Arch Pharm Res Vol 26, No 8, 631-637, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Cytochrome P450 2C8 and CYP3A4/5 are Involved in Chloroquine Metabolism in Human Liver Microsomes

Kyoung-Ah Kim, Ji-Young Park[1], Ji-Suk Lee, and Sabina Lim

Research Group of Pain and Neuroscience, East-West Medical Research Institute, Kyung Hee University, Seoul, Korea and [1]Department of Pharmacology, Gachon Medical School, Incheon, Korea

(Received May 7, 2003)

Chloroquine has been used for many decades in the prophylaxis and treatment of malaria. It is metabolized in humans through the *N*-dealkylation pathway, to desethylchloroquine (DCQ) and bisdesethylchloroquine (BDCQ), by cytochrome P450 (CYP). However, until recently, no data are available on the metabolic pathway of chloroquine. Therefore, the metabolic pathway of chloroquine was evaluated using human liver microsomes and cDNA-expressed CYPs. Chloroquine is mainly metabolized to DCQ, and its Eadie-Hofstee plots were biphasic, indicating the involvement of multiple enzymes, with apparent K_m and V_{max} values of 0.21 mM and 1.02 nmol/min/mg protein 3.43 mM and 10.47 nmol/min/mg protein for high and low affinity components, respectively. Of the cDNA-expressing CYPs examined, CYP1A2, 2C8, 2C19, 2D6 and 3A4/5 exhibited significant DCQ formation. A study using chemical inhibitors showed only quercetin (a CYP2C8 inhibitor) and ketoconazole (a CYP3A4/5 inhibitor) inhibited the DCQ formation. In addition, the DCQ formation significantly correlated with the CYP3A4/5-catalyzed midazolam 1-hydroxylation (r=0.868) and CYP2C8-catalyzed paclitaxel 6α-hydroxylation (r = 0.900). In conclusion, the results of the present study demonstrated that CYP2C8 and CYP3A4/5 are the major enzymes responsible for the chloroquine *N*-deethylation to DCQ in human liver microsomes.

Key words: Chloroquine, Desethylchloroquine, Cytochrome P450, CYP2C8, CYP3A4, CYP3A5

INTRODUCTION

Chloroquine has been used for the prophylaxis and treatment of malaria for many decades. Following the oral administration of single and multiple doses to healthy volunteers or malaria patients, chloroquine is metabolized through a dealkylation pathway, to monodesethylchloroquine (DCQ) and bisdesethylchloroquine (BDCQ) (Fig. 1).

The concentrations of DCQ and BDCQ in plasma reach 48 and 13% of the chloroquine level, respectively. Even though chloroquine has an extremely long half-life, it has a non-negligible total clearance, with equal contributions by the liver and kidneys in its elimination (Gustafsson *et al.*, 1983; Frisk-Holmberg *et al.*, 1984; Ette *et al.*, 1989; Augustijns *et al.*, 1992). It has been estimated that 30-50% of an administered dose of chloroquine is metabolized by the liver, presumably through cytochrome P450 enzymes (CYPs) (Ofori-Adjei and Ericsson, 1985). Previously, Ducharme and Farinotti (1997) reported that chloroquine is metabolized into DCQ and BDCQ in human liver microsomes, suggesting the involvement of CYPs in its metabolism. However, there has been no previous demonstration that CYP isoforms are involved in the metabolism of chloroquine.

This study was undertaken to identify the CYP isoforms responsible for the metabolism of chloroquine using human liver microsomes and cDNA expressing CYP isoforms.

Correspondence to: Sabina Lim, OMD, Ph.D., Research Group of Pain and Neuroscience, East-West Medical Research Institute, Kyung Hee University,1 Hoegidong, Dongdaemoongu, Seoul 130-701, Korea
Tel: 82-2-961-0324, Fax: 82-2-961-7831
E-mail: lms@khu.ac.kr

MATERIALS AND METHODS

Chemicals and reagents

The chloroquine, phenacetin, acetaminophen, dextromethorphan, quinidine, sulfaphenazole, ketoconazole, diethyldithiocarbamate (DDC), furafylline, chlorozoxazone, β-nicotinamide adenine dinucleotide phosphate (β-NADP),

β-nicotinamide adenine dinucleotide phosphate, reduced form of NADPH, EDTA, $MgCl_2$, glucose-6-phosphate (G-6-P) and glucose-6-phosphate dehydrogenase (G-6-PDH) were all purchased from Sigma Chemical Co. (St. Louis, MO). The desethylchloroquine (DCQ) and Bisdesethyl-chlroquine (BDCQ) were generously donated by the Malarial Research Centre in Indian, Council of Medical Research, India. The *S*-Mephenytoin, 4-hydroxymephenytoin, 6-hydroxychlorzoxazone and 1-hydroxymidazolam were purchased from Ultrafine Chemical Co. (Manchester, UK). The acetonitrile and methanol were provided by Fisher Scientific (Pittsburgh, PA). All other reagents and chemicals were of analytical or HPLC grade.

Human liver microsomes and cDNA-expressed P450s

Human liver microsomes (HG-42, HG-43, HG-56, HG-93) from four different donors were provided by BD Gentest Co. (Woburn, MA), which were stored frozen at 80°C until use. Eight different human cDNA expressing CYP450s, 1A2, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5 (Supersomes®), were purchased from BD Gentest Co. (Woburn, MA). The manufacturer supplied information regarding the protein concentration and CYP content.

Metabolism of chloroquine in human liver microsomes or cDNA-expressed P450 isoforms

All incubations were performed in duplicate, and the mean values were used. The incubation mixtures, containing either 25 μL of microsomes (1 mg protein/mL of stock, prepared from three different human liver microsomal preparations), or 25 μL of cDNA expressing CYP (diluted to 20-100 pmol/mL with phosphate buffer, pH 7.4), and various concentrations of chloroquine (12000 μM), reconstituted in 100 μM phosphate buffer (pH 7.4), were preincubated for 5 min at 37°C. The reactions were initiated by the addition of the NADPH-regenerating system (including 1.3 mM NADP, 3.3 mM G-6-P, 3.3 mM $MgCl_2$ and 1.0 U/mL G-6-PDH), and the reaction mixtures (final volume of 250 μL) incubated for 30 min at 37°C in a shaking water bath. The time of incubation, and the concentration of microsomes used, for each assay were determined to be in the linear range for the rate of the metabolite formation. The reactions were terminated by placing the incubation tubes on ice and adding 80 μL acetonitrile. The incubation mixtures were then centrifuged for 5 min at 10,000×g at 4°C. An aliquot of the supernatant fraction was subjected to analysis using high-performance liquid chromatography (HPLC).

HPLC analysis

The DCQ and BDCQ were analyzed using high-performance liquid chromatography (HPLC), with fluorescence detection. The HPLC analyses were carried out on a Capcell Pak C_{18} (250×1.5 mm, 5 μm, Shiseido Co, Tokyo, Japan) connected to a Shiseido SI-1 chromatographic system, consisting of a pump (model 2001), an autosampler (model 2003) and a fluorescence detector (model FP-2020, Jasco Co., Tokyo, Japan). Elution was performed at a flow rate of 150 μL/min, with a mobile phase composed of acetonitrile, methanol and 0.2 M potassium dihydrogen phosphate solution (5/30/65, v/v/v), with 1% triethylamine. Analytes were monitored by fluorescence detection (excitation 250 nm, excitation 380 nm). The data sampling and the peak integration were performed using the chromatography software, DsChrom2000 (Donam Instrument Co., Suwon, Korea).

Chemical inhibition study

The selective inhibitors of 9 major CYPs (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5) were chosen according to the results of previous studies (Bourrie *et al.*, 1996; Eagling *et al.*, 1998). Two concentrations of inhibitor were used: furafylline (CYP1A2; 10 and 50 μM), coumarin (CYP2A6; 100 and 200iM), orphenadrine (CYP2B6; 100 and 200 μM), quercetin (CYP2C8; 20 and 100 μM), sulfaphenazole (CYP2C9; 5 μM, 20 μM), omeprazole (CYP2C19; 10 μM, 100 μM), quinidine (CYP2D6; 1 and 10 μM), DDC (CYP2E1; 100 and 200 μM) and ketoconazole (CYP3A4/5; 1 and 10 μM). In the cases where the inhibitors had previously demonstrated mechanism-based inactivation of CYPs (furafylline, orphenadrine, and DDC), they were preincubated with microsomes and NADPH, at 37°C for 15 min, before the substrate was added. The control reactions for the orphenadrine, furafylline and DDC experiments included a pre-incubation without the inhibitor.

Correlation study

Correlation studies with 12 human microsomal samples were performed in duplicate, under the same conditions, with a chloroquine concentration of 25 μM. The human hepatic microsomal CYP activities were determined by HPLC. The correlation coefficients were determined by Pearson Moment correlations, using Prism Software (Graphpad Software Inc.).

Assays for the respective products of the CYP marker reactions were carried out using HPLC. The reaction probes used were phenacetin *O*-deethylation for CYP1A2 (Tassaneeyakul *et al.*, 1993), *S*-mephenytoin 4-hydroxylation for CYP2C19 (Wrighton *et al.*, 1993), dextromethorphan *O*-demethylation for CYP2D6 (Broly *et al.*, 1989), paclitaxel 6α-hydroxylation for CYP2C8 (Harris *et al.*, 1994) and midazolam 1-hydroxylation for CYP3A4/5 (Thummel *et al.*, 1994).

Data analysis

The results are expressed as the means±S.D. of estimates

obtained from four different liver microsome preparations, from duplicate experiments. The enzyme kinetic parameters were obtained by a nonlinear regression analysis (WinNONLIN; SCI, Inc., Apex, NC). The kinetic analyses of the metabolite formation were initially assessed by visual inspection of the Eadie-Hofstee plots. A single K_m value (rectangular hyperbolic) model was suggested by the presence of a straight-line plot. When the plot deviated from linearity, a multiple K_m was suggested. The Michaelis-Menten equation, $V = V_{max1} \cdot [S]/(K_{m1} + [S]) + V_{max2} \cdot [S]/(K_{m2} + [S])$, was fitted to the formation rates of the metabolite (DCQ) versus the substrate concentrations. V is the velocity of the reaction at substrate concentration [S], V_{max1} and V_{max2} are the maximum velocities and K_{m1} and K_{m2} the substrate concentrations at which the reaction velocity is 50% of V_{max}. The intrinsic clearance of the *in vitro* incubation was calculated as $Cl_{int} = V_{max}/K_m$.

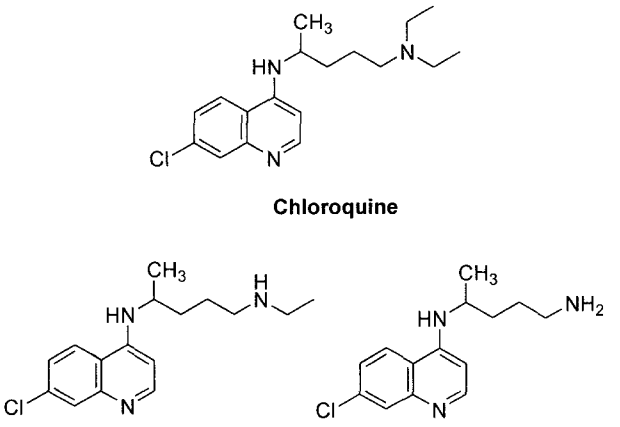

Fig. 1. Chemical structures of chloroquine, desethylchloroquine (DCQ), and bisdesethylchloroquine (BDCQ)

RESULTS

Chloroquine *N*-deethylation in human liver microsomes

Fig. 1 shows the DCQ formation in human liver microsomes. The Eadie-Hofstee plots for DCQ formation were biphasic (inset in Fig. 1), suggesting the involvement of multiple enzymes. The enzyme kinetic parameters for the chloroquine *N*-deethylation were therefore estimated according to a two enzyme model. The apparent K_m and V_{max} values of DCQ formation, along with intrinsic clearance (V_{max}/K_m) from four human liver microsomes, are summarized in Table I. The average K_m, V_{max}, and intrinsic clearance values for high and low affinity component were 0.21 mM, 1.02 pmol/min/mg protein and 4.94 μL/min/mg protein, and 3.43 mM, 10.47 pmol/min/mg protein and 3.09 μL/min/mg protein, respectively. The BDCQ was also found to be formed from an incubation study with chloroquine. However, the formation rate was relatively small (V_{max} = 0.53 pmol/min/mg protein), and the K_m value too high (K_m > 100 mM), compared with those of the DCQ formation, suggesting the minimal contribution of BDCQ formation in the metabolism of chloroquine.

Inhibition of DCQ formation by CYP-selective chemical inhibitors

Selective inhibitors of the nine major CYPs were used to identify the dominant CYPs that mediate the formation of DCQ (Fig. 3). Ketoconazole (CYP3A4/5) inhibited the formation of DCQ by 33 and 45% at concentrations of 1 and 10 μM, respectively. Quercetin (CYP2C8) also inhibited the formation of DCQ by 21 and 39% at concentrations of 20 and 100 μM, respectively. In contrast, furafylline (CYP1A2), coumarin (CYP2A6), orphenadrine (CYP2B6), sulfaphenazole (CYP2C9), (*S*)-mephenytoin (CYP2C19), quinidine (CYP2D6) and DDC (CYP2E1) showed weak or no inhibition of the formation of DCQ.

Chloroquine *N*-deethylation activity screening in cDNA-expressed CYPs

To evaluate the contribution of the major CYPs to the

Table I. Kinetic parameters for chloroquine *N*-deethylation to DCQ in human microsomes

	High affinity component			Low affinity component		
	V_{max} (nmol/min/mg protein)	K_m (mM)	Cl_{int} (μL/min/mg protein)	V_{max} (nmol/min/mg protein)	K_m (mM)	Cl_{int} (μL/min/mg protein)
HG-42	1.34	0.27	4.96	9.06	2.57	3.53
HG-43	1.49	0.30	4.97	12.6	4.12	3.06
HG-56	0.49	0.11	4.45	11.78	4.10	2.87
HG-93	0.75	0.14	5.36	8.42	2.91	2.89
Mean±S.D.	1.02±0.48	0.21±0.09	4.94±0.37	10.47±2.04	3.43±0.80	3.09±0.30

Cl_{int}, intrinsic clearance

V_{max} represents the maximal reaction velocity and K_m is the substrate concentration corresponding to 50% of V_{max}. Values were estimated from the nonlinear least regression analysis using WinNONLIN program.

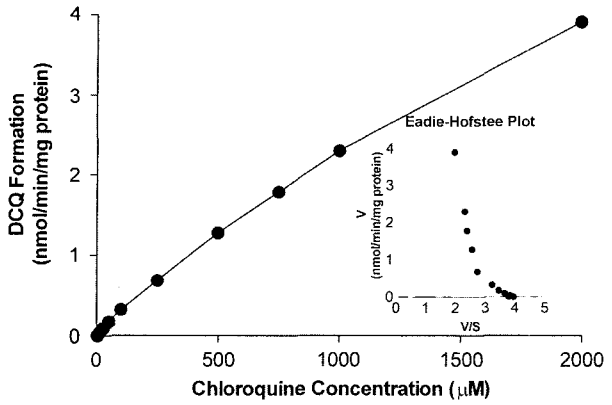

Fig. 2. Representative substrate saturation plot of chloroquine *N*-deethylation to DCQ in human liver (HG-56) microsomes with inset Eadie-Hofstee plot demonstrating biphasic kinetics

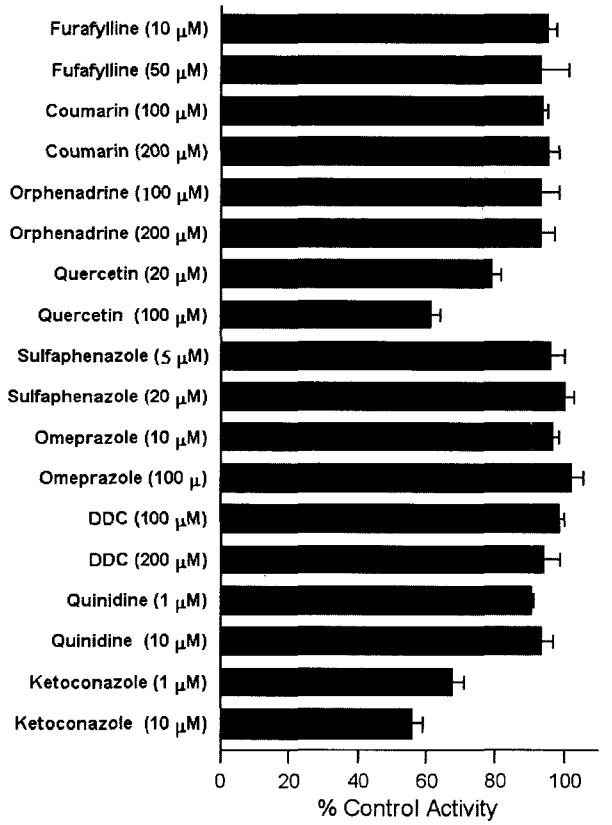

Fig. 3. Inhibition of chloroquine *N*-deethylation to DCQ in human liver microsomes by CYP-specific chemical inhibitors. Chemical inhibitors of CYPs were used in this experiment: furafylline (CYP1A2), coumarin (CYP2A6), orphenadrine (CYP2B6), quercetin (CYP2C8), sulfaphenazole (CYP2C9), omeprazole (CYP2C19), diethyldithiocarbamate (DDC, CYP2E1), quinidine (CYP2D6), and ketoconazole (CYP3A). The results represent the mean±S.D. of percentage of remaining activity relative to control DCQ formation rate (90.5±16.5 pmol/min/mg protein at 25 μM chloroquine).

chloroquine *N*-deethylation to DCQ, 25 μM chloroquine was incubated with a series of cDNA expressing CYPs

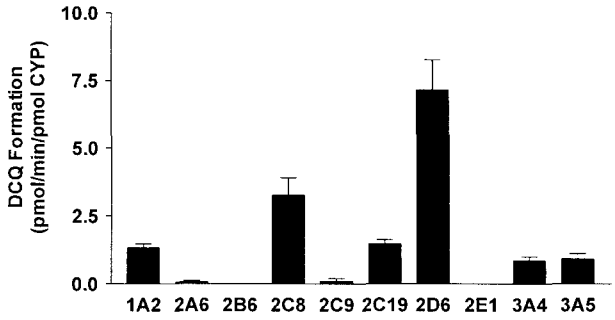

Fig. 4. Formation of DCQ after addition of 25 μM chloroquine in cDNA-expressed CYP isoforms. The results represent the mean (±S.D.) of a duplicate experiment done in each cDNA-expressed CYP. Formation rates are expressed per picomole of cDNA-expressed CYPs at a chloroquine concentration of 25 μM.

(1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5). As shown in Fig. 4, of all the CYPs tested, CYP2D6 displayed the greatest *N*-deethylation activity, followed by CYP2C8, 1A2, 2C19, 3A5 and 3A4, whereas little activity was observed with CYP2A6, 2C9 and 2E1.

Chloroquine *N*-deethylation activity in cDNA-expressed CYPs

Among the cDNA expressing CYPs tested, substrate saturation experiments were conducted in CYP1A2, 2C8, 2C19, 2D6, 3A4 and 3A5. The apparent K_m, V_{max}, and intrinsic clearance values of DCQ formation from chloroquine are summarized in Table II. Chloroquine showed the highest affinity in the CYP2C8 isoform and the highest capacity in the CYP2D6 isoform. The intrinsic clearance of chloroquine was highest in the CYP2D6 isoform, followed by 2C8, 2C19, 1A2, 3A5 and 3A4 (Table II).

Table II. Kinetic parameters for chloroquine *N*-deethylation to DCQ in cDNA-expressed CYP1A2, CYP2C8, CYP2D6, CYP3A4, and CYP3A5.

	Kinetic parameters of DCQ formation		
	V_{max} (pmol/min/pmol CYP)	K_m (mM)	Cl_{int} (μL/min/pmol CYP)
CYP1A2	230.0	4.48	0.051
CYP2C8	52.1	0.43	0.121
CYP2C19	113.2	2.39	0.047
CYP2D6	479.8	3.72	0.129
CYP3A4	166.5	5.00	0.033
CYP3A5	215.1	4.98	0.043

Cl_{int}, intrinsic clearance

V_{max} represents the maximal reaction velocity and K_m is the substrate concentration corresponding to 50% of V_{max}. Values were estimated from the non-linear least regression analysis using WinNONLIN program.

Table III. *Correlation of chloroquine N-deethylation activity with CYP isoform-specific activities in 12 human liver microsomes.*

Isoform-specific activities	Correlation Coefficient (r)
Midazolam 1-hydroxylation (CYP3A4/5)	0.900 (P=0.0004)
S-Mephenytoin 4-hydroxylation (CYP2C19)	0.056*
Dextromethorphan O-demethylation (CYP2D6)	0.342*
Paclitaxel 6α-hydroxylation (CYP2C8)	0.868 (P=0.0011)
Phenacetin O-deethylation (CYP1A2)	0.208*

*, not significant (P>0.05)

Correlation of chloroquine *N*-deethylation activity in a panel of human liver microsomes

The chloroquine *N*-deethylation activity, measured in human liver microsomes from 12 human livers, ranged from 7.47 to 25.25 pmol/min/mg protein. Correlations of these activities, from a panel of human liver microsomes, with their standard CYP isoform-specific activities, demonstrated a correlation with the CYP3A4/5-catalyzed midazolam 1-hydroxylation (r=0.900, P=0.0004) and CYP2C8-catalyzed paclitaxel 6-hydroxylation (r=0.868, P=0.0011) (Table III).

DISCUSSION

The *in vitro* metabolism of chloroquine in the presence of human liver microsomes and cDNA expressing CYPs have been demonstrated in this report for the first time. The CYP2C8 and CYP3A4/5 were observed to be the major enzymes in the formation of DCQ from chloroquine.

In this study, it was identified that the chloroquine was metabolized into DCQ in human liver microsomes; this is consistent with previous data (Ducharme and Farinotti, 1997). The Eadie-Hofstee plots of chloroquine *N*-deethylation in human liver microsomes were clearly biphasic, indicating the involvement of multiple enzymes. Consistent with this assumption, several CYP isoforms (i.e. CYP1A2, 2C8, 2C19, 2D6, 3A4/5) were found to be involved in the formation of DCQ using cDNA expressing CYPs. However, when the inhibition of the DCQ formation was estimated, by CYP-selective chemical inhibitors, only quercetin (a CYP2C8 inhibitor) and ketoconazole (a CYP3A4/5 inhibitor) showed significant inhibitions. The other CYP-selective chemical inhibitors showed no, or only weak inhibition, of the metabolism of chloroquine. A correlation study also supported the role of both CYP2C8 and CYP3A in the formation of DCQ in human liver microsomes. Altogether, CYP2C8 and CYP3A had a significant contribution to the formation of DCQ in human liver microsomes. The other CYPs that showed DCQ formation may also contribute to a minimal extent.

Interestingly, the DCQ formation was about two- and nine-fold greater in CYP2D6 than those in CYP2C8 and 3A4/5, respectively, when the CYP isoforms involved in the metabolism of chloroquine were screened and identified, using cDNA expressing CYPs. From this result, CYP2D6 might be considered the main isoform involved in the formation of DCQ. The substrate saturation experiments showed the highest capacity (V_{max}) and intrinsic clearance rate in CYP2D6, but its K_m value was relatively high, suggesting it to be one of low affinity components of chloroquine metabolism. Individual CYP data suggest that CYP2D6 is a main isoform of chloroquine metabolism, but different results were also found, indicating a relatively weak relationship between the CYP2D6 and the formation of DCQ in human microsomal preparations. Firstly, the chemical inhibitor studies showed that quinidine (a CYP2D6 inhibitor) did not inhibit the formation of DCQ. Secondly, the correlation study showed no significance between the DCQ formation, and the CYP2D6-catalyzed dextromethorphan *O*-demethylation, in a panel of human liver microsomes (r=0.342). Shimada *et al.* (1994) reported that the relative CYP3A4/5, 2C8/9/18/19, 1A2, 2E1 and 2A6 contents in 60 human samples are 29, 18, 13, 7 and 4%, respectively, with a CYP2D6 level of only 2%, suggesting a relatively small CYP2D6 content compared with those of the other CYPs. Based on the relatively small proportion of CYP2D6, and the chemical inhibitor and correlation studies, the CYP2D6 is unlikely to be involved in the formation of DCQ. Our *in vitro* results are in agreement with previous studies, which outlined the role of CYP2D6 in the metabolism of chloroquine in humans. Imipramine, a CYP2D6 substrate and inhibitor, was shown to elicit no difference in the plasma and urine profiles of chloroquine or its metabolites (Onyeji *et al.*, 1993). In contrast, the formation of DCQ from chloroquine was highly correlated with midazolam 1-hydroxylation. Ketoconazole, which is a potent inhibitor of CYP3A4/5, had a strong inhibitory effect on the formation of DCQ. Screening for chloroquine metabolism, with a panel of different cDNA expressing CYPs, indicated both CYP3A4 and 3A5 to be involved in the DCQ formation. Taken together, these studies confirmed the significant contribution of CYP3A4/5 in the metabolism of chloroquine. Even though the intrinsic clearance values of CYP3A4/5 are relatively lower than for the other CYPs tested, both isoforms should catalyze a significant portion of the DCQ formation as the respective proportions of the CYP3A4/5 contents are the largest (Shimada *et al.*, 1994). On the other hand, the formation of DCQ was also significantly correlated with the CYP2C8-catalyzed paclitaxel 6α-hydroxylation in a panel of human liver microsomes. Quercetin, a CYP2C8 inhibitor, showed an inhibitory effect on the DCQ formation (Bourrie *et al.*, 1996; Eagling *et al.*, 1998). With these results, it was confirmed that CYP3A4/5 as well as CYP2C8 have an important roles in the metabolism of chloroquine. Moreover, the substrate saturation experiments showed that the affinity

of the chloroquine for CYP2C8 was the highest of all the CYPs tested, with the intrinsic clearance of CYP2C8 for the DCQ formation also being significantly higher than the other CYPs, with the exception of CYP2D6.

Although chloroquine is still one of the most widely used antimalarial drugs, drug interaction studies, or case reports on drug interactions, on chloroquine are still lacking. One clinical study showed that pretreatment with cimetidine, an inhibitor of nonspecific CYP isoforms, especially CYP2C and CYP3A, resulted in a 50% increase in the half-life of chloroquine, and was associated with a 50% decrease in its clearance (Ette *et al.*, 1987). Because the area under the time-concentration curve (AUC) of DCQ increased by 47%, cimetidine probably decreased chloroquine metabolism by the inhibition of the CYPs. This suggests that the CYP-catalyzed chloroquine metabolism is affected by inhibition of the CYP. Although chloroquine has few side effects when used at the recommended oral doses, it has been established that pruritus, induced by the drug, is associated with the plasma chloroquine level (Ademowo *et al.*, 2000; Onyeji and Ogunboda, 2001). Because the metabolic activity of CYPs in catalyzing the drug correlates with their plasma concentrations, the activity of CYP2C8 and CYP3A4/5 may be related to chloroquine-induced pruritus. However, further studies will be necessary to deduce the mechanism(s).

In conclusion, our data demonstrated that CYP2C8 and CYP3A4/5 are the major enzymes responsible for the chloroquine *N*-deethylation into DCQ in human liver microsomes. Our results also suggested that CYP2D6, 1A2 and 2C19 have minor roles in the chloroquine *N*-deethylation.

ACKNOWLEDGMENT

This work was supported by Grant R03-2002-000-00028-0 from the Korea Science and Engineering Foundation.

REFERENCES

Ademowo, O. G., Sodeinde, O., and Walker, O., The disposition of chloroquine and its main metabolite desethylchloroquine in volunteers with and without chloroquine-induced pruritus: evidence for decreased chloroquine metabolism in volunteers with pruritus. *Clin. Pharmacol. Ther.*, 67(3), 237-241 (2000).

Augustijns, P., Geusens, P., and Verbeke, N., Chloroquine levels in blood during chronic treatment of patients with rheumatoid arthritis. *Eur. J. Clin. Pharmacol.*, 42(4), 429-433 (1992).

Bourrie, M., Meunier, V., Berger, Y., and Fabre, G., Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. *J. Pharmacol. Exp. Ther.*, 277(1), 321-332 (1996).

Broly, F., Libersa, C., Lhermitte, M., Bechtel, P., and Dupuis, B., Effect of quinidine on the dextromethorphan O-demethylase activity of microsomal fractions from human liver. *Br. J. Clin. Pharmacol.*, 28, 29-36 (1989).

Ducharme, J. and Farinotti, R., Rapid and simple method to determine chloroquine and its desethylated metabolites in human microsomes by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B Biomed. Sci. Appl.*, 698(1-2), 243-250 (1997).

Eagling, V. A., Tjia, J. F., and Back, D. J., Differential selectivity of cytochrome P450 inhibitors against probe substrates in human and rat liver microsomes. *Br. J. Clin. Pharmacol.*, 45(2), 107-114 (1998).

Ette, E. I., Brown-Awala, E. A., and Essien, E. E., Chloroquine elimination in humans: effect of low-dose cimetidine. *J. Clin. Pharmacol.*, 27(10), 813-816 (1987).

Ette, E. I., Essien, E. E., Thomas, W.O., and Brown-Awala, E.A., Pharmacokinetics of chloroquine and some of its metabolites in healthy volunteers: a single dose study. *J. Clin. Pharmacol.*, 29(5), 457-462 (1989).

Frisk-Holmberg, M., Bergqvist, Y., Termond, E., and Domeij-Nyberg, B., The single dose kinetics of chloroquine and its major metabolite desethylchloroquine in healthy subjects. *Eur. J. Clin. Pharmacol.*, 26(4), 521-530 (1984).

Gustafsson, L. L., Walker, O., Alvan, G., Beermann, B., Estevez, F., Gleisner, L., Lindstrom, B., and Sjoqvist, F., Disposition of chloroquine in man after single intravenous and oral doses. *Br. J. Clin. Pharmacol.*, 15(4), 471-479 (1983).

Harris, J. W., Rahman, A., Kim, B. R., Guengerich, F. P., and Collins, J. M., Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res.*, 54, 4026-4035 (1994).

Ofori-Adjei D. and Ericsson, O., Chloroquine in nail clippings. *Lancet.*, 2(8450), 331 (1985).

Onyeji, C. O. and Ogunbona, F. A., Pharmacokinetic aspects of chloroquine-induced pruritus: influence of dose and evidence for varied extent of metabolism of the drug. *Eur. J. Pharm. Sci.*, 13(2), 195-201 (2001).

Onyeji, C. O., Toriola, T. A., and Ogunbona, F. A., Lack of pharmacokinetic interaction between chloroquine and imipramine. *Ther. Drug Monit.*, 15(1), 43-46 (1993).

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., and Guengerich, F. P., Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.*, 270(1), 414-423 (1994).

Tassaneeyakul W., Birkett D. J., Veronese M. E., McManus M. E., Tukey R. H., Quattrochi L. C., Gelboin H. V., and Miners J. O. Specificity of substrate and inhibitor probes for human cytochromes P450 1A1 and 1A2. *J. Pharmacol. Exp. Ther.*,

265, 401-407 (1993).

Thummel, K. E., Shen, D. D., Podoll, T. D., Kunze, K. L., Trager, W. F., Hartwell, P. S., Raisys, V. A., Marsh, C. L., McVicar, J. P., Barr, D. M., Perkins, J. D., and Cariers, R. L., Use of midazolam as a human cytochrome P450 3A probe: I. In vitro-in vivo correlations in liver transplant patients. *J. Pharmacol. Exp. Ther.*, 271, 549-556 (1994).

Wrighton, S. A., Stevens, J. C., Becker, G. W., and Vandenbranden, M., Isolation and characterization of human cytochrome P4502C19: Correlation between 2C19 and *S*-mephenytoin 4'-hydroxylation. *Arch. Biochem. Biophys.*, 306, 240-245 (1993).