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Effect of *Plasmodium falciparum* malaria infection on the plasma concentration of α_1-acid glycoprotein and the binding of quinine in Malawian children

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1 We have measured plasma concentrations of α_1-acid glycoprotein (AGP) in 18 healthy children and 85 children with falciparum malaria in Malawi. In addition, we determined the degree of protein binding of quinine (QN) in the plasma of 52 of the patients and each of the healthy controls.

2 The mean plasma AGP concentration was higher in patients than in controls ($P < 0.0001$) and remained elevated 3 weeks after complete resolution of malaria infection.

3 The mean unbound QN fraction was significantly less ($P < 0.00001$) in patients with malaria (0.128 ± 0.037) than in controls (0.193 ± 0.051) and significantly higher ($P = 0.02$) in convalescence (0.153 ± 0.067) than during acute illness.

4 There were highly significant negative correlations between plasma AGP concentration and the free QN fraction in spiked plasma samples ($r = -0.534$, $P < 0.0001$, $n = 93$) and in clinical samples ($r = -0.484$, $P < 0.00001$, $n = 225$). There was a significant positive correlation between plasma concentrations of AGP and another acute phase reactant, C reactive protein ($P < 0.001$).

Keywords α_1-acid glycoprotein protein binding quinine malaria infection children

Introduction

α_1-acid glycoprotein (AGP) is an acute phase protein (Kremer *et al.*, 1988) which can bind basic drugs (Routledge, 1986). *Plasmodium falciparum* malaria infections are associated with increased plasma concentrations of AGP (Mansor *et al.*, 1990; Voulgari *et al.*, 1981). Severe falciparum malaria infections are treated with parenteral quinine (WHO, 1990), a drug which is highly protein-bound *in vivo*, and which binds preferentially to AGP over albumin *in vitro* (Mihaly *et al.*, 1987a). The free (unbound) concentration of quinine (QN) is likely to reflect the drug's efficacy and its toxicity. The free fraction fluctuates during malarial infection (Silamut *et al.*, 1985), but the relationship between plasma concentrations of AGP and the free fraction of QN has not previously been described.

In this study, we have examined the plasma protein binding of QN in Malawian children with malaria of various degrees of severity. We have also measured the plasma concentrations of AGP in these patients and have determined the relationship between the free fraction of QN and AGP at intervals during and after acute malaria illness.

Methods

The study was carried out in the Department of Paediatrics at the Queen Elizabeth Central Hospital in Blantyre, Malawi. Ethical approval was given by the Health Sciences Research Committee of the Malawi Government. Parents or guardians gave informed consent for the participation of children in the study. Patients were enrolled in the study if they presented with a febrile illness and *P. falciparum* parasitaemia, with no other identifiable cause of fever. All patients were considered to be sick enough to require admission to hospital so that initial treatment with parenteral quinine could be

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given. The severity of disease was further defined by the presence or absence of complications of falciparum malaria (WHO, 1990). Among comatose patients, the level of consciousness was assessed by a coma scale modified for use in children (Molyneux *et al.*, 1989).

Venous blood was drawn at the time of admission for full blood count (Coulter counter), blood culture (agar slants in trypticase soy broth), haematocrit (microcentrifuge) and microscopy for malaria parasites. Thick films were prepared with Field's A and B stains and thin films with Leishman's stain. Parasites were counted against 200 white cells on the thick film or 500 red cells on the thin film, the parasitaemia then being calculated from the total white cell count or red cell count respectively. After centrifuging the initial blood sample, plasma was frozen at −20° C for subsequent analysis of AGP concentration and quinine binding.

All patients were treated initially with parenteral quinine, according to a number of different regimens which were being evaluated in studies of quinine pharmacokinetics. These data have either been published (Mansor *et al.*, 1990) or will be published elsewhere. All received parenteral quinine eight-hourly until able to take oral treatment (one or two tablets of pyrimethamine-sulphadoxine [Fansidar]). Quinine was given either by slow intravenous infusion or by intramuscular injection. The first dose was either 10 mg kg^{-1} or 20 mg kg^{-1} of quinine dihydrochloride; all subsequent doses were 10 mg kg^{-1}. Progress during treatment was observed closely with vital signs and coma score being monitored every 2 h until the patient was afebrile and fully conscious.

Parasitaemia and haematocrit were recorded every 8 h. In addition to the pre-treatment sample, plasma was obtained at 16, 24, 48 and 56 h after the start of treatment, for measurement of AGP concentration and quinine binding. Patients were discharged from hospital when afebrile and aparasitaemic, and were reviewed after 1 month. At the follow-up visit a clinical assessment was made and blood was sampled for parasitaemia, haematocrit and plasma AGP and QN binding.

Control group

The control group consisted of children who were afebrile and aparasitaemic and who were taking part in a malaria survey in a school in the same region of Malawi.

Protein binding of quinine

The plasma protein binding of QN was measured by ultrafiltration. In pre-treatment and follow-up samples, binding of QN was measured by spiking the (QN-free) plasma with QN dihydrochloride (to achieve concentrations of 5 μg ml^{-1} and 10 μg ml^{-1}). In addition, plasma samples of the control group (healthy children) were spiked with QN dihydrochloride for measurement of plasma protein binding. In samples taken during the period of QN therapy, binding was assessed using QN already present in the plasma as a result of treatment. Centrifugation for 10–20 min at 2000 rev min^{-1} was carried out at room temperature and pH adjusted to 7.4 using 1 M HCl. Plasma protein free ultrafiltrate was prepared from 250–500 μl plasma producing a clear ultrafiltrate containing less than 0.1% protein. The ultrafiltrate was examined for protein leakage using Albustix® (Ames Division, Miles Laboratories, Slough U.K.); sensitivity 0.05 g l^{-1}. Binding was recorded as the ratio of bound QN to the total QN concentration.

Measurement of quinine

Concentrations of QN in plasma and ultrafiltrate were measured by a specific and sensitive h.p.l.c. method adapted from Mihaly *et al.* (1987b) and previously reported (Mansor *et al.*, 1990).

Binding of quinine to membrane

To determine whether QN was bound to YMT membranes (Amicon, Stonehouse, U.K.), QN solutions (5 μg ml^{-1} and 15 μg ml^{-1}) were prepared in distilled water. Aliquots (1.0 ml) were loaded into the reservoir of the ultrafiltration tube (MPS-1) kits. Following centrifugation (1000 *g* for 3 min), the concentrations of quinine in the filtered and unfiltered solutions were measured by h.p.l.c.

α_1-acid glycoprotein (AGP)

Plasma concentrations of AGP were estimated at the same time as measurements of concentrations of unbound QN using an immunodiffusion kit with a tolerance range of ± 15% (Nor-Partigen®, Behring Division, Hoechst Ltd, Hounslow, U.K.).

Statistical analysis

Comparisons of multiple means were made using a one-way analysis of variance. The relationship between concentration of AGP and unbound QN was examined by linear regression analysis.

Results

Eighty-five children with malaria (41 boys) and 18 controls (8 boys) were studied. The mean age (± s.d.) of the patients was 4.80 (± 2.8) years (range 0.75–12) and of the controls was 4.82 (± 1.32) years (range 3.3–9). The mean centile weight for height was similar in the two groups (patients 35.9 ± 32, controls 40.3 ± 23), but the mean centile weight for age was significantly lower in patients than in controls (patients 17.3 ± 22, controls 31.8 ± 23, $P = 0.002$, 95% CI −27–4).

In the patients the mean duration of fever prior to admission was 2 days (range 0.1–7), and the mean interval since last taking food was 1 day (range 0–5). There was a history of convulsions in 61 patients (72%) and convulsions were witnessed at the time of admission in 14 (16%). Malaria was uncomplicated in 18 patients (21%). The remaining 67 patients (79%) had altered consciousness, 39 (46% of all patients) having a coma score of two or less on a scale ranging from 0 (profound coma) to 5 (full consciousness) (Molyneux *et al.*, 1989). The mean rectal temperature on admission was 39.1 (± 1.05)°C (range 36.4–41.2). Three patients died. Among

survivors, three had neurological sequelae; the rest made a full recovery. The mean time to become afebrile was 39 (± 20) h and to become aparasitaemic it was 40 (± 11.4) h. Patients who were initially comatose took, on average, 18 (± 24) h to regain full consciousness.

α_1-acid glycoprotein (AGP)

In patients with malaria the mean plasma AGP concentration during acute illness, 1.81 (± 0.40) g l^{-1}, was significantly higher than the mean in 48 of these patients who attended for follow-up evaluation after 1 month (1.51 ± 0.58, $P < 0.05$; Figure 1). If the analysis is confined to the 48 patients who returned for follow-up, there is a highly significant difference between the acute and the convalescent AGP values (acute: 1.82 ± 0.46 *vs* convalescent: 1.51 ± 0.58, $P < 0.0001$, paired *t*-test). The mean AGP concentration in controls (0.82 ± 0.20 g l^{-1}) was significantly lower than in patients with malaria, both in the acute stage ($P < 0.0001$) and in convalescence ($P < 0.0001$; Figure 1).

Among patients with malaria, the mean AGP levels were similar in patients with uncomplicated illness and those with impaired consciousness. Among unconscious patients, AGP concentration was not related to the depth of coma. The mean AGP concentration on admission was significantly higher in 38 patients with hepatomegaly (1.99 ± 0.43 g l^{-1}) than in those without hepatomegaly (1.62 ± 0.35) ($P < 0.0001$). There was no correlation between AGP concentration and parasite density, or between AGP and total plasma protein or albumin concentration. There was, however, a significant correlation between levels of AGP and C reactive protein ($r = 0.582$, $P < 0.001$).

Quinine binding

The recovery of QN after repeated passage through the ultrafiltration equipment remained constant (i.e. 96 ± 1.9%; coefficient of variation (CV) = 2.0%; 96.4 ± 2.0%; CV = 2.2%) at 5 μg ml^{-1} and 15 μg ml^{-1} respectively. This was interpreted as a negligible loss of QN due to binding to plastic containers and membrane filters. The protein-binding of QN was measured in 52 patients during acute illness, in 36 of these in convalescence and in all the control children. Mean QN binding was significantly greater in patients with malaria (free QN fraction: 0.128 ± 0.037) than in controls (0.193 ± 0.051 $P < 0.00001$). QN binding was significantly less ($P = 0.02$) in convalescence (free QN fraction 0.153 ± 0.067) than during acute illness, but binding in convalescent samples remained significantly greater than in controls. At no time during treatment was there a correlation between total plasma QN concentration (range 4–20 mg l^{-1} and the free quinine fraction.

Sequential changes in AGP concentration and QN binding

Mean AGP concentrations and QN binding remained high throughout the first 56 h of treatment, but there were no significant differences among the AGP concentrations or free QN fractions measured before and at intervals during drug therapy (Figure 1). Significantly lower AGP concentrations and decreased QN binding were observed 1 month after the start of drug therapy in all patients (Figure 1).

Relationship between AGP concentration and QN binding

There was a highly significant negative correlation between plasma AGP concentration and the free QN fraction at all sampling times, in both parents and controls. This was the case both when binding was measured using QN-spiked plasma (patients before treatment and in convalescence, Figure 2) and when binding was estimated using the QN already present in plasma (patients receiving treatment, Figure 3).

The correlation coefficients for AGP concentration and free QN fraction were: patients pre-treatment $r = -0.870$ ($n = 42$, $P < 0.00001$); patients at times during

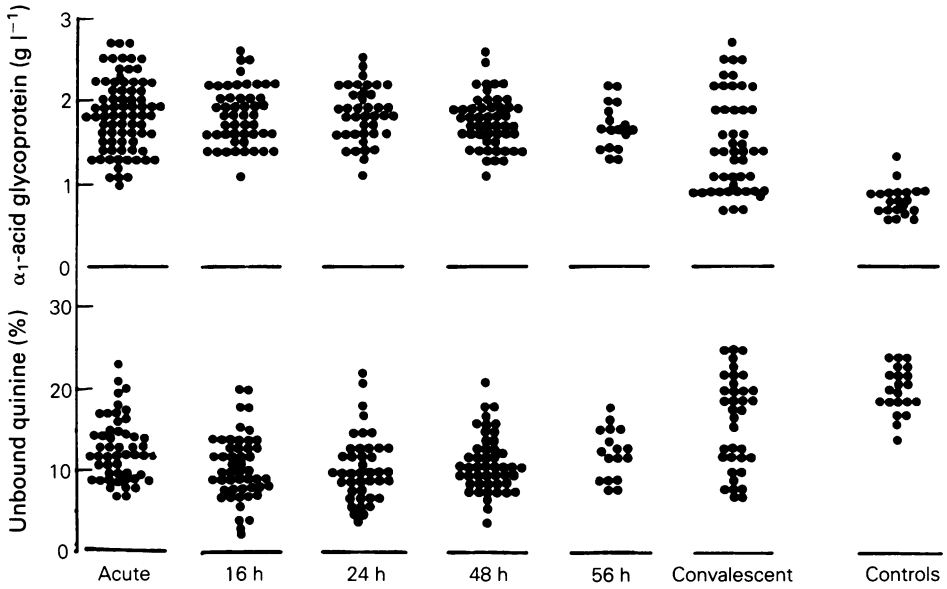

Figure 1 Mean percentage of quinine unbound and plasma concentrations of α_1-acid glycoprotein in controls and in Malawian children with falciparum malaria on admission, during quinine therapy (16, 24, 48, 56 h) and during convalescence (1 month after drug treatment).

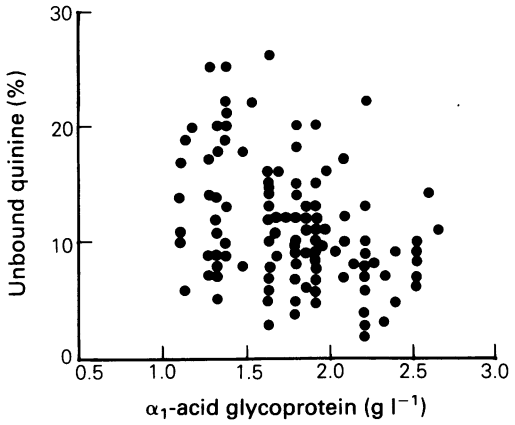

Figure 2 Correlation between percentage of quinine unbound in spiked samples and the plasma concentrations of α_1-acid glycoprotein (g l^{-1}) in Malawian children with falciparum malaria ($r = -0.534$, $P < 0.0001$, $n = 93$).

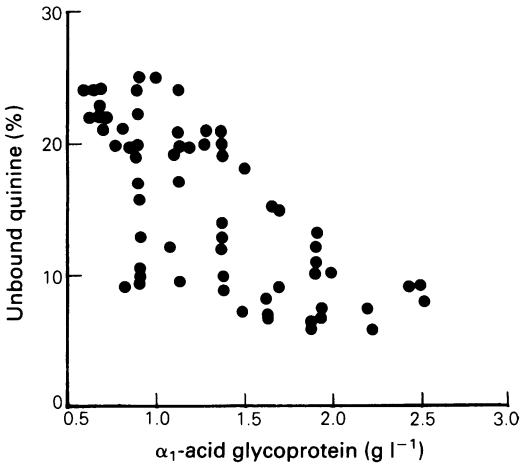

Figure 3 Correlation between percentage of quinine unbound and the plasma concentrations of α_1-acid glycoprotein (g l^{-1}) in 85 Malawian children receiving quinine treatment for falciparum malaria ($r = -0.484$, $P < 0.00001$, $n = 225$).

treatment, $r = -0.484$ ($n = 225$, $P < 0.00001$); patients in convalescence, $r = -0.576$ ($n = 36$, $P < 0.001$); controls, $r = 0.901$ ($n = 18$, $P < 0.00001$). There was no significant correlation between QN binding and either C reactive protein or total plasma protein concentration.

Mean fever clearance and parasite clearance times did not correlate significantly with plasma concentrations of AGP ($r = 0.047$ and 0.161) or with the degree of protein binding of QN ($r = 0.02$ and -0.22) measured on admission plasma samples. No clinical evidence of QN toxicity was encountered in any patient studied.

Discussion

The results of this study confirm the observations of Silamut *et al.* (1985) that QN binding in patients with malaria is greater than in healthy controls and that the increased binding in malaria persists for at least several weeks after recovery. We have extended our previous studies (Mansor *et al.*, 1990) to show that the plasma α_1-acid glycoprotein (AGP) concentration is increased in malaria, and have shown that the degree of quinine binding correlates strongly with the concentration of AGP, both in patients with malaria and in healthy controls. These findings provide further evidence for a possible role of AGP in the binding of quinine *in vivo*. While changes in the degree of binding might be expected to influence both the therapeutic efficacy and toxicity of QN, studies of the pharmacokinetics of QN in children (Shann *et al.*, 1985) suggest QN to be of low to intermediate clearance. Hence, free concentrations of QN may *not* increase in response to a change in the free fraction of QN. The significance of this is that the relatively high *total* plasma concentrations of QN in disease (White *et al.*, 1982) cause no additional toxicity.

Saturation of QN binding to a fixed concentration of AGP has been reported *in vitro* (Mihaly *et al.*, 1987a). In the present study we found no evidence of saturation of binding *in vivo*. Total plasma QN concentrations ranged from 4–20 mg l^{-1}. In the presence of binding saturation, the free fraction of QN should increase with increasing total plasma QN concentration. We found no such correlation at any time during treatment. In our patients there was a significant correlation between levels of AGP and levels of C reactive protein, another acute phase reactant. A simultaneous increase of both AGP and C reactive proteins has been observed in patients with hepatic amoebiasis (Monnet *et al.*, 1990).

The biological properties of AGP are diverse. It may be involved in tissue repair, normal coagulation and immunological processes (Kremer *et al.*, 1988). There is evidence that AGP may inhibit the multiplication of malaria parasites *in vitro* (Friedman, 1983; Friedman *et al.*, 1984). It is not known whether these functions of AGP have a role in pathogenesis, or contribute to host defence in malaria. The mechanism by which the concentration of AGP in plasma is increased in acute disease is unknown. Cytokines may have a role. In 68 of the patients in the present series, concentrations of tumour necrosis factor (TNF) were measured (mean 257; range 15–1860 pg ml^{-1}); the levels correlated with plasma AGP ($r = 0256$; $P < 0.05$; unpublished). The administration of TNF and interleukin-1 (IL-1) to rats leads to increased serum AGP concentrations and reduced hepatic microsomal cytochrome P-450 content (Bertini *et al.*, 1989, 1988; Chen *et al.*, 1990). In addition, plasma TNF concentrations are elevated in malaria and are particularly high in those patients with severe disease (Grau *et al.*, 1989). Further work is needed to study the relationship between these cytokines and the increase in circulating AGP in patients with malaria.

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