Short Communication

Efficacy of methylene blue monotherapy in semi-immune adults with uncomplicated falciparum malaria: a controlled trial in Burkina Faso

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Summary

Objective To assess the efficacy of methylene blue (MB) monotherapy in semi-immune adults with uncomplicated malaria in Burkina Faso.

Methods In an open-label controlled phase II study with 60 semi-immune adults with uncomplicated falciparum malaria in Nouna, north-western Burkina Faso, MB monotherapy (390 mg twice daily) was given sequentially to groups of 20 adults for 7 days (MB7), 5 days (MB5) and 3 days (MB3), respectively. The primary outcome was the rate of adequate clinical and parasitological response (ACPR) on day 28 of follow-up.

Results Of the study population, 27/58 (47%) and 5/51 (10%) patients still had parasites on days 2 and 3, respectively, of follow-up resulting in 9/58 (16%) early treatment failures. By day 14, no recrudescence was observed but in 4/19 (MB5) and 2/20 (MB3) individuals by day 28. The PCR-corrected rate of ACPR was 72%, 58% and 85% in groups 7, 5 and 3, respectively, by per protocol analysis. Self-limiting dysuria was the most frequent adverse event.

Conclusions MB acts slowly against the blood stages of P. falciparum. MB alone needs to be given for at least 7 days to be efficacious in the treatment of falciparum malaria but should be used in combination with a fast acting antimalarial.

Keywords Africa, falciparum malaria, methylene blue, efficacy, Burkina Faso

Introduction

Methylene blue (MB) was successfully used against malaria as early as a century ago (Schirmer et al. 2003). Recent trials on MB combined with different partner drugs and conducted in adults and children of rural Burkina Faso provide evidence that MB is safe and efficacious against falciparum malaria (Mandi et al. 2005; Meissner et al. 2005, 2006; Zoungrana et al. 2008). However, as MB monotherapy on P. falciparum has not been investigated in vivo since the early 20th century, and as recent studies in young children have shown partly insufficient efficacy of certain MB-based combination treatments (own unpublished observations), we re-assessed the specific effects of MB monotherapy. The primary objective thereby was to assess the efficacy of MB monotherapy in different dosing regimens in semi-immune adults with uncomplicated falciparum malaria in Burkina Faso.

Patients, materials and methods

The study was conducted during the rainy season in 2007 at the Centre de Recherche en Santé de Nouna (CRSN), in malaria-holoendemic north-western Burkina Faso (Müller
et al. 2001). Although artemisinin-based combination therapy is officially implemented since 2005, most febrile episodes were treated with chloroquine in 2007 (Kouyate et al. 2007).

The study was a single-centre, controlled phase II trial, with blinding only of laboratory technicians. The primary objective was to determine the efficacy of MB used in three different regimens in semi-immune adults. The primary end point was adequate clinical and parasitological response (ACPR) until day 28 with and without PCR correction for reinfection (WHO 2003). Secondary end points were incidence of adverse events, rates of early (ETF), late clinical (LCF), and late parasitological failures (LPF) and changes in haematocrit after 28 days compared to baseline. Participants with treatment failure received artemether-lumefantrine (CoArtém®).

The study protocol was approved by the Ethics Committee, Heidelberg University and the local Ethics Committee in Nouna, Burkina Faso. Study participants were asked for their written consent after having received detailed information in the local language. Participants were recruited from 10 villages of the CRSN study area (n = 46) and from Nouna town (n = 14). Inclusion criteria were male sex, age >17 and <56 years, uncomplicated falciparum malaria (axillary temperature ≥37.5 °C or a history of fever during last 48 h plus asexual parasites ≥1000/≤200 000/µl blood), Burkinabé nationality and informed consent. Exclusion criteria were severe malaria, haematocrit <21%, any apparent other disease and reported modern malaria treatment during the current disease episode.

Sixty adults (n = 20 per group) were assigned to receive MB monotherapy over 7 days (MB7), 5 days (MB5) and 3 days (MB3), respectively. MB (Uroleone Blue®, Star Pharmaceuticals, USA; 1 tablet = 65 mg) was given at a dose of 390 mg twice daily after breakfast and supper, respectively, under direct observation. In case of vomiting within 30 min, treatment was once repeated. Follow-up of patients (days 1–7, 14 and 28) used a slightly modified version of the respective WHO protocol (WHO 2003). Patients were encouraged to return any time between scheduled visits in case of unforeseen symptoms.

Finger-prick blood samples were taken on days 0, 2, 3, 14, 28 and during unscheduled visits. Malaria parasitaemia and haematocrit were determined using standard CRSN procedures (Müller et al. 2001). Asexual parasites were counted on thick blood films against 200 white blood cells, and parasite density was calculated assuming an average count of 8000/µl. Slides were declared negative if no parasites were seen in 400 high-power fields. DNA was extracted from filter paper blood spots (QiAmp, Qiagen, Germany). Recrudescences were differentiated from new infections by comparing PCR-generated P. falciparum msp1 and msp2 genotype patterns in matched pairs of isolates obtained on admission and on the day of reappearance of parasitaemia (Snounou et al. 1999). Discordance of lengths of allele-specific PCR fragments in initial and secondary isolate indicated recrudescence; complete discordance defined re-infection. Secondary isolates with both initial and new PCR fragments were classified as recrudescences.

The sample size of 20 per arm allows to demonstrate ACPR differences between MB7 and MB3 of 100% vs. 75% with 80% power at a 0.05 significance level. Exact unconditional tests were used to compare proportions and the Wilcoxon–Mann–Whitney test for comparison of metric or ordinal data (SAS release 9.1; SAS Institute Inc, Cary, USA).

Results

Patients were recruited sequentially over 7 weeks until each treatment group was complete. Recruitment started with MB7, followed by MB5, and finally MB3. All participants of MB7 and MB3 originated from the rural CRSN area, but most in MB5 from Nouna town. Apart from that, there were no major differences in baseline characteristics between the groups (Table 1). Two patients retrospectively met the exclusion criteria (prior intake of sulfamethoxazole/trimethoprim in MB7 and of chloroquine in MB5) and were excluded from efficacy analysis. There was only one loss to follow-up in the MB7 group between day 14 and day 28.

There was no serious adverse event. Self-limiting dysuria was the most frequent adverse event, followed by headache and gastro-intestinal symptoms (Table 2). There were no major differences in the number of adverse events (28/27/35) and their duration among the groups.

Efficacy results are given in Table 3. Four of 58 patients were febrile on day 2 (all ETF) but none on day 3 of follow-up. Parasite densities remained above the threshold defined by WHO (2003) in seven and two cases on days 2 and 3 of follow-up, respectively, resulting in 9/58 (16%) ETFs. By day 14, all MB7 and MB5 patients were parasite free, but there were 4/20 (20%) parasitaemic (afebrile) patients in the MB3 group. Genotyping identified these four as new infections. By day 28 and PCR corrected, there were 0, 4 and 2 LPFs in groups MB7, MB5 and MB3, respectively, and no LCFs. No recrudescence in MB7 (0/19) contrasted with the combined figure of groups MB5 and MB3 (6/39; P = 0.07). Because of the high rate of ETF, ACPR rates were low in all groups. Haematocrit changes over the follow-up period did not differ significantly between the study groups.
Table 1  Baseline characteristics of study patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MB7 (n = 20)</th>
<th>MB5 (n = 20)</th>
<th>MB3 (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venue</td>
<td>20 rural</td>
<td>6 rural, 14 urban</td>
<td>20 rural</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recruitment period (2007)</td>
<td>7.8–23.8</td>
<td>23.8–11.9</td>
<td>12.9–26.9</td>
<td></td>
</tr>
<tr>
<td>Age in years (median; range)</td>
<td>25.5; 18–53</td>
<td>24; 18–43</td>
<td>26.5; 18–55</td>
<td>0.596</td>
</tr>
<tr>
<td>Weight in kg (median; range)</td>
<td>59; 43–68</td>
<td>63; 48–87</td>
<td>57.5; 31–67</td>
<td>0.070</td>
</tr>
<tr>
<td>Length of current episode</td>
<td>2.5; 1–15</td>
<td>3.5; 1–14</td>
<td>2; 2–3</td>
<td>0.024</td>
</tr>
<tr>
<td>Prior treatment of episode (%)</td>
<td>5 (25)</td>
<td>10 (50)</td>
<td>3 (15)</td>
<td>0.045</td>
</tr>
<tr>
<td>Haematocrit in % (median; range)</td>
<td>38; 32–48</td>
<td>40; 34–46</td>
<td></td>
<td>0.117</td>
</tr>
<tr>
<td>P. falciparum density (median; range)</td>
<td>2640/μl; 1000–20 320</td>
<td>1860/μl; 1040–11 760</td>
<td>2400/μl; 1100–12 560</td>
<td>0.313</td>
</tr>
</tbody>
</table>

Table 2  Adverse events (AE) in study patients over the 28-day follow-up period by group

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>MB7 (n = 20)</th>
<th>MB5 (n = 20)</th>
<th>MB3 (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one AE (%)</td>
<td>17 (85)</td>
<td>18 (90)</td>
<td>18 (90)</td>
<td>0.851</td>
</tr>
<tr>
<td>Median duration (days; range)</td>
<td>5.5 (1–18)</td>
<td>5 (2–11)</td>
<td>4 (2–9)</td>
<td>0.158</td>
</tr>
<tr>
<td>Dysuria (%)</td>
<td>14 (70)</td>
<td>16 (80)</td>
<td>17 (85)</td>
<td>0.503</td>
</tr>
<tr>
<td>Headache (%)</td>
<td>5 (25)</td>
<td>2 (10)</td>
<td>5 (25)</td>
<td>0.392</td>
</tr>
<tr>
<td>Gastro-intestinal symptoms (%)</td>
<td>5 (25)</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>0.675</td>
</tr>
<tr>
<td>Other symptoms (%)</td>
<td>4 (20)</td>
<td>6 (30)</td>
<td>8 (40)</td>
<td>0.386</td>
</tr>
</tbody>
</table>

Discussion

This is the first study for at least 80 years in which MB monotherapy against falciparum malaria has been evaluated, for ethical reasons, in semi-immune adults. Given the evidence of efficacy from recent trials using MB-based combination therapy (Mandi et al. 2005; Meissner et al. 2005, 2006; Zoungrana et al. 2008), we studied the hypothesis that MB – an antimalarial with a short half-life of around 20 h (Walter-Sack et al. 2009) – would be able to eliminate all P. falciparum parasites when given for 7 days, compared to regimens of five and 3 days.

The main finding is that MB acts relatively slowly against P. falciparum blood stages which resulted in an unexpected number of ETFs (16%). These cases were defined as such because parasite density did not decline to below the threshold set by the WHO (2003) criteria which may have been oversensitive for the purpose of this study. Nevertheless, according to the study protocol, they had to be excluded from follow-up and given rescue treatment. The slow parasite clearance by MB can be explained in part on the basis of its biochemical action. With every dose, the damage caused by oxidative stress in the parasitized red blood cell increases (Buchholz et al. 2008). This interpretation is supported by findings from early clinical reports on this subject (Ferreira 1893). Another interpretation would be a lack of correlation between in vitro growth inhibition by MB and mechanisms of in vivo parasite clearance. In conclusion, it is obvious that because of its slow antiparasitic action, MB needs to be combined with a rapidly acting partner drug such as an artemisinin (International Artemisinin Study Group 2004; Greenwood et al. 2005).

All parasitological failures by day 14 occurred following 3 days of MB treatment and were re-infections. By day 28, confirmed recrudescences were seen only in the groups with three or 5 days MB treatment. This finding would support the notion that MB given over at least 7 days is able to eliminate all asexual P. falciparum parasites on a long-term scale and irrespective of the apparently slow action against parasitemia but needs to be supported in a study with a larger sample size.

Also, in this regard, the proportions of recrudescent infections in the small strata of patients need to be interpreted with caution, particularly considering the complex effects of immunity on this parameter (Sokhna et al. 2000).

The main adverse events were dysuria, headache and gastro-intestinal symptoms. While headache and gastro-intestinal symptoms may well be related to malaria illness itself (Müller et al. 1996; Greenwood et al. 2005), usually mild and self-limiting dysuria is known to be caused by MB (Zoungrana et al. 2008). Reassuringly, no signs of haemolysis and anaemia were observed in this population with and without G6PD deficiency, confirming previous results on the safety of MB in SSA populations (Mandi et al. 2005; Meissner et al. 2005, 2006).

This study has some limitations. First, the sample size was small and, consequently, small effects could not be
discovered. Second, patients were not randomized but recruited sequentially to the different treatment groups and in different proportions from the rural and urban study area, which could have introduced bias. However, all patients were recruited over a period of only 7 weeks at the height of the rainy season and the rural/urban proportion differed only for patients treated for 5 days. Third, the study did not have a placebo arm nor an active control group. It is thus not known to which extent *P. falciparum* parasites have been influenced by a pre-existing immunity of study patients.

In conclusion, this study demonstrates that MB acts rather slowly against the asexual *P. falciparum* parasites but appears to be able to clear the parasites, when given for 7 days. In the treatment of falciparum malaria, MB thus needs to be combined with an efficacious and rapidly acting partner drug.

**Acknowledgements**

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**References**


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