Increased Gastrointestinal Permeability in Patients with *Plasmodium falciparum* Malaria

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Sequential gastrointestinal permeability in patients with *Plasmodium falciparum* malaria was determined by measuring the permeation of sucrose, lactulose, and mannitol to assess both gastric and small intestine permeability. Sucrose absorption (gastroduodenal permeability) was increased in patients with severe malaria (defined as either >5% parasitemia or a serum bilirubin level of >50 μmol/L) and became normal by day 2 following treatment. A similar proportion of patients with uncomplicated malaria had increased gastroduodenal permeability initially, which resolved by day 7 of treatment. During acute malarial illness, the lactulose:mannitol (L:M) ratio in urine was increased and was found to be higher in patients with severe malaria than in those with uncomplicated malaria and in controls, but this ratio reverted to normal during convalescence. At the time of admission, the L:M ratios in patients with uncomplicated malaria were increased but became normal by day 2 after treatment. Conversely, the duration of increased L:M ratios in patients with severe malaria was longer. By day 7, the L:M ratios in patients with severe malaria were higher than those in patients with uncomplicated malaria and in controls. We conclude that gastrointestinal permeability is increased during severe and uncomplicated falciparum malaria but reverts to normal during convalescence.

The gastrointestinal mucosa provides a protective barrier between the external and internal milieus that allows the ingress of water, electrolytes, and nutrients into the portal circulation while excluding compounds that may be harmful to the host. Gastrointestinal permeability refers to the facility with which the epithelium allows molecules to pass through it by nonmediated diffusion (i.e., down a concentration or pressure gradient without the assistance of a carrier system) [1]. Increased gastric permeability has been described in patients with disorders associated with significant mucosal damage [2], while increased permeability across the small intestine is seen in patients with diseases associated with jejunal mucosa damage or functional impairment [3, 4]. As such, increased gastrointestinal permeability can be interpreted as mucosal damage.

The healthy gastrointestinal mucosa is relatively impermeable to disaccharides. With damage to the epithelium, increased permeation of these compounds occurs. This increased permeation can be detected by observing the presence of these compounds in the urine following an oral loading dose. The region of the gastrointestinal tract that has been damaged may be inferred from the disaccharide used. Sucrose is a disaccharide that is rapidly broken down in the small intestine by sucrase/ isomaltase and, therefore, is not found in appreciable concentrations within the small intestine. Thus, increased amounts of sucrose in the urine after an oral loading dose suggest gastric damage and have been demonstrated to correlate with endoscopically significant lesions [2].

Lactulose is not degraded by intestinal disaccharidase and can be used to assess the integrity of the small intestine. To correct for variability in the length of the intestine exposed to the probe, among other variables, mannitol is also administered. This monosaccharide utilizes a transcellular route for permeation and, therefore, reflects the surface area exposed to the two probe molecules.

The lactulose:mannitol (L:M) ratio thus reflects the degree of small intestine damage per unit of surface area [4, 5] and compares favorably with values of intestinal permeability obtained from determining the excretion of monosaccharide markers such as polyethylene glycol 400 [6]. The L:M ratio may be increased due to decreased mannitol absorption (i.e., decreased absorptive surface area) as in celiac disease [4] or increased lactulose absorption secondary to mucosal damage such as that which occurs after burn injury [7]. Using a ratio also controls for individual variations in nonmucosal factors such as gastric emptying rates, intestinal transit time, mucosal surface area, cardiac output, renal clearance, and completeness of urine collection [1, 8].

Gastrointestinal symptoms are common during the acute phase of malarial infection [9]. These symptoms include nau-
sea, vomiting, abdominal pain, and diarrhea. Impaired intestinal function, resulting in a transient reduction in the absorption of D-xylose, has been described in patients with Plasmodium falciparum malaria [10], thus suggesting the presence of intestinal damage. However, there are no data regarding serial changes in the permeability of either gastric or intestinal mucosa in these patients.

In the present study, we determined whether gastrointestinal damage occurred during acute malarial infection, whether it selectively occurred proximally or distally, and if the time for resolution of damage differed between severe and uncomplicated disease. To achieve these goals, we evaluated the sequential permeability of sucrose (gastroduodenal damage) and lactulose and mannitol (small intestine damage) in patients with P. falciparum malaria during both disease and convalescence. For the purpose of this study, and in accordance with the guidelines of the World Health Organization [9], we defined severe malaria as either >5% parasitemia or a serum bilirubin level of >50 μmol/L.

Methods

Patients

Twenty-one patients with acute slide-positive P. falciparum malaria and no other apparent illnesses who were admitted to the Bangkok Hospital for Tropical Diseases in Thailand were studied. None of the patients had taken antimalarial drugs before admission to the hospital. At the time of admission, urine samples were routinely tested for 4-aminoquinolones and sulfonamides, and only patients whose urine samples were negative for these agents were included in the study.

Patients were excluded from the study if they displayed evidence of diabetes mellitus or renal impairment (serum creatinine concentration, >133 μmol/L). At the time of admission, patients were questioned about their use of alcohol or nonsteroidal antiinflammatory agents, and if they admitted to use of these agents in the 4 weeks preceding and during admission, they were excluded from the study. It should be mentioned here that the most common antipyretic agent used by the outpatient population in Thailand is acetaminophen.

Patients were considered to have severe falciparum malaria if their blood level of parasites was >5%, if they were clinically jaundiced (total serum bilirubin concentration, >50 μmol/L), or both [9]. Pregnant women and children younger than 15 years of age were excluded from the study.

Controls

Eleven healthy subjects aged 16 to 36 years (mean age, 24.5 years) who were afebrile, had a negative blood smear for malaria, and had no history of gastrointestinal symptoms were studied as controls.

Clinical Laboratory Investigations

After rapid confirmation of the diagnosis and clinical assessment, baseline blood samples were drawn for determination of complete blood cell count, quantitative parasite count, electrolyte levels, blood urea nitrogen level, and creatinine level and for liver function tests.

Treatment

Patients with severe malaria were treated at the time of admission with an intravenous injection of artesunate (120 mg) followed by 60 mg twice daily for 4 days (total dose, 600 mg). Twelve hours after the last dose of artesunate, 750 mg of mefloquine was given orally followed by 500 mg 6 hours later (total dose, 1,250 mg). Patients with uncomplicated malaria were immediately treated with 100 mg of oral artesunate followed by 50 mg every 12 hours for 5 days (total dose, 600 mg). Twelve hours after the last dose of artesunate, 750 mg of oral mefloquine was administered followed by 500 mg 6 hours later (total dose, 1,250 mg) [11]. Vital signs were recorded every 4 hours until resolution of fever and then three times daily. Quantitative parasite counts were determined every 6 hours until parasites were no longer detected on a thick blood smear.

Gastrointestinal Permeability Tests

During admission (day 0), after a fast of at least 4 hours, patients were requested to drink 450 mL of a solution containing 100 g of sucrose, 2 g of mannitol, 7.5 mL of lactulose (concentration, 667 mg/mL), and a flavoring agent within 15 minutes. Before drinking the sugar solution, the urinary bladder was emptied, and a pretest urine sample was taken. Urine passed during the 5 hours following ingestion of the sugar solution was collected and pooled in a container with 5 mL of 10% thymol in isopropanol. Two aliquots were taken from the pooled urine, and the total volume of urine was recorded. Pretest and posttest urine samples were immediately frozen at −20°C and transported to Calgary, Alberta, Canada, on dry ice. Subjects were encouraged to drink fluids freely after the first hour of the test to maintain an adequate urinary output.

Urinary excretion of sucrose was measured as described previously [2, 12]. Measurements of gastric permeability were reported as the total mass of sucrose excreted over the 5-hour period of urine collection. The amounts of lactulose, mannitol, and sucrose in urine were analyzed simultaneously by HPLC with pulsed amperometric detection [2]. The percentages of urinary recovery of lactulose and mannitol were calculated, and the L:M ratio was computed as the percentage of urinary recovery of lactulose divided by the percentage of urinary recovery of mannitol. Absorption tests were repeated on days 2, 4, 7, and 21 following the start of antimalarial treatment.
Statistical Analysis

Differences between groups of patients or controls were tested by analysis of variance and paired or unpaired Student’s t-tests when appropriate. The results were expressed as means ± SEM.

Results

Clinical Course

The clinical and laboratory features of the patients are summarized in table 1. Seven patients had blood levels of parasites of >5%; three of these seven patients had both hyperparasitemia and jaundice. None of these patients had renal impairment. Fourteen patients had uncomplicated falciparum malaria. All patients were cured after antimalarial treatment. No recrudescence was observed during a 28-day observation period in the hospital. All patients with severe falciparum malaria and 12 of 14 patients with uncomplicated infection were restudied during convalescence. At this time, the patients were asymptomatic, afebrile, and aparasitemic, and their biochemical levels were normal. After ingestion of the sugar solution, none of the patients experienced vomiting, diarrhea, or any other adverse effects.

Gastroduodenal Permeability

The results of sucrose absorption tests are summarized in figure 1. At the time of admission (day 0), sucrose absorption was significantly greater in both patients with severe falciparum malaria (304.1 ± 82.4 mg) and patients with uncomplicated falciparum malaria (252.5 ± 43.7 mg) than in controls (94.2 ± 19.1 mg; significant differences (P < .05) between controls and both types of patients). However, there was no significant difference between sucrose absorption in patients with severe and uncomplicated falciparum malaria. By day 4, sucrose absorption in patients with uncomplicated falciparum malaria (186.7 ± 24.1 mg) remained greater than that in controls (0 mg; P < .05). Sucrose absorption returned to within normal limits by day 7 in both groups of patients.

Intestinal Permeability

At the time of presentation, the L:M ratio (figure 2) was significantly higher in patients with severe falciparum malaria (0.137 ± 0.002) than in either patients with uncomplicated infection (0.071 ± 0.002; P < .05) or controls (0.032 ± 0.007; P < .05). In both groups of patients, this ratio decreased during the hospital course and by day 21 had returned to normal. However, unlike the alterations observed in gastric permeability, intestinal permeability was increased to a greater degree in patients with severe infection, and the abnormality persisted for a longer time.

Although circulating parasite counts are not always a good reflection of total parasite burden, there was a reasonable correlation (r = .49) between the circulating parasite count and the L:M ratio at the time of admission. These data are shown in figure 3. A similar, slightly less significant, correlation (r = .43) existed between parasite count and sucrose excretion (data not shown). It is also apparent from figure 3 that patients with severe disease not only had higher circulating parasite counts but also tended to have greater intestinal permeabilities.

Finally, another useful item of information can be extracted from these data. It is clear that patients with falciparum malaria

Table 1. Clinical and laboratory features of patients with severe and uncomplicated falciparum malaria at the time of admission.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Severe (n = 7)</th>
<th>Uncomplicated (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SEM</td>
<td>21.9 ± 5.3</td>
<td>21.3 ± 4.2</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>1:6</td>
<td>11:3</td>
</tr>
<tr>
<td>Geometric mean of parasite count/µL (range)</td>
<td>234,268 (60,800–681,750)</td>
<td>16,631 (2,750–39,600)</td>
</tr>
<tr>
<td>Mean hematocrit ± SEM (%)</td>
<td>35.0 ± 11.1</td>
<td>29.4 ± 5.4</td>
</tr>
<tr>
<td>Mean blood urea nitrogen level ± SEM (mmol/L)</td>
<td>5.8 ± 1.5</td>
<td>4.3 ± 1.2</td>
</tr>
<tr>
<td>Mean serum creatinine level ± SEM (µmol/L)</td>
<td>122.0 ± 31.8</td>
<td>93.7 ± 21.2</td>
</tr>
<tr>
<td>Mean alanine aminotransferase level ± SEM (µmol/L)</td>
<td>33.9 ± 22.3</td>
<td>24.4 ± 11.2</td>
</tr>
<tr>
<td>Mean aspartate aminotransferase level ± SEM (µmol/L)</td>
<td>119.6 ± 68.1†</td>
<td>33.1 ± 15.6</td>
</tr>
<tr>
<td>Mean total serum bilirubin level ± SEM (µmol/L)</td>
<td>70.3 ± 17.4‡</td>
<td>19.7 ± 6.2</td>
</tr>
</tbody>
</table>

* According to the criteria of the World Health Organization [9].
† Significant difference, P < .05.
‡ Significant difference, P < .01.
Figure 1. Sucrose permeability. Urinary excretion of sucrose (mg) as a function of time following admission. Controls = open bars; patients with uncomplicated falciparum malaria = solid bars; patients with severe falciparum malaria = hatched bars; * = \( P < .05 \) compared with controls. Data are means ± SEM.

Figure 2. Lactulose:mannitol ratio (the percentage of urinary recovery of lactulose divided by the percentage of urinary recovery of mannitol) as a function of time following admission. Controls = open bars; patients with uncomplicated falciparum malaria = solid bars; patients with severe falciparum malaria = hatched bars; * = \( P < .05 \) compared with controls; ** = \( P < .05 \) compared with patients with uncomplicated falciparum malaria. Data are means ± SEM.

Figure 3. Intestinal permeability (lactulose:mannitol ratio) as a function of parasitemia (parasite count at the time of admission). Patients with severe falciparum malaria (closed circles) tended to have greater intestinal permeability and higher parasite counts than did patients with uncomplicated falciparum malaria (open circles).

Figure 4. Sucrose:lactulose ratio (the percentage of urinary recovery of sucrose divided by the percentage of urinary recovery of lactulose) as a function of time following admission. Controls = open bars; patients with uncomplicated falciparum malaria = solid bars; patients with severe falciparum malaria = hatched bars; * = \( P < .05 \) compared with controls. Data are means ± SEM.

have both gastric and intestinal damage at the time of presentation (day 0) (figures 1 and 2). Is this damage to the gastrointestinal tract more severe proximally (stomach) or distally? The outcome can be inferred from a careful consideration of sucrose:lactulose ratios. Consider a hypothetical patient who lacks sucrase activity in the small intestine. In this case, the amount of sucrose would not be substantially different from that of lactulose (i.e., sucrose would not be broken down within the small intestine and would cause intestinal damage in a fashion entirely analogous to lactulose). Under these conditions, the sucrose:lactulose ratio would approach one.

In practice, this finding is not observed, and the sucrose:lactulose ratio in controls in this study was on the order of 0.25 (figure 4). The interpretation of this observation is that sucrose is exposed to roughly 25% of the surface area that lactulose is exposed to.
is, assuming equivalent permeability characteristics per unit of surface area. From a consideration of the physiological handling of these molecules, we can reasonably infer that this 25% is the proximal portion of the gastrointestinal surface area.

If we know that gastrointestinal damage exists, then alterations in the sucrose:lactulose ratio can provide information regarding the location of this damage. If it is equally distributed along the proximal-distal axis of the intestine, then the sucrose:lactulose ratio will remain at 0.25. However, if proximal damage is greater than that observed distally, then sucrose permeability will increase to a greater extent than lactulose permeability, and the ratio will increase.

Conversely, with predominantly distal damage, lactulose excretion will increase out of proportion to sucrose excretion, and the ratio will decrease. The sucrose:lactulose ratios in patients presenting with acute malaria were significantly higher than those in controls (figure 4); this finding implies that although damage occurred diffusely along the gastrointestinal tract (figures 1 and 2), it was greater proximally than distally. However, this ratio rapidly returned to normal, thus suggesting that gastric or proximal intestinal damage was short-lived in comparison with the more distal intestinal damage.

Discussion

We have previously demonstrated that increased sucrose permeability in humans is useful in predicting the presence of clinically significant gastric disease such as severe gastritis [2]. The results of sucrose absorption tests in the present study demonstrate that patients with both severe and uncomplicated falciparum malaria had gastric mucosa damage. This finding supports the previous reports of histologically evident gastritis in these patients [13, 14]. However, since these tests are noninvasive and repeatable, we were also able to show that gastric damage became undetectable within 1 week of the initiation of treatment. This rapid resolution of gastric damage was slightly slower in patients with uncomplicated falciparum malaria than in those with severe disease.

A striking finding in this study was the marked decrease in permeation of mannitol in patients with severe falciparum malaria, which is consistent with a transient reduction in the absorptive area of the small intestine. Patients with severe malaria had higher L:M ratios than did patients with uncomplicated malaria during the acute phase of illness, but this ratio reverted to normal following adequate treatment. At the time of admission, patients with uncomplicated malaria also had higher L:M ratios than did controls.

These results demonstrate transient increased small intestine permeability in patients with both severe and uncomplicated malaria during the acute phase of illness. Patients with severe malaria had increased small intestine permeability for a longer time than did patients with uncomplicated malaria. Finally, there was a correlation between circulating parasite count and intestinal permeability as measured by the L:M ratio (figure 3).

An important issue to consider is whether these events are specific for malaria or are simply a reflection of acute systemic illness. While it is hard to control for this confounding variable, other researchers [15] have suggested that acute febrile infections due to other etiologies do not alter small intestine permeability. Another potential confounder might be alterations in intestinal transit time during malarial infection. However, as pointed out previously, one of the major advantages of using a dual sugar test is that the use of a ratio in reporting results compensates for any alteration in transit time. Permeability measurements are normalized to the intestinal surface area probed; therefore, alterations in transit time may have occurred but would not have interfered with our observations.

Several potential artifacts that may have influenced the data were considered. Neither group of patients had evidence of urinary tract infection. Furthermore, prompt freezing of urine samples and addition of a preservative meant that the potential for extraluminal fermentation of test sugars by bacterial contaminants was minimized [5]. To our knowledge, the antimalarial agents artesunate and mefloquine that were administered in this study have no effect on the gastrointestinal mucosa. Finally, in an attempt to ensure that our patients did not take other compounds likely to alter gut permeability, we excluded those patients who admitted to ingesting such agents before admission from the study.

While this methodology is not perfect, we are confident that most of our patients had not been taking nonsteroidal antiinflammatory agents before testing. Furthermore, the primary antipyretic agent used by our population of patients is acetaminophen, which is not recognized as altering gastrointestinal permeability.

Therefore, given the above-mentioned comments, we believe that the observed alterations in gastrointestinal permeability were directly attributable to malarial infection. The prolonged increase in small intestine permeability that was seen in patients with severe malaria may contribute to functional intestinal impairment. This observation of increased small intestine permeability during malaria lends support to the hypothesis that endotoxemia in patients with severe malaria originates from the small intestine by entry of endotoxin from the intestinal lumen to the systemic circulation and the failure of normal hepatic clearance mechanisms [16, 17].

Histological studies of the intestinal mucosa have demonstrated the presence of numerous parasitized erythrocytes in villous capillaries [18]. This sequestration may cause stasis and anoxia in the immediate vicinity of the enterocytes and tight junctions, thus leading to altered permeation of monosaccharides and disaccharides, respectively. This result has been clearly demonstrated in experimental models of intestinal injury due to ischemia-reperfusion.

It is also possible that cytokines, some of which are known to alter the cytoskeletal links comprising the tight junctions.
are responsible for altering small intestine permeability during acute malarial infection. With use of lactulose and rhamnose as permeability markers, altered small intestine permeability has been previously described in patients with severe malaria [10]. Our study supports the findings of this study and also demonstrates that increased small intestine permeability in patients with severe malaria persists for at least 7 days after treatment.

Our findings also extend these prior observations in an entirely different way. The human small intestine is not functionally homogeneous along the jejunal-ileal axis. Nutrient absorption predominates proximally, while absorption of specific nutrients such as vitamin B12 and bile acids occurs distally. A consequence of this regionalization of function is that proximal small intestine damage can have drastically different results than distal damage.

One of the deficiencies of traditional permeability testing is that by using only lactulose and mannitol it is impossible to tell whether small intestine damage is proximal, distal, or both. From a permeability perspective, a patient with Crohn’s disease of the distal ileum is indistinguishable from a patient with celiac disease of the proximal small intestine. However, by adding sucrose to the solution for permeability testing, these patients can be distinguished (figure 4).

It was quite apparent that patients with acute malarial infection have both gastric and small intestine damage at the time of presentation (figures 1 and 2). It can be appreciated that sucrose permeability was increased far out of proportion to lactulose permeability at the time of admission (figure 4), thereby indicating a preponderance of damage proximally. However, this discrepancy rapidly resolved by the second day of therapy, at which time the damage was evenly distributed down the small intestine as suggested by the normal sucrose/lactulose ratios. The fact that small intestine damage still existed was proven by the continuously abnormal L:M ratios (figure 2). Currently, we do not believe that these abnormal ratios have any therapeutic implications, but they may provide clues in future studies aimed at elucidating the mechanism of malaria-induced small intestine damage.

Finally, the patients with severe falciparum malaria included in the present study were limited to those with hyperparasitemia and jaundice. We recognize that this subset may not be representative of other patient populations; however, the selection of this group of patients was necessitated by the methodological techniques used in the assessment of intestinal permeability. It seems likely that this selection process would have excluded the most severely ill patients; therefore, if anything, we have probably underestimated the severity of gastrointestinal alterations that occur in association with severe malaria. The alterations in gastrointestinal permeability, in all likelihood, occur in patients with other complications such as cerebral malaria, renal insufficiency, and pulmonary dysfunction. This impairment of intestinal function must be taken into consideration in the clinical management of these patients.

References