Quinine interactions with tryptophan and tyrosine in malaria patients, and implications for quinine responses in the clinical setting

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Objectives: Recent work with the yeast model revealed that the antiprotozoal drug quinine competes with tryptophan for uptake via a common transport protein, causing cellular tryptophan starvation. In the present work, it was hypothesized that similar interactions may occur in malaria patients receiving quinine therapy.

Patients and methods: A direct observational study was conducted in which plasma levels of drug and amino acids (tryptophan, tyrosine and phenylalanine) were monitored during quinine treatment of malaria patients with Plasmodium falciparum infections.

Results: Consistent with competition for uptake from plasma into cells, plasma tryptophan and tyrosine levels increased ≥2-fold during quinine therapy. Plasma quinine levels in individual plasma samples were significantly and positively correlated with tryptophan and tyrosine in the same samples. Control studies indicated no effect on phenylalanine. Chloroquine treatment of Plasmodium vivax-infected patients did not affect plasma tryptophan or tyrosine. During quinine treatment, plasma tryptophan was significantly lower (and quinine significantly higher) in patients experiencing adverse drug reactions.

Conclusions: Plasma quinine levels during therapy are related to patient tryptophan and tyrosine levels, and these interactions can determine patient responses to quinine. The study also highlights the potential for extrapolating insights directly from the yeast model to human malaria patients.

Keywords: competitive inhibition, antiparasitic drugs, amino acid uptake, aromatic amino acids, antimalarial drugs

Introduction

Quinine has been a mainstay of drug therapy against the malaria parasite, the protozoan Plasmodium spp. However, the safety profile and effectiveness of the drug differs from one patient to another. An understanding of the molecular basis for these differences in patients could potentially improve treatment strategies.

Recently, a high throughput screening approach in yeast gave new insight to the mode of quinine action. Yeast deletion strains defective for tryptophan biosynthesis were susceptible to quinine, and this was suppressed by exogenous tryptophan. Mutants defective for biosynthesis of tyrosine, another aromatic amino acid, were also quinine susceptible and these were rescued by tyrosine addition. Quinine was shown to inhibit tryptophan uptake via the yeast Tat2p transporter competitively. It was concluded that, owing to their structural relatedness, quinine interferes with tryptophan metabolism in cells causing tryptophan starvation, a novel mechanism of quinine toxicity.

There is close evolutionary conservation between yeast and higher eukaryotes including humans. Here we set out to test the hypothesis that tryptophan may also influence the outcome of quinine therapy in malaria patients. We show that plasma quinine levels are related to tryptophan and tyrosine levels, and that this can influence patient responses to quinine.

Methods

Clinical studies

An observational study of patients during malaria treatment was performed between May and December 2010. The methodology was adapted from the WHO guide on antimalarial efficacy. Patients were recruited with appropriate consent from four Ministry of Health hospitals within the Klang Valley in Selangor and Kuala Lumpur, Malaysia. The study was registered under the National Medical Research Registration, with ethical approval from the Medical Research Ethics Committee Malaysia (ID: NMRR-10-313-5172). The study group comprised adults...
diagnosed with a positive parasitological malaria infection and treated with quinine or chloroquine (88% of the latter received chloroquine in combination with doxycycline or primaquine). Patients without parasitaemia records or who had been treated with antimalarials during the 2 weeks preceding admission were not included. Parasite levels and treatment efficacy were monitored according to local hospital treatment protocols. The day a patient was confirmed for malaria was designated ‘day 0’. Blood samples were collected before treatment on day 0, and within 3 h of treatment on subsequent days for at least 5 days (quinine) or 4 days (chloroquine). Daily treatments of 600 mg of quinine or 300 mg of chloroquine were administered orally, and repeated in patients who vomited within 30 min. Patients requiring more than one repeat dose were excluded from the study. Blood samples were centrifuged (1000 g, 10 min) within 2 h of collection, and the plasma supernatant was stored at −20°C.

Patients were screened daily for treatment failure (increased parasitaemia), and for symptoms that emerged after treatment, including known adverse drug reactions (ADRs). Suspected ADRs were assessed by the Malaysian Adverse Drug Reaction Centre (MADRAC) for causality, based on the WHO-UMC categories of ADRs.4

**Analysis of blood plasma**

Plasma tryptophan, tyrosine and phenylalanine were analysed with a Waters 600 Controller HPLC under gradient conditions.5 Quinine and chloroquine were analysed with a Varian 320-MS liquid chromatography mass spectrometer.6 The two mobile phases were 20 mM ammonium formate and 50% (v/v) acetonitrile, both with 0.5% (v/v) formic acid. The gradient conditions were: 0–5 min, 80% to 50% ammonium formate; 5–5.5 min, 50% to 80% ammonium formate; and 5.5–10 min, 80% ammonium formate.

**Statistical analyses**

Data were analysed using SPSS v.16 (SPSS Inc., Chicago, IL, USA). Correlations between plasma amino acids versus treatment days and plasma quinine and chloroquine were analysed using a Pearson correlation (R). Unpaired t-tests were used to test for differences in plasma amino acid or drug concentrations between patients who did and did not experience ADRs.

**Results**

**Plasma quinine is correlated with plasma tryptophan and tyrosine**

A total of 51 patients met the study inclusion criteria (Table S1, available as Supplementary data at JAC Online). Of these, 18 received quinine treatment for *Plasmodium falciparum* infection, while the other 33 received chloroquine treatment for *Plasmodium vivax*. *P. falciparum* was treated with quinine monotherapy (*n* = 8), or in combination with doxycycline or sulfadoxine/pyrimethamine (*n* = 10). None of these patients had prior medication.

The main hypothesis was that quinine and tryptophan or tyrosine compete for uptake from plasma into cells. If that is the case, high plasma quinine should decrease amino acid uptake (i.e. increase retention in the plasma). Therefore, the hypothesis predicted that quinine and tryptophan or tyrosine levels in the plasma should be correlated. Chloroquine-treated patients were infected with a different *Plasmodium* species, and chloroquine was predicted not to interact with uptake of endogenous tryptophan or tyrosine.2 Plasma drug levels in patients were within the recommended therapeutic ranges: 4–34 μM quinine (1.3–11.0 mg/L) and 0.3–10.6 μM chloroquine (0.1–3.4 mg/L) (Figure 1).

Mean plasma tryptophan levels increased significantly (≅3-fold; *P* = 0.023, one-tailed; *n* = 18) during quinine treatment (Figure 1a), but not in patients receiving chloroquine (Figure 1g). Similarly, plasma tyrosine increased significantly (≅2-fold) during quinine (Figure 1c) but not chloroquine (Figure 1f) treatment. The coefficient of correlation with quinine were relatively low (Figure 1a and c), consistent with quinine not being the only factor affecting patient tryptophan and tyrosine levels. Previously, there was no evidence for a relationship between the related aromatic amino acid phenylalanine and quinine in yeast.2 Similarly, quinine had no significant effect on plasma phenylalanine (Figure 1e).

Tryptophan and other amino acid levels are notoriously variable between individuals, depending partly on diet.7,8 Such variability (also in plasma drug concentrations) was evident here (Figure 1). We exploited this variability to probe further the relationship between plasma quinine and amino acids, analysing data collected for each patient on each day. Plasma quinine was significantly positively correlated with plasma concentrations of tryptophan and tyrosine (Figure 1b and d). Phenylalanine was not correlated with quinine (Figure 1f), and none of these amino acids correlated with chloroquine (Figure 1h, j and l). We also examined within-patient correlations between drug and amino acid levels across days of treatment for individual patients. Although the limited numbers of days meant small sample sizes, plasma quinine and tryptophan were positively and significantly (*P* < 0.05, one-tailed) correlated in 50% of the patients. No such relationship with phenylalanine was discernible in any of the patients.

**ADRs correlate positively with plasma quinine and negatively with plasma tryptophan**

It was previously suggested that tryptophan or tyrosine might influence ADRs commonly associated with quinine treatment.2 Here, ADRs were recorded for 50% of the quinine-treated patients. Several experienced more than one ADR during treatment, totalling 25 reported ADRs (Figure 2a). The most common ADRs were gastrointestinal. ADR frequency did not differ between monotherapy and combination treatment (Fisher’s exact test, two-tailed *P* > 0.05). We compared plasma quinine and amino acid levels between patients who did, and did not, experience an ADR, examining each treatment day independently. Mean quinine was ≅50% higher in samples from patients reporting an ADR (Student’s t-test, one-tailed *P* = 0.002) (Figure 2b). Consistent with the hypothesis, tryptophan was lower (t-test, *P* = 0.040) in samples from patients experiencing an ADR. This inverse relationship was discernible despite plasma tryptophan correlating positively with plasma quinine (Figure 1a and b), which itself correlated positively with ADR incidence (Figure 2b). Therefore, this apparent effect of tryptophan on ADRs probably underestimates the real influence of tryptophan, independent of quinine. ADRs did not correlate significantly with tyrosine or phenylalanine levels (Figure 2b). An apparent increased mean phenylalanine in patients experiencing an ADR was primarily due to one outlier with unusually high phenylalanine. Quinine and amino acid levels did not differ between patients receiving...
Figure 1. Relationship between aromatic amino acids and quinine in blood plasma. Plasma levels of tryptophan, tyrosine or phenylalanine for each patient are presented either according to the day of quinine (a, c and e) or chloroquine (g, i and k) treatment, or by comparison with quinine (b, d and f) or chloroquine (h, j and l) levels in each patient sample. The 0 plasma drug values (b, d, f, h, j and l) refer to patient samples taken just before drug treatment on day 0. The indicated $R$ and $P$ (one-tailed) values were determined by Pearson correlations. Points are means from three replicate analyses.
Discussion

Previously we established that quinine competes with tryptophan and tyrosine for transport into yeast cells, a likely consequence of these molecules’ structural relatedness. The present study extrapolates this to quinine therapy in malaria patients. The patients received monotherapy or combination therapy involving quinine for *P. falciparum*. The outcomes for monotherapy and combination therapy did not differ, according to parasite clearance (not shown), ADR rates and plasma drug and amino acid levels. Therefore, the results of interest could be assigned primarily to quinine.

Phenylalanine and tryptophan are essential in the human diet. Tyrosine can be synthesized from phenylalanine, and is also dietary. Plasma quinine was correlated with plasma tryptophan and tyrosine, consistent with the yeast data. The lack of a relationship with phenylalanine indicates that the effect was not generic, e.g. related to dietary differences among patients, or return of appetite during treatment. This phenylalanine internal control was further supported by the lack of correlation between plasma levels of chloroquine and tryptophan or tyrosine. The possibility that tryptophan and tyrosine levels changes during quinine treatment might be related to decreasing parasitaemia (infected erythrocytes reportedly take up more amino acids) was countered by the fact that parasites were cleared within a few days in both quinine- and chloroquine-treated patients, but only quinine was linked to tryptophan and tyrosine. The effect appeared to be quinine specific, although we cannot fully discount the possibility of a species-specific (*P. falciparum* versus *P. vivax*) effect of decreasing parasitaemia.

The positive correlations between plasma quinine and tryptophan or tyrosine levels fulfilled a key prediction of the hypothesis that these molecules compete for uptake from plasma into blood cells. In yeast, quinine competed competitively with tryptophan at the Tat2 permease. In human erythrocytes, aromatic amino acids are taken up via Na\(^+\)-independent transporters, the L and T systems. These differ in their relative amino acid affinities, perhaps explaining the differing relationships with quinine. The T system is important for tryptophan (like yeast Tat2p) and the L system is important for phenylalanine. There was no evidence for chloroquine uptake by tryptophan permeases in yeast. The lack of relationship between chloroquine and the amino acids in patients was consistent with that.

The ADRs in this study were classified as ‘possible ADRs’, as they were observed only after drug therapy commenced, and subsided once completed. The classification ‘possible’ rather than ‘probable’ was because of potential similarities to disease symptoms and because we could not re-challenge with drugs, as is not atypical for this type of study. That the ADRs were quinine-associated was reinforced by that fact that patients experiencing an ADR exhibited significantly higher plasma quinine on the relevant day(s). Plasma tryptophan was another determinant of ADR susceptibility. Patients experiencing an ADR had decreased tryptophan, despite tryptophan being positively correlated with quinine, which in turn correlated positively with ADRs. This evidence suggests that low tryptophan could predispose patients to ADRs, commonly associated with quinine. This is important as human tryptophan levels are highly variable, and readily affected by diet. Dietary tryptophan supplements could potentially help to suppress adverse quinine reactions,
making this a safer drug. One concern would be suppression of antiparasitic quinine action. However, unlike ADRs, our preliminary data do not suggest a relationship between tryptophan and parasite clearance by quinine (F. Islahudin, R. J. Pleass, S. V. Avery and K.-N. Ting, unpublished data). The tryptophan–quinine relationship uncovered in yeast may extrapolate more faithfully to humans than to the malaria parasite.

This work supports the validity of yeast as a model for gaining insights to antimalarial action. Yeast is also being employed to help discover antiparasitic agents. The present approach circumvents animal experimentation, adding further value to the strategy. This study supports the hypothesis that quinine safety is closely related to tryptophan and tyrosine levels in malaria patients.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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Transparency declarations
None to declare.

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