REDUCTION OF PARASITE LEVELS IN PATIENTS WITH UNCOMPPLICATED MALARIA BY TREATMENT WITH HE2000

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Abstract. 16α-Bromoepiandrosterone (HE2000) is a synthetic androstane steroid that has immune effects in pre-clinical models of malaria, tuberculosis, and infection with human immunodeficiency virus. In pilot studies, 42 patients with confirmed uncomplicated Plasmodium falciparum malaria were treated with a seven-day course of HE2000 by either buccal administration or intramuscular injection. Of the 42 patients, 41 showed a 50% reduction in blood levels of parasites, the primary endpoint of the study. Of these, 32 (76%) cleared malaria parasites below detectable levels. All febrile patients became afebrile by the end of treatment. There was no reduction in gametocyte forms. Adverse events were transient and mild to moderate in intensity. The anti-malarial response was generally similar with either the intramuscular or buccal routes of administration. HE2000 shows a safety profile and pharmacologic activity worthy of further investigation to understand its role in the treatment of malaria, perhaps in combination with anti-malarial agents.

INTRODUCTION

Drugs that improve the host’s immune control of malarial infection are needed because of their potential for broad-spectrum anti-infective activity, their promise to enhance the effectiveness of combination therapy, and their potential to successfully treat multidrug-resistant strains.1 Widespread drug resistance has been reported with single-agent therapy, consequently this has resulted in combination therapies becoming the standard for malaria treatment.2 Unfortunately, increased exposure to combination drugs also leads to resistance.3 In the setting of malaria, an effective immune agent has the potential to assist the infected host in clearing parasites and to enhance protective long-term immunity. However, low-cost clinically effective agents have been elusive.

The androsten/androstane steroids have been reported to have potent immune modulation effects in pre-clinical models of a variety of pathogens.4–13 However, this phenomenon has yet to translate into meaningful activity in human clinical trials. Previously, 16α-bromoepiandrosterone (HE2000) was reported to show inhibition of growth in vitro against chloroquine resistant strains of Plasmodium falciparum malaria and activity in vivo against a rat model of P. berghei malaria. Immune modulation was cited as the probable mode of action.12 We report the results of two clinical studies that explore the activity of HE2000 in patients infected with P. falciparum malaria from the Myanmar-Thai border, a region with multidrug-resistant malaria.14 The objectives of these studies were to assess the safety, tolerance, and early signs of anti-malaria activity of HE2000 that might warrant further research.

METHODS

Study drug. 16α-bromo-3β-hydroxy-5α-androstan-17-one (16α-bromoepiandrosterone, HE2000) was prepared by chemical synthesis (Hovione, Loures, Portugal) and formulated as a solution for intramuscular injection. The study drug consists of 50 mg/mL of HE2000 in a mixture of polyethylene glycol 200, propylene glycol, benzyl benzoate, and benzyl alcohol (College of Pharmacy, Health Science Center, University of Tennessee; Memphis, TN) supplied in 1-mL aliquots in 2-mL amber glass vials. Tablets for transmucosal administration that rapidly disintegrate in the buccal cavity were approximately 5 mm in diameter and consisted of 25 mg of 16α-bromoepiandrosterone blended with lactose, crospovidone, mannitol, magnesium stearate, and silica excipients (Nateco Pharma, Ltd., Hyderabad, India).

Clinical studies. HE2000 was studied for safety, tolerance, and activity in the acute treatment of uncomplicated P. falciparum malaria. Two separate open-label, phase-I/II type clinical trials were conducted using either intramuscular injection or buccomucosal administration. The protocols planned to enroll 21 individuals per study who were field screened and recruited from malaria-endemic regions of Thailand. Both studies were similar in design and were conducted by the Department of Tropical Medicine, Mahidol University in Bangkok, Thailand. Both the Mahidol University Institutional Ethics Committee and Ministry of Public Health reviewed and approved the studies. The United States and/or Thailand Food and Drug Administration approved the drug for importation.

Patients were asymptomatic at enrollment with no chills or myalgia. By protocol design and consistent with the World Health Organization (WHO) consensus opinion, body temperature was not considered a deciding sign and therefore not an exclusion criterion.15 Each initially asymptomatic patient, diagnosed with a parasitemia < 2% measured by peripheral blood smear and determined to be at low risk of developing complications from malarial infection, was provided transportation to the hospital and admitted for treatment. Blood screening test kits that detected both P. falciparum and P. vivax were used for field screening (Optimal®; DiaMed, Cressier sur Morat, Switzerland). No P. vivax infections were detected. Trained microscopists at Mahidol University confirmed test kit results. Individuals with a malaria infection that did not meet protocol criteria were treated with standard of care.

After oral and written informed consents were obtained, patients were hospitalized and given seven consecutive daily 100-mg doses of HE2000 without concomitant anti-malarial drugs. Intramuscular injections were given as one or two injections in different locations in the deltoid muscle. Buccomucosal dosing was administered with four tablets (25 mg each) inserted between the lower gingival surfaces of the gum and buccal mucosa of the cheek. Patients were hospitalized for the seven-day treatment period and then followed-up for
three weeks. Determinations of the blood parasitemia and the febrile status (axillary) were performed at the investigator’s discretion; however, at least four readings were performed per day for the first three days, twice per day through day seven, at least once per day until day 14, and then at each subsequent patient visit (at a minimum of at least once a week until study day 29). Patients with residual parasitemia measured on day 7 and patients who experienced recrudescence after day seven were treated with the anti-malarial agents Coartem® (Novartis Pharma, Basel, Switzerland), Artecom® (Benchmark Pharma Limited, Guangzhou, Guangdong, People’s Republic of China), Artek® (Guangzhou Holleykin Pharmaceuticals Co. Ltd., Guangzhou, People’s Republic of China), Artezun® (Benchmark Pharma Limited), and Mefloquine® (Roche Laboratories, Nutley, NJ). Patients that cleared their parasitemia were released from the hospital after day seven at the earliest. Use of all concomitant medications, including vitamins and dietary supplements, was recorded. Safety observations included clinical signs and symptoms, vital signs, and changes in clinical chemistry and hematology laboratory parameters. Chemistry and hematology were checked daily during the first week, then at days 10, 14–17, and finally weekly to study day 29.

Adverse events were coded using MedRA version 5.1 (Maintenance and Support Services Organization, Reston, VA) preferred terms and analyzed by body systems. All safety analyses included comparison of baseline, treatment, and post-treatment laboratory values.

Study procedures. Parasitemia was evaluated by standard microscopy of thick and thin film peripheral blood smears and expressed as the number of parasites/microliter of blood or parasites per thousand red blood cells, respectively, and expressed as percent of infected red blood cells. Each patient was evaluated for signs and symptoms at every visit, especially those related to malaria. Clinical chemistry and hematology results were determined using central laboratory facilities.

Data management and statistical methods. Data processing was conducted centrally. Case report forms (CRFs) and edit checks were designed and validated based on previously stipulated protocol requirements. The completed CRFs were faxed from the site and were read by character-recognition software (TeleForm™, Vista, CA) that translates images of data into electronic database fields. Trained data management staff verified the translation by checking 100% of the database fields. The database was audited for quality assurance, completeness, and accuracy.

Fever was defined as axillary temperature ≥ 37.5°C. Parasite clearance corresponded to a zero parasite count using thick film peripheral blood smears. Results for binary variables are expressed as percentages, with exact 95% confidence intervals calculated by the Blyth-Still-Casella formula. Counts are summarized as medians and interquartile ranges. Time to first event was estimated by the Kaplan-Meier method. Statistical analyses were performed with SAS® software (SAS Institute, Cary, NC). Patient response to treatment was classified using the WHO 2003 criteria adapted to 7 days of treatment.

RESULTS

Patient characteristics. Twenty-one adult patients recruited from the multidrug-resistant P. falciparum endemic region of the Myanmar-Thailand border were enrolled into each study. Study period and baseline characteristics of the patients according to study assignment are shown in Table 1. All patients were confirmed to have uncomplicated P. falciparum malaria with a parasitemia < 2%, and 10 of 42 were febrile (temperature > 38°C) at baseline. Of the 32 non-febrile cases, 6 became febrile during the treatment week. No patients were co-infected with P. vivax.

Evolution of parasitemia and fever after treatment with HE2000. The main features regarding clearance of parasites and resolution of fever during the treatment week are shown in Table 2. Within the treatment period, 21 of 21 patients treated with intramuscular HE2000 and 20 of 21 patients treated with buccal HE2000 achieved 50% parasite clearance, the primary end point of the study. The median time to 50% clearance was six hours in both instances. There was no reduction in gametocyte forms after seven days of therapy with either route of administration. Of the 42 patients tested, 17 (81%) of 21 (95% confidence interval [CI] = 58.1–94.6%; exact 95% CI for the population percentage) receiving intramuscular HE2000 and 15 (71.4%) of 21 (95% CI = 47.8–88.7%) receiving buccal HE2000 achieved total clearance of malarial parasites during study treatment with a median clearance time of 36 hours and 50 hours, respectively. Figure 1 shows a detailed account by cohort of how time to total parasite clearance unfolds during the treatment week. It can be seen that profiles are similar, although the intramuscularly-treated patients appear to attain complete parasite clearance slightly earlier than those receiving the buccal formulation. At baseline 17 of 21 (intramuscular) and 12 of 21 (buccal) patients were febrile (temperature ≥ 37.5°C) and all had a normal temperature with a median time of 24 hours in both cases. The mean time to initiation of treatment with other medications from the last dose of HE2000 was 4 days (median = 1.0 day, interquartile range = 1–8 days, range = 1–15 days) for the intramuscular study and 4 days (median = 1.0 day, interquartile range = 1–3 days, range = 1–24 days) for buccal administration. One patient did not recrudesce. Pa-

<table>
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<tr>
<th>Characteristic</th>
<th>Intramuscular study (n = 21)</th>
<th>Buccal study (n = 21)</th>
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<tbody>
<tr>
<td>Enrollment period</td>
<td>9/11/01–11/10/01</td>
<td>4/30/02–6/13/02</td>
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<tr>
<td>Completion period</td>
<td>10/2/01–12/1/01</td>
<td>5/29/02–6/12/02</td>
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<tr>
<td>Median (range) follow-up, days</td>
<td>29 (22–43)</td>
<td>29 (26–49)</td>
</tr>
<tr>
<td>Age, years</td>
<td>17 (81.0)</td>
<td>16 (76.2)</td>
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<tr>
<td>Prior malaria treatment (%)</td>
<td>27 (22.5–36.0)</td>
<td>24 (19.2–32.3)</td>
</tr>
<tr>
<td>Febrile patients (≥ 37.5°C) (%)</td>
<td>13 (61.9)</td>
<td>14 (66.7)</td>
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<tr>
<td>Febrile patients (&gt; 38°C)†</td>
<td>17 (81.0)</td>
<td>12 (57.1)</td>
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<tr>
<td>Temperature °C</td>
<td>37.7 (37.5–38.0)</td>
<td>37.5 (37.0–38.0)</td>
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<td>Pulse rate per minute*</td>
<td>84 (76–88)</td>
<td>86 (76–90)</td>
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<tr>
<td>Degree of Plasmodium falciparum parasitemia</td>
<td>4.942</td>
<td>5.651</td>
</tr>
<tr>
<td>Hemoglobin, g/dL‡</td>
<td>11.2 (9.9–12.2)</td>
<td>13 (11.9–13.9)</td>
</tr>
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</table>

* Median (interquartile range limits).
† Fever controlled at enrollment with paracetamol, nonsteroidal anti-inflammatory drugs, or aspirin.
tient responses to HE2000 are classified using an adapted WHO 2003 criteria and are shown in Table 3. Early treatment failures were observed in 3 of 21 and 5 of 21, clinical failures were observed in 6 of 18 and 6 of 16, parasitologic failures were observed in 4 of 12 and 5 of 10, and adequate clinical and parasitologic responses were observed in 8 of 21 and 5 of 21 in the intramuscular and buccomucosal studies, respectively.

Drug safety. HE2000 was generally well tolerated (Table 4). The most frequent adverse events were injection site pain and elevated creatine phosphokinase (CPK) levels in the intramuscular study. These events were transient, mild to moderate in severity, and resolved over the course of the study. No patient withdrew the study because of irritation at the injection site. Serious adverse events were reported by 11 patients. Grade 3 toxicities included elevated blood CPK levels in the intramuscular administration cohort and thrombocytopenia in the buccal administration cohort.

**DISCUSSION**

Evidence indicates that HE2000 may be useful to treat diseases such as infection with human immunodeficiency virus (HIV), tuberculosis, and malaria, and other intracellular infections that bias the host immune response towards Th2 immunity. The present studies evaluated safety, tolerance, and activity of HE2000 by intramuscular and buccomucosal administration and are the first clinical data that demonstrate an anti-malarial effect of this compound in patients with uncomplicated malaria.

HE2000 may provide a distinctive set of features to aid in

![Figure 1. Distribution of time to total parasite clearance (% patients) as a function of hours from baseline to total parasite clearance.](image-url)
the management of malaria infection. A review of the literature shows that many pathogens including HIV and those that cause malaria and tuberculosis subvert host Th1 immunity as the disease enters into a chronic phase. 18-22 Effective agents that stimulate immunity are considered an important addition to future drug treatment combinations. Available data indicate that HE2000 has anti-inflammatory properties and induces cellular immunity that may aid the control of infection (Stickney DR and others, unpublished data). 8,9,12 Innate immune control of malaria parasites has been reported through a macrophage CD36-mediated pathway in rodents. 23 Immune phenotype studies in a phase 1 clinical trial of HE2000 has demonstrated increased CD36 + macrophage phenotypes in the blood (Hollis-Eden Pharmaceuticals, unpublished data). This suggests that a similar pathway may control early immune elimination of parasitized erythrocytes in humans. In extensive immunologic studies in HIV patients, dendritic cells and activated HIV peptide specific CD8 T cells increased. This increase in innate and adaptive cellular immunity was associated with a statistically significant decrease in viral load. Resistance to malaria infection may also be improved with antigen-specific cellular responses that enhance adaptive host immunity. Through this mechanism, HE2000 may be a useful addition to treatment strategies for drug resistant malaria strains. 24-26

Although the studies were exploratory in design and not formally powered to demonstrate efficacy, it is surprising that some parameters of drug activity were already detectable at the low sample sizes considered. In these studies, a decrease in parasite counts was observed with both routes of administration. Although the lack of an experimental control group prevents any formal proof of efficacy, the magnitude of the response seen cannot be dismissed as a placebo effect in these malaria patients. Of the 42 patients, 41 achieved a 50% reduction in blood parasites during study treatment, the primary endpoint of the study. In addition, 32 of 42 patients cleared malaria parasites below detectable levels. The treatment effect, however, was transitory. Of the 42 patients, 41 recrudesced during the study period and were consequently treated with anti-malarial drugs. Because HE2000 has an immune effect rather than a direct anti-parasitic effect, relapses can be attributed to periodic release of sequestered parasites that rise above detectable levels resulting in a recrudescence event. As a safety precaution for these first HE2000 clinical trials in malaria, patients were treated with anti-malarial drugs immediately on recrudescence after day 7. The median time to anti-malarial treatment of both studies was 1 day after the seven-day treatment course with HE2000.

The WHO classification was developed to assess and compare anti-malarial efficacy between different agents. Application of these criteria to HE2000 results in early treatment failures in only 14.3% (3 of 21) of the intramuscular cohort and 23.8% (5 of 21) of the buccal cohort. HE2000 showed an adequate clinical and parasitologic response against P. falciparum-parasitized erythrocytes in 8 of 21 intramuscular- and 5 of 21 buccal-treated patients. In these studies, the adverse event profile of the drug was consistent with the safety of HE2000 already noted elsewhere, a generally safe moiety, with mild-to-moderate intramuscular injection site reactions. 15,17 Elevated levels of CPK were observed more frequently with intramuscular than with buccal administration. In studies of HE2000 in HIV-infected patients that used a comparator arm, exercise was found most often responsible for unexpectedly elevated CPK levels. 17 This may explain the two unexpected events in this buccal study compared with the 19 expected events in the intramuscular study. Consequently,
both the buccal and intramuscular formulations have competitive potential for further development with the balance appearing to be slightly in favor of the intramuscular route.

The data from these studies pose the question of whether HE2000 in combination with standard anti-malarial therapy might not shorten the duration of anti-malarial therapy and therefore reduce the toxicity associated with standard therapy regimens, such as artesisinin anti-malarial drugs. To explore this possible combination, studies with longer follow-up are first needed to determine if the administration of HE2000 yields a stronger cellular immunity to malaria, consequently, preventing reinfection. Thus, HE2000 shows a profile potentially useful to enhance immune activity against malaria in those who are semi-immune and perhaps in those who are symptomatic when combined with approved anti-malarial drugs.

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