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Identification of quinine metabolites in urine after oral dosing in humans

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Abstract

A gas chromatographic–mass spectrometric method was used to separate quinine and its metabolites present in urine after oral dosing of 300 mg quinine in humans. The technique allowed the separation of quinine and ten metabolites. Four of these metabolites were definitely identified as 3-hydroxyquinine, 2′-quinone, *O*-desmethylquinine and 10,11-dihydroxy-dihydroquinine, by comparing their methane chemical ionization mass spectra with those of authentic standards prepared by organic synthesis. Six other metabolites are described for the first time in human urine. From their electron impact and chemical ionization mass spectra, we propose these compounds to be 3-hydroxy-2′-quinone, *O*-desmethyl-2′-quinone, *O*-desmethyl-3-hydroxyquinine, *O*-desmethyl-3-hydroxy-2′-quinone, 10,11-dihydroxydihydro-2′-quinone and 10,11-dihydroxydihydro-*O*-desmethylquinine. These secondary metabolites probably arose from further biotransformation of the four primary metabolites.

Keywords: Quinine

1. Introduction

Quinine is an alkaloid obtained from the bark of the cinchona tree and is a levorotatory diastereomer of quinidine (Fig. 1). Quinine is used for the treatment of malaria, the prevention of nocturnal leg cramps, and the reversal of multidrug resistance during chemotherapy [1–3]. Quinine and quinidine are metabolized in a very similar fashion in man. The resulting metabolites are the products of oxidation reactions taking place on either the quinoline or the quinuclidine moieties (Fig. 2). Oxidation of the quinuclidine moiety occurs preferentially at the C-3 position but also at N-1 and on the vinyl side chain [4–11]. In vitro studies with human liver microsomes have shown that quinidine oxidation to the (3*S*) 3-hydroxy and N-oxide products appears to be catalysed primarily by cytochrome *P*450-3A4 [12–14]. Both quinine and quinidine also undergo oxidation on the vinyl side chain to give the 10,11-dihydroxydihydro derivatives [8–10]. In the rat bile, both the (*R*)- and (*S*)-forms of 10,11-dihydroxy-dihydroquinidine have been identified [10]. In one study, quinine-10,11-epoxide was also found in human urine, suggesting that the dihydroxydihydro derivatives originated from the epoxide by ring

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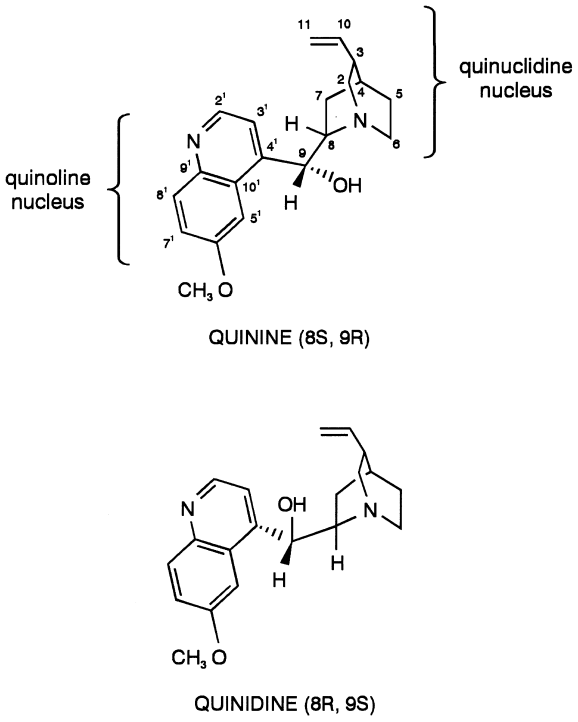

Fig. 1. Chemical structures of quinine and quinidine.

fission [9]. A secondary metabolite oxidized at two different sites on the molecule, 10,11-dihydroxydihydroquinidine N-oxide, was found in human urine

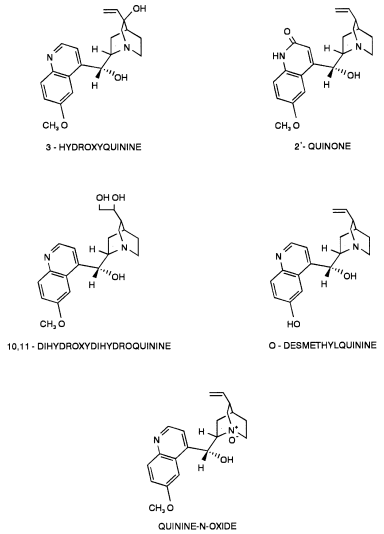

Fig. 2. Structures of the primary metabolites of quinine.

[15] and was also obtained by organic synthesis [16]. Oxidation of the quinoline ring has been shown to yield the 2′-oxo derivatives (Fig. 2) [4,11,15]. In guinea pigs and rabbits, Beedham et al. [17] have shown that hepatic aldehyde oxidase was the enzyme responsible for the production of this metabolite. The products of demethylation of the quinoline ring, the *O*-desmethyl derivatives, were first described by Drayer et al. [18]. Although found in significant concentrations in rat urine [8], these derivatives represented only minor metabolites in human urine [9].

HPLC with fluorescence detection techniques have been widely preferred for measuring quinine or quinidine and their metabolites in blood or urine [19–25]. In the current study, we present a gas chromatographic–mass spectrometric method to separate quinine from its metabolites after derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide. New metabolites resulting from biotransformation at more than one site on the molecule are described.

2. Materials and methods

2.1. Chemicals and reagents

Optima grade methylene chloride, 2-propanol, methanol and pyridine were obtained from Fisher Scientific (Nepean, Ontario, Canada). *N*,*O*-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS), quinine hemisulfate and cinchonine were purchased from Sigma–Aldrich (Oakville, Ontario, Canada). 3-Hydroxyquinine, 2′-quinone, *O*-desmethylquinine, 10,11-dihydroxydihydroquinine and quinine *N*-oxide were synthesized from quinine as described by Diaz-Arauzo et al. [16].

The purity of these compounds was confirmed by TLC, NMR and MS analyses. 10,11-Dihydroxydihydroquinine was obtained as two pure diastereoisomers [P. Yu and J.M. Cook, unpublished results].

2.2. Samples

Eight healthy volunteers (five men and three women, aged 27–47 years) ingested 300 mg of quinine sulfate (Parke-Davis, Scarborough, Ontario,

Canada) orally. Their urines were collected as three separate collections, 0–8 h, 8–24 h and 24–48 h and portions were kept frozen at −70°C until use. Urine samples collected before administration of quinine were also obtained. The study was approved by the local institution ethics committee, and a written informed consent was obtained from each volunteer prior to the study.

2.3. Procedure

2.3.1. Solvent extraction

Urine (0.2 ml) was mixed with 0.1 ml of a methanolic solution of cinchonine 0.1 g/l (internal standard) in a glass-stoppered tube with a PTFE-lined screw cap. Samples were alkalinized with 0.2 ml of 0.1 *M* NaOH, vortexed for 10 s, and 6 ml of methylene chloride–2-propanol (8:2, v/v) were added. The mixture was shaken for 20 min on an alternative agitator, then centrifuged at 2000× *g* for 10 min. The aqueous layer was discarded and the organic layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 37°C.

2.3.2. Derivatization

The residues were dissolved in 0.05 ml of pyridine and 0.05 ml of BSTFA–TMCS (99:1, v/v) was added. The tubes were heated at 90°C for 2 h and 1-μl volumes were injected for GC–MS analysis.

2.4. Instrumentation

We used a Varian Star 3 400 gas chromatograph equipped with an automated sampler 8100 and interfaced with a Varian Saturn II ion trap mass spectrometer (Varian, Mississauga, Canada). Separations were performed on a DB5MS capillary column 15 m×0.25 mm I.D., 0.25 μm film thickness (J&W Scientific, Folson, CA, USA) coupled to the injection port liner by a retention gap of deactivated fused-silica tubing 1 m×0.53 mm (I.D.)× 0.8 mm (O.D.). Ultra pure-grade helium was used as the carrier gas at a flow-rate of 1.5 ml/min. The injection port was initially at 100°C for 0.1 min and programmed to 300°C at 200°C/min. The initial oven temperature was 100°C for 1.1 min and then programmed to 275°C at 5°C/min and to 300°C at 15°C/min.

The transfer line temperature was held at 300°C. The mass spectrometer was operated either in the electron impact mode or in the methane chemical ionization mode using the Silchrom ion trap electrode at a scan range of 10–650 a.m.u.

3. Results and discussion

Fig. 3 shows a typical chromatogram obtained in the electron impact mode from a urine sample collected 0–8 h after quinine ingestion, and the proposed identity of the peaks is listed in Table 1. Track A of Fig. 3 shows the reconstructed total ion current chromatogram while tracks B, C and D show the single ion chromatograms for m/z values of 136, 224 and 314, respectively. These ions are fragments derived from the quinuclidine ring system. In the electron impact mode, they represent the only peaks present in the mass spectra for quinine and for most of its metabolites except those exhibiting the 10,11-dihydroxydihydro derivative. In the latter case, a fragment peak derived from the quinoline ring plus C-9 (m/z 261) and peaks of higher m/z values including the molecular ion, are also present [8,10].

The single ion chromatograms of Fig. 3 underscore three classes of metabolites; firstly those with an unmodified quinuclidine moiety of m/z value 136, which are represented by quinine itself, *O*-desmethylquinine and 2′-quinone; secondly, those with an hydroxyl group at C-3 resulting in a m/z value of 224 after trimethylsilylation (e.g. 3-hydroxyquinine) and thirdly, those hydroxylated at C-10 and C-11 on the vinyl side chain, resulting in a m/z value of 314 after trimethylsilylation (e.g. 10,11-dihydroxydihydroquinine). As seen in Fig. 3, numerous metabolites are visible in the three different tracks. These metabolites were further characterized by operating the mass spectrometer in the methane chemical ionization (CI) mode. Methane CI mass spectra recorded for the TMS derivatives of quinine and its metabolites are summarized in Table 2.

The CI mass spectra typically showed a readily identifiable protonated molecular ion and fragment ions representing the elimination of a methyl group and of TMSOH from the protonated molecular ion.

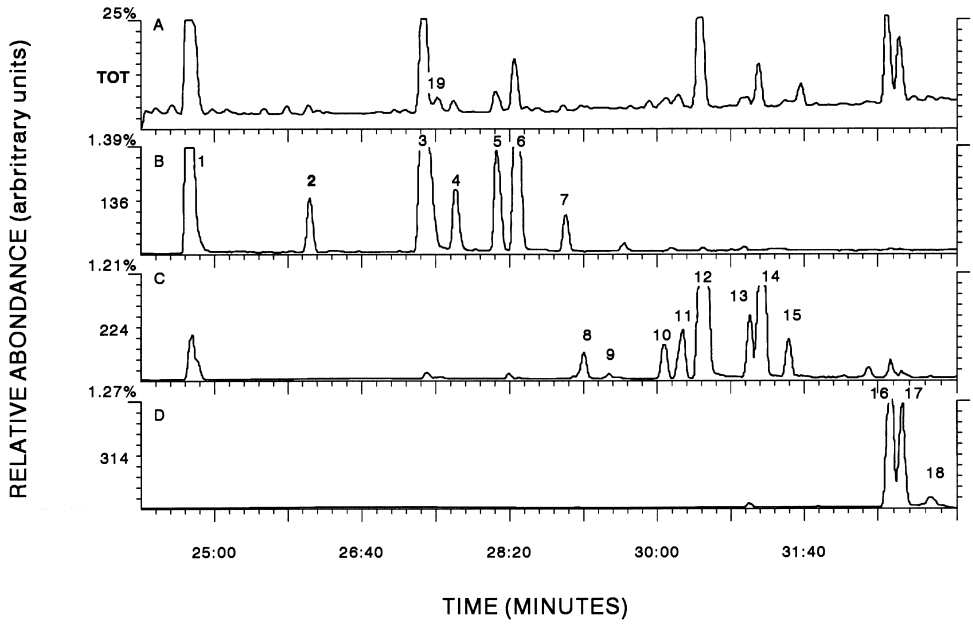

Fig. 3. GC–MS chromatogram of a urine sample obtained after the administration of 300 mg of quinine sulfate p.o. (A) Total ion current chromatogram; (B) single ion current chromatogram, 136 m/z value; (C) single ion current chromatogram, 224 m/z value; (D) single ion current chromatogram, 314 m/z value.

Fragments derived from the quinuclidine ring were also diagnostic. In addition, most metabolites also yielded CI adduct ions at M+29 ($M+C_2H_5^+$) which confirmed their molecular mass. The five following primary metabolites, namely 3-hydroxyquinine, 2′-quinone, *O*-desmethylquinine, 10,11-dihydroxy-

Table 1
Proposed identity of compounds present in chromatogram of Fig. 3 and their relative retention times (t_{RR})

Peak number	Compounds	t_{RR}
1	Cinchonine (internal standard)	1.000
2	Unknown	1.055
3	Quinine	1.106
4	Unknown	1.121
5	*O*-Desmethylquinine	1.139
6	2′-Quinone	1.149
7	*O*-Desmethyl-2′-quinone	1.171
8	Unknown	1.179
9	(3*R*)-3-Hydroxyquinine	1.190
10	Unknown	1.216
11	Unknown	1.224
12	(3*S*)-3-Hydroxyquinine	1.233
13	*O*-Desmethyl-3-hydroxyquinine	1.254
14	3-Hydroxy-2′-quinone	1.260
15	*O*-Desmethyl-3-hydroxy-2′-quinone	1.272
16	10,11-Dihydroxydihydroquinine	1.318
17	10,11-Dihydroxydihydro-2′-quinone	1.324
18	10,11-Dihydroxydihydro-*O*-desmethylquinine	1.337
19	Dihydroquinine	1.113

Table 2
Principal ions of quinine and its proposed metabolites as their trimethylsilyl derivatives using methane chemical ionization mass spectrometry

Compounds	MH^+	$(M-CH_3)^+$	$(MH-TMSOH)^+$	Q[b]
Quinine[a]	397	381	307	136
O-Desmethylquinine[a]	455	439	365	136
2′-Quinone[a]	485	469	395	136
O-Desmethyl-2′-quinone	543	527	453	136
3-Hydroxyquinine[a]	485	469	395	224
O-Desmethyl-3-hydroxyquinine	543	527	453	224
3-Hydroxy-2′-quinone	573	557	483	224
O-Desmethyl-3-hydroxy-2′-quinone	631	615	–	224
10,11-Dihydroxydihydroquinine[a]	575	559	485	314
10,11-Dihydroxydihydro-*O*-desmethylquinine	633	617	543	314
10,11-Dihydroxydihydro-2′-quinone	–	647	573	314

[a] Spectra of these compounds were confirmed using authentic compounds.
[b] Fragments derived from the quinuclidine ring system.

dihydroquinine and quinine *N*-oxide, were prepared by organic synthesis, derivatized with BSTFA and examined using identical chromatographic conditions with detection in the methane CI mode. For each of the first four authentic compounds, the mass spectrum of their TMS derivatives was identical to that of the presumed metabolite.

In this system, 3-hydroxyquinine (peak 12) was always the most abundant. It was demonstrated by Carroll et al. [4] that 3-hydroxyquinidine isolated from the urine of man had the 3*S* configuration with the 3-hydroxyl *syn* to C-5. By analogy, we assumed that peak 12 was the TMS derivative of (3*S*)-3-hydroxyquinine. Peak 9, a very minor peak, exhibited an identical CI mass spectrum and we presumed that it was the TMS derivative of (3*R*)-3-hydroxyquinine. The compound obtained by organic synthesis in the present study was predominantly (3*R*)-3-hydroxyquinine and included only a small amount of (3*S*)-3-hydroxyquinine. The second most abundant metabolite was 10,11-dihydroxydihydroquinine (peak 16). As C-11 is asymmetric, it follows that this compound also exists as a pair of diastereoisomers. Barrow et al. [8] have shown that both diastereoisomers could be separated by HPLC from rat urine. In the present study, the two authentic stereoisomers were prepared in pure form. In our chromatographic system, both their TMS derivatives were shown to comigrate and to exhibit identical electron impact and CI mass spectra, and thus could not be differentiated.

The third most abundant metabolite was 2′-quinone (peak 6). No other peak in the chromatogram exhibited a similar CI mass spectrum. The fourth primary metabolite, *O*-desmethylquinine (peak 5) was the least abundant. Peak 2 also exhibited an identical CI mass spectrum but, as *O*-desmethylquinine contains no asymmetric carbon other than carbon 9, the identity of this peak remains unknown. Finally, authentic quinine *N*-oxide comigrated with quinine in our system and exhibited a CI mass spectra identical to that of quinine. In this case, we cannot rule out the possibility that this compound was reconverted to quinine during the extraction procedure or during the derivatization step. Such conversion of the *N*-oxide back to the parent moiety as a result of the experimental conditions was shown to occur for chlorpromazine N-oxide which is converted back to chlorpromazine under alkaline conditions [26,27].

The extraction efficiency of quinine and the five primary metabolites was also studied. A fixed amount of the authentic metabolites was added to normal urine, extracted and derivatized as described in Section 2. The area under the peaks were compared to those of the unextracted metabolites. Extraction efficiency was within the 95-99% range ($n=3$) for all metabolites except quinine *N*-oxide. The latter showed an apparent extraction efficiency well over 100%, in agreement with the hypothesis that it was reconverted to quinine or to an unknown compound with better detector response during the

extraction procedure. The extraction efficiency of 10,11-dihydroxydihydroquinine, the most polar metabolite, was lower when the urine to organic solvent ratio was higher.

As shown in Table 2, six additional metabolites are described for the first time in human urine. Of these, only 3-hydroxy-2′-quinone (peak 14) had been previously reported; it was identified by Beedham et al. [17] from the incubation of quinine with rabbit liver 10 000× *g* supernatant fraction. Presumed 10,11-dihydroxydihydro-2′-quinone (peak 17) was also found in significant amounts in the urine of the volunteers. The high molecular mass of its TMS derivative (calculated M_r=662) precluded the identification of its protonated molecular ion. However, other diagnostic fragments as well as two additional observations supported its identity. First, its chromatographic behaviour was similar to that of other metabolites containing the 2′-oxo group, all of them exhibiting significant tailing when the retention gap was dirty. Second, this compound was absent from the urine of two volunteers who also presented an absence of 2′-quinone and 3-hydroxy-2′-quinone.

Three more presumed secondary metabolites, namely, *O*-desmethyl-2′-quinone (peak 7), *O*-desmethyl-3-hydroxyquinine (peak 13) and 10,11-dihydroxydihydro-*O*-desmethylquinine (peak 18) were also present in small amounts in the urine of most of the volunteers. Peak 8 exhibited a CI mass spectrum identical to that of the presumed TMS derivative of *O*-desmethyl-3-hydroxyquinine (peak 13). This observation should be interpreted with caution in light of the fact that peak 2 and peak 5 (TMS derivative of *O*-desmethylquinine) also exhibited identical CI mass spectra. Finally, a presumed tertiary metabolite, *O*-desmethyl-3-hydroxy-2′-quinone (peak 15) was also found.

Three more compounds (peaks 4, 10 and 11) in the chromatogram of Fig. 3 were also suspected to be metabolites of quinine. Peak 4 exhibited a fragment with a *m*/*z* value of 136 and a protonated molecular ion with *m*/*z* value at 575. This compound would then have to be a metabolite with two hydroxyl groups incorporated in the quinoline moiety. Both peak 10 and 11 exhibited a fragment at *m*/*z* value of 224, indicating that they were probably hydroxylated on C-3. These compounds were not detected in blank urine samples. Dihydroquinine (peak 19) and dihydrocinchonine (t_{RR}=1.003) which were probable contaminants of quinine and cinchonine respectively, were also identified in the chromatogram of Fig. 3. The system even allowed identification of some presumed metabolites of dihydroquinine, namely 2′-dihydroquinone (t_{RR}=

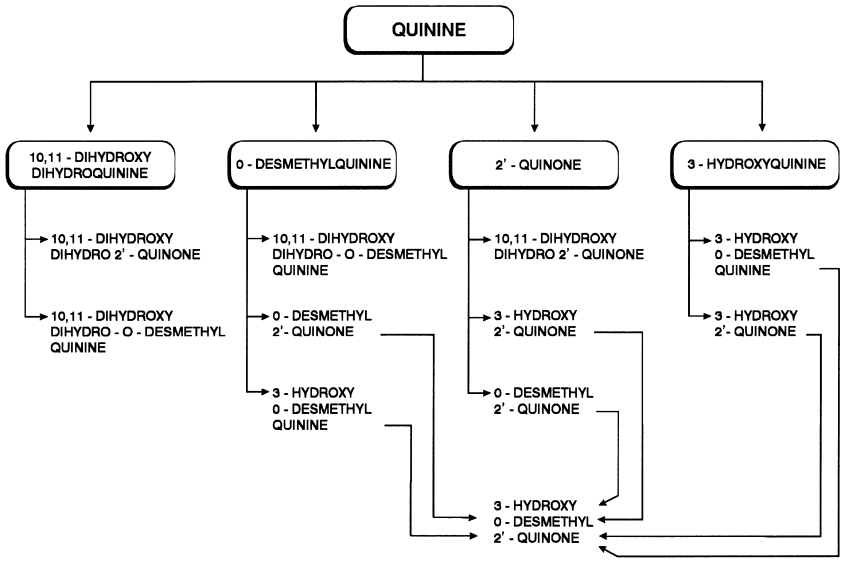

Fig. 4. Proposed pathways of quinine metabolism in man.

1.155) and 3-hydroxydihydroquinine (t_{RR}=1.252). The presence of quinine-10,11-epoxide and 10,11-dihydroxydihydroquinine *N*-oxide could not be verified under our assay conditions.

4. Conclusions

This study has allowed us to acquire a more comprehensive view of the biotransformation of orally administered quinine in humans. A proposed scheme for quinine metabolism is shown in Fig. 4.

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