Evidence for involvement of human CYP3A in the 3-hydroxylation of quinine

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Aims Our previous studies using *in vitro* hepatic microsomal preparations suggested that the hepatic metabolism of quinine to form the major metabolite 3-hydroxyquinine is most likely catalysed by human P450 3A (CYP3A). The present study was carried out to investigate the kinetics and to identify and further characterise the human liver CYP isoforms involved in the metabolism of quinine.

Methods *In vitro* human microsomal techniques were employed.

Results The mean apparent K_m value for 3-hydroxyquinine formation was 83 ± 19 (s.d.) μM , ranging from $57 \mu M$ to $123 \mu M$ in microsomes from ten human livers. There was a 6.7-fold variation in V_{max} values (mean 547 ± 416 pmol min⁻¹ mg⁻¹). Quinine 3-hydroxylation was inhibited by the specific CYP3A inhibitors, troleandomycin, midazolam and erythromycin. Inhibitors selective for CYP1A1/2, CYP2D6, CYP2E1, CYP2C9/10 or CYP2C19 had little or no effect on quinine 3-hydroxylation. Using microsomes from a panel of livers, significant correlations were found only between 3-hydroxyquinine activity and other CYP3A activities (caffeine 8-oxidation, omeprazole sulphoxidation, midazolam 1'-hydroxylation and midazolam 4-hydroxylation) and immunoreactive CYP3A content. There were no statistically significant correlations with activities selective for CYP1A2, CYP2C9 and CYP2E1. Competitive inhibition of quinine 3-hydroxylation was observed with a substrate known to be specifically metabolized by human CYP3A, i.e. midazolam, with an apparent K_i value of $11.0 \mu M$.

Conclusions The present results strongly indicate that the conversion of quinine to 3-hydroxyquinine is the major metabolic pathway in human liver *in vitro* and that the reaction is catalysed by CYP3A isoforms.

Keywords: quinine, drug metabolism, CYP3A, human liver microsomes

Introduction

The antimalarial quinine still remains the drug of choice for treatment of severe and complicated malaria. Its importance has even increased during recent decades due to the spread of chloroquine resistant falciparum malaria [1, 2]. Despite its long history in the treatment of malaria, the metabolism of quinine in humans has not been fully elucidated. By contrast, the metabolism of its diastereoisomer, quinidine, has been well studied. Major metabolites of quinidine appear to be 3-hydroxyquinidine, quinidine-*N*-oxide and 2'-quinidinone [3, 4]. The *in vitro* hepatic microsomal metabolism of quinidine to form 3-hydroxyquinidine and quinidine-*N*-oxide metabolites was shown to be catalysed by CYP3A4 [3]. These authors also reported a polar product of quinine formed after incubation with human liver microsomes which had a similar retention time to 3-hydroxyquinidine, but this product was not identified [3]. Liddle *et al.* [5] had identified 3-hydroxyquinine and *O*-desmethylquinine in human urine after administration of quinine.

Cytochrome P450 (CYP) plays an important role in the biotransformation of numerous endogenous compounds

and xenobiotics, including steroids, fatty acids, drugs and carcinogens. Quinine is extensively metabolized by hepatic enzymes [6]. The human CYP forms responsible for the biotransformation of quinine have not been identified. Our preliminary studies [7, 8] using *in vitro* hepatic microsomal preparations have shown that the hepatic metabolism of quinine to form the major metabolite 3-hydroxyquinine (3-OH.Q) is most likely catalysed by human CYP3A. However, previous study has shown that cigarette smoking enhances the elimination of quinine [9], metabolism assumed to be catalysed by CYP3A. These findings could not be predicted by *in vitro* microsomal studies as it is well known that smoking mainly induces the CYP1A enzyme family [10–12]. This suggests that the metabolism of quinine may be catalysed by either CYP3A or CYP1A or both. To better understand and predict metabolic drug interactions with quinine, the present study aimed to identify and further characterise the human liver CYP isoform(s) involved in the metabolism of quinine using an *in vitro* human liver microsomal technique.

This work was presented in part at the 28th Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Auckland, New Zealand, December 1994.

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Methods

Chemicals and reagents

Quinine bisulphate was kindly supplied by Kimia Pharma, Indonesia. Synthetic 3-hydroxyquinine was kindly donated by Dr P. Winstanley, University of Liverpool, UK. Furaflavine was a gift from Dr R. Gasser, Hoffman-LaRoche, Basle, Switzerland. Midazolam, 1'-hydroxymidazolam, 4-hydroxymidazolam and debrisoquine were kindly provided by Roche (New Zealand) Ltd. Erythromycin, NADPH, caffeine, troleandomycin (TAO), diethyldithiocarbamate, α -naphthoflavone, phenacetin and tetrabutylammonium bromide (TBA) were purchased from Sigma Chemical Co (St Louis, MO, USA). Tolbutamide was kindly provided by Hoechst Australia (Melbourne, Australia). Sodium dodecylsulphate (SDS) was purchased from BDH Lab (Poole, England). All solvents and reagents used were of the highest purity grade commercially available.

Human liver microsomes

Human liver samples were obtained from registered organ donors. The use of such tissue in these studies had local Ethics Committee approval. Both patient 1 (H1) and 2 (H2) were male Caucasians aged 50 years who had met traumatic deaths. They were on no medications and had no significant past medical history. The clinical details of the donors of the other liver samples (H5–10, H12 and H13) used in this study have been published elsewhere [13]. Liver samples were stored at -80°C until used. Microsomes were prepared by differential centrifugation as described previously [14]. The protein content of the microsomal suspensions was determined by Sigma BCA reagent as described by the manufacturer. Standard calibration was made with bovine serum albumin.

Analytical procedures

3-Hydroxyquinine (3-OH.Q) concentration in microsomal samples was assayed by an h.p.l.c. method [15] using ion-paired chromatography. Detection of quinine and the metabolite 3-OH.Q was accomplished with the use of a fluorescence detector, with excitation and emission wavelengths of 350 and 450 nm, respectively. The detection limit of the method is 0.02 mg l^{-1} ($0.06\text{ }\mu\text{M}$). The inter-assay coefficient of variation was less than 6% over the concentration range of 0.02 to 10 mg l^{-1} (0.06 to $31\text{ }\mu\text{M}$). The mobile phase consisted of an acetonitrile–aqueous phosphate buffer (10 mM, pH 2.1) mixture (40:60, v/v) containing 10 mM SDS and 0.1 mM TBA.

Identification of quinine metabolites

Initially, as synthetic quinine metabolites are still not available (except 3-hydroxyquinine), the metabolites of its diastereoisomer quinidine were used as reference compounds in order to identify possible metabolites of quinine. Quinidine metabolites, 2'-oxoquinidine (2'-oxoquinidine), quinidine-N-oxide, 3-hydroxyquinidine and O-

desmethylquinidine (6'-hydroxycinchonidine) were kindly provided by Professor I. A. Blair, Vanderbilt University of Medicine, Tennessee, USA. The authentic 3-hydroxyquinine (3-OH.Q) was used as a reference for identification of chromatographic peaks and for quantitative analysis.

In vitro hepatic microsomal metabolism of quinine

0.5 ml incubation mixtures comprised the substrate quinine ($100\text{ }\mu\text{M}$, unless otherwise stated), 1 mg ml^{-1} of microsomal protein and 1 mM NADPH in phosphate buffer (0.067 M, pH 7.4). The reaction was initiated by the addition of NADPH to a final concentration of 1 mM. In blank incubations, quinine solution was replaced by a corresponding volume of phosphate buffer or organic solvent used for dissolving inhibitors as appropriate, i.e. without inhibitors. Incubations were performed at 37°C in a shaking water bath for 30 min. The reaction was terminated by the addition of cold methanol (1 ml) and cooling on ice. The samples were vortex mixed briefly and centrifuged at 1500 g for 10 min. The resultant supernatant fraction was diluted 1 in 4 with h.p.l.c. water and an aliquot (30 μl) of this diluted supernatant was injected onto the h.p.l.c. column. Dilution was carried out to ensure the detection of 3-OH.Q was within the appropriate attenuation of the h.p.l.c. integrator.

Kinetics of quinine metabolism

Different concentrations of quinine, ranging from 12.5 to $500\text{ }\mu\text{M}$, were used in the kinetic experiments. Ten different human livers (H1–2, H5–10, H12–13) were studied and apparent K_m and $V_{\max}$ values for the formation of 3-hydroxyquinine were determined. The Michaelis-Menten parameters were estimated initially by graphical analysis of Eadie-Hofstee plots. These values were subsequently used as initial estimates for a least square non-linear modelling programme, MINIM (courtesy Dr R. Purves, Department of Pharmacology, University of Otago, Dunedin, New Zealand). The kinetics of the inhibition of 3-OH.Q formation by the CYP3A inhibitors, TAO, midazolam and erythromycin, were visualized by fitting the curves obtained by non-linear regression analysis to the Lineweaver-Burk equation. The data were fitted using conventional relationships for competitive, non-competitive, uncompetitive and mixed type inhibition [16].

Inhibition experiments

The effects of a range of CYP isoform selective inhibitors/activators were determined at two inhibitor concentrations in microsomes from two livers (H1 and H2) using substrate concentrations approximating the apparent K_m value for quinine hydroxylation determined previously in these livers. The isoform selective inhibitors or alternative substrates were caffeine (CYP1A1/2) [17]; α -naphthoflavone (CYP1A inhibition at low concentrations and normally CYP3A activation at higher concentrations) [13]; furaflavine (CYP1A2) [18]; sulphaphenazole (CYP2C9/10) [19]; R,S-mephenytoin (CYP2C19) [20]; tolbutamide (CYP2C9) [21];

diethyldithiocarbamate (CYP2E1) [22]; debrisoquine (CYP2D6) [23]; troleandomycin (CYP3A) [20]; midazolam (CYP3A) [24]; erythromycin (CYP3A) [24]. The putative inhibitors were studied at two concentrations chosen to be selective for the respective CYP isoforms on the basis of published K_i or K_m values. All inhibitors/activators were dissolved in methanol, except for mephenytoin, furafylline and 7-ethoxycoumarin which were dissolved in phosphate buffer. In all cases, inhibited activities were compared with activities in control incubations (a final volume of 500 μ l) containing either 5 μ l (1% v/v) of methanol or phosphate buffer (5 μ l) as appropriate. TAO was preincubated with microsomes and the NADPH for 10 min at 37° C before the reaction was started by addition of the substrate quinine. Otherwise, the incubations were started by adding the NADPH.

Correlation experiments

The rates of quinine hydroxylation to 3-OH.Q using microsomes from eight human livers (H5–10, H12 and H13) from the Flinders Medical Centre liver bank were correlated with activities towards the metabolism of other isoform selective substrates. The data for the high affinity component of phenacetin *O*-deethylation [25], tolbutamide methylhydroxylation [21], 4-nitrophenol hydroxylation [26], caffeine 8-oxidation [17] and omeprazole sulphone formation [27] have been published previously. Midazolam 1'-hydroxylation and 4-hydroxylation were determined by the h.p.l.c. method of Kronbach *et al.* [28] with slight modifications. The modifications included use of a C18-Nucleosil (5 μ m, 4.6 mm i.d. $\times$ 15 cm) h.p.l.c. column and a mobile phase of 10 mM potassium phosphate buffer (pH 7.4)/acetonitrile/methanol (400:200:100, v/v/v) delivered at 1 ml min⁻¹ with u.v.-detection. Under these conditions, retention times were 4-hydroxymidazolam, 5.1 min; 1'-hydroxymidazolam, 6.1 min; internal standard (diazepam), 8.1 min; and midazolam 10.6 min. Coefficients of variation with incubations using the same liver microsomes were 6.8% at 100 μ M midazolam and 5.3% at 30 μ M midazolam. Quinine hydroxylation rates were also correlated with immunoreactive CYP3A protein contents determined previously by Western blot analysis [13].

Expression of CYP1A1 and CYP1A2 in COS-7 cells

CYP1A1 or CYP1A2 cDNAs were isolated, subcloned into the pCMV4 expression vector and transfected into COS-7 cells according to procedures described elsewhere [17]. Cells were harvested 48 h post-transfection, resuspended in 0.1 M phosphate buffer (pH 7.4) containing 20% v/v glycerol and stored at -70° C until required. Expressed CYP1A1 and CYP1A2 proteins were immunoadsorbed with polyclonal anti-rabbit CYP1A2 antibody, which recognised both CYP1A1 and CYP1A2. In this manner, the relative expression of CYP1A1 and CYP1A2 was shown to be 1.3:1 [25]. Both expressed human CYP1A1 and CYP1A2 were demonstrated to actively catalyse the *O*-demethylation of phenacetin, a known CYP1A substrate [25].

Incubation of quinine with cDNA expressed CYP1A1 and CYP1A2

Metabolism of quinine by expressed CYP1A1 and CYP1A2 was determined by a method described previously for the study of caffeine biotransformation *in vitro* [17]. A 0.5 ml incubation contained COS cell protein (2 mg ml⁻¹), the phosphate buffer (0.067 M, pH 7.4), NADPH (1 mM) and quinine (0.5 mM) as substrate. Reactions were initiated by the addition of NADPH and carried out at 37° C. The incubation was performed for 30 min and reactions were terminated by the addition of cold methanol (1 ml) and cooling on ice. After centrifugation at 2000 *g* for 10 min, 20 μ l supernatant was injected onto an h.p.l.c. column and analysed by using the modified mobile phase [15].

Statistical analysis

Results are expressed as mean $\pm$ s.d. Statistical evaluation of results was performed using Student's *t*-test. When variances of data were unequal, the comparison was assessed by Welch's test. In all cases, $P < 0.05$ was considered the minimum level of statistical significance.

Results

Metabolism of quinine in human liver microsomes

Blank samples of human liver microsomes showed no interference with the h.p.l.c. assay. After microsomal incubation with quinine, both at 100 μ M and 500 μ M for 30 min, the major metabolite peak observed had a retention time of 19.3 min, which corresponded to authentic standard 3-OH.Q. This confirms that the major metabolite of quinine in human liver microsomes is 3-OH.Q (Figure 1).

The rate of 3-OH.Q formation was linear for microsomal protein concentrations of 0.25 to 3 mg ml⁻¹ up to 45 min. Therefore, 1 mg ml⁻¹ human microsomal protein and an incubation time of 30 min were used in all experiments.

Kinetics of quinine metabolism

A substrate *vs* velocity plot for formation of 3-OH.Q by human microsomes from liver H1 is shown in Figure 2, with the corresponding Eadie-Hofstee plot. In microsomes from ten livers studied (H1–2, H5–10, H12–13), all Eadie-Hofstee plots were linear over the substrate (quinine) concentration range 12.5 to 500 μ M (Figure 2). The apparent K_m values ranged from 57 μ M to 123 μ M and there was a 6.7-fold variation in apparent V_{max} values (range 222 to 1490 pmol min⁻¹ mg⁻¹) (Table 1). Estimates of relative intrinsic clearance (CL_{int}) of quinine, based on V_{max}/K_m calculations, revealed a very large variation (8-fold) (Table 1).

Inhibition experiments

The effects of various inhibitors/activators on the formation of 3-OH.Q are shown in Figure 3. After incubation of quinine with troleandomycin (TAO), 3-OH.Q formation was markedly inhibited at both concentrations of the substrate (100 μ M and 500 μ M). When midazolam and

Figure 1 H.p.l.c. chromatograms showing the formation of the major metabolite (3-hydroxyquinine) by human liver microsomes. (a) Quinine (100 μM) was incubated with human liver microsomes, H1 (1 mg ml⁻¹); and (b) Quinine (500 μM) was incubated with H1 liver microsomes (1 mg ml⁻¹). The reaction was initiated by addition of 1 mM NADPH, at 37° C and was terminated after 30 min. Peak identification: 3-OH=3-hydroxyquinine and Q=quinine.

erythromycin were separately added into the incubations, 3-OH.Q formation was moderately inhibited. Consistent with this, α -naphthaflavone 50 μM (which usually activates isoforms of the CYP3A subfamily) activated 3-OH.Q formation by 44% at 500 μM quinine. However, at a lower quinine concentration of 100 μM , α -naphthoflavone (50 μM) caused a moderate inhibition of 3-OH.Q formation in both livers, H1 and H2. Sulphaphenazole, mephentoin and tolbutamide (specific CYP2C inhibitors), slightly decreased the rate of 3-OH.Q formation. There was no or only slight inhibition in both livers by caffeine (CYP1A1/2), phenacetin (CYP1A1/2), furafylline (CYP1A2), 7-ethoxycoumarin (CYP1A2), debrisoquine (CYP2D6) and diethyldithiocarbamate (CYP2E1). Similar patterns of inhibition and activation were seen with microsomes from human liver (H2) at a quinine concentration of 100 μM . Experiments with a high substrate (quinine) concentration at 500 μM were not performed with microsomes from liver H2 due to insufficient sample.

In vitro inhibition of quinine 3-hydroxylation by midazolam

In order to determine the nature of inhibition of 3-OH.Q formation by midazolam, a CYP3A specific inhibitor,

human hepatic microsomes were incubated with increasing quinine concentrations ranging from 12.5 to 500 μM , in the absence or the presence of various midazolam concentrations. The rates of formation of 3-OH.Q were assessed and analysed according to the Dixon method. The Dixon plots showed that midazolam inhibited 3-OH.Q formation competitively (Figure 4) with an apparent inhibitory constant (K_i) of 11.0 μM . V_{max} remained unchanged in the absence or presence of midazolam (data not shown).

Correlation experiments

Table 2 shows the correlations between quinine hydroxylation to form 3-OH.Q and CYP isoform selective activities. Correlations with CYP1A2 activity (phenacetin *O*-deethylation), CYP2C9 activity (tolbutamide methylhydroxylation) and CYP2E1 activity (4-nitrophenol hydroxylation) were not statistically significant. Significant correlations were observed only with the CYP3A activities, caffeine 8-oxidation, omeprazole sulphone formation, midazolam 1'-hydroxylation and midazolam 4-hydroxylation. The formation of 3-OH.Q also correlated significantly ($r=0.96$, $P<0.01$) with immunoreactive CYP3A content (Figure 5) in eight human liver microsomal samples studied (H5-H10,

Figure 2 Substrate *vs* velocity and Eadie-Hofstee plots for the formation of 3-hydroxyquinine using H1 liver microsomes.

H12 and H13). Livers H1 and H2 were not included in the analysis as the technique for determination of CYP3A content is not presently available in our laboratories.

Quinine was incubated with the COS-cells transfected with human cDNA expressed CYP1A1 and CYP1A2. A control experiment was performed by incubation of quinine with the vector alone (COS-cells). No quinine metabolites were observed with control cells or with cells expressing CYP1A1 or CYP1A2.

Table 2 Correlations between quinine hydroxylation to 3-hydroxyquinine and CYP isoform selective activities.

Activity or isoform	Correlation coefficient
Tolbutamide methylhydroxylation (2C9)	0.010
Phenacetin O-deethylation (1A2)	0.247
4-Nitrophenol hydroxylation (2E1)	0.205
Caffeine 8-oxidation (3A)	0.868*
Omeprazole sulphone formation (3A)	0.965*
Midazolam 1'-hydroxylation (3A)	0.989*
Midazolam 4-hydroxylation (3A)	0.981*

* $P < 0.01$.

Discussion

After incubation of quinine with human liver microsomes, 3-hydroxyquinine is the major metabolite formed *in vitro* and this is consistent with our *in vivo* results obtained from urine data [8]. At least seven metabolites were detected in human urine after quinine administration with three identified as 2'-oxoquinone, quinine glucuronide and 3-hydroxyquinine [8]. No peak with a retention time corresponding to quinine-*N*-oxide was detected from *in vitro* studies suggesting that this is not formed as a metabolite of quinine in humans. *In vivo* quinine excreted unchanged in urine accounts for approximately 8% of the dose, quinine glucuronide 1.2% and 3-hydroxyquinine 6% which leaves 85% of the dose unaccounted for, but we have observed at least four other metabolites, one of which has been identified as 2'-oxoquinone [8]. These *in vitro* results provide evidence for the possibility of differences in the metabolism of the diastereoisomers, quinine and quinidine. O-desmethylquinine was found to be the metabolite of quinine after incubation with rabbit or guinea pig liver microsomes [29]. It also has been identified in human urine [5]. Our *in vivo* study showed that urine samples from all subjects had no peak corresponding to O-desmethylquinine [8] and this is consistent with the present results from the *in vitro* system. However, this interpretation may be masked by the assumption that O-desmethylquinine has a similar fluorescence response to 3-hydroxyquinine and lack of a synthetic equivalent.

Table 1 Computer derived K_m and V_{max} values for formation of 3-hydroxyquinine in human liver microsomes from ten livers.

Liver number	Sex	Apparent K_m (μM)	Apparent V_{max} ($pmol\ min^{-1}\ mg^{-1}$)	V_{max}/K_m ($\mu l\ min^{-1}$)
H1	M	85	1490	17.5
H2	M	57	1035	18.2
H5	M	72	734	10.2
H6	M	63	270	4.3
H7	F	91	467	5.1
H8	M	123	274	2.2
H9	M	72	222	3.1
H10	F	77	299	3.9
H12	M	99	658	6.6
H13	F	91	293	3.2
Mean $\pm$ s.d.		83 $\pm$ 19	574 $\pm$ 416	7.4 $\pm$ 5.9

Figure 3 Effects of various specific CYP inhibitors and/or substrates on the formation of 3-hydroxyquinine (a) at a low substrate quinine concentration (100 μM); and (b) at a high substrate quinine concentration (500 μM) in human liver microsomes (H1). Each bar represents the average value of duplicate measurements.

Figure 4 Dixon plots for the inhibition of 3-hydroxyquinine formation by midazolam. $\square$ Quinine 25 μM ; $\bullet$ quinine 50 μM ; $\blacktriangle$ quinine 200 μM ; $\circ$ quinine 500 μM .

Figure 5 Correlation between the formation of 3-hydroxyquinine and immunoquantified levels of CYP3A for eight human liver microsomal samples.

Cytochrome P450 (CYP) exists as multiple isoforms which respond differently to specific inducers and inhibitors [30–32]. Within each mammalian species there are estimated to be over 20 different CYP gene products that have been characterized with regard to their properties and catalytic specificities [32, 33]. Many useful approaches are available for determining which CYP isoforms make major contributions to the metabolism of a particular drugs, and include assessing the effect of specific inhibitors and antibodies in microsomal preparations.

The kinetics of 3-hydroxyquinine formation *in vitro* by human liver microsomes followed Michaelis–Menten kinetics as demonstrated by linear Eadie–Hofstee plots (Figure 2). The monophasic linear kinetics are consistent with the involvement of a single CYP isoform in the biotransformation of quinine to 3-hydroxyquinine. The results presented in this report strongly indicate that *in vitro* quinine 3-hydroxylation to form 3-hydroxyquinine is mediated by CYP3A. This conclusion was drawn from the following experiments: (i) Quinine 3-hydroxylation was inhibited by the specific CYP3A inhibitors, troleandomycin, midazolam and erythromycin, while inhibitors selective for CYP1A1/2, CYP2D6, CYP2E1, CYP2C9 or CYP2C19 had little or no effect on quinine 3-hydroxylation. (ii) Significant correlations were found only between 3-hydroxyquinine formation and the other CYP3A activities (caffeine 8-oxidation, omeprazole sulphone formation, midazolam 1'-hydroxylation and midazolam 4-hydroxylation). (iii) Immunoreactive CYP3A content of microsomes from a liver bank correlated significantly with 3-hydroxyquinine activities. (iv) Competitive inhibition of quinine 3-hydroxylation was observed with a substrate known to be specifically metabolized by human CYP3A, i.e. midazolam [24, 34]. The K_i value of midazolam found in this study is in a range reported by others [35, 36]. CYP3A4 appears to be the major CYP3A enzyme expressed in adult liver [30, 37, 38] and is involved in the metabolism of many drugs, including nifedipine, erythromycin, midazolam, quinidine and troleandomycin [37, 39].

The biotransformation of quinine by human liver microsomes showed a broad inter-individual variability. The apparent K_m values for the formation of 3-hydroxyquinine ranged from 57 μM to 123 μM . There was a 6.7-fold variation in apparent V_{max} values. The *in vitro* intrinsic clearance (CL_{int}) of quinine, i.e. V_{max}/K_m , varied considerably (8-fold), similar to *in vivo* variation in CL_{int} (4-fold) [40]. The variability of *in vitro* intrinsic clearance reported in the present study is consistent with previous data on variations of other CYP3A substrates, eg. the rate of 4- and 1'-hydroxylation of midazolam varied 15-fold and 24-fold, respectively, in eighteen adult liver samples [34]. It is also in agreement with the 5-fold variability of total CYP3A contents observed in fourteen human liver microsomes [41].

It is evident from the present study that the metabolism of quinine is catalysed by CYP3A4 enzyme. Previous findings of a marked increase in quinine clearance in smokers [9] would suggest that CYP3A4 may be inducible by cigarette smoking. To our knowledge, there are no examples of CYP3A4 catalysed drugs whose metabolism is induced by cigarette smoking. As cigarette smoking mainly induces cytochrome CYP1A1 and CYP1A2 [10–12],

another possible explanation of the increased clearance of quinine [9] is the involvement of the CYP1A enzyme family. However, our results indicate that both cDNA expressed CYP1A1 and CYP1A2 are not involved in the metabolism of quinine. This is consistent with a recent study [42] showing that quinine is a potent inhibitor of CYP1A1 but is not metabolised by cDNA expressed CYP1A1. Our results also suggest that the increased metabolism of quinine in cigarette smokers found in the previous study is not due to enhanced activity of the CYP1A family.

In conclusion, this study provides evidence that 3-hydroxylation is the major CYP-mediated metabolic pathway of quinine and that 3-hydroxyquinine formation is catalysed selectively by CYP3A isoforms. Isoforms CYP1A1 and CYP1A2 are unlikely to be involved in the metabolism of quinine. There is a possibility that human CYP3A is inducible by cigarette smoking and this may explain why smoking enhances the elimination of quinine.

The studies were financially supported by the Otago Research Committee, University of Otago, New Zealand.

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(Received 23 January 1996,
accepted 14 November 1996)