Plasma glutamine and glutamate concentrations in Gabonese children with Plasmodium falciparum infection

T. PLANCHE1, A. DZEING2, A.C. EMMERSON1, M. ONANGA2, P.G. KREMSNER3,4, K. ENGEL4, M. KOMBILA2, E. NGOU-MILAMA5 and S. KRISHNA1

From the 1Department of Infectious Diseases, St George’s Hospital Medical School, Cranmer Terrace, London, UK, 2Département de Parasitologie, Mycologie et Médecine Tropicale and 3Département de Biochimie, Faculté de Médecine et des Sciences de la Santé, Libreville, Gabon, 4Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon, 4Sektion Humanparasitologie, Institut für Tropenmedizin, Universitaet Tübingen, Tübingen, Germany

Received 18 September 2001 and in revised form 31 October 2001

Summary

Background: Low plasma glutamine levels in critical illness, neonates and burns patients are associated with poor outcome and increased risk of intercurrent infection.

Aim: To investigate the relationship between plasma glutamine/glutamate levels and severity/outcome of malaria.

Design: Two-hospital prospective study, with both febrile and healthy controls.

Methods: We measured plasma glutamine and glutamate concentrations in 239 Gabonese patients: 145 children with malaria (86 with severe, 36 with moderate and 23 with uncomplicated disease), 42 healthy children, 44 febrile controls and eight healthy adults, and related findings to conventional markers of disease severity such as plasma lactate.

Results: Median (IQR) plasma glutamine was lower in uncomplicated falciparum malaria and in moderate malaria than in healthy controls: 353 (287–474) and 379 (293–448) vs. 485 (428–531) µmol/l, respectively; \( p < 0.01 \) for both malaria groups vs. controls. In contrast, plasma glutamine was within the normal range in those with severe malaria and in febrile control children: 431 (342–525) and 472 (338–547) µmol/l, respectively. Furthermore, plasma glutamine was significantly higher in the children who died with malaria than in survivors: 514 (374–813) \((n = 12)\) vs. 399 (316–475) µmol/l \((n = 133)\), respectively; \( p = 0.001 \). There were no significant differences in plasma glutamate concentrations between any of the study groups.

Discussion: In severe malaria, there was a positive correlation between plasma glutamine and lactate levels \((p = 0.009, r = 0.281)\). This correlation may reflect impaired gluconeogenesis. Glutamine supplementation is probably not justified in severe P. falciparum infection.

Introduction

There are 1–2 million deaths from malaria each year, most of them occurring in children aged <5 years in sub-Saharan Africa. The case fatality rate of children admitted to hospital with cerebral malaria is ~20%, and has remained unchanged for the past 40 years.1 A better understanding of
the pathophysiology of severe infection may suggest new treatments aimed at reducing this unacceptably high mortality.

Glutamine is a conditionally essential amino acid that is required by rapidly dividing cells such as leucocytes and enterocytes, particularly in catabolic states. Glutamine has a wide spectrum of other biological activities, such as renal ammonia genesis, glutathione synthesis and carbohydrate metabolism.

Low plasma glutamine levels have been reported in a number of conditions, including burns, critically ill adults, trauma, sepsis and premature infants. Glutamine supplementation reduces the incidence of sepsis and late mortality in critically ill adults as well as in infants with very low birth weight. Plasma glutamine concentrations fall, and plasma glutamate concentrations rise, in response to extreme physical exertion. An elevation in the ratio of these two amino acids indicates a state of over-exertion. Physiological changes in malaria, induced by sequestration of parasitized red blood cells and tissue hypoxia, may be similar to those observed following extreme exertion.

We have previously reported that plasma glutamine levels are almost halved in acute (predominantly uncomplicated) P. falciparum infection. The magnitude of this fall is similar to that observed in sepsis, and may contribute to some of the complications of malaria such as anaemia or secondary infection. However, our previous study was not designed to examine relationships between plasma glutamine levels, disease severity and mortality. This study now compares severity and mortality of malaria with plasma glutamate and glutamine levels, using plasma lactate concentrations as a marker of disease severity in African children.

Methods

This study was conducted in two hospitals: the Albert Schweitzer Hospital in Lambaréné and the Centre Hospitalier de Libreville between September 1999 and July 2000. The study was approved by the Ethics Committees of the Albert Schweitzer Hospital in Lambaréné and the Gabonese Ministry of Health and the University of Tübingen.

Inclusion criteria

Admitting physicians referred children aged 12–71 months (inclusive) with a suspected diagnosis of malaria to the study team. These children were screened, with a history, physical examination, blood film examination and measurement of blood lactate, glucose and haematocrit, within an hour of admission to hospital. Malaria was defined as a history of fever within 24 h of presentation and the presence of asexual P. falciparum in the thick or thin blood film. The severity of malaria and control groups was defined as follows:

Severe malaria was malaria with one or more of the following features: Blantyre coma score (BCS) ≤ 2; repeated witnessed convulsions (three or more observed); lactate in whole blood or capillary > 5 mmol/l, glucose in whole blood or capillary ≤ 2.2 mmol/l, and severe anaemia (Hb < 5 g/dl or haematocrit < 15%).

Moderate malaria was defined as malaria without the features of severe disease with one or more of the following features: hyperparasitaemia > 250 000 asexual forms of P. falciparum/μl, obtundation (BCS 3–4), inability to tolerate an oral route for treatment and inability to sit or stand unaided (prostration), as appropriate for age.

Uncomplicated malaria was defined as malaria without any of the features of severe or moderate malaria.

Febrile controls were children aged 12–71 months admitted to hospital with a core temperature (tympanic) of ≥ 37.5 °C with a negative blood film examination and no history suggestive of treated malaria.

Healthy adults and children were adults or children (aged 12–71 months) who were afebrile, aparasitaemic and reported no ill health.

Patients with sickle anaemia were excluded retrospectively by the results of haemoglobin electrophoresis.

Procedures

Parents or guardians gave informed consent for children; adults gave informed consent themselves. A blood sample (2 ml) was taken into a heparinized tube. Whole blood glucose and lactate measurements were made on site with a YSI 2300 analyser (YSI Instrument Co.) and haematocrit was measured with a microhaematocrit centrifuge (Hawksley). Thick and thin blood films were stained with a Field’s stain and counted on thin films per 1000 red cells or by the Lambaréné method on thick films. Films were defined as negative if there were no asexual malaria parasites in 100 high-power fields of the thick film.

Plasma was separated and stored at −80 °C before being transported within a few weeks on dry ice for assay. Children with severe and moderate malaria were treated with parenteral quinine; those with uncomplicated malaria were treated with oral sulphadoxine/pyrimethamine.
Assays for glutamine and glutamate measurements

Glutamine and glutamate concentrations were measured using a YSI 2700 (YSI Instrument Co.). This analyser uses a platinum electrode to measure the current generated on two enzyme-impregnated membranes. The glutamate membrane generates electrons by the glutamate oxidase reaction, and the current measured is proportional to the glutamate concentration. The glutamine electrode uses the reaction catalysed by glutaminase to convert glutamine to glutamate, and measures the current generated by glutamate oxidase to measure total glutamine and glutamate concentration. Glutamine concentrations are thus calculated as the concentration measured by the glutamate electrode minus that detected by the glutamine electrode. We used 1 mM glutamate and glutamine standards to calibrate the machine every four measurements (sample size 65 l). All plasma samples were measured in duplicate, and the mean was used in calculations.

Within-day coefficients of variation (CV) for standard solutions containing the following concentrations of glutamine were: 0.4 mmol/l, 12%; 0.6 mmol/l, 10%; 1 mmol/l, 9% (n = 6). For glutamate, the within-day CVs were: 0.4 mmol/l, 9%; 0.6 mmol/l, 8%; 1 mmol/l, 14% (n = 6). The between-day CVs for the same solutions were (glutamine): 0.4 mmol/l, 13% (n = 11); 0.6 mmol/l, 12% (n = 9); 1 mmol/l, 9% (n = 9); and (glutamate) 0.4 mmol/l, 9% (n = 11); 0.6 mmol/l, 9% (n = 9); 1 mmol/l, 9% (n = 9). Plasma transaminase measurements and creatine phosphokinase (CPK) were analysed on autoanalysers in the Department of Biochemistry, St George’s Hospital Medical School.

Statistical analyses

Statistical analyses used Systat (version 5.2) and Stata Statistical Software (Release 6.0). After checking distributions with the Shapiro-Wilks W test, and transforming the data logarithmically (parasitaemia only) or by Box-Cox transformations, as appropriate, we analysed normally distributed data by two tailed Student’s T test (paired if appropriate) and non-parametric data with the Mann-Whitney U statistic. Proportions were compared with Fisher’s exact test, and correlations were assessed by Pearson linear regression analysis. ANOVA tests were used for multiple comparisons, with Tukey’s post hoc test.

Results

Demographic and clinical data

Glutamine and glutamate were assayed in 239 patients: 145 children with P. falciparum parasitaemia, 44 febrile controls (one fatality), 42 healthy children and eight healthy adult controls.

In the malaria group, 86 children had severe disease (12 fatalities), 36 children had moderate malaria and 23 children had uncomplicated disease. The admission characteristics of the patients are shown in Table 1. Healthy control children were older than children with malaria: median (IQR) age 54 (35–65) months vs. 25 (18–37) months. The age of the febrile controls was similar to that of the children with malaria. BMI values of children in all study groups were comparable.

Laboratory data

Plasma lactate

Plasma lactate levels were higher in children with malaria than in healthy controls: median (IQR) plasma lactate 2.4 (1.6–4.0) vs. 1.26 (0.1–1.7) mmol/l (Figure 1). Elevations in plasma lactate concentrations were proportional to disease severity: median (IQR) plasma lactate was 3.4 (2.3–5.8) mmol/l in severe malaria, 1.8 (1.5–3.3) mmol/l in moderate malaria and 1.5 (1.2–1.9) mmol/l in uncomplicated malaria (Figure 1). A χ² test for trend confirmed a significant positive correlation between lactate concentrations and severity in these groups (p = 0.0001).

Plasma glutamine

The median (IQR) plasma glutamine concentrations were lower in children with malaria than in healthy or febrile controls: 413 (323–487) μmol/l, 485 (428–531) μmol/l and 472 (338–547) μmol/l, respectively (p < 0.001). Plasma glutamine concentrations in the six diagnostic groups are shown in Figure 2a, and differences between these groups are summarized in Table 2. The plasma concentrations of glutamine of the children with moderate malaria and uncomplicated malaria were lower than those in the healthy or febrile control groups. Children with severe malaria had plasma glutamine concentrations similar to those in the control groups and higher than those with moderate malaria (p = 0.026).

There was a positive correlation between plasma lactate concentrations and plasma glutamine in patients with severe malaria (r = 0.281, p = 0.009). In contrast, there was a negative correlation between plasma glutamine and plasma lactate concentrations in children with moderate malaria.
Table 1  Admission characteristics of study patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy children (n = 42)</th>
<th>Healthy adults (n = 8)</th>
<th>Febrile controls (n = 44)</th>
<th>All malaria (n = 145)</th>
<th>Uncomplicated malaria (n = 23)</th>
<th>Moderate malaria (n = 36)</th>
<th>Severe malaria (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (months)</td>
<td>54 (36–65)</td>
<td>281 (243–302.5)</td>
<td>29 (18–49.5)</td>
<td>25 (18–37)</td>
<td>28 (17–37)</td>
<td>30.5 (20.5–39)</td>
<td>24 (18–36)</td>
</tr>
<tr>
<td>Male:female</td>
<td>28/14</td>
<td>6/2</td>
<td>20/24</td>
<td>70/75</td>
<td>13/10</td>
<td>15/21</td>
<td>42/44</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>101.5 (90–111)</td>
<td>170.5 (160.5–174.5)</td>
<td>87 (82–101)</td>
<td>84.5 (79–94)</td>
<td>93 (77–98)</td>
<td>88 (81–96)</td>
<td>82 (79–91)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>15.5 (12.3–17.9)</td>
<td>65.6 (53.5–74.2)</td>
<td>11.7 (10–14.7)</td>
<td>11 (9.7–13)</td>
<td>11 (9.6–14.36)</td>
<td>11.5 (10.1–13.3)</td>
<td>10.7 (9.4–13)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>15.3 (13.3–16.6)</td>
<td>22 (20.8–24.7)</td>
<td>15.5 (14.4–6.4)</td>
<td>15.4 (14.2–16.4)</td>
<td>14.9 (14.4–16.1)</td>
<td>15 (14–16)</td>
<td>15.6 (14.5–16.5)</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.7 (0.37)</td>
<td>36.9 (0.345)</td>
<td>38.4 (0.71)</td>
<td>38.3 (1.24)</td>
<td>37.4 (1.3)</td>
<td>38.2 (1.2)</td>
<td>38.5 (1.2)</td>
</tr>
<tr>
<td>Pulse (per min)</td>
<td>104 (94–120)</td>
<td>74 (66–79)</td>
<td>126 (120–144)</td>
<td>148 (126–160)</td>
<td>120 (112–152)</td>
<td>132 (124–146)</td>
<td>160 (140–160)</td>
</tr>
<tr>
<td>BCS ≤ 2(%)</td>
<td>NA</td>
<td>NA</td>
<td>20.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34.9</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasitaemia (μ/l)</td>
<td>0</td>
<td>0</td>
<td>36386 (7146–119 760)</td>
<td>26000</td>
<td>116 808</td>
<td>31 148</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.3 (1.0–1.7)</td>
<td>0.9 (0.8–1.3)</td>
<td>1.3 (1.0–1.8)</td>
<td>2.4 (1.6–4.0)</td>
<td>1.5 (1.2–1.9)</td>
<td>1.8 (1.5–3.3)</td>
<td>3.4 (2.3–5.8)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.5 (4.1–4.9)</td>
<td>4.5 (4.0–4.9)</td>
<td>4.6 (4.2–5.4)</td>
<td>5.0 (4.2–6.2)</td>
<td>4.4 (4.0–5.1)</td>
<td>4.8 (4.3–5.8)</td>
<td>5.4 (4.4–6.6)</td>
</tr>
<tr>
<td>Glutamate (μmol/l)</td>
<td>45 (36–64)</td>
<td>32 (28–43)</td>
<td>54 (36–82)</td>
<td>44 (3–74)</td>
<td>36 (25–81)</td>
<td>52 (32–112)</td>
<td>43 (29–69)</td>
</tr>
</tbody>
</table>

All values are given as median (IQR) or as proportions except for temperature which is given as mean (standard deviation).
(r = −0.345, p = 0.039) and in children with uncomplicated malaria (r = −0.421, p = 0.045). There was no relationship between plasma glutamine concentrations and age, sex, coma score, haematocrit, peripheral parasitaemia or any other demographic, clinical or laboratory variable.

**Plasma glutamate**

Figure 2b shows plasma glutamate concentrations in all study groups. There were no significant differences in plasma glutamate concentrations between any of the groups in this study. There was a weak positive correlation between plasma glutamate and glutamine (r = 0.299, p = 0.001). There were no differences between plasma glutamate/glutamine ratios in any of the six groups.

**Plasma lactate and glutamine concentrations and outcome**

Plasma lactate concentrations were significantly higher in the children with malaria who died than in those who survived: median (IQR) 7.9 (5.2–11.2) vs. 2.3 (1.6–3.6) mmol/l (p = 0.0001). Plasma glutamine concentrations in the 12 children who died with severe malaria were significantly higher than in those who survived their malaria infection: median (IQR) 514 (374–813) vs. 399 (316–475) μmol/l (p = 0.001).

**Plasma aspartate transaminase (AST), creatinine and creatine phosphokinase (CPK) levels**

Overall, plasma creatinine levels were not significantly higher in children with malaria than in healthy children or febrile controls: median (IQR) 41 (31–54) vs. 35 (31–43) μmol/l (p = 0.135) or 37 (28–43) μmol/l (p = 0.281), respectively. However, plasma CPK levels were elevated (≥210 IU/l) in a higher proportion of children with severe malaria (25%, n = 21) than healthy children (7%, n = 3), febrile controls (7%, n = 3), uncomplicated (0%) or moderate malaria (11%, n = 4) (p = 0.006) (Figure 3). Elevations in CPK were unrelated to plasma glutamine levels. Plasma AST levels were significantly higher in malaria patients than in healthy children or febrile controls: median (IQR) 38 (28–65) IU/l vs. 25 (22–31) IU/l (p < 0.001) or 29 (23–41) IU/l (p = 0.003), respectively. There was no significant difference in AST levels between children with severe malaria and those with uncomplicated malaria (p = 0.498).

AST levels were correlated with plasma creatinine (r = 0.421, p < 0.001), glutamine (r = 0.392, p < 0.001), glutamate (r = 0.535, p < 0.001) and lactate (r = 0.298, p < 0.001), in all cases.

**Discussion**

Glutamine is an important substrate for cells with rapid turnover such as gut epithelial cells and white blood cells.19 In vitro studies have demonstrated impaired function in white cells cultured in media containing relatively low glutamine concentrations.20 Plasma glutamine plays an important role as a carrier of nitrogen, carbon and energy between organs21 and is used for hepatic urea synthesis,
Figure 2. a Plasma glutamine concentrations in different study groups. b Plasma glutamate concentrations in different study groups. HC, healthy children; HA, healthy adults; FC, febrile controls; UM, uncomplicated malaria; MM, moderate malaria; SM, severe malaria. Filled circles indicate a fatal outcome. Horizontal lines show median values.

Table 2 Comparison of plasma glutamine concentrations between study groups

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Healthy children</th>
<th>Healthy adults</th>
<th>Febrile controls</th>
<th>Uncomplicated malaria</th>
<th>Moderate malaria</th>
<th>Severe malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy children</td>
<td>NA</td>
<td>0.94</td>
<td>0.79</td>
<td>0.008</td>
<td>0.000</td>
<td>0.48</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>NA</td>
<td>0.57</td>
<td>0.03</td>
<td>0.01</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Febrile controls</td>
<td>NA</td>
<td>NA</td>
<td>0.17</td>
<td>0.037</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Uncomplicated malaria</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.0</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Moderate malaria</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Severe malaria</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*p values for post hoc tests are shown.*
renal ammonia genesis,\textsuperscript{4} gluconeogenesis\textsuperscript{22} and nucleotide biosynthesis, and glutamine is a preferential fuel for many cells.\textsuperscript{3} Glutamine is not classed as an essential amino acid, but becomes conditionally essential in catabolic states. Plasma glutamine levels are low in patients with critical illness,\textsuperscript{9,23} post trauma,\textsuperscript{24} in burns patients\textsuperscript{8} and in pre-term infants.\textsuperscript{11,25} In these circumstances, glutamine supplementation improves survival, and it decreases the risk of intercurrent infection in pre-term neonates\textsuperscript{11} and adults with critical illness.\textsuperscript{12,13} Stable isotope studies have shown that low plasma glutamine levels are a result of higher glutamine utilization in catabolic tissues, which is not compensated by for increased proteolysis.\textsuperscript{23} Single measurements of plasma glutamine concentrations do not necessarily give an indication of total body glutamine or glutamine metabolic flux. Changes in plasma glutamine may result from changes in volume of distribution, cellular uptake and release of glutamine, as well as changes in utilization and synthesis.

We have previously demonstrated that plasma glutamine concentrations are significantly lowered in children with moderate malaria.\textsuperscript{15} This study confirms our previous finding that plasma glutamine levels are low in uncomplicated and moderate malaria infection (the magnitude of these falls was comparable). Glutamine concentrations in healthy control children in this study (median (IQR) 485 (428–531) μmol/l) are also in good agreement with reported normal data. There was no relationship between age, plasma glutamate, glutamine or lactate concentrations, suggesting that the higher age of our healthy control children did not influence these values significantly.

In contrast to uncomplicated and moderate malaria, plasma glutamine concentrations were not decreased in children with severe malaria. Moreover, children with severe malaria who subsequently died ($n=12$) had significantly higher plasma glutamine levels than those who survived ($n=74$) ($p=0.001$). There are several possible explanations for this finding: there may be increased glutamine release into plasma in severe malaria; plasma clearance of glutamine may be decreased; the volume of distribution of glutamine may have decreased; or a combination of mechanisms may operate. Previous studies of critically ill patients have described falls in plasma glutamine concentrations,\textsuperscript{8,10,12} which are probably due to increased tissue utilization.\textsuperscript{23} A recent study of 80 severely ill adults showed a relationship between low plasma glutamine concentrations on admission and probability of death. Patients with a plasma CPK $>150$ IU/l had significantly higher plasma glutamine levels than those with plasma CPK $\leq 150$ IU/l.\textsuperscript{9} As glutamine levels are within the normal range in children with severe malaria, it is possible that intermediary metabolism is affected in a different way in this condition compared with other critical illnesses.

Plasma lactate concentrations in this study agree well with previous observations that correlate...
elevations in blood lactate with clinical assessment of disease severity, as well as with fatal outcome.\textsuperscript{16,17,26} These data confirm that lactate measurements are a valuable and useful objective method for defining severity of malaria for uncomplicated, moderate and severe malaria.

We also measured CPK as an indicator of muscle damage and potential source of glutamine into the plasma, and AST and creatinine to assess liver and kidney impairment. There were no relationships between plasma CPK, creatinine and plasma glutamine concentrations, suggesting that the raised plasma glutamine concentrations in severe malaria are unrelated to either muscle damage or renal dysfunction as assessed by these crude plasma indicators. The relationship between plasma glutamine and disease severity in malaria is complex, and further investigation of intermediary metabolism in severe childhood \textit{P. falciparum} infection is needed before the issue of glutamine supplementation in malaria can be resolved.

### Acknowledgements

We would like to thank the medical, nursing, and laboratory staff of the Albert Schweitzer Hospital, and of the Centre Hospitalier de Libreville, especially Dr Josseaume, Dr Tchoua, Professor Ngaka and Professor R. Tchoua for advice and aid in conducting this study. We would also like to thank M. Obiang Nestor, Batchelili Batchelili, Ekoumembia Michel, Mozogho Emmanuel and Mbandinga Frankie for their assistance. We thank Martin Hurl of YSI for the loan of the YSI2700 analyser. This work is a part of a program for research in tropical medicine based at St George’s Hospital Medical School, London and funded by the Wellcome Trust. TP was funded by the Special Trustees of St Georges Hospital Medical School.

### References


