A randomized, parallel group, study of the safety and efficacy of 45 mg primaquine versus 75 mg bulaquine as gametocytocidal agents in adult patients of acute uncomplicated falciparum malaria

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ABSTRACT

Background
The study was carried out to assess the efficacy of a single dose of 45 mg primaquine versus 75 mg Bulaquine as a gametocytocidal agents in adult patients of uncomplicated *Plasmodium falciparum* malaria in Mumbai.

Methods:
The protocol was approved by the ethics committee and written; informed consent was obtained from all subjects. Inclusion for the study were presence of gametocytes> 55/µl within the first 72 hours. Patients were divided two groups and randomized either to receive bulaquine or primaquine. The patients were followed up to Day 29 for gametocytemia and gametocyte viability as determined by exflagellation.

Results:
Analysis for patients sensitive to schizonticidal drugs in the uncomplicated malaria group showed that on Day 8, 20/31, (64.5%) who received primaquine versus 7 /59 (11.86%) who received bulaquine showed persistence of gametocytes (P<0.05).

Conclusion:
The study thus indicates that 75 mg Bulaquine is superior to WHO recommended single dose 45 mg primaquine in clearing gametocytes.
Introduction
Malaria remains the most important parasitic infection with 300-500 million people affected yearly and 1.5-2.7 million deaths each year. World over, malaria control has focused on pharmacological intervention, vector control, curtailing irrational and indiscriminate use of antimalarials, and the development of malaria vaccines. Of all these strategies, pharmacological intervention in malaria still remains the most effective way to combat malaria. Among the drugs used, the 8 aminoquinolines such as primaquine are unique antimalarials in that they exhibit activity against multiple life cycle stages of *Plasmodia* that infect humans. Primaquine is currently recommended by the World Health Organization to be used in a single dose of 45 mg for its gametocytocidal activity in *P. falciparum* and in the dose of 15 mg/day for 14 days for its antirelapse activity in *P. vivax*. The NAMP of India (National Antimalarial programme) also recommends a single dose of 45 mg primaquine for its gametocytocidal action. However, a previous study by Gogtay et al has shown that the efficacy of this 45 mg dose may not be one hundred percent.

During the past several years, attempts have been made to modify the basic primaquine structure to produce drugs that retain antimalarial activity with lower toxicity. Bulaquine (formerly called CDRI 80/53) is one such compound which differs from primaquine only by the 2,4 dihydrofuran group present in the basic side chain anchored onto the quinoline nucleus in the 8 position. The drug is currently licensed only for use in India as anti-relapse for vivax malaria in the dose of 25 mg/day for 5 days. A preliminary report of this paper has been published earlier and has shown that 75 mg Bulaquine is superior to 45 mg primaquine for gametocytocidal effect. The present study was carried out in a larger sample to confirm the efficacy of 75 mg Bulaquine as a gametocytocidal agent in adult patients of uncomplicated falciparum malaria.

Materials and Methods
The study protocol was approved by the institutional ethics committee and the Drugs Controller General of India. Written informed consent was obtained from all participating subjects or guardians. Adult patients who were 16 years and above with *Plasmodium falciparum* infection irrespective of asexual parasite count were studied after obtaining informed consent if they had a gametocyte count more than 55/µl in the first 72 hours of illness. Patients who were pregnant or lactating, had received antimalarial treatment in the preceding 2 weeks, had co-infection with *Plasmodium vivax*, or had a history of allergy to primaquine or bulaquine were excluded. The gametocyte count of 55/µl was taken as the cut off based on infectivity to mosquitoes. G6PD status was also determined for all patients and those who were G6PD deficient were excluded from the study.

Initial diagnosis of malaria was done using thick and thin blood films stained using the Jaswant Singh and Bhattacharji (JSB) field stain. Giemsa stain was used for parasite count and asexual and sexual parasites were counted/µl assuming a standard white blood cell (WBC) count of 8000 WBCs per mm3.
Day 1 was taken as the day of admission and diagnosis. Patients of uncomplicated falciparum malaria were treated with quinine orally 10 mg/kg/day thrice daily for a total of 7 days and doxycycline 100 mg once daily for 7 days. Patients were then randomly allocated into 2 groups. One group received primaquine, while the other received bulaquine. Both drugs used were from a single batch only. A 1:2 randomization was chosen for the study in view of the superior efficacy of bulaquine as demonstrated earlier, with twice the number of patients receiving bulaquine. Supervised 45 mg primaquine or 75 mg bulaquine was given on Day 4 for all patients. Smears were prepared twice a day for the first 72 hours and once a day thereafter until discharge. Patients were then asked to follow up on Day 8, 15, 22 and 29. The medical officer who treated the patient and the malaria technician who read the peripheral smears were blinded as to the nature of the treatment.

Efficacy of therapy was assessed by gametocytemia on all follow up days and gametocyte viability as assessed by the modified Shute’s technique, where exflagellating microgametes were observed in blood films that were kept moist at 21-25°C for 1 hour with complete RPMI medium and AB positive serum before being dried, fixed and stained with Giemsa stain.

**Statistical analysis**

The sample size for the study was calculated based on the results of the previous study by Gogtay et al comparing the two drugs. Assuming a 30% difference in efficacy at 5% significance and 90% power, a sample size of 28 patients and 56 patients respectively were required in the primaquine and bulaquine group to demonstrate the superiority of bulaquine.
Results
The study was carried out from Jan 2002 to April 2004.
A total of 93 male patients of uncomplicated falciparum malaria were enrolled in the study. There were three drop outs, 2 in Bulaquine arm and one in primaquine arm. The age of the patients ranged from 16-72 years with a mean ± SD(31.47 ± 11.62).

There was no significant difference between the baseline gametocytaemia within two groups. A total of 31 patients received primaquine. Of these 20/31 (64.5%) were positive for gametocytes and 16/31(51.61%)patients had viable gametocytes on Day 8. (Table). As against this, in 59 bulaquine treated cases, 19/59 (32.2%) were positive for gametocytes and 7/59(11.86%) had viable gametocytes on Day 8, none on Day 15, Day 22 and Day 29. The difference between the number of patients with viable gametocytes in peripheral blood in the two groups was statistically significant (P < 0.05) on Day 8.

Discussion
A single dose of 45mg primaquine is given along with or after schizonticidal therapy in areas where malaria endemic as a transmission blocking strategy and is the only option available for this indication. In volunteer studies, primaquine both reduced the number of gametocytes as well as sterilized them. The present study carried out in 91 cases of uncomplicated Plasmodium falciparum malaria assessed the efficacy of a primaquine analog bulaquine in the single dose of 75 mg for its gametocytocidal effect. On day 8 of therapy, fewer patients with bulaquine had gametocytes as compared to those who received 45mg primaquine.

Assessment of true gametocytocidal efficacy of any drug will depend upon demonstration of the ability to block transmission to mosquitoes. This in turn can be assessed by checking for the presence/absence of the oocyst and ookinete in the mosquito midgut. In this study, the Shute technique, which measures the ability of the male gametocyte to exflagellate was used as a surrogate marker for assessing transmission blocking. When primaquine is given along with anti-malarials such as quinine that have a short half-life, the gametocyte kill is likely to be more complete. However, with anti malarials like chloroquine and mefloquine that have longer half lives, the single dose of primaquine because of its short lived action may not completely eliminate gametocytes and thus some remaining trophozoites will continue to form gametocytes.

Among the schizonticidal drugs, artemisinin derivatives are particularly useful, since they kill early stage gametocytes. They are however more useful in areas of low transmission rather than high transmission. They also need to be used in combination with longer acting anti-malarials and are not adopted by many countries as treatment options. Under these circumstances, bulaquine in a single dose of 75mg may represent an important treatment option for gametocytocidal effect and the current licensing of the drug in the country could be changed to include gametocytocidal effect apart from anti-relapse effect. It is possible that increasing the dose of primaquine itself from 45 to 60mg will improve gametocytocidal efficacy. This study is currently ongoing.
Table 1: Gametocytaemia of patients at different days of follow-up in the two groups

<table>
<thead>
<tr>
<th>Days of follow-up</th>
<th>Primaquine n=31. Gametocyte density/µl(mean ± SD)</th>
<th>Bulaquine n=59. Gametocyte density/µl(mean ± SD)</th>
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<tbody>
<tr>
<td>1</td>
<td>80-6040(1342±182.80)</td>
<td>80-4160(1064.2±182.80)</td>
</tr>
<tr>
<td>4</td>
<td>120-8000(1494± 385.04)</td>
<td>80-3260(1003.7±118.35)</td>
</tr>
<tr>
<td>8</td>
<td>80-2120(543±128.75)</td>
<td>32-1820(161.01±32.76)</td>
</tr>
<tr>
<td>15,22,29</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Table 2: Results of Efficacy.

<table>
<thead>
<tr>
<th>Days of follow-up</th>
<th>No of patients positive for gametocytes</th>
<th>Patients sample positive for exflagellation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PQ N=31</td>
<td>BQ N=59</td>
</tr>
<tr>
<td>1</td>
<td>31/31 100%</td>
<td>59/59 100%</td>
</tr>
<tr>
<td>4</td>
<td>31/31 100%</td>
<td>59/59 100%</td>
</tr>
<tr>
<td>8</td>
<td>20/31 64.5% *</td>
<td>19/59 32.2% *</td>
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<td></td>
<td></td>
<td>16/31 51.6% *</td>
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<td>15</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>22</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>29</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

= statistically significant P<0.05

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References


