Arterolane, a New Synthetic Trioxolane for Treatment of Uncomplicated *Plasmodium falciparum* Malaria: A Phase II, Multicenter, Randomized, Dose-Finding Clinical Trial

Neena Valecha,¹ Sornchai Looareesuwan,² Andreas Martensson,³ Salim Mohammed Abdulla,¹ Srivicha Krudsood,¹ Noppadon Tengpukdee,³ Sanjib Mohanty,² Saroj K. Mishra,² P. K. Tyagi,³ S. K. Sharma,² Joerg Moehrle,³ Anirudh Gautam,⁴ Arjun Roy,⁴ Jyoti K. Paliwal,⁴ Monica Kothari,⁴ Nilanjan Saha,⁴ Aditya P. Dash,¹ and Anders Björkman⁶,⁷

¹National Institute of Malaria Research, Indian Council of Medical Research, Delhi; ²Ispat General Hospital and ³Field Station, Malaria Research Centre, Rourkela, and ⁴Ranbaxy Laboratories, Gurgaon, India; ⁵Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; ⁶Karolinska University Hospital, Stockholm, Sweden; ⁷Zanzibar Malaria Research Unit of Karolinska Institute, Zanzibar, and ⁸District Hospital, Ifakara Health Research and Development Centre, Bagamoyo, Tanzania; and ⁹Medicines for Malaria Venture, Geneva, Switzerland

**Background.** Drug-resistant *Plasmodium falciparum* malaria necessitates development of novel drugs for treatment. The present study assessed the efficacy and safety of 3 dose levels of arterolane (RBx 11160), a synthetic trioxolane, for treatment of acute uncomplicated falciaparum malaria.

**Methods.** In this randomized, double-blind, multicenter, parallel-group, dose-finding, phase II trial, 230 patients from 4 centers in Thailand, India, and Tanzania (mainland and Zanzibar) received either 50 mg (n = 78), 100 mg (n = 76), or 200 mg (n = 76) of arterolane once daily for 7 days. Patients (aged 13–65 years) with asexual parasite density of 1000–100,000 parasites/μL were included and were followed up for 28 days. The median time to 90% parasite clearance (PC₉₀) was evaluated.

**Results.** The median PC₉₀ was longer in the group receiving the 50-mg dose (19.4 h), compared with the groups receiving the 100-mg dose (12.8 h) and 200-mg dose (12.6 h) (P < .01). The polymerase chain reaction–corrected adequate clinical and parasitological responses on day 28 were 63%, 71%, and 72% for the groups receiving the 50-mg, 100-mg, and 200-mg doses, respectively, by intention-to-treat analysis (odds ratio, 1.55; 95% confidence interval, 0.78–3.06, for comparison of the 200-mg and 50-mg dose groups). Treatment was generally well tolerated. No patient died or experienced any serious adverse event. Mild complaints were reported in <10% of the patients and were similar in the 3 groups. Biochemistry and hematological analyses did not show any sign of drug toxicity in any patient.

**Conclusion.** Arterolane at daily doses of 100 and 200 mg is a rapidly acting, effective, and safe synthetic antimalarial drug, which may potentially represent an alternative to artemisinin derivatives in antimalarial combination therapy.

**Trial registration.** ClinicalTrials.gov identifier NCT00362050.
Table 1. Disposition of Subjects

<table>
<thead>
<tr>
<th>Measure</th>
<th>Arterolane maleate dose once daily for 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mg</td>
</tr>
<tr>
<td>All randomized patients</td>
<td>78 (100)</td>
</tr>
<tr>
<td>ITT/safety population</td>
<td>78 (100)</td>
</tr>
<tr>
<td>Per-protocol population</td>
<td>77 (99)</td>
</tr>
<tr>
<td>Reason for early termination</td>
<td></td>
</tr>
<tr>
<td>Insufficient therapeutic effect (ie, recrudescence)</td>
<td>35 (45)</td>
</tr>
<tr>
<td>Adverse event/coexistent infection</td>
<td>0</td>
</tr>
<tr>
<td>Consent withdrawn</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1</td>
</tr>
<tr>
<td>Other reasonb</td>
<td>1</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients. ITT, intention to treat.

a Ten patients were not included in the per-protocol population: 5 patients had <1000 asexual parasites/μL at the time of screening, 2 patients had >100,000 asexual parasites/μL at screening, and 3 patients did not complete the recommended dosing schedule of 7 days.

b Other reasons included *Plasmodium vivax* malaria, instead of *Plasmodium falciparum* malaria (1 subject in the 50-mg dose group and 1 subject in the 100-mg dose group), and receipt of exclusionary medication (1 subject in the 100-mg dose group).

thetic antimalarial drugs with similar antimalarial activity [4, 5]. Arterolane (RBx 11160) maleate is a new, fully synthetic 1,2,4-trioxolane with a peroxidic pharmacophore and is a rapidly acting oral antimalarial drug [6].

Arterolane has shown in vitro potency higher than that of chloroquine, mefloquine, artemether, and artesunate against K1 (chloroquine-resistant) and NF54 (chloroquine-susceptible) strains of *P. falciparum*. In vivo, arterolane is highly effective against *Plasmodium berghei* in mice (unpublished data on file with the sponsor, Medicines for Malaria Venture, and Ranbaxy). Pharmacokinetic studies indicate that arterolane has an elimination half-life (t1/2) between 1 and 3 h in different animal species. In a phase I study involving healthy subjects in the United Kingdom, single doses of up to 600 mg of arterolane and once-daily doses of up to 200 mg of arterolane for 7 days have been well tolerated.

The pharmacodynamics of arterolane were evaluated after single doses of 0, 25, 50, 75, 100, and 200 mg in 72 adult Thai patients with uncomplicated *P. falciparum* malaria (unpublished data on file with the sponsor, Medicines for Malaria Venture, and Ranbaxy). The patients also received mefloquine (at 25 mg/kg) 6 h after arterolane. The design of the study was similar to that of the study by Angus et al [7]. The median times to 50% parasite clearance (PC50) for doses of 0–200 mg of arterolane were 5.5–12 h, and the respective median times to 90% parasite clearance (PC90) were 14–24 h. The 200-mg dose group showed significantly shorter PC90 values (P = 0.0071) and PC50 values (P = .048), compared with the control group. Median T_max ranged from 2 to 4 h postdose. Thereafter, plasma arterolane concentrations decreased, with t1/2 ranging from 0.8 to 4.2 h for individual patients. Between-patient (interindividual) variability in area under the concentration curve (AUC) for 0–24 h was moderate to high, with a coefficient of variation ranging from 31% to 89%. The values of the pharmacokinetic parameters (Cmax and AUC) of arterolane, however, were nearly one-third of those observed in healthy volunteers. All doses of the drug were well tolerated. Adverse events reported were mild or moderate and were generally similar for placebo and arterolane-receiving groups.

In the current multicenter, phase II trial, multiple doses of 50, 100, and 200 mg of arterolane were evaluated to assess the antimalarial activity and safety of arterolane administered once daily for 7 consecutive days to patients with acute uncomplicated *P. falciparum* malaria. The study was intended to provide an evidence base for selecting an optimal dose regimen that could be considered for combination therapy with a selected partner drug.

**METHODS**

**Study design.** This double-blind, multicenter, randomized, parallel-group, dose-ranging study was conducted in Bangkok, Thailand; Rourkela, Orissa State of India; Bagamoyo, Tanzania Mainland; and Kivunge, Zanzibar, from June through December 2006. The sites varied in terms of malaria transmission and treatment guidelines. In Thailand, the transmission is low and seasonal, and artesunate/mefloquine is the first-line treatment. In Rourkela, the transmission is high and perennial, and artesunate/sulfadoxine-pyrimethamine is the treatment of choice. In Tanzania and Zanzibar, malaria is perennial, and artemether/lumefantrine and artesunate/amodiaquine are the first-line treatments.

The primary end point of the trial was PC90. The secondary
Patients receiving arterolane maleate dose

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>50 mg (n = 78)</th>
<th>100 mg (n = 76)</th>
<th>200 mg (n = 76)</th>
<th>All patients (n = 230)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (77)</td>
<td>62 (82)</td>
<td>53 (70)</td>
<td>175 (76)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (23)</td>
<td>14 (18)</td>
<td>23 (30)</td>
<td>55 (24)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>21 (27)</td>
<td>20 (26)</td>
<td>19 (25)</td>
<td>60 (26)</td>
</tr>
<tr>
<td>Asian</td>
<td>57 (73)</td>
<td>56 (74)</td>
<td>57 (75)</td>
<td>170 (74)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.4 ± 12.21</td>
<td>26.2 ± 9.9</td>
<td>27.3 ± 9.82</td>
<td>26.6 ± 10.68</td>
</tr>
<tr>
<td>Median</td>
<td>22</td>
<td>23</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Min; Max</td>
<td>13; 65</td>
<td>13; 52</td>
<td>13; 55</td>
<td>13; 65</td>
</tr>
<tr>
<td>Age &lt;18 years</td>
<td>21 (27)</td>
<td>14 (18)</td>
<td>11 (14)</td>
<td>46 (20)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
<td>50.5</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Min; Max</td>
<td>33.8; 90.2</td>
<td>37; 80</td>
<td>31.5; 90.2</td>
<td>31.5; 90.2</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>20.4 ± 2.88</td>
<td>20.3 ± 2.96</td>
<td>20.2 ± 2.71</td>
<td>20.3 ± 2.84</td>
</tr>
<tr>
<td>Median</td>
<td>19.9</td>
<td>19.8</td>
<td>19.9</td>
<td>19.9</td>
</tr>
<tr>
<td>Min; Max</td>
<td>15.4; 30.9</td>
<td>15.4; 29.4</td>
<td>14.5; 28.9</td>
<td>14.5; 30.9</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. The demographic profiles for particular sites were consistent and did not vary with the dose level assigned. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); Max, maximum; Min, minimum.

end points included PC_{50}, 100% parasite clearance time (PCT), fever clearance time (FCT), and polymerase chain reaction (PCR)–corrected adequate clinical and parasitological response (ACPR) rate on day 28.

The study was approved by the local ethics committees and/or institutional review boards of all participating sites. Written informed consent was obtained from patients or parents or legally accepted representatives or guardians. The trial was conducted in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice, the Declaration of Helsinki, and current World Health Organization (WHO) guidelines for assessment of antimalarial drug efficacy [8] and safety, in addition to the applicable regulations of the participating countries.

**Patients.** Eligible patients were male and female individuals aged 13–65 years with body weight ≥30 kg who have acute uncomplicated *P. falciparum* monoinfection with a parasite density of 1000–100,000 asexual parasites/μL of blood and an axillary temperature ≥37.5°C or oral temperature ≥38°C. *P. falciparum* infection was confirmed by microscopic examination of blood smear. Clinical exclusion criteria were severe malaria, any significant comorbidity, pregnancy, lactation, any antimalarial or other treatment within 2 weeks prior to screening, history of allergic reactions to artemisinins, or previous participation in an investigational drug study during the past 30 days.

Patients were randomized using a centralized computer-generated list to receive 1 of the 3 arterolane dose amounts (50, 100, or 200 mg). Patients were hospitalized, and arterolane was administered once daily under observation for 7 consecutive days (days 0–6) at approximately the same time each day irrespective of the time of food intake. Patients were discharged after completion of the 7 days of treatment and thereafter were followed up on days 14, 21, and 28. The patients were told to visit the study site whenever they experienced any clinical symptom during the follow-up period.

**Outcome measures.** Clinical signs and symptoms, including disease severity, were evaluated at screening and on days 0–6, 14, 21, 28, and any other day that a patient spontaneously returned with fever. Parasitological assessments were performed on thick and thin blood smears from venous or finger prick samples at screening and on days 0, 1, 2, 3, 6, 14, 21, and 28, as well as any other day that a patient returned with clinical symptoms. On days 0, 1, and 2, thick blood smears were collected at 6-h intervals for at least 72 h after the first dose of study medication or until 2 consecutive negative smears were recorded in the previous 24-h period.

Blood smears were stained with Giemsa and were independently examined by 2 technicians. Asexual and sexual parasites...
Table 3. Time to 50%, 90%, and 100% Clearance of Parasites and Fever Clearance for the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Measure</th>
<th>Arterolane maleate dose once daily for 7 days</th>
<th>Comparison of treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>PC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients with PC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>78 (100)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>No. (%) censored</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median PC&lt;sub&gt;90&lt;/sub&gt; (95% CI), h</td>
<td>19.4 (17.4–23.8)</td>
<td>12.8 (11.8–15.0)</td>
</tr>
<tr>
<td>25th–75th Percentiles, h</td>
<td>10.1–31.0</td>
<td>7.3–20.7</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.607 (1.16–2.22)</td>
<td>1.46 (1.23–1.73)</td>
</tr>
<tr>
<td>Log-rank &lt;i&gt;P&lt;/i&gt;</td>
<td>.004</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wilcoxon &lt;i&gt;P&lt;/i&gt;</td>
<td>.006</td>
<td>.001</td>
</tr>
<tr>
<td>PC&lt;sub&gt;50&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients with PC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>78 (100)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>No. (%) censored</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median PC&lt;sub&gt;50&lt;/sub&gt; (95% CI), h</td>
<td>12.2 (10.4–17.1)</td>
<td>9.0 (7.9–11.9)</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48 (1.06–2.05)</td>
<td>1.37 (1.15–1.62)</td>
</tr>
<tr>
<td>Log-rank &lt;i&gt;P&lt;/i&gt;</td>
<td>.018</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wilcoxon &lt;i&gt;P&lt;/i&gt;</td>
<td>.018</td>
<td>.009</td>
</tr>
<tr>
<td>PCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients with PCT</td>
<td>78 (100)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>No. (%) censored</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median PCT (95% CI), h</td>
<td>48.3 (42.5–55.3)</td>
<td>32.1 (25.8–38.2)</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50 (1.08–2.07)</td>
<td>1.46 (1.23–1.74)</td>
</tr>
<tr>
<td>Log-rank &lt;i&gt;P&lt;/i&gt;</td>
<td>.014</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wilcoxon &lt;i&gt;P&lt;/i&gt;</td>
<td>.013</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients with FCT</td>
<td>78 (100)</td>
<td>76 (100)</td>
</tr>
<tr>
<td>No. censored</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median FCT (95% CI), h</td>
<td>2.9 (1.8–6.0)</td>
<td>5.8 (1.7–6.0)</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83 (0.60–1.15)</td>
<td>1.02 (0.87–1.19)</td>
</tr>
<tr>
<td>Log-rank &lt;i&gt;P&lt;/i&gt;</td>
<td>.250</td>
<td>.814</td>
</tr>
<tr>
<td>Wilcoxon &lt;i&gt;P&lt;/i&gt;</td>
<td>.617</td>
<td>.411</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; FCT, fever clearance time; PC<sub>50</sub>, time to 50% parasite clearance; PC<sub>90</sub>, time to 90% parasite clearance; PCT, time to 100% parasite clearance.

* From Cox proportional hazards regression analysis.

were counted against 200 white blood cells, and the average of the 2 readings was recorded after adjustment of parasite density to the patient’s white blood cell value. Central quality control for parasitological examination was conducted by the Swiss Tropical Institute, Basel, Switzerland.

Blood for PCR genotyping was collected at each blood sampling on FTA Elute cards, which were stored in sealed plastic bags with desiccant until processing. The PCR genotyping was done at the Swiss Tropical Institute using procedure, as described by Felger and Beck [9], based on the <i>P. falciparum</i> genes merozoite surface protein 1 (msp-1), merozoite surface protein 2 (msp-2), and glutamate-rich protein (glurp). Recurrent parasitemia was defined as new infection if alleles in the post-treatment sample from the patient were completely different from those in the pretreatment sample and as recrudescence if at least 1 allele was common between the 2 paired samples.

Body temperature (°C) was recorded by axillary or oral routes at screening; immediately before and after dosing on day 0; at 6 h intervals (or adjusted to the closest possible 6-h interval to make the schedule consistent with routine care) for at least 72 h following the first dose of study medication or until temperature normalized for at least 48 h, whichever was later; on days 3–6; and at all follow-up visits.

A single dose of paracetamol (≤1 g) was administered with the first dose of arterolane for fever and general body aches unless paracetamol or another antipyretic had been taken in the previous 6 h. Paracetamol could be repeated at intervals ≥6 h if fever or body aches persisted. Use of any other antimalarial during the study period resulted in patient withdrawal.

Safety assessments were performed at the screening, throughout the 7-day hospitalization, and at follow-up visits. Safety assessments included laboratory parameters, physical examinations, electrocardiograms (ECGs), vital signs, and monitoring of clinical events. The laboratory evaluations (hematological
Table 4. Adequate Clinical and Parasitological Response (ACPR) and Polymerase Chain Reaction (PCR)–Corrected ACPR for the Intention-to-Treat Population

<table>
<thead>
<tr>
<th>Arterolane maleate dose once daily for 7 days</th>
<th>Comparison of treatment groupsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg (n = 78)</td>
<td>100 mg vs 50 mg</td>
</tr>
<tr>
<td>100 mg (n = 76)</td>
<td>200 mg vs 50 mg</td>
</tr>
<tr>
<td>200 mg (n = 76)</td>
<td>200 mg vs 100 mg</td>
</tr>
<tr>
<td><strong>ACPR on day 28</strong></td>
<td></td>
</tr>
<tr>
<td>Success, no. of patients</td>
<td>37</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>42</td>
</tr>
<tr>
<td>OR (95% CI) for success</td>
<td>1.37 (0.73–2.58)</td>
</tr>
<tr>
<td><strong>PCR-corrected ACPR on day 28</strong></td>
<td></td>
</tr>
<tr>
<td>Success, no. of patients</td>
<td>49</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>54</td>
</tr>
<tr>
<td>OR (95% CI) for success</td>
<td>1.45 (0.74–2.86)</td>
</tr>
<tr>
<td><strong>ACPR on day 14</strong></td>
<td></td>
</tr>
<tr>
<td>Success, no. of patients</td>
<td>47</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>46</td>
</tr>
<tr>
<td>OR (95% CI) for success</td>
<td>1.01 (0.53–1.93)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; OR, odds ratio.

* OR and associated CI were calculated using logistic regression.

analysis, liver function tests, renal function tests, metabolic parameters, and urine analysis) were performed at screening and on day 6, day 28, and any other day that a patient returned with symptoms.

Venous blood samples (2 mL) were collected on day 0 prior to dosing and at 3 ± 0.5 h and 8 ± 0.5 h after dosing, on day 1 prior to dosing, and on day 6 both prior to dosing and at 3 ± 0.5 h and 8 ± 0.5 h after dosing, for pharmacokinetic evaluation. Arterolane concentrations in plasma were determined using a validated liquid chromatography–tandem mass spectrometry method with a lower limit of quantification for arterolane of 1.16 ng/mL.

**Statistical analysis.** The primary efficacy analyses were performed using the intention-to-treat (ITT) population that included all randomized patients who received at least 1 dose of arterolane. Efficacy analyses were also repeated using the per-protocol population as confirmation of the primary efficacy results. The safety population was used to evaluate safety.

The primary efficacy outcome—that is, the median PC₉₀, was defined as the interval from the time of the first dosing of arterolane to the time when 90% of parasites were cleared relative to baseline parasite counts. Each pair of arterolane dose groups (50, 100, and 200 mg dose groups) was compared with respect to PC₉₀ using the log-rank and generalized Wilcoxon (Gehan-Breslow) tests at a 2-sided significance level of 0.05. The effect of dose on PC₉₀ was also investigated using Cox proportional hazards regression analysis. The estimated hazard ratios for comparison of pairs of dose groups and 95% confidence intervals were provided on the basis of the Cox proportional hazards regression analysis. Secondary end point analyses (PC₅₀, PCT, and FCT) were summarized and analyzed as described for PC₉₀.

Parasitological cure rates—that is, ACPR and PCR-adjusted ACPR by day 28—were assessed for each dose group. Point estimates and 95% confidence intervals for the 3 pairwise odds ratios of treatment success were calculated on the basis of the logistic regression model. The overall incidence of treatment-emergent adverse events was coded using the Medical Dictionary for Regulatory Activities, version 8.0.

The arterolane plasma concentrations were quantified in ng/mL, with a lower limit of quantification of 1.16 ng/mL. The pharmacokinetic parameter AUC₀–₈₇ (3-point exposure determined from 0-, 3-, and 8-h concentrations) on day 0 and day 6 was estimated by noncompartmental analysis using validated WinNonlin Professional software (version 4.1; Pharsight). Actual time of blood collection was used to determine the AUC. Plasma concentrations below the lower limit of quantification of the assay were taken as zero for all calculations.

**Trial registration.** This trial was registered with ClinicalTrials.gov (identifier NCT00362050).

**RESULTS**

A total of 230 patients were randomized at the 4 sites and received the study drug arterolane; 78 patients received 50-mg doses, 76 patients received 100-mg doses, and 76 patients received 200-mg doses. Each of these 230 randomized patients received at least 1 dose of study drug and was included in the safety and ITT populations. Ten patients were not included in the per-protocol population because of deviations from the...
protocol, including 1 patient from the 50-mg dose group, 3 from the 100-mg dose group, and 6 from the 200-mg dose group (Tables 1 and 2).

**Efficacy.** Parasite clearance was generally fast in all 3 dose groups, with slightly longer median PC\textsubscript{50} values in the 50-mg dose group (19.4 h), compared with the 100-mg and 200-mg dose groups (12.8 h and 12.6 h, respectively; \(P<.01\)) (Table 3). Indeed, in the ITT population, PC\textsubscript{50}, PC\textsubscript{90}, and PCT were shorter in the 100- and 200-mg dose groups than in the 50-mg dose group, and the differences were statistically significant. No significant difference in PC\textsubscript{50} or PC\textsubscript{90} was observed between the 50- and 200-mg dose groups, but PCT was statistically significantly shorter in the 200-mg dose group on the basis of the log-rank test. No statistically significant differences in median FCT were observed between any of the groups. Similar results were seen for the per-protocol population. The PCR-corrected ACPR on day 28 was 63%, 71%, and 72% in the 50-, 100-, and 200-mg dose groups (Table 4). In both the ITT and the per-protocol populations, the PCR-corrected ACPR and noncorrected ACPR on day 28 and the ACPR on day 14 were generally higher in the 100-mg and 200-mg dose groups than in the 50-mg dose group (Table 4).

**Safety.** No patient died or experienced any serious adverse event during the study. Only mild clinical adverse events were reported after initiation of the treatment. The percentage with complaints did not exceed 10% in any of the groups, and complaints included headache (3%), vomiting (3%), abdominal pain (2%), diarrhea (1%), and vertigo (1%). There was no significant difference between the dose groups. Laboratory examinations provided no evidence of any toxic reactions to the treatments. Mean hemoglobin concentrations decreased by day 6 but were above baseline (day 0) values by day 28 in all 3 groups. No individual patient developed severe anemia (hemoglobin level, <8.0 g/L). Mean alanine aminotransferase and aspartate aminotransferase concentrations remained slightly elevated by day 6 and were similar in the 3 dose groups. By day 28, only 5 patients continued to have elevated alanine aminotransferase levels (2, 2, and 1 patients in the 50-, 100-, and 200-mg groups, respectively), and 15 patients had elevated aspartate aminotransferase levels (5, 8, and 2 patients in the 50-, 100-, and 200-mg groups, respectively). No patient experienced liver enzyme concentrations \(\geq 3\) times the upper limit of normal during the study. There were no statistically significant differences in any laboratory measurements between the dose groups. ECG investigations revealed only 2 significant changes on day 6: 1 change each in a patient receiving the 200-mg dose and a patient receiving the 100-mg dose. QT interval prolongations from 402 to 462 ms and from 406 to 464 ms were observed. The QT intervals subsequently decreased to 451 and 446 ms, respectively, and were not considered clinically significant but were possibly related to the study drug.

**Pharmacokinetics.** Plasma concentrations of arterolane are presented in Table 5. The mean 3-h and 8-h concentrations increased with higher dose, from 50 to 200 mg. The mean AUCs were 6.0 times higher (day 0) and 5.2 times higher (day 6) in the 200-mg dose group, compared with the 50-mg dose group. In addition, within the respective dose groups, the mean and individual AUC\textsubscript{0–24 h} values on day 6 were 1.4–2.0-fold higher than those on day 0, indicating increased exposure of arterolane in patients as they tended to become aparasitemic.

**DISCUSSION**

Arterolane maleate in combination with long-acting piperazine phosphate is under development as an antimalarial product in line with WHO-recommended combination therapy for the treatment of uncomplicated *P. falciparum* malaria. In this study, however, safety and efficacy data were generated for arterolane maleate in combination with long-acting piperazine phosphate.
terolane as monotherapy before evaluation of the combination with piperaquine.

All 3 doses of arterolane were well tolerated by the patients, and the only significant observations, ECG findings of prolonged QT intervals in 2 patients, were considered to be most likely related to malaria, as has been reported earlier [10, 11], and a relationship to arterolane could not be ascertained. Parasite clearance was observed with all 3 doses. The 100-mg and 200-mg doses cleared 90% of the parasites in <24 h. The median PCTs after the 100- and 200-mg doses (both ~32 h) are comparable to findings after monotherapies with different artemisinin derivatives (mean, 43 h; range, 38–104 h) [12]. This suggests that arterolane may have a rapid parasiticidal effect similar to that of artemisinin derivatives, thereby leading to fast reduction of parasite biomass, which is critical for the drug’s potential as an alternative to artemisinin in a 3-day combination treatment regimen.

Recrudescence rates after PCR adjustment were observed to be between 28% and 37% in our study groups. Reported treatment failures after artemisinin monotherapy vary from 3% to 50%, depending on the duration of treatment in studies; these studies, however, did not differentiate recrudescence from repeat infection [13–15]. In a recent study in Central African Republic, PCR correction was performed, and the cure rate after a 7-day course of artesunate was estimated to be 95% [16]. This may be compared with our findings of PCR-corrected cure rates for arterolane at the 2 African trial sites, which were between 84% and 100% for the 3 doses (Table 6). However, little conclusion can be drawn from such comparisons because not only the different treatment durations but also factors such as different levels of immunity [17] will affect treatment outcomes.

In malaria treatment guidelines, the WHO identified the rationale of combining antimalarials that have different modes of action, to prevent development of resistance and to optimize antimalarial therapy [18]. In 3-day artemisinin-based combination therapy regimens, the artemisinin component is present in the body during only 2 asexual parasite life-cycles, which reduces the number of parasites in the body. The complete clearance of parasites is dependent on the partner medicine being effective and persisting at parasiticidal concentrations until most of the infecting parasites have been killed. The relatively high rate of recrudescence with arterolane 3-day monotherapy highlights the need to combine the drug with long-acting drugs, such as lumefantrine, piperaquine, or mefloquine.

The 200-mg dose of arterolane appears to be the optimal regimen. A classic sigmoid relationship between AUC_{0–24h} and efficacy was observed with maximal effect using the 3 prospectively defined parameters PC_{90}, PC_{90}, and PCT. Median PC_{90} (12.6 h) was <24 h, and PCTs were less variable and were statistically significantly shorter in the 200-mg dose group, compared with the 100-mg dose group, on the basis of the log-rank test. These results are similar to the findings of Angus et al [7], who demonstrated a similar dose-response relationship with oral doses of arsunate.

Following the present dose range–finding trial of arterolane alone, further development is already ongoing for arterolane in combination with piperaquine, an already established long-acting partner drug to dihydroartemisinin [19]. In view of the emerging resistance against the existing antimalarial agents and increased PCTs following artemisinin–based combination therapy [20, 21], there is an urgent need to develop new alternative drugs. A fully synthetic drug such as arterolane, which has an activity profile similar to that of the artemisinins, provides an important potential in such an endeavor.

Acknowledgments

We are grateful to the patients who agreed to participate in this trial; Paragon Biomedical, for statistical analysis; Swiss Tropical Institute, Switzerland, for their work in PCR analysis and their dedicated involvement in the project management activities; and all the regulatory authorities that granted permission to undertake the clinical trial.

Financial support. Medicines for Malaria Venture, Geneva, Switzerland.

Potential conflicts of interest. J.M. is employed at Medicines for Malaria Venture. A.G., A.R., J.K.P., M.K., and N.S. are employed by Ranbaxy Laboratories, which is developing the product. All other authors: no conflicts.

References


