Skeletal Muscle Involvement in Falciparum Malaria: Biochemical and Ultrastructural Study

Timothy M. E. Davis, Emsri Pongponratan, Wichai Supanaranond, Sasithorn Pukrittayakamee, Timothy Helliwell, Paul Holloway, and Nicholas J. White

Biochemical evidence of skeletal muscle damage is common in malaria, but rhabdomyolysis appears to be rare. To investigate the relationship between serum creatine kinase and myoglobin levels, muscle histology, and renal function in *Plasmodium falciparum* infections, we studied 13 patients with uncomplicated malaria, 13 with severe noncerebral malaria, and 10 with cerebral malaria. A muscle biopsy specimen was obtained from each patient for light microscopy and electron microscopy. Mean serum creatine kinase concentrations ± SD were raised but similar for the three groups (258 ± 277, 149 ± 158, and 203 ± 197 U/L, respectively; *P* = .5). The mean serum myoglobin level ± SD was highest in cerebral malaria (457 ± 246 vs. 170 ± 150 and 209 ± 125 ng/mL in uncomplicated and severe malaria, respectively; *P* < .01) and correlated with the mean serum creatinine level (r = .39 for 36 patients; *P* = .02). The number of intravascular parasites, proportion of mature forms, and glycogen depletion were highest in biopsy specimens from patients with cerebral malaria. Myonecrosis was not observed. Muscle appears to be an important site for *P. falciparum* sequestration, which could contribute to metabolic and renal complications.

Evidence of skeletal muscle damage in falciparum malaria has been found in African children, a patient group in whom increased serum creatine kinase (CK) and myoglobin concentrations parallel clinical severity [1]. Serum CK concentrations are also raised in adults with complicated malaria [2]; this concentration is mostly made up of skeletal muscle isoenzyme. However, myonecrosis severe enough to cause myoglobinuria and renal failure is rare [2–6]. *Plasmodium falciparum* has been reported to sequester in muscle [7], but to our knowledge, no formal ultrastructural assessment has been reported. Nevertheless, examination of cardiac muscle specimens obtained after death from patients with cerebral malaria has revealed that, in contrast to other samples of tissue such as brain, the sequestered intravascular parasitized erythrocytes are not tightly packed and that there is no associated endothelial damage [8]. Consistent with these findings, myocardial dysfunction is unusual in severe malaria [9, 10].

Renal failure is a common and life-threatening complication of severe malaria in adults [7]. It is possible that parasite sequestration may damage muscle without inducing overt rhabdomyolysis and still contribute to renal impairment in severely ill patients. To provide in vivo ultrastructural data relating to cytoadherence in muscle and to assess its pathophysiological consequences, we performed histological assessment of muscle biopsy specimens from patients with well-characterized malaria infections. The results suggest that sequestration in skeletal muscle parallels that in brain and other vital organs. In addition, renal dysfunction is associated with increased serum myoglobin concentrations even when there is no clinical or histological evidence of rhabdomyolysis.

Patients and Methods

Patients. The study included 36 Thai adults with slide-positive falciparum malaria (table 1). All patients had been admitted to Pholpolpayahasena Hospital, Kanchanaburi, Thailand. Patients with a history of recent seizure or intramuscular injection or who had clinical or laboratory evidence of a bleeding tendency were excluded from the study. Each subject or, in the case of those in comas, a first-degree relative was asked to provide witnessed informed consent for needle muscle biopsy. Study procedures were approved by the Faculty of Tropical Medicine Ethics Committee, Mahidol University, Bangkok, Thailand.

Methods. After initial clinical assessment, routine laboratory tests, and confirmation of the diagnosis by microscopy, all patients were treated with strict bed rest, ventilatory support, and fluids. Antimalarial treatment with quinine was given either intravenously or orally. Vital signs were monitored fre-
Table 1. Findings for three groups of patients with falciparum malaria at the time of admission to the hospital in Thailand.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Group 1 (n = 13)</th>
<th>Group 2 (n = 13)</th>
<th>Group 3 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (y) ± SD</td>
<td>29 ± 10</td>
<td>31 ± 10</td>
<td>35 ± 14</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>100</td>
<td>85</td>
<td>80</td>
</tr>
<tr>
<td>Mean oral temperature (°C) ± SD</td>
<td>38.3 ± 0.8</td>
<td>38.2 ± 1.2</td>
<td>37.7 ± 1.0</td>
</tr>
<tr>
<td>Mean hemoglobin level (g/dL) ± SD</td>
<td>12.0 ± 1.7</td>
<td>11.3 ± 1.8</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td>Median parasite density per µL (range)</td>
<td>22,470 (3,900–129,310)</td>
<td>238,000* (76,900–736,750)</td>
<td>54,180 (4,320–680,100)</td>
</tr>
<tr>
<td>Mean serum creatinine level (µmol/L) ± SD</td>
<td>123 ± 36</td>
<td>145 ± 147</td>
<td>180 ± 51*</td>
</tr>
<tr>
<td>Mean serum bilirubin level (µmol/L) ± SD</td>
<td>20 ± 15</td>
<td>68 ± 75*</td>
<td>93 ± 85*</td>
</tr>
<tr>
<td>Serum creatine kinase level (U/L) ± SD</td>
<td>258 ± 277</td>
<td>149 ± 158</td>
<td>203 ± 197</td>
</tr>
<tr>
<td>Serum myoglobin level (ng/mL) ± SD</td>
<td>170 ± 150</td>
<td>209 ± 125</td>
<td>457 ± 246*</td>
</tr>
</tbody>
</table>

NOTE. ANOVA = analysis of variance; group 1 = patients with uncomplicated malaria; group 2 = patients with severe noncerebral malaria; group 3 = patients with cerebral malaria.

* P < .05 vs. group 1 by ANOVA.
† P < .05 vs. group 2 by ANOVA.

Quently, and complications were managed as described previously [11]. An additional venous blood sample was obtained for assay of serum CK and myoglobin levels by standard automated techniques. Normal ranges for Caucasian adults were <80 ng/mL (males) and <60 ng/mL (females) for serum myoglobin levels and <50 U/L (males) and <30 U/L (females) for serum CK levels. Once the patient was hemodynamically stable and euglycemic, muscle biopsy was performed.

Each biopsy was carried out by using a standard protocol. With the patient lying supine, a small area of skin overlying the lateral musculus quadriceps femoris was shaved if necessary, and antiseptic solutions of iodine and 70% ethanol (vol/vol) were applied. A local anesthetic (1% lignocaine with adrenaline) was administered, and a small horizontal incision was made with a scalpel. A University College Hospital biopsy needle [12] was introduced into the incision by using an aseptic technique and pushed rapidly into the muscle, and the cutting cylinder was advanced quickly. The insertion was repeated if an inadequate sample was obtained. The skin was closed with sterile adhesive tape, and an ice pack was applied firmly over the muscle for 10 minutes.

The biopsy specimen was divided into three sections. For histochemical studies, a specimen was oriented on a cork disk, set in mounting medium, and placed promptly in liquid nitrogen. A second specimen was fixed in glutaraldehyde for electron microscopy. The remaining tissue sample was fixed in formaldehyde for light microscopy.

The biopsy incision was inspected daily as part of regular clinical review. When patients were able to take medications by mouth, quinine therapy was supplemented with tetracycline for a total of 7 days. Patients were discharged when they were afebrile and there were no parasites in the blood.

Light microscopy. Five frozen tissue specimens were stained with hematoxylin-eosin for the assessment of necrosis and pigment granules in vessels. The mean number of granules per vessel was determined from 49–354 (median, 188) vessels per specimen by using oil immersion microscopy at a magnification of × 1,000. Specimens were stained by the periodic acid-Schiff method for assessment of glycogen content, and results were compared with those for samples of control muscle stained in the same batch. Glycogen depletion was assessed subjectively as normal, mild (score of 1; <33% of fibers depleted), moderate (2; 33%–67% of fibers depleted), or severe (3; >67% of fibers depleted).

Electron microscopy. Each biopsy specimen was minced and fixed with 2.5% glutaraldehyde in Sorensen’s buffer, pH 7.4, for 2 hours. After fixation, the tissue sample was washed with buffer and fixed again for a further 2 hours in 1.5% OsO4. Dehydration was carried out in acetone, and the tissue specimen was stained with 2% uranyl acetate in 70% acetone before infiltration with propylene oxide. Specimens were embedded in Epon 812 (Shell Chemicals, Houston, TX). Sections were stained with lead citrate before examination with an H-7000 electron microscope (Hitachi, Tokyo). The number of intravascular erythrocytes was counted, and the proportion containing asexual parasites was determined together with scoring by the stage of development. The electron microscopist (E.P.) was blind to the clinical state of the patient from whom the biopsy sample was obtained.

Statistical analysis. Data were analyzed by parametric tests for continuous variables and by nonparametric tests for discontinuous variables or those not conforming to a normal distribution (SPSS for Windows; SPSS, Chicago). Data are reported as mean ± SD, geometric mean ± SD (range), or median (range). Two-sample comparisons were performed by means of the Student’s t test or Mann-Whitney-Wilcoxon test. Multiple comparisons were made by analysis of variance and Bonferroni modified least significant difference post hoc test or the Kruskal-Wallis test. Associations between variables were assessed by using Pearson’s product-moment or Spearman’s rank correlation coefficients.

Results

Clinical course. Thirteen patients had uncomplicated falciparum malaria [11] (group 1) at the time of admission. These
patients were fully conscious, had serum creatinine levels of <256 μmol/L after fluid therapy, serum bilirubin levels of <50 μmol/L, and <5% peripheral parasitemia (defined as <250,000 parasites/μL of whole blood). Thirteen patients had severe noncerebral malaria (group 2) and had renal impairment, jaundice, and/or hyperparasitemia; these patients were not in comas. Another 10 patients had cerebral malaria (group 3) as defined by the absence of a purposive response to painful stimuli [11]. Clinical and laboratory findings for the three patient groups are shown in table 1. The highest serum creatinine concentrations were in group 3 (P < .05 vs. group 1), but parasite densities were highest in group 2 (P < .05 vs. group 1).

All group 1 patients responded well to treatment and were discharged an average of 3 days (range, 1–7 days) after admission. One patient in group 2 died of septicemia on the second day of treatment, and a second patient required peritoneal dialysis but made a full recovery. The other patients were discharged well after a mean of 5 days (range, 2–7 days) in the hospital. Three group 3 patients (30%) died in the hospital within 3 days of admission, one of whom also required dialysis. The remaining patients with cerebral malaria recovered consciousness within 72 hours and were discharged after an average of 8 days (range, 5–21 days).

Muscle biopsy was well tolerated by all patients. No wound infections, muscle hematomata, or other complications occurred. When patients became ambulant, there was minor discomfort and stiffness at the biopsy site in some cases, but these symptoms were transient and did not limit mobility.

Serum CK and myoglobin levels. Patients in the three groups had similar serum CK concentrations at admission (P = .53; table 1). Serum myoglobin concentrations were significantly higher in patients with cerebral malaria (P < .01 for group 3 vs. groups 1 and 2; table 1 and figure 1). There was a significant correlation between serum CK and myoglobin levels in the series as a whole (r = .49 for 28 patients; P = .004). The serum myoglobin concentration also correlated with the simultaneous serum creatinine level (r = .39; P = .02; figure 2), and this association remained in a stepwise linear regression model with serum creatinine level as the dependent variable and serum myoglobin level, oral temperature, hemoglobin level, serum bilirubin level, and parasite density as independent variables (P = .04).

Two patients had dark urine, hemoglobinuria determined by dipstick urinalysis, and hemosiderinuria identified by Prussian blue staining of urine obtained at presentation. The first patient was initially classified as having uncomplicated malaria; his serum creatinine level was 204 μmol/L, and his serum myoglobin level was 387 ng/mL. Although his serum creatinine concentration rose to a peak of 469 μmol/L on the third day of treatment, he was kept well hydrated, maintained satisfactory urinary output, and did not require peritoneal dialysis. The second patient had serum creatinine and myoglobin concentrations of 97 μmol/L and 108 ng/mL, respectively. His serum creatinine level did not rise above 137 μmol/L at any stage.

Light microscopy and electron microscopy. There was no evidence of muscle necrosis in any of the 21 biopsy specimens examined. Of the erythrocytes visible within small blood vessels, significantly more were parasitized in specimens from patients with cerebral malaria than in those from patients in the other two groups (P = .016; table 2). By contrast, parasite densities in peripheral blood were greatest in group 2 (table 1).
In group 3, the proportion of intravascular parasites that were mature trophozoites or schizonts was also significantly higher than that in the other two groups, whether expressed as a proportion of the total number of RBCs examined or as a proportion of those containing parasites ($P < .05$; table 2). The infected RBCs were not usually tightly packed. Most of the erythrocytes containing mature forms had visible knobs projecting from the cell membrane (figure 3).

Consistent with greater sequestration of mature parasite forms in cerebral malaria, the density of $P. falciparum$ pigment granules was also greatest in group 3 ($P < .05$; table 2). The glycogen depletion score also tended to be higher in group 3 than in the other two groups ($P = .12$; table 2) and correlated significantly with the serum myoglobin level in the series as a whole ($r_s = .39$ for 27 patients; $P = .022$). However, neither glycogen depletion score or serum myoglobin level was associated with the proportion of parasitized erythrocytes or the proportion of mature parasite forms in intravascular RBCs that were identified by using electron microscopy ($-0.22 < r_s < .14$; $P \geq .2$).

Discussion

The present observations suggest that cerebral malaria is associated with parasite sequestration in skeletal muscle and muscle damage, both of which may have clinical implications. Although myonecrosis and rhabdomyolysis did not occur, lesser degrees of muscle damage may have contributed to renal dysfunction in our patients with severe malaria. Thus, a markedly raised serum myoglobin concentration may confirm the diagnosis of rhabdomyolysis, but milder elevations might still direct management aimed at preserving renal function. Patients with severe malaria can have highly synchronous infections in which most parasites are sequestered in the microvasculature and very few are in the peripheral blood. Muscle biopsy may therefore reveal $falciparum$ malaria as the cause of coma in a patient for whom a blood smear is negative for parasites.

There are limited reported data on skeletal muscle involvement in falciparum malaria. In African children, serum CK and myoglobin concentrations correlated with infection severity [1], but renal failure, the most common and serious sequela of muscle damage, is an uncommon complication of malaria in this age group. Serum CK concentrations that could reflect rhabdomyolysis (>1,000 U/L) have been described in association with renal failure in a small number of adults with malaria.

Table 2. Results of electron microscopy and histochemical analysis of muscle biopsy specimens from three groups of patients with falciparum malaria in Thailand.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Median (range)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitized intravascular erythrocytes (%) of total</td>
<td>0 (0–18)*</td>
<td>5 (0–13)†</td>
<td>14 (0–50)‡§</td>
<td></td>
</tr>
<tr>
<td>Intravascular mature trophozoites (%) of total no. of erythrocytes</td>
<td>0 (0–8)*</td>
<td>0 (0–5)†</td>
<td>11 (0–50)‡ §</td>
<td></td>
</tr>
<tr>
<td>Intravascular mature trophozoites (%) of parasitized erythrocytes</td>
<td>0 (0–100)*</td>
<td>0 (0–100)†</td>
<td>38 (0–100)‡ ‡</td>
<td></td>
</tr>
<tr>
<td>Glycogen depletion score</td>
<td>0 (0–2)*</td>
<td>0 (0–2)**</td>
<td>1 (0–3)*</td>
<td></td>
</tr>
<tr>
<td>No. of pigment granules per vessel</td>
<td>0 (0–0.17)*</td>
<td>0.10 (0–0.34)**</td>
<td>0.13 (0.01–0.69)**</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Group 1 = patients with uncomplicated malaria; group 2 = patients with severe noncerebral malaria; group 3 = patients with cerebral malaria.

* Nine patients.
† Five patients.
‡ $P < .05$ vs. group 1 by the Kruskal-Wallis test.
§ $P < .05$ vs. group 2 by the Kruskal-Wallis test.
\# Seven patients.
\$ Eleven patients.
** Twelve patients.

Figure 3. Electron micrograph of parasitized erythrocytes (PEs) within an interstitial vessel (v) in a muscle tissue (m) specimen from a patient with falciparum malaria. One PE is a growing trophozoite with pigment granules in a damaged food vacuole (large arrow). Cytoadherence of the PE to the endothelium via knobs (k) is present (small arrows). The endothelium (e) appears intact, and there is no evidence of myonecrosis (original magnification, $\times$ 18,000).
However, serum CK levels in larger studies did not predict whether severely ill patients developed renal failure, even those in the range associated with rhabdomyolysis [2]. Serum CK levels were unrelated to renal dysfunction in the present study.

By contrast, and after allowing for factors that might influence renal function including pyrexia and anemia, serum myoglobin and creatinine concentrations in our patients correlated positively. Although creatinine is itself a muscle breakdown product, it is likely that raised serum concentrations primarily reflect renal impairment. Myoglobin can influence renal function in several ways [13]. Precipitation of myoglobin or uric acid in the loop of Henle seems unlikely in our patients. Hyperuricemia has not been reported in malaria, and even when the serum CK level is >1,000 U/L, myoglobinuria is infrequent [2], probably because serum concentrations of >15,000 ng/mL are required before myoglobin becomes detectable in the urine [13]. Myoglobin-induced renal vasoconstriction and direct nephrotoxicity might, however, underlie the association between serum myoglobin and creatinine levels in our patients, acting with dehydration and renal microvascular sequestration to increase the risk of oliguria and the need for dialysis. The patient with the highest serum myoglobin level (820 ng/mL) required dialysis from admission until death 4 days later.

Whether serum myoglobin concentrations should guide clinical management is uncertain. In addition to fluid therapy, and as recommended in other conditions in which the serum myoglobin concentration is significantly raised, the urine could be alkalized to pH ≥7.0 with sodium bicarbonate. This strategy would increase the risk of hypocalcemia but may counteract acidosis. Where a raised serum myoglobin level and hemoglobinuria occur together, as in the first of our two patients presenting with dark urine, mannitol infusion might be beneficial, especially if the urinary output cannot be maintained at >100 mL/h [13].

Consistent with serum CK and myoglobin concentrations below the range associated with rhabdomyolysis and the favorable response to treatment in most of our patients, neither light microscopy nor electron microscopy revealed myonecrosis in any biopsy specimen. Nevertheless, parasite sequestration and pigment accumulation associated with *P. falciparum* were visible, especially in cases of cerebral malaria. These findings suggest that muscle sequestration parallels that in brain and, because these patients also had the highest serum creatinine concentrations, in other organs such as kidneys. Although there are no previously reported studies comparing skeletal muscle sequestration with that in other tissues, the parasitized erythrocytes were not tightly packed, and endothelial morphology was normal, consistent with features found previously in postmortem cardiac muscle samples from patients with cerebral malaria [8]. Nevertheless, skeletal muscle has a large mass compared with other tissues and may therefore be an important site for parasite maturation to schizogony. The significant correlation between the serum myoglobin level and the forearm depletion score in our cases and the observation that forearm lactate production is increased in severe malaria [14] suggest that glycogenolysis and anaerobic glycolysis are also consequences of skeletal muscle involvement.

The present cross-sectional study provides evidence that skeletal muscle is involved in a variety of the complications of severe *falciparum* malaria in adults. Muscle may harbor large-scale sequestration, thus facilitating parasite development that, in turn, increases lactate production and elevates serum myoglobin concentrations. These changes can occur without myonecrosis but may have significant effects on vital organs, especially kidneys. Muscle biopsy was easy to perform and well tolerated in our cases and could be done serially to examine further metabolic and ultrastructural aspects of malaria during treatment.

**Acknowledgments**

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**References**