16α-BROMOEPIANDROSTERONE, A DEHYDROEPIANDROSTERONE (DHEA) ANALOGUE, INHIBITS PLASMODIUM FALCIPARUM AND PLASMODIUM BERGHEI GROWTH


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Abstract. Dehydroepiandrosterone (DHEA) and its analogue, 16α-bromoepiandrosterone (α-epi-Br), may have activity against viral and parasitic infections, including human immunodeficiency virus (HIV) and Cryptosporidium parvum. Therefore, we evaluated its antimalarial effects on Plasmodium falciparum and Plasmodium berghei. In vitro, chloroquine (CQ)-sensitive and resistant strains of \( P. falciparum \) parasitized red blood cells were incubated with escalating doses of α-epi-Br or CQ. In vivo, 62 rats were infected with \( P. berghei \) and treated with CQ or α-epi-Br. At the highest doses tested against a CQ-sensitive strain, parasitemias decreased from 25.4% in the saline control group to 4.3% and 4.8% in the α-epi-Br and CQ groups, respectively \((P < 0.05)\). Against two CQ-resistant strains, parasitemias decreased from 22.3–28.8% and 24.8–30% in the CQ and saline groups, respectively, to 2.5–2.7% in the α-epi-Br groups \((P < 0.05)\). These data demonstrate that α-epi-Br shows activity against CQ-sensitive and resistant strains of \( P. falciparum \) in vitro. At the doses tested against \( P. berghei \) in vivo in rats, α-epi-Br is comparable to CQ.

INTRODUCTION

Dehydroepiandrosterone (DHEA) has been shown to have immune-modulating effects in experimental \( C. parvum \) infection and in human immunodeficiency virus (HIV) and human T-cell lymphotropic virus type I infection.\(^1\)\(^-\)\(^2\) In rodents with \( C. parvum \) infection, DHEA decreased intestinal parasite colonization and fecal oocyst shedding resulting in less severe \( C. parvum \) infection.\(^3\)\(^-\)\(^5\) \( 16α \)-bromoepiandrosterone (α-epi-Br), a DHEA analogue without significant androgenic activity, may be an immune modulator. In HIV infection, α-epi-Br may increase circulating acetylated immune effector cells and shift cytokine production to a Th1 phenotype. In HIV and simian HIV (SHIV) infection, α-epi-Br may decrease viral load (RNA polymerase chain reaction [PCR]) (Reading C and Frincke J, unpublished data).

We hypothesized that these immune-modulating effects may be therapeutically relevant in malaria as well. In addition, because DHEA and α-epi-Br are inhibitors of glucose 6-phosphate dehydrogenase (G6PDH),\(^1\)\(^-\)\(^4\) a direct cytotoxic mechanism of action may also be important. Here we sought to assess α-epi-Br's antimalarial activity against chloroquine-sensitive and resistant \( P. falciparum \) strains in vitro and against chloroquine-tolerant \( P. berghei \) in vivo in rats.

METHODS

In vitro. Three strains of \( P. falciparum \) were tested (CQ-sensitive [wild type, WT], CQ-resistant [Dd2], and CQ- and mefloquine-resistant [W,M,MEF]). Saline or escalating doses of α-epi-Br or CQ was added to 50 \( \mu \)L of \( P. falciparum \)-parasitized red blood cells (RBCs), RPMI culture medium with NaHCO\(_3\) and HEPES buffer (N-[2-hydroxyethyl] piperazine-N’-[4 butanesulfonic acid] (Sigma, St. Louis, MO), gentamicin sulphate (4 \( \mu \)g/mL), and Albumax II (Gibco Life Technologies, Auckland, New Zealand), to a total volume of 150 \( \mu \)L per well. Using uninfected human RBCs and culture medium, parasitemia and hematocrit were adjusted to 1% and 7%, respectively. The mixtures were incubated in 96-well culture plates in an anaerobic chamber (CO\(_2\) 5%, O\(_2\) 2%, and N\(_2\) 90%) at 37°C.\(^6\)

The culture medium/drug mixture was changed at 24, 48, and 72 hr. α-epi-Br was tested at concentrations of 30, 15, 7.5, 3.75, 1.875, and 0.938 \( \mu \)M and CQ at 2.0, 0.2, and 0.02 \( \mu \)M. Each drug concentration was tested in triplicate and assays for WT and Dd2 strains were repeated. Pooled data (mean parasitemia) from both runs are reported. Parasitemia was assessed at 96 hr by preparing thin smears fixed with methanol and stained with Giemsa. Fifty-six hundred RBCs were counted on each slide. The microscopist was blinded to the study arm.

In vivo. Sixty-two Lewis rats (Charles River Lab, Wilmington, MA), five to six weeks of age and weighing approximately eighty-five grams, were intraperitoneally (IP) inoculated with \( 5–30 \times 10^6 \) \( P. berghei \) (ANKA strain)-infected RBCs. All experiments were approved by University of Vermont’s Institutional Animal Care and Use Committee.

Two hours after the IP injection, rats were randomized and intravenously injected with one of four treatments: 1) saline control \((n = 16)\), 2) CQ 40 mg/kg \((n = 16)\), 3) low dose \((LD)\) α-epi-Br 30 mg/kg \((n = 16)\), and 4) high dose \((HD)\) α-epi-Br 60 mg/kg \((n = 14)\). These doses were tested because unpublished preliminary work in a mouse model suggested an observed effect with a dose of 100 mg/kg. When adjusted for body surface area, this corresponds to our maximum dose of 60 mg/kg in rats. This treatment was repeated at 24, 48, and 72 hr after the initial IP injection for a total of four injections. In between therapies, rats were returned to their housing, fed standard lab chow, and allowed free access to water.\(^7\)

Rats were bled on Days 4, 11, 17, and 28 post-inoculation. Parasitemia, hematocrit, and weight were measured. Rats were observed twice daily looking for evidence of progressive disease or an adverse drug reaction, defined as loss of...
at least 20% of the rat’s original weight or listlessness. The rats were followed for 28 days in two groups of 20 and 42 rats each, with data pooled for the two runs.

Statistical analysis. Pooled results are reported as the mean (± the standard error of the mean [SEM]). Repeated measures analysis of variance and Scheffe’s test were used for in vivo data. Due to the small sample sizes, rank methods were used. The Kruskal-Wallis and rank sum tests with a Bonferroni adjustment were used to compare parasitemia for in vitro data at the highest drug doses tested. Statistical significance was determined using α = 0.05. Analyses were performed using Stata statistical software (Stata, College Station, TX).

RESULTS

In vitro. Both α-epi-Br and CQ inhibited parasite growth of the WT strain of P. falciparum (a CQ-sensitive strain). The IC50 (drug concentration that inhibited parasitemia by fifty percent) of α-epi-Br against the WT strain was 7.5 µM, α-epi-Br, tested at concentrations of 30, 15, 7.5, 3.75, 1.875, and 0.938 µM, resulted in parasitemias of 4.3, 8.8, 13.3, 15.8, 22, and 22.3%, respectively. Chloroquine tested at concentrations of 2.0, 0.2, and 0.02 µM, produced parasitemias of 4.8, 8.6, and 11.5%, respectively, and saline control resulted in parasitemia of 25.4% (Figure 1). At the highest dose tested, compared to saline control, both α-epi-Br and CQ significantly inhibited parasite growth (P = 0.003 and P = 0.007, respectively). There was no difference between α-epi-Br and CQ (P = 0.30).

Corresponding parasitemias against the CQ-resistant Dd2 strain were: 2.5, 6.3, 10.7, 11.7, 17.3, and 17.7% for α-epi-Br; respectively, in an IC50 of 3.0 µM. Chloroquine-treated Dd2 strain produced parasitemias of 28.8, 27.8, and 30%, and saline treatment resulted in parasitemia of 29.7%, respectively. At the highest doses tested, in comparison to saline control, CQ did not decrease parasitemia (P = 0.20), as expected. However, α-epi-Br significantly inhibited parasite growth (P = 0.003).

Against the multi-drug resistant W2MEF strain, only α-epi-Br decreased parasitemia: 2.7, 9.7, 11.5, 21.5, 23.0, and 20.7% for α-epi-Br, 22.3, 20.2, and 20.7% for CQ, and 24.8% for saline control, respectively (P = 0.003 and P = 0.085 for comparisons of α-epi-Br with saline, and CQ and saline, respectively). The IC50 was approximately 6.75 µM. Mefloquine was not tested.

In vivo. At baseline, all groups had similar weights and hematocrits. There were no significant differences in weight throughout the experiment in any of the arms. Although only minimally increased, hematocrit was significantly greater in the α-epi-Br HD arm, as compared to saline control: 44.7% versus 42.1%, respectively (P = 0.01). All rats survived the twenty-eight day experiment.

Four days after inoculation of rats with P. berghei parasites were as follows: saline control (23%), CQ (12%), α-epi-Br LD (12%), and α-epi-Br HD (9%). This decrease in parasitemia on Day 4 in the CQ, α-epi-Br LD, and α-epi-Br HD arms, as compared to the saline-treated control group was significant (P < 0.001). On Day 11, there was an even greater difference in parasitemia (P < 0.001): saline (31%), CQ (12%), α-epi-Br LD (10%), and α-epi-Br HD (6%). However, there was no difference between the CQ, α-epi-Br LD, and α-epi-Br HD arms (P > 0.05). By Days 17 and 28, there were no significant differences in any of the four groups (P > 0.05) (Figure 2).

DISCUSSION

16α-Bromoepiandrosterone, a DHEA analogue, is a 17-keto steroid with significant antimalarial activity against chloroquine-sensitive and resistant strains of P. falciparum in vitro. Its activity against P. berghei in vivo in rats is comparable to that of CQ with the current dosing regimens. When compared to the saline control, α-epi-Br decreased
parasitemia in all the *P. falciparum* strains tested in vitro and *P. berghei* in vivo, maintained rat growth, and slightly increased hematocrit. At the two in vivo doses tested (30 and 60 mg/kg daily for four days), suppression of parasitemia appeared dose-independent (*P* > 0.05).

To the best of our knowledge, α-epi-Br is not androgenic. In vitro, it binds weakly to the androgen receptor with a dissociation constant of 10 μM, and in vivo, there has been no evidence of androgenic activity based on early toxicology studies. Furthermore, preliminary results of a clinical HIV trial have not demonstrated serious adverse effects other than mild intramuscular injection site soreness (Frincke J and others, unpublished data). Thus, as α-epi-Br appears safe and has efficacy against different strains of *Plasmodium* in preclinical studies, it may turn out to be a useful antimalarial in clinical practice either alone or in combination with other drugs.

Although the mechanism of action of α-epi-Br was not evaluated in these pilot studies, potential mechanisms include an antimalarial cytotoxic and/or an immune-modulating effect. Because the drug is effective in vitro, where immune cells are either absent or in extremely low numbers, an antiplasmodial cytotoxic effect is likely responsible, at least in part.

Marks and Banks showed that DHEA inhibits G6PDH, and Henderson and others demonstrated that α-epi-Br is sixty times more potent as a G6PDH inhibitor. Furthermore, Cappadoro and others recently showed that inhibition of G6PDH is associated with increased in vitro phagocytosis of ring-stage *P. falciparum* parasitized erythrocytes, although this was not seen with trophozoite or schizont-infected erythrocytes. They hypothesized that malaria resistance in G6PDH-deficient populations may be related to decreased ring-stage erythrocyte antioxidant defense, increased membrane damage, and consequent enhanced phagocytic removal and decreased maturation to later-stage parasites. Further studies demonstrated that α-epi-Br also enhances phagocytosis of ring-stage infected erythrocytes; however a significant effect on G6PDH was not documented (i.e., pentose-phosphate shunt flux or reduced glutathione) (Ayi K and others, unpublished data). Thus, enhanced phagocytosis of ring-stage infected erythrocytes may also play a role in α-epi-Br’s antimalarial activity.

Dehydroepiandrosterone has also been studied in *Cryptosporidium parvum* infection in various rodent models. In *C. parvum*-infected immunosuppressed rats and mice, DHEA increased B and T cell blastogenic response to lipopolysaccharide and to concanavalin A, respectively, and increased serum IgG and splenic CD4 and CD8 T cells. Importantly, both prophylactic (in hamsters) and therapeutic administration of DHEA decreased oocyst shedding and parasite colonization. Metabolites of DHEA, 5-androsten-3β,17β-diol (AED), and 5-androsten-3β,7β,17β-triol (AET), appear to be responsible for DHEA’s in vivo activity, and are approximately 1,000 to 10,000 times more potent than DHEA in pathogen protection assays in mice. It may be that DHEA’s immune-modulating effects in *C. parvum*, another coccidian parasite, is relevant to α-epi-Br’s antimalarial effect through common or related metabolites. Interestingly, DHEA-SO₃ has been demonstrated to ameliorate *Schistosoma mansoni* infection in mice, although an immune-mediated effect was not seen.

Dehydroepiandrosterone has also been studied in several viral infections. Exogenous administration of DHEA has been reported to decrease mortality in mice with systemic coxsackievirus B4 infection and with herpes simplex type 2 encephalitis. Decreased DHEA levels have been inversely correlated with progression of disease in HIV and human T-cell lymphotropic virus type I (HTLV-1) infections. Furthermore, decreased DHEA levels in the elderly have been implicated, at least in part, in age-related immunosenescence.

We are not aware of any published data with regard to α-epi-Br’s antimicrobial effects. However, treatment of simian HIV (SHIV 229)-infected macaques has been reported to decrease viral load and to increase survival (Frincke J and others, unpublished data). Furthermore, preliminary results of a clinical HIV trial have not demonstrated serious adverse effects other than mild intramuscular injection site soreness. Therefore, DHEA appears safe and has efficacy against different strains of *Plasmodium* in preclinical studies, it may turn out to be a useful antimalarial in clinical practice either alone or in combination with other drugs.

**FIGURE 2.** Effect of 16α-bromoeiandrostone (α-epi-Br) and chloroquine (CQ) versus saline control on in vivo growth of *Plasmodium berghei* (ANKA strain) in juvenile Lewis rats (*P* < 0.001 for Days 4 and 11) (mean ± SEM).
others, unpublished data). In HIV infection, α-epi-Br decreases viral load, increases activated CD8, LAK, NK, ADCC, and dendritic cells, and shifts cytokine production to a Th1 response (Reading C and others, unpublished data). Thus, DHEA and α-epi-Br appear to modify host defense against viral infections as well, and DHEA may also be relevant to age-related decreases in host immunity (i.e., immunosenescence).

In summary, α-epi-Br is a novel antimarial agent with in vitro efficacy against chloroquine-sensitive and resistant strains of *Plasmodium falciparum* and *Plasmodium berghei* in rats. Potential mechanisms of action include G6PD inhibition with enhanced phagocytosis of ring-stage infected erythrocytes, an immune modulating effect, or other undefined mechanisms. Further investigation, including dose finding, synergy, mechanism of action, and clinical studies are warranted.

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REFERENCES