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Metabolism of Quinine in Man: Identification of a Major Metabolite, and Effects of Smoking and Rifampicin Pretreatment

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Abstract

Our previous studies have shown that cigarette smoking and rifampicin pretreatment enhance the elimination of quinine, metabolism assumed, by analogy with quinidine, to be carried out by CYP3A (P450IIIA). This finding is unexpected since it has been shown that smoking induces the CYP1A rather than the CYP3A enzyme family, suggesting that the metabolism of quinine may be catalysed by CYP1A. Therefore, we conducted this study to identify possible quinine metabolites in human urine and to determine which metabolic pathway is induced by cigarette smoking and rifampicin pretreatment. A specific HPLC procedure was employed to identify metabolites of quinine in urine samples collected from healthy volunteers after an oral dose of 600 mg quinine sulphate.

The results showed that there were at least seven possible metabolites of quinine detected in human urine. Three of these were identified as 2′-oxoquininone, quinine glucuronide and 3-hydroxyquinine. The 3-hydroxyquinine appeared to be a major metabolite of quinine in urine samples from every subject who took an oral dose of quinine.

Although cigarette smoking and rifampicin pretreatment were shown to cause a marked increase in the elimination of quinine there were no significant changes in the formation of 3-hydroxyquinine as measured in the urine samples. This suggests that the effects of smoking and rifampicin are more complicated than we expected and require further investigation.

Use of quinine for the treatment of malaria decreased after synthetic antimalarials such as chloroquine, amodiaquine and mefloquine had been introduced. However, in recent decades, *Plasmodium falciparum* resistance to chloroquine and mefloquine has been rapidly increasing in both degree and prevalence in endemic areas such as Southeast Asia, South America and East Africa (Wernsdorfer 1991). This has resulted in an increased use of quinine, either singly or in combination with other antimalarials, for the treatment of malaria (White 1985; Wernsdorfer 1991).

Despite its long history in the treatment of malaria, the metabolism of quinine in man has not been fully elucidated. Quinine is extensively metabolized by hepatic enzymes (Brodie et al 1951). By contrast, the metabolism of its diastereoisomer, quinidine, has been well investigated. Major metabolites of quinidine appear to be 3-hydroxyquinidine, quinidine-*N*-oxide and 2′-quinidinone, while minor metabolites are *O*-desmethylquinidine, quinidine-10,11-dihydrodiol and its *N*-1′-oxide (Tamai et al 1985; Guengerich et al 1986). The in-vitro hepatic microsomal metabolism of quinidine to form 3-hydroxyquinidine and quinidine-*N*-oxide metabolites was shown to be catalysed by CYP3A4 (Guengerich et al 1986). These investigators also found a polar product of quinine after incubation with human liver microsomes which had a similar retention time to 3-hydroxyquinidine; however, this product was not identified (Guengerich et al 1986). Liddle et al (1981) have identified 3-hydroxyquinine and *O*-desmethylquinine in human urine after administration of quinine.

The human P450 (CYP) forms responsible for biotransformation of quinine have not been identified. Our recent studies (Wanwimolruk et al 1993, 1995) have shown that cigarette smoking and rifampicin pretreatment enhance the elimination of quinine (i.e. clearance was increased 77% by smoking, and 690% by rifampicin pretreatment) with metabolism assumed to be catalysed by CYP3A. These findings could not be anticipated as it is known that smoking induces the CYP1A rather than the CYP3A enzyme family (Sesardic et al 1988; Combalbert et al 1989; Ged et al 1989), which suggests that the metabolism of quinine may be catalysed by CYP1A. Therefore, we conducted this study to identify possible quinine metabolites in human urine and to determine which metabolic pathway is induced by smoking and rifampicin pretreatment.

Materials and Methods

The studies conducted in humans were approved by the Southern Regional Health Authority Ethics Committee (Otago), Dunedin, New Zealand.

Reagents and chemicals

All chemicals were of analytical grade. Quinine bisulphate was kindly supplied by Kimia Pharma, Indonesia. Cinchocaine hydrochloride (an internal standard) was obtained from Orgamol Evionnaz, Switzerland. Tetrabutylammonium bromide (TBA) and β-glucuronidase (type VII-A)

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were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium dodecyl sulphate (SDS), HPLC-grade methanol and acetonitrile were purchased from BDH Chemicals Ltd (Poole, UK). All water was double glass-distilled and MilliQ-filtered.

A stock solution of 1 mg mL^{-1} quinine free base was prepared in 50% (v/v) methanol-water. This solution was stored at −20°C until required. Standard solutions of plasma and urine with known concentrations of quinine were prepared by mixing stock quinine solutions and drug-free samples. The internal standard solution of cinchocaine (30 mg L^{-1}) was prepared in methanol and kept cold at −20°C until required.

Analytical procedures

Quinine concentrations in plasma, urine and microsomal samples were assayed by a HPLC procedure described previously (Zoest et al 1990), unless otherwise specified. Detection of quinine, its possible metabolites and quinidine metabolites (used as reference compounds) was accomplished with the use of a Hitachi fluorescence detector, with excitation and emission wavelengths of 350 and 450 nm, respectively. The detection limit of the method was 0·02 mg L^{-1} (0·06 μM). The inter-assay coefficient of variation was less than 8% over the concentration range of 0·02 to 10 mg L^{-1} (0·06–30·8 μM). Plasma and urine collected before administration of quinine tablets (i.e. blank samples) showed no endogenous sources of interference with the analytical assay.

Identification of quinine glucuronide metabolites in human urine: β-glucuronidase hydrolysis

To 200 μL plasma or urine sample, 400 μL 0·1 M phosphate buffer (pH 6·8) containing 2500 int. units mL^{-1} β-glucuronidase was added. The samples were incubated at 37°C in a shaking water bath for 2 h. Another 200 μL plasma or urine sample was treated and incubated under the same conditions without β-glucuronidase and served as a control. The β-glucuronidase used contains no activity for sulphatase. After incubation, the samples (200 μL) were treated with 400 μL cold methanol solution containing the internal standard (cinchocaine 30 mg L^{-1}) to precipitate the proteins. The samples were vortexed, centrifuged at 2000 *g* for 3 min and analysed by HPLC as above. As a glucuronide standard was not available, the amount of quinine glucuronide metabolite formed was deduced from the increase in quinine recovery.

Modification of the mobile phase

As the original mobile phase (Zoest et al 1990) used to analyse plasma and urine samples did not provide a satisfactory separation of quinine and its metabolites in human urine samples, the HPLC mobile phase was modified to consist of an acetonitrile-aqueous phosphate buffer (10 mM) mixture (40 : 60, v/v) containing 10 mM SDS and 0·1 mM TBA and adjusted to pH 2·1 with orthophosphoric acid.

Identification of urinary metabolites of quinine

Initially, as synthetic quinine metabolites are still not available, the metabolites of its diastereoisomer quinidine were used as reference compounds to identify possible metabolites of quinine in the human urine samples. Quinidine metabolites, 2′-oxoquinidine (2′-oxoquinidinone), quinidine-*N*-oxide, 3-hydroxyquinidine and *O*-desmethylquinidine (6′-hydroxycinchonidine) were kindly provided by Professor I. A. Blair, Vanderbilt University School of Medicine, Tennessee, USA. Later the synthetic 3-hydroxyquinine (kindly donated by Dr P. Winstanley, University of Liverpool, UK) was available and was therefore used as a reference.

Stock solutions of 10 mg L^{-1} of each metabolite of quinidine (2′-oxoquinidinone, quinidine-*N*-oxide, 3-hydroxyquinidine and *O*-desmethylquinidine) were prepared in 96 : 4 (v/v) methanol-water. The solutions were further diluted with distilled water and mixed to give a final concentration of 0·1 mg L^{-1} of all the metabolites, except for *O*-desmethylquinidine where the final concentration was 6·3 mg L^{-1}. The mixture of metabolites was injected into the HPLC column and the analysis was performed using the modified mobile phase.

Determination of 3-hydroxyquinine in urine samples

Urine samples previously obtained from non-smokers, smokers and subjects before and after rifampicin pretreatment (Wanwimolruk et al 1993, 1995) were analysed by the modified mobile phase for 3-hydroxyquinine concentrations. The standard curve for 3-hydroxyquinine was prepared in drug-free urine. The amount of 3-hydroxyquinine excreted in the urine was calculated for each subject from the observed urinary 3-hydroxyquinine concentration and urine volume. A fractional urinary recovery of 3-hydroxyquinine (f_{3-OH}) was calculated as the percentage of quinine dose administered. Partial metabolic clearance (CL_{3-OH}) to 3-hydroxyquinine was estimated as:

$$CL_{3-OH} = f_{3-OH} \cdot CL \quad (1)$$

where CL is total plasma clearance of quinine calculated from the plasma quinine concentration-time data as described previously (Wanwimolruk et al 1993, 1995).

Statistical analysis

Results are expressed as mean ± 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, $P < 0{\cdot}05$ was considered the minimum level of statistical significance.

Results

Evidence of quinine glucuronide metabolite

To identify any glucuronide metabolites formed as a result of quinine biotransformation, plasma samples were hydrolysed with β-glucuronidase. No significant changes in the peak heights of quinine and its possible metabolites in plasma were observed between those samples (n = 8) before and after hydrolysis with β-glucuronidase. There was no significant difference in plasma quinine concentrations before (1·07 ± 0·06 mg L^{-1}, n = 9) and after (1·07 ± 0·59 mg L^{-1}, n = 9) hydrolysis in all samples studied ($P > 0{\cdot}8$).

The same hydrolysis procedure was carried out with urine samples (0–48 h) obtained from subjects who participated in

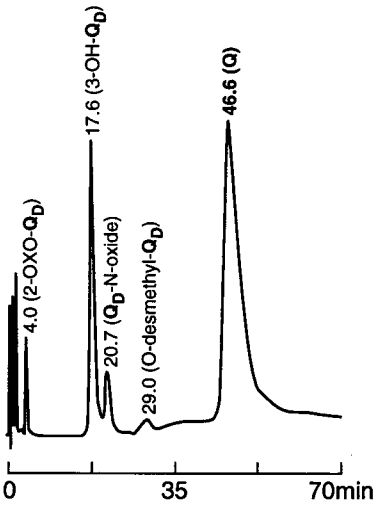

FIG. 1. HPLC separation of quinine and metabolites of quinidine using a modified mobile phase which consisted of an acetonitrile-aqueous phosphate buffer (10 mM, pH 2·1) mixture (40:60, v/v) containing 10 mM SDS and 0·1 mM TBA. Concentrations of 2′-oxoquinidine, quinidine-*N*-oxide, 3-hydroxyquinidine and quinine were 0·1 mg L^{-1} and concentration of *O*-desmethylquinidine was 6·3 mg L^{-1}. Peak identification: 2-OXO-Q_D = 2′-oxoquinidine; 3-OH-Q_D = 3-hydroxyquinidine; Q_D-*N*-oxide = quinidine-*N*-oxide; *O*-desmethyl-Q_D = *O*-desmethylquinidine; and Q = quinine. Retention time (min) for each compound is given on top of the peak.

our previous study after they took a single dose of 600 mg quinine sulphate (Wanwimolruk et al 1993). After hydrolysis with β-glucuronidase, one of the peaks of quinine metabolites disappeared. There was a corresponding increase in the quinine peak which indicates a higher concentration of quinine had resulted from the hydrolysis of quinine glucuronide metabolites. The mean amount of quinine increase after hydrolysis with glucuronidase was 1·2% of the quinine dosage, with a range of 0·5–1·8%. The intra-sample coefficient of variation for measuring quinine concentration after β-glucuronidase hydrolysis was 2·3% (n = 4).

Analysis of urine samples from our previous study revealed that the percentage increase of urinary quinine concentrations after β-glucuronidase hydrolysis was similar in smokers (18·6 ± 8·2%, n = 8) and non-smokers (17·1 ± 3·4%, n = 8). Also, there was no significant difference in the amount of glucuronide formed between the two groups (smokers: 10·2 ± 2·8 mg vs non-smokers: 8·4 ± 4·5 mg; $P > 0·5$).

Development of HPLC method for a simultaneous determination of quinine and its metabolites

An initial attempt had been made to identify possible metabolites of quinine in human urine by using the original HPLC mobile phase (Zoest et al 1990). Metabolites of quinidine were employed as reference compounds, as the metabolites of quinine are not yet available. An assumption was made that there is a similarity in the retention times of corresponding metabolites of quinine and quinidine. This was supported by the evidence that quinine and quinidine had very similar retention times of 8·2 and 8·3 min, respectively, when the original mobile phase was used.

Under the original chromatographic conditions (Zoest et al 1990), the peaks of 3-hydroxyquinidine and quinidine-*N*-oxide were very close to each other, with retention times of 3·9 and 4·5 min. Chromatograms of urine samples showed that there is a major peak eluted at 4·1 min, which was detected in every human urine sample analysed. However, it is difficult to identify whether this peak is 3-hydroxyquinine or quinine-*N*-oxide or both, because the original chromatographic conditions do not provide a good resolution of these metabolites. Therefore, the composition of the original mobile phase was modified to give a better separation between 3-hydroxyquinidine and quinidine-*N*-oxide. The mobile phase, modified as mentioned in the methods section, 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, which was chosen as it provides a good resolution between 3-hydroxyquinidine and quinidine-*N*-oxide. Optimum separations among other quinidine metabolites (*O*-desmethylquinidine and 2′-oxoquinidine) were also achieved using this modified mobile phase (Fig. 1). When a mixture containing all four metabolites of quinidine and quinine was injected into the modified HPLC system, 2′-oxoquinidine was eluted first, followed subsequently by 3-hydroxyquinidine, quinidine-*N*-oxide, *O*-desmethylquinidine and quinine. Their retention times were 4·0, 17·6, 20·7, 29·0 and 46·6 min, respectively. The synthetic 3-hydroxyquinine was co-eluted with 3-hydroxyquinidine under these chromatographic conditions. Quinine and quinidine also have very similar retention times of 46·6 and 46·8 min, respectively, when this modified mobile phase was used. The fluorescence response of *O*-desmethylquinidine was much poorer than other metabolites as a higher concentration was used (Fig. 1).

Identification of quinine metabolites in human urine

Urine samples of the subjects from the previous study (Wanwimolruk et al 1993) were re-analysed with the modified mobile phase described above. The metabolites of quinine were identified by comparing their retention times with those of the synthetic 3-hydroxyquinine and quinidine metabolites. Fig. 2 shows chromatograms of representative human urine samples. No endogenous sources of interference were observed in the blank urine samples. There were at least seven possible metabolites of quinine detectable in the human urine samples obtained from 0–48 h after the subjects took a single oral dose of 600 mg quinine sulphate (Fig. 2B). More peaks were seen when compared with those analysed by using the original mobile phase. The retention times of the peaks were also prolonged. Quinine was eluted at 46·6 min, which is much longer than seen previously with the original mobile phase (8·2 min). Some of the peaks were not well separated from each other. For instance, the retention times for the two metabolites (peak 6) before the major peak were too close together. The peak with retention time of 17·5 min (peak 4) was observed to be the major metabolite peak (Fig. 2B, C) in all urine samples from 16 subjects studied. Its retention time was similar to that of 3-hydroxyquinine (17·6 min), suggesting that this peak corresponds to 3-hydroxyquinine.

Other possible metabolites of quinine were eluted before the major metabolite. Peak 1 had a retention time of 4·0 min (Fig. 2B) which was similar to that of 2′-oxoquinidine (2′-quinidinone) suggesting that peak 1 is 2′-oxoquininone.

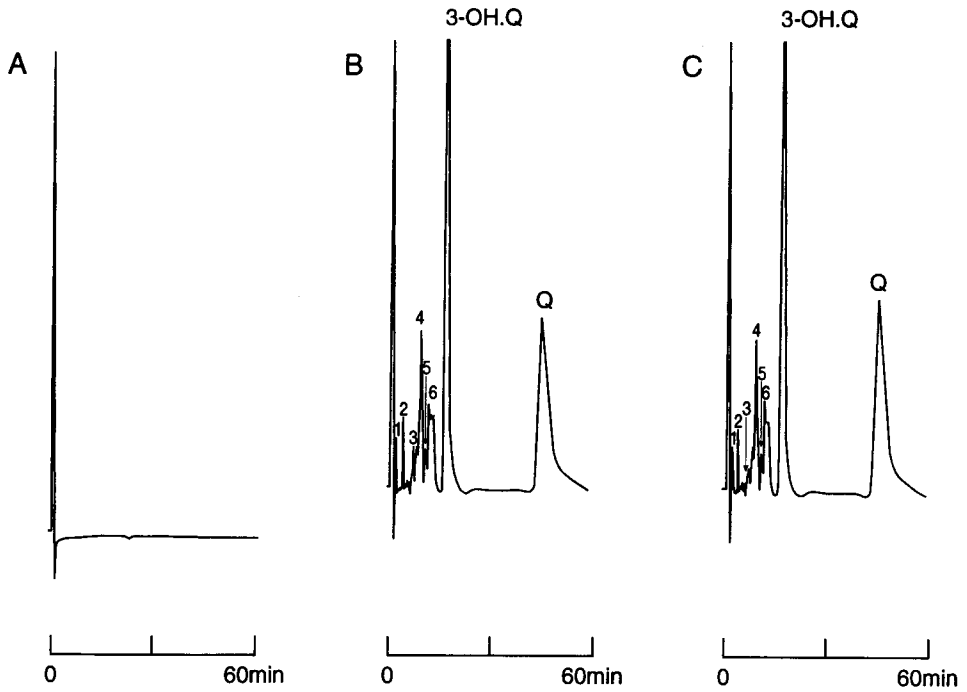

FIG. 2. HPLC analysis of quinine and its metabolites in subject F's urine samples (0–48 h) after a single dose of 600 mg quinine sulphate, using a modified mobile phase. A. Blank urine, B. before hydrolysis with β-glucuronidase, C. after hydrolysis with β-glucuronidase (2500 int. units mL^{-1}). Peak identification: 1 = 2'-oxoquininone; 3 = quinine glucuronide; 3-OH.Q = 3-hydroxyquinine; 2, 4, 5 and 6 = unidentified metabolites of quinine; and Q = quinine.

Peaks 2, 3, 4, 5 and 6 were smaller peaks compared with the major metabolite peak, with retention times of 5·3, 7·4, 9·8, 12·8 and 13·6 min, respectively. None of them has a retention time corresponding to those of quinidine-*N*-oxide (20·7 min) or *O*-desmethylquinidine (29·0 min). These four (possibly minor) metabolite peaks observed were small, their peak heights varied among individuals and were not detected in the samples from all subjects. Urine samples before and after hydrolysis with β-glucuronidase were re-analysed using the modified mobile phase (Fig. 2B, C). Peak 3, with a retention time of 12·8 min, disappeared after hydrolysis with β-glucuronidase. This indicates that peak 3 may be the glucuronide of quinine which has been identified previously using the original HPLC mobile phase. Peaks 2, 4, 5 and 6 remain unidentified. In addition, when the urine samples were mixed with a known amount of quinidine metabolite standards, only two peaks (2'-oxoquininone and 3-hydroxyquinine) were co-eluted with the corresponding quinidine metabolite standards.

Effects of smoking and rifampicin pretreatment on the formation of 3-hydroxyquinine

Later when synthetic 3-hydroxyquinine was available, the formation of 3-hydroxyquinine was measured in urine samples obtained from our previous studies on effects of smoking and rifampicin pretreatment (Wanwimolruk et al 1993, 1995). The within-day coefficient of variation of the HPLC assay for identical samples (n = 4) did not exceed 7% for both 3-hydroxyquinine and quinine. The effects of smoking and rifampicin pretreatment on quinine total plasma clearance (CL) and on the metabolic clearance to 3-hydroxyquinine (CL_{3-OH}) are summarized in Table 1. The clearance of quinine was significantly increased in smokers compared with non-smokers. The fraction of quinine dosage excreted as 3-hydroxyquinine (f_{3-OH}) in Thai non-smoker subjects was 5·6 ± 4·8%, with a range of 1·9 to 14·2%. The fraction of quinine dosage excreted as 3-hydroxyquinine in smokers did not differ significantly from the non-smokers ($P > 0·4$, Table 1). The partial metabolic clearance to 3-hydroxyquinine in smokers and non-smokers was not significantly different. Similarly, rifampicin pretreatment caused a marked increase in the clearance of quinine but the fraction of quinine dosage excreted as 3-hydroxyquinine and partial clearance to 3-hydroxyquinine in the subjects pretreated with rifampicin was not significantly different from the controls who received quinine alone (Table 1).

Table 1. Effects of cigarette smoking and rifampicin pretreatment (600 mg/day for 2 weeks) on metabolic clearance to 3-hydroxyquinine (CL_{3-OH}) in healthy volunteers[a].

Effects of smoking		
	Non-smokers (n = 9)	Smokers (n = 10)
CL[b] ($L\,h^{-1}\,kg^{-1}$)	0·106 ± 0·047	0·189 ± 0·075*
Fraction to 3–OH	0·056 ± 0·048	0·043 ± 0·025
CL_{3-OH} ($L\,h^{-1}\,kg^{-1}$)	0·006 ± 0·005	0·007 ± 0·004
Effects of rifampicin pretreatment (n = 8)		
	Quinine alone	Quinine + rifampicin
CL[b] ($L\,h^{-1}\,kg^{-1}$)	0·141 ± 0·049	0·873 ± 0·348**
Fraction to 3–OH	0·056 ± 0·051	0·049 ± 0·066
CL_{3-OH} ($L\,h^{-1}\,kg^{-1}$)	0·008 ± 0·008	0·045 ± 0·059

[a] Results are presented as mean ± s.d. *$P < 0·05$, **$P < 0·001$.
[b] Total clearance (CL) of quinine was obtained from previous studies (Wanwimolruk et al 1993, 1995), except one subject sample was missing for non-smokers, and one for rifampicin study.

Discussion

Quinine is extensively metabolized by hepatic enzymes (Brodie et al 1951) and many oxidation metabolites were identified in human urine after administration of quinine (Brodie et al 1951; Liddle et al 1981). The present study has developed a suitable and specific HPLC assay to determine quinine and its metabolites in human urine. The newly developed HPLC assay with a modified mobile phase is also applicable to quantify metabolite formation in studies of in-vitro hepatic microsomal metabolism of quinine. The internal standard (cinchocaine) used in the original HPLC assay for plasma (Zoest et al 1990) had too long a retention time (82 min) when the modified mobile phase was used. Therefore, the internal standard was not employed in the modified method. Despite this, the reproducibility of the assay was good with a coefficient of variation for intra-assay of less than 7% for both 3-hydroxyquinine and quinine, because the sample preparation required only a direct protein precipitation.

Using this modified analytical method, the results demonstrate that at least seven possible metabolites of quinine can be detected in human urine. The results revealed that no glucuronide conjugates of quinine were present in the plasma samples. Glucuronide conjugates are highly water soluble and thus they are likely to be excreted rapidly from the circulation. One of the quinine metabolites in the urine from subjects following an oral dose of quinine was identified as a glucuronide metabolite. The results were derived from both chromatographic systems using the original mobile phase and the modified mobile phase. Even though a glucuronide conjugate of quinine is formed, this glucuronide conjugate is a minor metabolite of quinine as it accounted for less than 2% of the dose excreted in the urine. Previous studies have shown that glucuronidation of some drugs, such as paracetamol and oxazepam, are induced by 3-methylcholanthrene-type inducers or cigarette smoking (Ochs et al 1981; Bock et al 1987). However, the results from our study showed no significant difference in the amount of quinine glucuronide formed between the smoker and non-smoker groups, suggesting cigarette smoking is unlikely to induce glucuronide conjugation of quinine. In this study, non-availability of authentic standards of all quinine metabolites makes it difficult to identify the possible metabolites detected by HPLC. Based on the comparison of retention times with those of 3-hydroxyquinine and quinidine metabolites, another two metabolites of quinine were identified in the urine samples (0–48 h) from the subjects after an oral administration of 600 mg quinine sulphate. One of them, eluting with a retention time corresponding to 3-hydroxyquinine, appeared to be a major metabolite and was detected in urine samples from every subject. This suggests that a major metabolite of quinine in human urine is 3-hydroxyquinine. The percentage of dose excreted as 3-hydroxyquinine in the Thai non-smokers was $5{\cdot}6 \pm 4{\cdot}8\%$. 3-Hydroxyquinine was previously identified in human urine and reported to be the major urinary metabolite of quinine (Liddle et al 1981; Bolaji et al 1991). Another small peak (peak 1) found in the urine was identified as 2′-oxoquininone (2′-quininone). Only a very small peak of 2′-oxoquininone was detected. This suggests that the metabolic pathway to form 2′-oxoquininone makes little contribution to the metabolism of quinine, assuming the fluorescence detector has a similar response to this metabolite as it has to 3-hydroxyquinine. In-vitro studies, however, have shown that 2′-oxoquininone was the major metabolite of quinine after the incubation of quinine with rabbit liver aldehyde oxidase (Beedham et al 1992). Being a non-quantitative study through lack of reference compounds, we cannot rule out the possibility that other metabolites of quinine were not detected in some urine samples because of dilution in a large volume.

One might also expect quinine-*N*-oxide as a possible metabolite of quinine, based on the in-vitro hepatic microsomal study which had demonstrated that quinidine-*N*-oxide was one of the major metabolites of quinidine after incubation with human liver microsomes (Guengerich et al 1986). Our in-vitro hepatic microsomal study has also confirmed the same observation that, after incubation of quinidine with human liver microsomes, quinidine-*N*-oxide was detected as one of the metabolites of quinidine (Wanwimolruk et al unpublished data). However, under our chromatographic conditions, no peak was detected from human urine samples at the retention time corresponding to quinidine-*N*-oxide. This suggests that quinine-*N*-oxide was not formed as a metabolite of quinine in man and provides 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 (Beedham et al 1992). It also has been identified in human urine (Liddle et al 1981). In our study, there was no peak from the urine samples eluted at the retention time corresponding to that of *O*-desmethylquinidine. This suggests that *O*-desmethylquinine may not be formed as a metabolite of quinine. However, the possibility of the presence of *O*-desmethylquinine in the urine samples cannot be ruled out since this compound had a much lower fluorescence intensity than the other metabolites. In addition there was a peak eluted after quinine in chromatograms of some plasma and urine samples. This peak was thought to be dihydroquinine. Contamination of quinine with dihydroquinine in commercial and pharmaceutical preparations has been reported (Smith et al 1973). Some of its metabolites (6′-hydroxydihydrocinchonidine and 3-hydroxydihydroquinine) have been identified in human urine (Liddle et al 1981). Therefore, some of the unidentified peaks observed in our chromatograms of urine samples could be the metabolites of this contaminant.

The metabolic pathways of quinine have not been well elucidated. In-vitro hepatic microsomal studies have shown that quinidine, a diastereoisomer of quinine, is metabolized by CYP3A4 (Guengerich et al 1986). The effect of cigarette smoking on quinidine disposition has been studied by Edwards et al (1987), who reported no significant effect of cigarette smoking on the metabolism of quinidine. Surprisingly, our previous study has shown that there was a marked increase in the clearance (by 77% vs non-smokers) of quinine in Thai cigarette smokers (Wanwimolruk et al 1993). Our recent studies have also demonstrated that co-administration of rifampicin with quinine caused a marked increase in the clearance of quinine (by 690%). If the

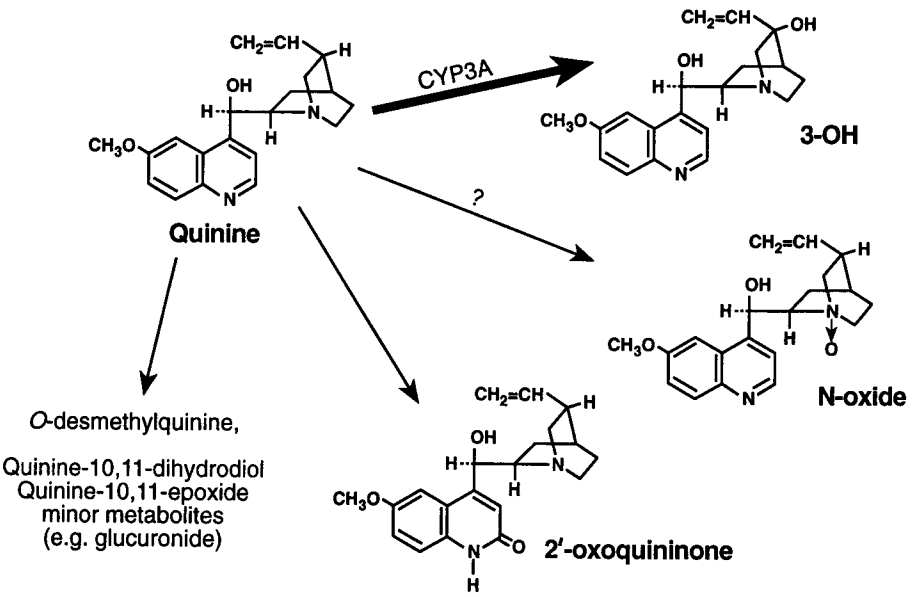

FIG. 3. Proposed scheme for quinine metabolism in man showing the CYP3A (P450IIIA) enzyme involved in the formation of a major metabolite 3-hydroxyquinine (3-OH). Other metabolites including quinine-*N*-oxide (*N*-oxide), *O*-desmethylquinine (*O*-desmethyl), 2'-oxoquininone are possible and have been reported (Brodie et al 1951; Liddle et al 1981; Bolaji et al 1991).

assumption that metabolism of quinine is also catalysed by CYP3A4 is true, our results would suggest that CYP3A4 may be inducible by cigarette smoking. To our knowledge, there are no such examples of CYP3A4-catalysed drugs whose metabolism is induced by cigarette smoking. For example, it has been reported that cigarette smoking has no significant effect on metabolism of CYP3A-mediated drugs including quinidine, midazolam and triazolam (Ochs et al 1985, 1987; Edwards et al 1987; Kronbach et al 1989). One of the possible explanations for the findings of our previous study could be that quinine, like quinidine, is catalysed by the human CYP3A4 enzyme and CYP3A4 could be induced by cigarette smoking. It is known that cigarette smoking mainly induces cytochrome CYP1A1 and CYP1A2 (Sesardic et al 1988; Combalbert et al 1989; Ged et al 1989). Therefore, another possible explanation of the increased clearance of quinine found in the previous study (Wanwimolruk et al 1993) could be that quinine is metabolized by the CYP1A enzyme family. Recently we have demonstrated that 3-hydroxyquinine appeared to be the major metabolite of quinine formed in in-vitro hepatic microsomal preparations (Wanwimolruk et al 1994). This is consistent with our in-vivo results obtained from the urine data. Evidence from the inhibition studies also revealed that the formation of 3-hydroxyquinine was catalysed by CYP3A (unpublished data). To determine whether smoking and rifampicin pretreatment induce the formation of a major metabolite of quinine, the urinary fraction and partial clearance to 3-hydroxyquinine was quantitated. Unexpectedly our results showed that the increase in quinine clearance due to smoking was not due to enzyme induction in the 3-hydroxyquinine metabolic pathway. This is because there were no significant differences in the fraction of quinine dosage excreted as 3-hydroxyquinine and partial clearance to 3-hydroxyquinine between the smokers and non-smokers. Although rifampicin is known to be a potent inducer of CYP3A4 (Combalbert et al 1989; Ged et al 1989) no significant changes in the formation of 3-hydroxyquinine were detected after rifampicin pretreatment. These results indicate that cigarette smoking and rifampicin may not induce the formation of 3-hydroxyquinine. However, as other metabolites were not determined the possibility of inducing 3-hydroxyquinine formation by smoking and rifampicin cannot be excluded. The formation of 3-hydroxyquinine in smokers and subjects pretreated with rifampicin may be masked by 3-hydroxyquinine being further metabolized to secondary metabolites, such as sulphate and glutathione conjugates. Large variations in the formation of 3-hydroxyquinine and the partial clearance to 3-hydroxyquinine may also contribute to this outcome.

In conclusion, this study provides evidence that 3-hydroxylation is a major metabolic pathway of quinine. Based on the results of this study, and other studies (Brodie et al 1951; Liddle et al 1981; Bolaji et al 1991), the metabolic pathways of quinine in man are proposed as shown in Fig. 3.

Acknowledgements

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