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SHORT REPORT: *IN VITRO* ACTIVITY OF ARTEMISONE COMPARED WITH ARTESUNATE AGAINST *PLASMODIUM FALCIPARUM*

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Abstract. Artemisinins show the potential for neurotoxicity in preclinical studies. Artemisone is a leading candidate of second-generation semi-synthetic artemisinin derivatives for antimalarial therapy devoid of neurotoxicity. Artemisone showed 3–5-fold higher *in vitro* activity (50% effective concentration (EC_{50}) = 0.14 nmol/L, EC_{90} = 2.55 nmol/L) than artesunate against fresh *Plasmodium falciparum* isolates from Gabon and a high-activity correlation indicates a shared drug target.

Artemisinin-based combination therapy is recommended by the World Health Organization as first- or second-line antimalarial treatment in most malaria-endemic countries.[1,2] Other than the rare occurrence of idiosyncratic allergic reactions, artemisinins are remarkably well-tolerated.[3,4] However, experimental evidence and data from toxicology studies have shown the potential for inducing neurologic damage.[5,6] Although these findings have not shown clear correlations in humans, there is still some concern about the safety of artemisinins. Recently, a renewed interest in the clinical importance of neurologic side effects of artemisinin derivatives has evolved because of case reports of toxic brainstem encephalopathy after the administration of artemisinin derivatives and a report on hearing loss in patients treated with a combination of artemether and lumefantrine.[7,8]

The pharmaceutical company Bayer Healthcare and the Hong Kong University of Science and Technology initiated a collaborative program to develop artemisinin derivatives with improved efficacy, stability, pharmacokinetic behavior, and reduced neurotoxicity. Artemisone (proposed international nonproprietary name: artemifone, BAY 44-9585; molecular weight = 401.53; structural formula: $C_{19}H_{31}NO_6S$) was selected as the lead candidate of a class of second-generation semi-synthetic artemisinin derivatives because of its lack of neurotoxicity, increased efficacy, and comparably low costs of production. First, animal studies in *Aotus trivirgatus* monkeys suggested a 10–30 times increased activity based on pharmacokinetic and efficacy data against *Plasmodium falciparum* compared with artesunate.[9] However, it is not clear whether the increased activity in animal models is due to improved pharmacokinetic or to pharmacodynamic characteristics. The aim of this study was therefore to assess the *in vitro* activity of artemisone compared with artesunate against fresh African *P. falciparum* isolates.

The study took place at the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné, Gabon, between August and October 2003. This hyperendemic region is characterized by a high degree of resistance to chloroquine, a considerable degree of resistance to antifolate drugs,and an increasing number of isolates showing borderline resistance to mefloquine.[10–12]

Outpatients were considered eligible if they presented with uncomplicated *P. falciparum* malaria, a reported minimum of four weeks without antimalarial drug intake, and if informed consent was provided. Ethical clearance was obtained from the Ethics Committee of the International Foundation for the Albert Schweitzer Hospital in Lambaréné. Venous blood was drawn from patients presenting with microscopically confirmed *P. falciparum* monoinfection of a parasitemia > 1,000 parasites per microliter of peripheral blood. Parasitemia was adjusted with uninfected O^+ erythrocytes to a final concentration of 0.02%. The blood medium mixture was adjusted to a final hematocrit of 1.5%.

Artemisone (10 mmol/L; Bayer Healthcare, Leverkusen, Germany), artesunate (10 mmol/L; Sanofi Synthelabo, Paris, France), and mefloquine hydrochloride (1 mmol/L; Hoffmann-La Roche AG, Basel, Switzerland) were dissolved in ethanol. Chloroquine-diphosphate (Sigma, St. Louis, MO) was dissolved in double-distilled water. All stock solutions were further diluted with double-distilled water. Test plates were predosed with ascending concentrations of artemisone (0.003–10 nmol/L), artesunate (0.01–30 nmol/L), and chloroquine (20–2,560 nmol/L). Test plates were dried under sterile conditions and kept at 4°C for up to three months prior to use. Drug activity was measured using the histidine-rich protein 2 (HRP2) assay as described previously.[13] A cut-off level of at least a four-fold increase in HRP2 concentration was set as exclusion criterion for further analysis.

Cumulative effective concentrations (ECs) were calculated using a log-concentration/probit-response model.[14] Individual ECs were obtained by non-linear regression analysis, and Spearman's rank test was used for correlation analysis of drug activities. The Wilcoxon rank sum test was used to compare EC values of artemisone and artesunate. All tests were performed at a two-sided significance level of $\alpha = 5\%$ ($P < 0.05$).

Forty patients participated in this study and had a median parasitemia at inclusion of 23,000 asexual-form parasites per microliter of blood (25% percentile = 10,950/μL and 75% percentile = 123,253/μL). Twenty-seven isolates showed sufficient growth in the artemisone assay. Log-probit analysis of cumulative EC values showed a good fit of the dose-response model with $\chi^2 = 4.95$ (maximal permitted = 11.07). The EC values for artemisone were 0.14 nmol/L (95% confidence interval [CI] = 0.08–0.25 nmol/L) at the 50% level and 2.55 nmol/L (95% CI = 0.89–7.32 nmol/L) at the 90% level (Table 1). Twenty-seven isolates were eligible for log-probit

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TABLE 1

In vitro activity (nmol/L) of artemisone and artesunate against 27 fresh *Plasmodium falciparum* isolates in Gabon*

	EC_{50}	Lower 95% CI	Upper 95% CI	EC_{90}	Lower 95% CI	Upper 95% CI
Artemisone	0.14	0.08	0.25	2.55	0.89	7.32
Artesunate	0.73	0.40	1.34	13.62	4.69	39.60

* EC_{50} = 50% effective concentration; CI = confidence interval.

analysis for artesunate activity ($\chi^2 = 5.32$;). The 50% effective concentration (EC_{50}) was 0.73 nmol/L (95% CI = 0.40–1.34 nmol/L) and the EC_{90} concentration was 13.62 nmol/L (95% CI = 4.69–39.60 nmol/L).

Based on individual non-linear regression analysis, EC_{50} values of artesunate were 2.96 times higher (median interquartile range = 2.35–6.40, n = 23) than those of artemisone. This difference was more pronounced at the EC_{90} level (median = 4.73, interquartile range = 3.70–22.56, n = 23). The difference in the EC_{50} and EC_{90} values of artemisone and artesunate against individual *P. falciparum* isolates was statistically significant (EC_{50}: $P < 0.001$; n = 23 and EC_{90}: $P < 0.001$; n = 14).

Correlation analysis was based on EC values for individual isolates. As expected for representatives of the same chemical class, artemisone and artesunate showed a high level of activity correlation at the EC_{50} and EC_{90} levels (EC_{50}: n = 23, $\rho = 0.65$, $P < 0.001$ and EC_{90}: n = 14, $\rho = 0.49$, $P = 0.08$; Table 2). Conversely, no activity correlation was seen for artemisone or artesunate with either mefloquine or chloroquine (Table 2).

Due to the recent policy change in artemisinin-based combination therapy, there was a projected need of 120 million courses of artemisinin containing therapies for 2005.[15,16] In light of possible supply shortages and open questions concerning the safety of current artemisinin derivatives, considerable efforts have been made to identify semi-synthetic second-generation artemisinin derivatives.[5–8] Artemisinin derivatives have shown the potential to cause neurotoxicity in preclinical *in vitro* studies and in animal models. However, these data are in contrast to the extensive experience with artemisinin-containing regimens in clinical studies and in clinical practice showing a particularly favorable tolerability and safety profile despite the occurrence of rare idiosyncratic reactions.[4] Similarly, conflicting results have been reported from clinical studies assessing the potential neurologic impact of artemisinin containing regimens in humans.[8,17] In the context of this ongoing discussion, artemisone was chosen as the lead candidate for further development because of its lack of neurotoxicity and markedly increased antimalarial activity as observed in first animal studies.[9]

TABLE 2

Correlation analysis of *in vitro* activity (EC_{50}) of artemisone, artesunate, chloroquine, and mefloquine against *Plasmodium falciparum* isolates*

	Artesunate	Mefloquine	Chloroquine
Artemisone	n = 23, rho = 0.62, $P = 0.0008$	n = 20, rho = 0.00, $P = 1.00$	n = 15, rho = –0.09, $P = 0.74$
Artesunate		n = 17, rho = 0.19, $P = 0.46$	n = 13, rho = 0.36, $P = 0.23$

* EC_{50} = 50% effective concentration.

In our study, artemisone showed a significantly increased *in vitro* activity against fresh *P. falciparum* isolates compared with artesunate. The EC_{50} and EC_{90} values were three and five times lower for artemisone compared with artesunate, respectively. Thus, artemisone is the most active antimalarial used today. However, the 3–5-fold enhanced *in vitro* activity is well below the reported 10–30-fold increase of *in vivo* efficacy against multidrug-resistant *P. falciparum* strain in *Aotus trivirgatus* monkeys.[9] This discrepancy might be due to the added impact of differing pharmacokinetic characteristics on *in vivo* drug efficacy besides increased antiplasmodial activity. However, differences in dosing and modes of administration might also play a role.

Our data show a close association of *in vitro* activity of the two sesquiterpene lactones artesunate and artemisone. These results point to the fact that artemisone and artesunate share a common mechanism of action. The SERCA-like PfATP6 acts most likely as the specific target of artemisone, as it was previously shown for other artemisinin derivatives.[18] Interestingly, Uhlemann and others showed a reduced enzyme inhibition constant (K_i) of artemisone for PfATP6, which indicated a distinctly increased affinity.[19] This finding might serve as a functional explanation for the increased *in vitro* activity of artemisone and its close activity correlation with artesunate. Given the improved safety profile and the increased activity of artemisone, further clinical development seems promising.

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