, 250 mM sucrose, 0.25 mM EDTA). Cells were treated with 0.5 mg/mL lysozyme prior to disruption by French press (1000 psi, 3 repeats). The membranes containing the human CYPs were isolated by ultracentrifu-

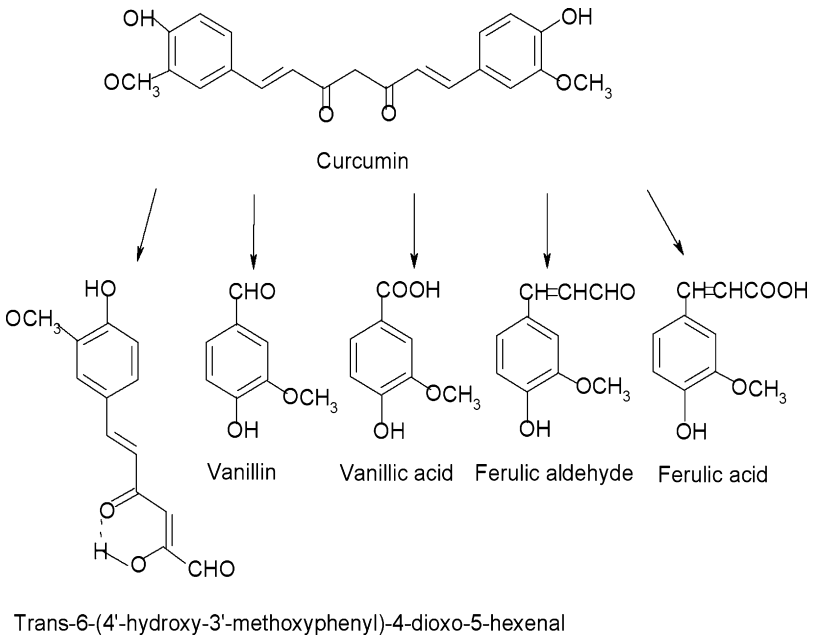

Fig. 1. Chemical structures of curcumin and its decomposition products at pH 7.4.

gation in a Beckmann 50.2Ti rotor (60 min, 40,000 rpm, 4 °C), resuspended in TSE buffer and stored at −80 °C until use.

2.3. Decomposition of curcumin

Curcumin decomposition was performed according to the method of Wang et al. (1997) in phosphate buffer of pH 7.4. Briefly, aliquots of 20 μL of 5 mM curcumin (dissolved in methanol) were added to 980 μL of 0.1 M potassium phosphate buffer pH 7.4. After 1 h incubation at 37 °C samples were analyzed by HPLC (Jasco Separations FP 1575). Vanillin, vanillic acid, ferulic aldehyde and ferulic acid were used as standards. Gradient reversed-phase HPLC separations were performed using a C18 column (150 mm × 3.2 mm, 5 μm particle size, Phenomenex) and a mobile phase consisting of 0.1% acetic acid (solvent A) and 98% methanol with 0.1% acetic acid (solvent B). HPLC-gradient separation was carried out with a linear gradient eluting from 15 to 95% of solvent B in 60 min. The carrier flow rate was 0.4 mL/min and chromatographic peaks of decomposition products were monitored by UV detection (λ = 280 nm). Under these chromatographic conditions curcumin and commercially available reference compounds of possible degradation products eluted at: 28.5 min (curcumin), 14.3 min (vanillic acid), 15.2 min (vanillin), 17.9 min (ferulic acid) and 18.5 min (ferulic aldehyde).

2.4. CYP inhibition assays

2.4.1. 7-Methoxyresorufin, 7-benzyloxyresorufin, dibenzylfluorescein, 7-benzyloxy-4-trifluo-romethylcoumarin and 7-benzyloxyquinoline O-dealkylation

Inhibition of the activities of the human CYP isoforms 1A2, 3A4 and 2B6, by curcumin and its decomposition products was determined using microplate fluorimetric assays (Burke et al.,

Table 1
Experimental conditions for fluorescence CYP assays

CYP	Enzyme concentration (nm)	Incubation time (min)	Substrate	Substrate concentration (μM)	Excitation wavelength (nm)	Emission wavelength (nm)
1A2	13.2	10	MRes	5.0	530	586
3A4	14.3	30	BRes	5.0	530	586
	2.8	10	DBF	0.5	485	535
	14.3	20	BFC	80.0	410	538
	14.3	30	BQ	40.0	410	538
2B6	15.3	30	BRes	20.0	530	586

MRes, methoxyresorufin; BRes, benzyloxyresorufin; DBF, dibenzylfluorescein; BQ, 7-benzyloxyquinoline; BFC, 7-benzyloxy-4-trifluoromethylcoumarin.

1985; Stresser et al., 2000). Incubation conditions (e.g. enzyme concentration, substrates, incubation time) and wavelengths for detection for each assay are shown in Table 1. Under these conditions, the product formation was linear over the periods indicated [data not shown]. The inhibitory activity of curcumin towards CYP3A4 was tested using four different substrates of CYP3A4, namely DBF, BFC and BQ, because inhibition of CYP3A4 activity is known to be substrate-dependent (Stresser et al., 2000).

In general, the microsomal incubations were carried out in a total volume of 200 μL and in the presence of 100 μM NADPH (freshly prepared) in a black coaster 96-well plate. Microsomes were preincubated for 5 min at 37 °C with 0.1 M potassium phosphate buffer (pH 7.4), substrates and inhibitors (curcumin and its decomposition products) with DMSO at a final concentration of 0.5% (v/v) or less in all the CYP assays. At these DMSO concentrations, the activities of the studied human CYPs are only affected to a small extent (Busby et al., 1998). Uninhibited incubations, set at 100%, always contained the same concentration of DMSO as the inhibited incubations. Stability of curcumin in phosphate buffer pH 7.4, has been reported to be strongly improved by addition of rat liver microsomes or cytosol, glutathione (GSH), *N*-acetyl L-cysteine (NAC) or ascorbic acid (Oetari et al., 1996). Therefore, enzymes were always added before the incorporation of curcumin in all inhibition assays performed.

Initially, the inhibitory effect of curcumin and its decomposition products on the CYP isoforms was studied at a concentration of 300 μM. To study the effect of the total mixture of curcumin decomposition products, curcumin was first added to phosphate buffer pH 7.4 to a final concentration of 300 μM; under these conditions curcumin is known to rapidly decompose (Wang et al., 1997). After 1 h, enzyme, substrate and NADPH were added to determine enzyme activity in presence of decomposition products derived from 300 μM curcumin.

For IC_{50} determinations, concentration ranges for curcumin used were from 0.9 to 100 μM and for the decomposition products, from 2.5 to 1000 μM. Incubations were commenced by the addition of 100 μM NADPH, maintained at 37 °C for the periods defined. Reactions were terminated with 75 μL of 80% acetonitrile and 20% 0.5 M Tris solution or 2N NaOH in the case of dibenzylfluorescein (DBF). Product formation was linear for all incubation times. Concentrations of the probe substrates in all reaction mixtures were chosen near the Michaelis–Menten's (K_m) value for each of the CYPs tested. The K_m values obtained using the alkoxyresorufins and all other substrates were within the range of reported literature values (Staskal et al., 2005). All measurements were performed in triplicate. Metabolite formation was measured spectrophotometrically on a Victor^2 1420 multilabel counter.

2.4.2. Diclofenac hydroxylation

For the CYP2C9 inhibition assay, reaction mixtures in 500 μL total volume consisted of 49 nM enzyme, 100 μM NADPH, 0.1 M potassium phosphate buffer (pH 7.4), 6 μM diclofenac and inhibitor. Screening for inhibitory effects of 300 μM concentrations of curcumin, its decomposition mixture and the individual decomposition products on the CYP2C9 was done. For IC_{50} determinations, curcumin concentrations used were of the range 0.4–100 and 3.9–2000 μM for the individual decomposition products, vanillin, vanillic acid, ferulic aldehyde and ferulic acid. After preincubation for 5 min at 37 °C, reactions were initiated by adding NADPH and terminated after 10 min with the addition of 200 μL methanol. The reaction mixtures were centrifuged at 14,000 rpm for 3 min. Product formed was measured using an isocratic HPLC method (Walsky and Obach, 2004). A C18 column (150 mm × 3.2 mm, 5 μm particle size, Phenomenex) was used and the carrier flow rate was 0.6 mL/min. The mobile phase consisted of 60% (v/v) 20 mM potassium phosphate buffer (pH 7.4), 22.5% (v/v) methanol and 17.5% (v/v) acetonitrile. Peaks were monitored at the wavelength of 280 nm. Retention times for 4-hydroxydiclofenac and diclofenac were 5.0 and 24.1 min, respectively.

2.4.3. Dextromethorphan O-demethylation

Inhibition of CYP2D6 activity by curcumin and its decomposition products was evaluated by the method described by Ko et al. (2000). Inhibitory effects of 300 μM concentrations of curcumin, its decomposition mixture and the individual decomposition products on the CYP2D6 were first assessed. The reaction mixture had a total volume of 500 μL and consisted of 18.2 nM enzyme, 4.5 μM dextromethorphan, 90.9 μM NADPH, 0.1 M potassium phosphate buffer and inhibitor. For IC_{50} determination, curcumin concentration range used was 0.4–181.8 μM and the decomposition products 3.6–1818.2 μM. Reactions were initiated by the addition of NADPH and allowed to proceed for 45 min before termination with the addition of 60 mM zinc sulphate solution. Product formed was measured using an isocratic HPLC fluorescence detection method and a C18 column (100 mm × 3 mm, 5 μm particle size, Chromspher). The mobile phase consisted of 24% (v/v) acetonitrile and 0.1% (v/v) triethylamine adjusted to pH 3 with perchloric acid. The carrier flow rate was 0.6 mL/min. Peaks were monitored at 280 nm (excitation) and 310 nm (emission). The retention times of dextrorphan and dextromethorphan were 3.4 and 24.5 min, respectively.

2.5. Type of inhibition

To determine the types of inhibition occurring in the reactions involving CYP1A2 and CYP3A4, the substrate concentrations used were ranging from 0.65 to 10 μM for both methoxyresorufin and benzyloxyresorufin. In the case of the other CYPs, substrate concentration ranges were from 3.1 to 50 μM benzyloxyresorufin (CYP2B6), 0.3 to 20 μM diclofenac (CYP2C9) and 1.4 to 22.7 μM dextromethorphan (CYP2D6). Five different substrate concentrations were used in each assay. The concentration of curcumin used for the assays are indicated in Table 3. Reactions were carried out as described above for all CYPs.

2.6. Mechanism-based inhibition

The potential of curcumin for mechanism-based inhibition of CYP1A2, CYP3A4 and CYP2B6 was evaluated according to the method of Heydari et al. (2004) with slight modifications. Briefly preincubation mixtures of total volumes 600 μL contained 13–16 nM CYP enzymes, 100 μM NADPH and curcumin solution (0, 10 and 50 μM). The preincubations were performed for 20 min at 37 °C, and at 5 min intervals 100 μL aliquots were taken to determine the remaining CYP activity. The aliquots of preincubation mixtures were added to tubes containing 400 μL of the respective substrates (5 μM methoxyresorufin for CYP1A2, 5 μM benzyloxyresorufin for CYP3A4 and 20 μM benzyloxyresorufin for CYP2B6) and NADPH (100 μM), and incubated as described above. After stopping the reactions the remaining activities were determined using a fluorescence spectrophotometer (Perkin-Elmer, Model 3000). Duplicate experiments were performed.

For CYP2D6 and CYP2C9, preincubation tubes contained 40–49 nM CYP enzyme, 100 μM NADPH, 50 and 20 μM curcumin solution. Preincubations were performed for 20 min at 37 °C and at time intervals of 5 min the remaining activities of the enzymes were re-assessed. Aliquots (100 μL) of the 600 μL preincubation mixtures were transferred into tubes containing 400 μL dextromethorphan (4.5 μM) or diclofenac (6.0 μM) and NADPH (100 μM) and incubated at 37 °C for 30 or 10 min to determine remaining activity of CYP2D6 or CYP2C9, respectively.

2.7. Data analysis

Percent inhibition of CYP activity by curcumin, its decomposition products were calculated from the ratio of the activity of treated to control samples. Statistical analysis was performed using the Student's *t*-test. The enzyme kinetic parameters (K_m and V_{max}) for metabolism of the various substrates and IC_{50} values were analyzed using GraphPad Prism 4.0 version (GraphPad Prism Software Inc., San Diego, CA). The inhibitor constant (K_i) values for competitive inhibition were calculated according to the following equation: for competitive inhibition, $K_i = K_{m(inhibited)}[I]/(K_{m(uninhibited)} - K_{m(inhibited)})$, where substrate concentration is equal to the K_m, and for non-competitive inhibition, $K_i = V_{max(inhibited)}[I]/(V_{max(uninhibited)} - V_{max(inhibited)})$, where K_m, V_{max}, S and [I] are Michaelis constant, maximal enzyme activity, substrate concentration and inhibitor concentration, respectively.

3. Results

3.1. Decomposition of curcumin

Degradation of curcumin under various pH conditions, and the stability of curcumin in physiological matrices have been previously reported (Wang et al., 1997). To identify the inhibitory potentials of the decomposition products of curcumin towards human CYPs, decomposition experiments were also performed in the present study. Curcumin was treated with 0.1 M phosphate buffer of pH 7.4 at 37 °C for 1 h, according to the procedure described by Wang et al. (1997). Fig. 2 shows the HPLC chromatogram of the resulting mixture of decomposition products of curcumin. Chromatographic peaks observed included a major peak and seven minor peaks, four of which were identified as vanillin, vanillic acid, ferulic aldehyde and ferulic acid, by co-eluting with commercially available reference compounds [data not shown]. This major decomposition product with retention time of 16.9 min most likely represents *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-4-dioxo-5-hexenal, as the same procedure of decomposition was used as that described by Wang et al. (1997).

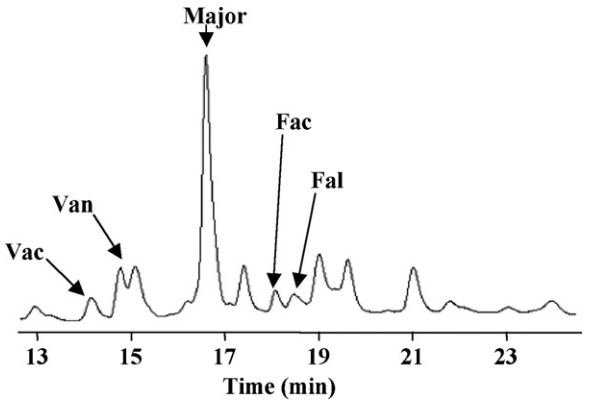

Fig. 2. HPLC chromatogram of the decomposition mixture of curcumin after incubation for 1 h at pH 7.4: identified products are indicated by arrows—vanillin (Van), vanillic acid (Vac), ferulic aldehyde (Fal), ferulic acid (Fac). The major peak most likely corresponds to *trans*-6-(4′-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal (Wang et al., 1997).

3.2. CYP inhibition and types of inhibition

The inhibitory effects of 300 μM concentrations of curcumin, four of the individual decomposition products and a complete mixture of decomposition products, on the activities of CYP1A2, CYP3A4, CYP2D6, CYP2C9 and CYP2B6 are shown in Fig. 3. At this concentration, curcumin appeared to inhibit almost completely the activities of CYP3A4, CYP2C9, and CYP1A2, and 72 and 69.1% of the activities of CYP2D6 and CYP2B6, respectively. The complete decomposition mixture and the four decomposition products, vanillin, vanillic acid, ferulic aldehyde and ferulic acid, showed milder inhibitory effects on the above CYPs. Percentages of inhibition of CYP activities in the range 1–50% were observed in assays with the decomposition products

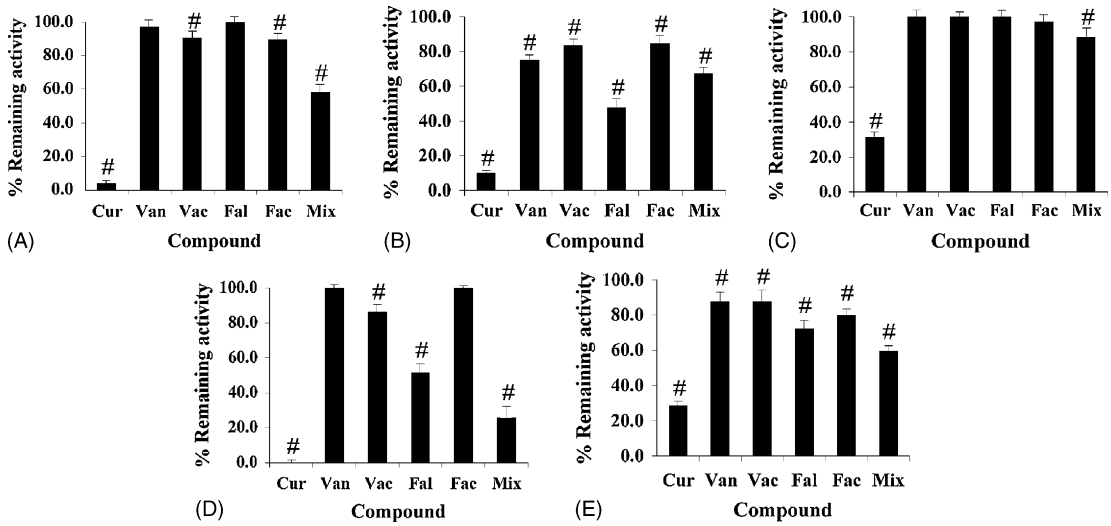

Fig. 3. Inhibition of CYP3A4 (A), CYP1A2 (B), CYP2B6 (C), CYP2C9 (D) and CYP2D6 (E) activities by curcumin, four of its decomposition products (each at a concentration of 300 μM) and a mixture of decomposition products derived from 300 μM curcumin after incubation for 1 h at 37 °C (pH 7.4). General assay conditions are described under Section 2. The charts represent mean and standard deviations of triplicate experiments. The symbol '#' indicates statistically significant difference ($P < 0.05$) from uninhibited reactions, as determined by Student's *t*-test. Cur, curcumin; Van, vanillin; Vac, vanillic acid; Fal, ferulic aldehyde; Fac, ferullic acid.

(Fig. 3). The decomposition mixture caused 57.4 and 74.8% inhibition of CYP3A4 and CYP2C9, respectively.

For the decomposition products showing more than 50% inhibition of enzyme activity at 300 μM, the IC_{50} values were determined (Table 2). The four decomposition products of curcumin that exhibited inhibition of the CYP isoforms, appeared to be rather weak inhibitors as shown in Table 2. For curcumin, the inhibition of the CYPs in decreasing order of potency was CYP2C9 > CYP3A4 > CYP2B6 > CYP1A2 > CYP2D6. Results obtained on inhibition of CYP3A4 by curcumin, using the substrates 7-benzyloxyquinoline (BQ) and 7-benzyloxy-4-trifluoromethylcoumarin (BFC) could not be analyzed due to interference by curcumin at the wavelength for detection of the metabolites.

Table 2
Concentrations of curcumin and four of its decomposition products required to reduce the activities of five different human CYPs by 50% (IC_{50} value, μM)

Enzyme	Curcumin	Ferulic aldehyde
CYP1A2	40.0 ± 12.7	227.5 ± 23.4
CYP3A4	16.3 ± 1.7[a]	nd
	13.9 ± 3.4[b]	nd
CYP2D6	50.3 ± 2.0	nd
CYP2C9	4.3 ± 0.8	259.3 ± 22.8
CYP2B6	24.5 ± 0.8	nd

All values are the means ± standard deviation (S.D.) of at least two experiments as described in Section 2. nd: not determined due to low percent inhibition observed (<50%).
[a] Substrates used, benzyloxyresorufin.
[b] Substrates used, DBF.

Enzyme kinetic parameters for all CYPs and the corresponding types of inhibition with curcumin are shown in Table 3. In the MROD (methoxyresorufin O-deethylase, with CYP1A2) and BROD (benzyloxyresorufin O-debenzylase, with CYP3A4, CYP2B6) assays, the presence of curcumin resulted in an increase of the respective K_m values, while the V_{max} values did not change significantly when compared to the control experiment, thus indicating competitive inhibition according to Michaelis–Menten's kinetics. However, with respect to CYP2D6 and CYP2C9 curcumin caused significant decreases in V_{max} values with no significant changes in the K_m values, thus indicating non-competitive inhibition.

3.3. Mechanism-based inhibition

The effects of preincubation of curcumin with NADPH-fortified CYP isozymes on inhibition potency was also evaluated with all five human CYPs. Preincubation of curcumin for 20 min with NADPH-supplemented CYP did not increase inhibition of CYP activities by curcumin in any of the CYP isoforms (data not shown). Therefore, mechanism-based inhibition does not seem to occur with any of the CYPs studied.

Table 3
Enzyme kinetic parameters of the effects of curcumin on human CYP activities

Enzyme	Curcumin (μM)	V_{max} (nM/min/nM)	K_m (μM)	K_i (μM)	Type of inhibition
CYP1A2	0.0	2.43 ± 0.51	5.0 ± 1.6	43.3	Competitive
	25.0	2.27 ± 0.04	7.8 ± 0.8	± 10.9	
CYP3A4[a]	0.0	0.21 ± 0.02	2.8 ± 0.2	7.4	Competitive
	2.5	0.16 ± 0.03	3.8 ± 0.1	± 3.5	
CYP2D6	0.0	1.68 ± 0.01	3.2 ± 0.1	51.0	Non-competitive
	45.5	0.89 ± 0.04	3.3 ± 0.1	± 3.9	
CYP2C9	0.0	7.83 ± 0.05	6.1 ± 1.2	11.5	Non-competitive
	6.0	5.14 ± 0.07	6.4 ± 0.2	± 0.8	
CYP2B6	0.0	0.13 ± 0.04	34.0 ± 10.4	33.2	Competitive
	50.0	0.11 ± 0.01	69.0 ± 12.8	± 14.0	

Values are means ± S.D. of at least two experiments as described in Section 2. Curcumin concentrations used in the experiments are indicated in the table.
[a] The substrate used in this CYP3A4 inhibition assay was benzyloxyresorufin.

4. Discussion

The purpose of this study was to evaluate the inhibitory potential of curcumin and its decomposition products on the five important human drug-metabolizing CYPs, namely CYP1A2, CYP3A4, CYP2B6, CYP2C9 and CYP2D6. Earlier reports on the inhibition of rat liver microsomal CYPs by curcumin showed that curcumin is a strong inhibitor of CYP1A and CYP2B (Oetari et al., 1996; Thapliyal and Maru, 2001). In the present study, curcumin and its decomposition products were first screened at a high concentration of 300 μM, for their inhibitory potential towards the five CYPs used. At the high concentration, compounds exhibiting less than 50% inhibitory activities were excluded from further tests with the particular CYPs. Curcumin was found to possess higher inhibitory potentials against human CYP1A2, CYP2B6, CYP3A4, CYP2D6 and CYP2C9 than the decomposition products. However, in contrast to CYP inhibition data on rat mentioned above, curcumin is a less potent inhibitor of human CYP1A2 and CYP2B6. These results support findings that animal data may be poorly predictive of the human situation (Eagling et al., 1998). A recent study on inhibitory activities of Indonesian medicinal plants, showed plant extracts of three curcuma species possessing 65–73% inhibitory activities towards CYP3A4 and 30–54% inhibition towards CYP2D6 (Usia et al., 2006). Although these activities are due to the whole extracts with components including curcumin they lend support to our findings that curcumin significantly inhibits CYP3A4 but less significantly inhibits CYP2D6. Curcumin inhibited CYP1A2, CYP2B6 and CYP3A4 competitively, while non-competitive inhibition was observed with CYP2C9 and CYP2D6. The structural difference in active sites of the enzymes used may have contributed to the two different types of inhibition observed. The insignificant inhibitory activities of the decomposition products towards the tested CYPs, clearly indicate that the decomposition products of curcumin are not likely to cause drug–drug interaction at the level of major drug-metabolizing CYPs. Moreover, decomposition of curcumin is not likely to occur significantly at the low pH in the gut, in addition to the presence of the stabilizing factors such as enzymes and GSH (Oetari et al., 1996).

The inhibitory potential of curcumin towards CYP3A4 (IC_{50} = 16.3 μM and K_i = 7.4 μM) could have implications for drug–drug interactions in the intestines because of the direct exposure of the intestines to curcumin upon oral administration and the high levels of CYP3A4 in the intestinal epithelial cells. Inhibition of CYP3A4 in the intestines upon co-administration of drugs could result in a significantly increased bioavailability of drugs, and consequently increased plasma concentrations of drugs, with the potential result of adverse drug reactions. Inhibition of CYP3A4 by co-administered drugs has been shown to result in adverse clinical drug–drug interactions, including fatalities (Honig et al., 1993). For example, concomitant intake of grapefruit juice with drugs has also been shown to increase plasma concentrations of many drugs in humans (Fuhr, 1998). This effect appears to be mediated mainly by the inhibition of CYP3A4 in the intestinal wall. It is worth noting that inhibitors of CYP3A4 are often also known to inhibit P-glycoprotein (P-gp) function (Wacher et al., 1998), both phenomena have been suggested to synergistically influence bioavailability of orally administered agents. Inhibition of P-gp by curcumin at an effective concentration of 15 μM has also been documented (Chearwae et al., 2004). It is antici-

pated that inhibition of CYP3A4 and P-gp by curcumin may be advantageous in mitigating first pass elimination of orally administered drugs (Kuppens et al., 2005).

It is still unknown whether inhibition of the activities of the presently tested human CYPs in the liver may cause significant systemic drug–drug interactions. As yet there are no reports on interaction between curcumin and drugs at the level of hepatic CYPs in humans. Currently available human pharmacokinetic data show an extremely low exposure of the liver to curcumin even at very high doses. Plasma concentrations of curcumin and its metabolites in humans were found to be in nanomolar ranges. High concentrations of curcumin were found in the faeces (Sharma et al., 2004). This implies that the inhibitory effect of curcumin on activities of the CYPs in the liver may be insignificant. The inhibition parameters determined in the present study, including IC_{50} and K_i values, which are important to estimate the CYP-inhibitory potential of curcumin (Bapiro et al., 2001) are relatively high (in micromolar ranges) compared to the anticipated amounts of curcumin in the liver. However, in recent rodent studies it was demonstrated that repeated oral administration of curcumin resulted in a two-fold up-regulation of both CYP3A and P-gp in the liver, whereas a down-regulation of these proteins was observed in the intestines (Zhang et al., 2006). The significant increase in the area-under-curve and decreases of oral clearances of midazolam and celiprolol, suggest that the effects on the intestinal activities was more significant than those on the hepatic activities.

In conclusion, curcumin appears to inhibit five of the important human CYPs, with the increasing order of potency as CYP2D6 < CYP2B6 < CYP1A2 < CYP3A4 < CYP2C9. Our results suggest that inhibition of CYP3A4, and to a lesser extent CYP2C9, by curcumin has the potential to cause clinically significant and harmful drug–drug interactions upon oral co-administration of curcumin and other drugs metabolized by these CYPs. Further investigation is required to evaluate the in vivo relevance of the inhibitory activities of curcumin observed in the present in vitro study.

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