Quinacrine (QA), an antimalarial drug used for over seven decades, has been found to have potent antiprion activity in vitro. To determine whether QA can be used to treat prion diseases, we investigated its metabolism and ability to traverse the blood-brain barrier in mice. In vitro and in vivo, we identified by liquid chromatography-tandem mass spectrometry the major metabolic pathway of QA as N-desethylation and compared our results with an authentic reference compound. The major human cytochrome (P450) isoforms involved in QA mono-desethylation were identified as CYP3A4/5 by using specific chemical and antibody inhibition as well as cDNA-expressed P450 studies. QA transport from the basolateral to apical side in multidrug resistance protein 1 gene (MDR1)-transfected Madin-Darby canine kidney (MDCK) cells was markedly greater than in control MDCK cells and was inhibited by the potent P-glycoprotein (P-gp) inhibitor GG918 (N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-iso-1-quinolynyl)-ethyl]-phenyl)-5,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamine). In MDR1-knockout (KO) mice, QA brain levels were 6 to 9 times higher after a single i.v. dose of 2 mg/kg QA and 49 times higher after multiple oral doses of 10 mg/kg/day QA for 7 days, compared with those in wild-type (WT) FVB mice. In contrast, the QA levels in plasma, liver, spleen, and kidney were similar after a single 2 mg/kg i.v. dose and <2 times greater after 10 mg/kg oral doses in MDR1-KO mice compared with WT mice. These results indicate that P-gp plays a critical role in transporting QA from the brain.

Quinacrine (QA) is an acridine derivative introduced for malarial therapy in 1930 and was widely used before and after World War II. QA as an antimalarial has been superseded by other drugs with fewer side effects (Goodman and Gilman, 1975). At present, uses of QA include the treatment of giardiasis, systemic lupus erythematosus, and rheumatic diseases (Goodman and Gilman, 1975; Wallace, 1989). In addition, QA has been used as an anthelmintic (Goodman and Gilman, 1975) and for female sterilization in certain countries (Zipper and Kessel, 2005). Recent years, QA has been found to have a potent antiprion activity in vitro (Doh-ura et al., 2000; Korth et al., 2001). The effective QA concentration for half-maximal inhibition (EC50) for the formation of PrPSc (the abnormal isoform of the prion protein) in scrapie-infected neuroblastoma cells was found to be 300 nM (120 ng/ml) (Korth et al., 2001). In preliminary clinical observations of QA to treat Creutzfeldt-Jakob disease, a transient improvement of the symptoms was reported (Kobayashi et al., 2003; Nakajima et al., 2004). However, several studies showed negative results for QA in the treatment of prion diseases (Collins et al., 2002; Barret et al., 2003; Benito-Leon, 2004; Doh-ura et al., 2004; Gayrard et al., 2005). Currently, clinical trials are being undertaken at University of California San Francisco and in London, England, to determine whether treatment with QA has an effect on the symptoms and course of prion disease. Thus far, no drug has been found to be effective in treating prion diseases.

Despite these clinical uses of QA, its pharmacokinetics is not well understood. Most pharmacokinetic studies of QA were conducted in the 1940s and 1950s, which indicated that QA is very readily absorbed from the intestinal tract, extensively distributed into tissues, and eliminated slowly from the body (Shannon et al., 1944; Goodman and Gilman, 1975). However, the metabolic fate of this drug in the body and the enzymes involved in its metabolism remain unknown (Goodman and Gilman, 1975; Bjorkman et al., 1998). To understand better the action of QA for the treatment of prion diseases, it is necessary to elucidate the metabolism of QA and its ability to traverse the blood-brain barrier (BBB). In a previous paper (Yung et al., 2004), we measured QA brain concentrations in mice after oral doses of 37.5 and 75 mg/kg/day for 4 weeks. Here, we have identified the metabolic

**ABSTRACT:**

Quinacrine (QA), an antimalarial drug used for over seven decades, has been found to have potent antiprion activity in vitro. To determine whether QA can be used to treat prion diseases, we investigated its metabolism and ability to traverse the blood-brain barrier in mice. In vitro and in vivo, we identified by liquid chromatography-tandem mass spectrometry the major metabolic pathway of QA as N-desethylation and compared our results with an authentic reference compound. The major human cytochrome (P450) isoforms involved in QA mono-desethylation were identified as CYP3A4/5 by using specific chemical and antibody inhibition as well as cDNA-expressed P450 studies. QA transport from the basolateral to apical side in multidrug resistance protein 1 gene (MDR1)-transfected Madin-Darby canine kidney (MDCK) cells was markedly greater than in control MDCK cells and was inhibited by the potent P-glycoprotein (P-gp) inhibitor GG918 (N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-iso-1-quinolynyl)-ethyl]-phenyl)-5,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamine). In MDR1-knockout (KO) mice, QA brain levels were 6 to 9 times higher after a single i.v. dose of 2 mg/kg QA and 49 times higher after multiple oral doses of 10 mg/kg/day QA for 7 days, compared with those in wild-type (WT) FVB mice. In contrast, the QA levels in plasma, liver, spleen, and kidney were similar after a single 2 mg/kg i.v. dose and <2 times greater after 10 mg/kg oral doses in MDR1-KO mice compared with WT mice. These results indicate that P-gp plays a critical role in transporting QA from the brain.

**ABBREVIATIONS:** QA, quinacrine; ACMA, 9-amino-6-chloro-2-methoxyacridine; LC/MS/MS, liquid chromatography-coupled tandem mass spectrometry; CID, collision-induced dissociation; HLM, human liver microsome; RLM, rat liver microsome; P450, cytochrome P450; mAb-CYP3A4/5, monoclonal antibody to human CYP3A4/5; P_app, apparent permeability; BBB, blood-brain barrier; PrPSc, abnormal isoform of the prion protein; MDCK, Madin-Darby canine kidney; MDR1-MDCK, Madin-Darby canine kidney cells transfected with multidrug resistance protein 1 gene; P-gp, P-glycoprotein; A to B, apical to basolateral; B to A, basolateral to apical; KO, knockout; WT, wild-type; HPLC, high-performance liquid chromatography; MRM, multiple reaction monitoring; amu, atomic mass unit(s).
pathway of QA and investigated the role of P-glycoprotein (P-gp) in the elimination of QA from the brain.

Materials and Methods

Materials. QA hydrochloride USP reference standard was obtained from U.S. Pharmacopeia (Rockville, MD). QA dihydrochloride (purity, 98.6%) used for dosing animals was purchased from Fluka (Milwaukee, WI). Mono-desethyl-QA dihydrochloride (purity, 98.0%) was synthesized by Toronto Research Laboratory (Toronto, ON, Canada). Its structural identity was confirmed by NMR and mass spectral analysis. 9-Amino-6-chloro-2-methoxyacridine (ACMA) (purity, 99%) was obtained from Molecular Probes, Inc. (Eugene, OR), GD918 (N-[4-(2,1,2,3-tetrahydro-6,7-dimethoxy-2-isoleuquinolinyl)-ethyl]-phenyl)-9,10-di hydro-5-methoxy-9-oxo-4-acridine carboxylic acid) was a gift from GlaxoSmithKline (Triangle Park, NC). NADPH and isocitrate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol, and other solvents or reagents were HPLC grade or analytical grade. The pooled male human liver microsomes (HLMs), male Sprague-Dawley rat liver microsomes (RLMs), recombinant human cytochrome P450 (P450) isozymes, and mouse monoclonal antibody (mAb) to human CYP3A4/5 were obtained from BD Biosciences (Woburn, MA).

Microsome and Hepatocyte Incubations. HLM and RLM incubation mixtures were composed of 0.5 mg/ml microsomal protein, 25 μM QA, and a cofactor regenerating system containing 10 mM NADPH, 2 units of isocitrate dehydrogenase, and 10 mM magnesium chloride in 0.2 M phosphate buffer, pH 7.4. The reaction was initiated by adding 1 mM NADPH after preincubation at 37°C for 5 min and stopped after 30 min by the addition of 2 volumes of ice-cold acetonitrile containing internal standard (chlorphenamine, isocitrate dehydrogenase, and 10 mM magnesium chloride in 0.2 M phosphate buffer). After vortexing for 1 min, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Enzyme kinetics experiments were performed on either the apical or the basolateral side of the monolayers contained 25 μg/ml of QA. Monolayers were treated with 25 μM QA for 30 min at 37°C. The monolayers were then incubated with 1 mM NADPH at 37°C for up to 1 h. After preincubation for 5 min, the reaction was stopped after 30 min by the addition of 2 volumes of ice-cold acetonitrile containing internal standard (chlorphenamine). After vortexing for 1 min, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was analyzed by LC/MS/MS.

Transepithelial Transport of QA across MDR1-MDCK Cell Monolayers. Madin-Darby canine kidney (MDCK) and MDCK transfected with multidrug resistance protein 1 gene (MDR1-MDCK) cell lines were kindly provided by Dr. Ira Pastan, National Institutes of Health (Bethesda, MD) (Pastan et al., 1988). The cells were maintained in culture in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum with (MDCK) or without (MDR1-MDCK) 80 ng/ml colchicine. The colchicine was used as a selection marker for MDR1. For transport experiments, MDCK and MDR1-MDCK cells were grown as epithelial layers by seeding onto permeable filter matrices of Transwell cluster plates (Corning Inc., Coming, NY) for 5 days. The integrity of the cell monolayers was assessed by transepithelial electrical resistance measurements with a Millipore Millicell-ERS resistance system (Millipore Corp., Bedford, MA).

Measurement of Bidirectional Transepithelial Fluxes of QA. The experimental procedure was as described by Hunter et al. (1993). First, 1.5-ml and 2.5-ml aliquots of transport medium (Hanks’ balanced salt solution including 1% fetal calf serum and HEPES, pH 7.4) were pipetted into insert culture (solution) and six-well plates (basolateral solution), respectively. The medium on either the apical or the basolateral side of the monolayers contained 25 μg/ml QA. Monolayers were then incubated at 37°C for up to 1.5 h. After 30 min, 100 μl of solution was taken from the receiver side and replaced with an identical volume of the transport medium. The sample QA concentration was analyzed by LC/MS/MS.

In Vivo Studies. QA Brain Distribution in MDR1-Knockout and Wild-Type FVB Mice. The protocol was approved by the University of California San Francisco animal care and use committee. Five-week-old male MDR1-knockout (KO), MDR1a/b (−/−, −/+ ) mice, and male wild-type (WT) FVB mice (Taconic Farms, Germantown, NY) were housed with free access to food and water, and were maintained on 12-h light/dark cycles for 1 week before dosing studies. Each group of 12 mice were randomly assigned to one of four treatment conditions: i.p. injection of QA, i.m. injection of QA, oral administration of QA solution, or oral administration of QA suspension in 5% carboxyl methylcellulose solution. The samples were sacrificed by decapitation at 30 min or 3 h postdose (n = 6 mice/group). Plasma samples were obtained by centrifuging the blood samples with heparin as anticoagulant at 10,000 rpm for 5 min. Brain, liver, spleen, and kidney were harvested and stored at −20°C before analysis.

The QA concentrations in plasma and tissues were measured by the LC/MS/MS method described below. To prepare samples for analysis, 50 μl of plasma was added with 100 μl of acetonitrile containing internal standard (chlorphenamine, 50 ng/ml). Tissue was homogenized with distilled water (10 ml/g tissue), and then 200 μl of the homogenate was taken to a test tube, then added with 400 μl of acetonitrile containing internal standard. Both plasma and tissue samples were vortexed for 1 min and centrifuged at 10,000 rpm for 5 min. The supernatant was transferred to a vial and injected into the LC/MS/MS apparatus for analysis.

Collection of Rat Urine and Bile for Metabolite Identification. Male adult Wistar rats (Charles River Laboratory, Wilmington, MA) were given oral
doses of QA at 50 mg/kg/day for 4 days. The urine was collected with a metabolic cage at different days after the drug administration. Urine samples were centrifuged at 3000 rpm for 10 min and then directly injected into the LC/MS/MS apparatus for analysis. Bile was collected with a cannulated tube from rats under anesthesia at 2 h after a single oral dose of QA at 50 mg/kg. The bile samples were precipitated with acetonitrile before LC/MS/MS analysis.

Liquid Chromatography/Tandem Mass Spectrometry. LC/MS/MS was used for analysis of QA and its metabolites. The LC/MS/MS system consisted of Shimadzu LC-10 AD pumps, a Waters Intelligent Sample Processor 717 Plus autosampler, a Shimadzu SPD10A UV detector, and a Micromass Quattro LC Ultima triple quadrupole tandem mass spectrometer. The mass spectrometer was set to electrospray ionization in positive-ion mode. For metabolite detection, full scan, and the parent ion scan of QA-specific fragment ions, 259 m/z and 142 m/z under collision-induced dissociation (CID) conditions were performed. The consistent neutral loss scan of 176 m/z or 80 m/z was used for searching glucuronide- or sulfate-conjugated metabolites, respectively. The daughter ion scan was conducted to identify the metabolites. For QA, the sample cone voltage and collision energy were 25 V and 20 eV, respectively, and the source block and desolvation temperature were 100°C and 400°C, respectively. The column was a Beta Basic-18 (150 x 4.6 mm) from Hypersil-Keystone. The mobile phase was composed of solvent A (100% H2O containing 0.1% formic acid) and solvent B (80% CH3CN containing 0.1% formic acid). The gradient elution was as follows: 0 to 5 min, 0 to 30% solvent B; 5 to 13 min, 30 to 35% solvent B; 13 to 20 min, 35 to 100% solvent B; 20 to 21 min, 100 to 0% solvent B. The flow rate was set at 1 ml/min with a 1/4 split into the mass spectrometer and a 3/4 split into the UV detector.

For quantification of QA and mono-desethyl-QA concentrations, multiple reaction monitoring (MRM) was performed as 372.2 > 114.2 m/z for QA, 372.2 > 114.2 m/z for mono-desethyl-QA, and 277.2 > 142.2 m/z for internal standard (chlorphenamine). The column was a Betasil C18 column (50 x 4.6 mm) from Hypersil-Keystone and the mobile phase consisted of CH3OH/H2O/trifluoroacetic acid (45:55:0.05) with 1 mM ammonium formate. The flow rate was 0.8 ml/min. The interday and intraday variations (CV) for quantification of QA and mono-desethyl-QA were less than 15%.

Statistics. Student’s t test was used for two-group comparisons.

Results

Identification of QA Metabolites. Figure 1 shows the CID daughter ion mass spectrum of protonated quinacrine at m/z 400.

FIG. 1. Quinacrine structure and the daughter ion mass spectrum of protonated quinacrine at m/z 400.

Fig. 2. HPLC-UV detection of the major metabolites of QA in human liver microsomes after incubation at 37°C for 30 min without (A) and with (B) NADPH. Quinacrine concentration, 25 μM; UV wavelength, 280 nm. M1, M3, and M2 were named for peak retention times at 9.3 min, 9.5 min, and 9.9 min, respectively. The 244 m/z fragment was generated by loss of a NH group from the 259 m/z fragment. The 327 m/z fragment was QA lost from a N-(C2H5)2 group and one proton, which was supposed to be transferred from its neighbor CH2. To identify QA metabolites in vitro, we first incubated QA in RLMs or HLMs with or without NADPH. After incubation, the reaction mixture was purified by acetonitrile; then, the supernatant was injected into the liquid chromatograph coupled with the UV detector and the tandem mass spectrometer. Without adding NADPH, no metabolite peak was found. In contrast, several metabolite peaks, namely, M1, M3, and M2, at retention times of 9.3, 9.5, and 9.9 min, respectively, were observed at UV 280 nm in a sample after adding NADPH (Fig. 2). By using a full scan, the molecular ion ([M + H]+) of the major metabolite peak (M2) was 372 m/z, 28 amu less than QA, suggesting loss of an ethyl (C2H4) group. The CID daughter ion mass spectrum of the 372 m/z fragment is shown in Fig. 3A. The other tricyclic fragments, 327, 259, and 244 m/z, were the same as with the parent drug, whereas a 114 m/z fragment of 28 amu less than the previously identified 142 m/z fragment suggests that a side chain was lost. To confirm the structure, a mono-desethyl-QA was chemically synthesized and analyzed by LC/MS/MS, which shows identical chromatographic behavior and mass spectra to M2 (Fig. 3B). M2 therefore was identified as mono-desethyl-QA.

Using the same strategy, the molecular ion ([M + H]+) of M1 was 386 m/z, corresponding to a loss of a methyl (CH3) group, which suggests that it is demethylated QA. The daughter ion scan of 386 m/z
is shown in Fig. 4A. The fragment of 142 m/z was observed, which indicates that the side chain did not change, whereas the fragments 327, 259, and 244 m/z were converted to 313, 245, and 230 m/z, respectively; all 14 amu less than the corresponding fragments. These results indicate that M1 represents O-demethylation of QA. Mono-desethyl-QA could lose another ethyl group to produce di-desethyl-QA (M3). The molecular ion (M + H)⁺ of M3 was 344 m/z, indicating loss of two ethyl groups. Its daughter ion mass spectrum is shown in Fig. 4B. There was an 86 m/z fragment instead of 142 m/z, suggesting that two ethyl groups were removed from the side chain. The 327, 259, and 244 m/z fragments were still observed, which indicated that the tricyclic moiety had not changed. With loss via mono-desethylation and O-demethylation, QA could produce another metabolite, M4, which was detected in the HLM QA incubation at retention time 9.3 min. When incubated with standard mono-desethyl-QA in HLMs, both M3 and M4 were formed (data not shown), which confirms that M3 and M4 were sequentially metabolized from M2.

M1, M2, M3, and M4 were also detected in rat urine after dosing QA. In addition, another metabolite, M5 at retention time 16.5 min, was observed in the parent ion scan of 259 m/z. Its molecular positive ion (M + H)⁺ and negative ion (M − H)⁻ were 359 m/z and 357 m/z, respectively. Figure 4C shows its positive daughter ion mass spectrum. The side chain fragment of 142 m/z was changed to 101 m/z, which was tentatively identified as a carboxyl acid QA metabolite. We also observed another QA metabolite (M6) in rat urine at retention time 12.7 min. Its mass spectra and retention time were the same as the reference standard ACMA (Fig. 5). However, M6 was a very minor product in microsome and hepatocyte incubations. No glucuronide- or sulfate-conjugated QA metabolites were found in urine or in bile by using a consistent neutral loss scan of 176 m/z and 80 m/z, respectively.

Based on our data, the proposed metabolic pathways of QA in vivo are shown in Scheme 1. All of these metabolites in urine, blood, and tissue could be detected simultaneously by MRM in a gradient HPLC elution. Figure 6 shows the MRM-chromatographic profiles of QA incubation in HLMs and rat urine sample after oral doses. In rat hepatocyte incubation, and in rat blood and bile after oral dosing QA, the major QA metabolites were also found to be M2 and M3, which indicated that N-desethylation was the major metabolic pathway of QA. A small M5 peak was detected in microsomal incubation in MRM mode. In contrast, a higher M5 peak was observed in rat hepatocyte incubation. In RLM and HLM incubations, a similar metabolic profile was obtained, which suggests that rats and humans have similar QA metabolic pathways.

**Human P450 Isoforms Involved in QA N-Desethylation.** The QA major metabolic pathway, N-desethylation, was investigated in microsomal preparations from pooled human livers. The mono-desethyl-QA and O-demethyl-QA formations increased linearly with time and protein concentration. A monophasic curve was found for
QA mono-desethylation by Eadie-Hofstee plot analysis. The apparent $K_m$ and $V_{max}$ values from pooled male HLMs were 26 μM and 157 pmol/min/mg protein, respectively. To investigate the P450 isoform involved in the QA major metabolic pathway, different P450 chemical inhibitors were present in the HLM incubation mixture. The coincubations of 40 μM QA with orphenadrine (CYP2B6 inhibitor), sulfaphenazole (CYP2C9 inhibitor), quercetin (CYP2C8 inhibitor), quindine (CYP2D6 inhibitor), and omeprazole (CYP2C19 inhibitor) resulted in less than 30% inhibition of mono-desethyl-QA formation (Fig. 7A). When incubated in the presence of ketoconazole (1 μM), a CYP3A4-specific inhibitor, mono-desethyl-QA formation was inhibited by 67%. Furthermore, the inhibition of QA mono-desethylation was ketoconazole concentration-dependent (Fig. 7B). At 5 μM ketoconazole, QA mono-desethylation activity was almost completely inhibited (94% inhibition). When QA was incubated with the recombinant human CYP1A1, CYP1A2, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP2C8, CYP2C9, CYP2C19, CYP19, CYP2D6*1, and CYP2E1, the CYP3A4 and CYP3A5 showed the greatest capacity to catalyze the formation of mono-desethyl-QA (Fig. 8A). The CYP1A1 also showed modest ability to catalyze mono-desethyl-QA formation. The other P450 isoforms exhibited poor metabolic activities. When HLM was treated with mAb CYP3A4/5, mono-desethyl-QA formation was inhibited, in a concentration-dependent manner (Fig. 8B). In 100 μg of HLM protein, 10 μl of mAb CYP3A4/5 could inhibit QA mono-desethylation by approximately 78%. These results indicate that CYP3A4 and CYP3A5 are the major P450 isoforms responsible for QA mono-desethylation.

QA Transporting across MDR1-MDCK Cell Monolayers. As Fig. 9 shows, the QA transport from basolateral to apical (B to A) was greatly increased in MDR1-MDCK cells compared with parent MDCK cells. This increase was completely inhibited by the potent P-gp inhibitor GG918 at 1 μM. The apparent permeability ($P_{app}$) values of QA through MDCK and MDR1-MDCK cells were low, which indicates that QA does not readily cross the cell membrane. The $P_{app}$ of MDR1-MDCK from B to A was 22.5 times higher than that of MDCK from B to A (Table 1). These results indicate that QA is a P-gp substrate.

QA Brain Distribution in MDR1-KO Mice. To evaluate the
importance of P-gp in determining the brain distribution of QA in vivo, the brain QA concentrations were measured after a single i.v. injection (Table 2) or multiple oral doses of QA (Table 3) into MDR1-KO and WT FVB mice. The QA plasma concentration was very low. In contrast, the QA levels in tissues were high. This is consistent with previous reports (Yung et al., 2004; Gayrard et al., 2005), indicating that QA is highly tissue bound. After a single i.v. dose of 2 mg/kg, the brain QA concentrations at 0.5 h and 3 h were 6 to 9 times higher, respectively, in MDR1-KO mice than those in WT FVB mice. However, QA concentrations were not significantly different in plasma, liver, kidney, and spleen between the two strains of mice. In MDR1-KO and WT FVB mice given oral doses of 10 mg/kg/day QA for 7 days, we measured QA levels 20 h after the last oral dose (Table 3). The QA brain concentration in MDR1-KO mice was $7.8 \pm 1.5 \mu g/g$, whereas the brain QA concentration in WT FVB mice was approximately 49 times lower, at $0.16 \pm 0.056 \mu g/g$. The QA levels in liver, kidney, and spleen were increased substantially in MDR1-KO mice compared with WT FVB mice, but the ratios were less than 2 times. In plasma, no significant change in the QA concentration between the two strains of mice was observed.

Mono-desethyl, di-desethyl, and O-demethyl QA metabolites were also distributed throughout mouse brain. The percentages of their respective peak areas versus QA peak areas in FVB mice at 20 h after
Discussion

Despite the clinical use of QA for more than 70 years, its metabolic fate has been unknown. In this study, we identified by LC/MS/MS the primary metabolic pathway of QA as N-desethylation and O-demethylation. Mono-desethyl-QA was the major metabolite of QA in vitro and in vivo, which was confirmed by comparison with the authentic reference compound. Mono-desethyl-QA was further metabolized to di-desethyl-QA. At present, it is unclear whether these metabolites have antiprion activities. Di-desethyl QA was further oxidized to a carboxyl acid derivative (MS), probably through an unstable aldehyde (Koppel et al., 1987; Baker et al., 1990). In addition to P450, other enzymes such as
Monoamine oxidase might be involved in oxidative deamination (Baker et al., 1990). This was supported by the observation that a higher M5 was formed in rat hepatocyte incubation than in rat microsomal incubation. The carboxyl acid metabolite seems to be more easily excreted into urine, because a higher M5 proportion was found in the urine than in the tissues. Another minor metabolic pathway was cleavage of the whole side chain of QA and of its metabolites to form the ACMA metabolite. ACMA has strong fluorescence and was used as a molecular probe for DNA studies because it could intercalate into a DNA helix (Fukui and Tanaka, 1996). Although very little ACMA was biotransformed in microsomal and hepatocyte incubations, we could not exclude the ACMA formation in the body, especially since QA accumulates in tissues for a long time (Goodman and Gilman, 1975).

![Figure 8](https://example.com/fig8.png)  
**Fig. 8.** Mono-desethyl-QA formation in recombinant human P450 isozymes (A) and in HLMs after incubation with mAb CYP3A4/5 (B). Incubation with 40 μM QA at 37°C for 30 min. mAb CYP3A4/5 volumes are expressed in 100-μg HLMs. The values are the mean of duplicate experiments.

![Figure 9](https://example.com/fig9.png)  
**Fig. 9.** Bidirectional transepithelial transport of quinacrine across MDR1-MDCK cell and MDCK cell monolayers. Quinacrine concentration was 25 μM. The concentration of GG918, a P-gp inhibitor, was 1 μM. Solid squares, A to B transport in control MDCK cells; solid circles, B to A transport in control MDCK cells; ticked line, A to B transport in control MDR1-MDCK cells; solid triangles, B to A transport in control MDR1-MDCK cells; solid diamonds, A to B transport in MDR1-MDCK cells incubated with GG918; patterned squares, B to A transport in MDR1-MDCK cells incubated with GG918. Values are the mean ± S.D. of triplicate experiments.

### Table 1

**The permeability of quinacrine through the cell monolayer**

<table>
<thead>
<tr>
<th>Cell</th>
<th>Direction</th>
<th>Control/Inhibitor*</th>
<th>$P_{exp}$</th>
<th>$cmls \times 10^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCK</td>
<td>A to B</td>
<td>Control</td>
<td>0.15 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>MDCK</td>
<td>B to A</td>
<td>Control</td>
<td>0.20 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>MDR1-MDCK</td>
<td>A to B</td>
<td>Control</td>
<td>0.20 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>MDR1-MDCK</td>
<td>A to B</td>
<td>Inhibitor</td>
<td>0.30 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>MDR1-MDCK</td>
<td>B to A</td>
<td>Control</td>
<td>4.5 ± 0.19***</td>
<td></td>
</tr>
<tr>
<td>MDR1-MDCK</td>
<td>B to A</td>
<td>Inhibitor</td>
<td>0.12 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.001, compared with MDCK B to A control by Student’s t test.
*Control, without inhibitor; inhibitor, P-glycoprotein inhibitor GG918 at concentration 1 μM.

### Table 2

**Quinacrine tissue distribution in MDR1-KO and WT FVB mice after a single intravenous administration at 2 mg/kg.**

Mice (n = 5) were sacrificed at 0.5 h or 3 h after dose. The values are expressed as mean ± S.D.

<table>
<thead>
<tr>
<th>Time</th>
<th>Tissue</th>
<th>MDR1-KO</th>
<th>FVB</th>
<th>MDR1-KO/FVB Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h</td>
<td>Brain</td>
<td>1.8 ± 0.52**</td>
<td>0.32 ± 0.26</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>0.038 ± 0.010</td>
<td>0.033 ± 0.009</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>1.8 ± 0.56</td>
<td>1.8 ± 1.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>4.6 ± 1.3</td>
<td>5.4 ± 2.0</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2.2 ± 0.70</td>
<td>4.4 ± 2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3 h</td>
<td>Brain</td>
<td>1.8 ± 0.42**</td>
<td>0.20 ± 0.11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>0.019 ± 0.013</td>
<td>0.016 ± 0.0060</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>1.0 ± 0.41</td>
<td>1.1 ± 0.48</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>4.5 ± 0.45</td>
<td>4.0 ± 0.73</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>4.1 ± 0.63</td>
<td>4.9 ± 1.6</td>
<td>0.84</td>
</tr>
</tbody>
</table>

**P < 0.01 compared with FVB mice by Student’s t test.

### Table 3

**Quinacrine tissue distribution in MDR1-KO and WT FVB mice after oral administration of 10 mg/kg/day for 7 days.**

Mice (n = 6) were sacrificed 20 h after the last dose. The values are expressed as mean ± S.D.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MDR1-KO</th>
<th>FVB</th>
<th>MDR1-KO/FVB Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>7.8 ± 1.5***</td>
<td>0.16 ± 0.056</td>
<td>49</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.019 ± 0.0031</td>
<td>0.018 ± 0.012</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>8.6 ± 2.2*</td>
<td>4.5 ± 1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.4 ± 1.2*</td>
<td>2.9 ± 1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>8.4 ± 1.8*</td>
<td>4.7 ± 1.1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*P < 0.05 and ***P < 0.0001, compared with FVB mice by Student’s t test.
that QA and its other metabolites might be finally degraded to ACMA by removal of the whole side chain, similar to chloroquine degradation to 4-aminooquinoline (O’Neill et al., 1998). The proposed metabolic pathway is shown in Scheme 1. In a preliminary study, the accumulated amount of unchanged QA in rat urine for 28 days was found to be less than 1% of the dose, which indicated that the renal excretion of QA was a very minor elimination route. QA metabolism in liver was considered to play a major role in the elimination of QA in vivo.

The human P450 isoforms involved in the QA mono-desethylation were then examined by using specific chemical inhibition and cDNA-expressed P450 studies. Mono-desethyl-QA formation was inhibited by the CYP3A4-specific inhibitor ketoconazole (Fig. 7) as well as mAb CYP3A4/5 (Fig. 8B). Furthermore, cDNA-expressed CYP3A4 and CYP3A5 revealed the highest activities in mono-desethyl-QA formation among 11 P450 isoforms (Fig. 8A). These results indicate that CYP3A4 and CYP3A5 are the major P450 isoforms responsible for QA mono-desethylation.

Because achieving high brain concentrations is an important prerequisite to deploying QA in the treatment of prion disease, we investigated whether QA was a P-gp substrate. QA transport from the basolateral to apical side in MDR1-MDCK cells was markedly greater than in control MDCK cells and was inhibited by the potent P-gp inhibitor GG918, indicating that QA is a P-gp substrate. Dohgu et al. (2004) recently reported that A to B transport of QA in mouse brain endothelial cells was increased in the presence of a P-gp inhibitor, cyclosporine or verapamil, which suggests that QA transport at the BBB is mediated by P-gp (Dohgu et al., 2004). In a preliminary clinical observation, coadministration of the P-gp inhibitor verapamil was reported to have reduced QA hepatotoxicity in sporadic Creutzfeldt-Jakob disease patients while not affecting its efficacy (Satoh et al., 2004). Unfortunately, the QA levels in cerebrospinal fluid before and after coadministration of verapamil were not measured. To evaluate the role of P-gp in eliminating QA accumulation in the brain, we compared QA brain levels in MDR1-KO mice and in WT mice after a single i.v. dose of 2 mg/kg QA or multiple oral doses of 10 mg/kg/day QA for 7 days. In MDR1-KO mice, we observed a remarkable accumulation of QA in brain after both i.v. and oral administration (Tables 2 and 3). In contrast, QA concentrations in plasma, liver, spleen, and kidney between the two strains of mice were not changed or increased much (<2 times at oral dose). These results indicate that P-gp plays a critical role in excluding QA from brain in vivo.

At present, it is not clear why QA shows strong antiprion activity in vitro, but not in vivo. Although numerous factors may affect the discrepancy between in vitro and in vivo results, the inability of QA to treat prion disease effectively may be due to its pharmacokinetics. Gayrard et al. (2005) recently reported that free QA concentration in brain was lower than the EC50 reported in vitro, even though the total brain QA concentration was high, which suggests a pharmacokinetics, not a pharmacodynamics, problem (Gayrard et al., 2005). In our previous QA pharmacokinetic studies, the brain and liver QA concentrations in normal mice were found to be approximately 1.5 μg/g and 300 μg/g, respectively, at doses of 75 mg/kg/day for 4 weeks (Yung et al., 2004). However, in MDR1-KO mice, the QA brain level was up to 7.8 μg/g, whereas the QA liver concentration was 8.6 μg/g at doses of 10 mg/kg/day for 1 week. The hepatotoxicity of QA might be less in MDR1-KO mice at low doses. When given oral doses of QA at 10 mg/kg/day for 7 days, we observed neither body weight changes nor abnormal behaviors in these mice. The selective increase of brain QA concentration in MDR1-KO mice suggests that this animal model might be used to test whether QA has any antiprion activity in vivo or not.

In summary, QA metabolites were identified by LC/MS/MS. N-desethylation was the major metabolic pathway of QA in vitro and in vivo. The major P450 isoforms involved in QA mono-desethylation were CYP3A4/3A5. QA was also found to be a typical P-gp substrate and P-gp in the BBB plays a very important role in eliminating QA from brain.

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References