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Letter to the Editor

Atorvastatin as a Potential Antimalarial Drug: In Vitro Synergy in Combinational Therapy with Dihydroartemisinin[▽]

Atorvastatin (AVA) is a synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A. AVA has lipid-lowering ability, a long plasma half-life, and an excellent safety record. In addition to the previously mentioned positive aspects of AVA, this drug has also shown *in vitro* antimalarial activity (10). AVA showed no *in vitro* cross-resistance with the commonly used antimalarials. In addition, the 50% inhibitory concentrations (IC_{50}s) of AVA were found to be unrelated to mutations occurring in transport protein genes involved in quinoline antimalarial drug resistance, including the *pfcrt*, *pfmdr1*, *pfmrp*, and *pfnhe-1* genes (8). Nevertheless, AVA treatment alone, at a dose of 20 mg/kg of body weight, failed to prevent death due to cerebral malaria and was not able to affect parasitemia in infected mice (1). One possibility is that AVA could act as an adjuvant therapy. Wong and Davis recently showed that AVA did not potentiate the *in vitro* activity of dihydroartemisinin (DHA) in the 3D7 strain of *P. falciparum* (13) and also posited that AVA had no clinical relevance because AVA IC_{50}s were well above the plasma concentrations achievable in humans taking high-dose AVA (0.1 to 0.5 μM) (2).

In the present study, we tested the effects of AVA at six concentrations (0.1, 0.5, 1, 2, 4, and 8 μM). We used 13 well-established strains of *P. falciparum* for the analysis (Table 1) (6). The methodology of the *in vitro* potentiation test was previously described (5). The combination of AVA and DHA had synergistic effects (Fig. 1). The differences in DHA IC_{50} between AVA concentrations groups have first been tested using analysis of variance for repeated measures to take into account the fact that observations made within each strain were not independent. Using the most conservative correction for interdependence between observations (i.e., Box's conservative epsilon), the differences in DHA IC_{50} were highly significant ($P < 0.001$) in the range of AVA plasma concentrations achievable in patients taking 80 mg of AVA daily (Table 1). Using a random-effect linear regression approach, the regression coefficients for the log-transformed DHA IC_{50} indicated that the mean fold change in the DHA IC_{50} when adding AVA at concentrations of 0.1, 0.5, and 1.0 μM (0.90, 0.82, and 0.72, respectively) were also highly significant ($P < 0.001$). The DHA IC_{50} was reduced by 10% (range, 0 to 24%; 95% confidence interval [CI], 5 to 15%), 18% (range, 8 to 38%; 95% CI, 14 to 22%), and 28% (range, 15 to 43%; 95% CI, 24 to 32%) in the presence of AVA at concentrations of 0.1, 0.5, and 1.0 μM, respectively. These reductions were all significant ($P < 0.001$). Another finding was that the synergy of the effects of DHA was AVA dose dependent. The reductions in DHA IC_{50} were also significant ($P < 0.001$) between 0.1 and 0.5 μM AVA and between 0.5 and 1.0 μM AVA.

In contrast to previously published studies on 3D7 (13), we showed that AVA had a synergistic effect on DHA activity. These controversial findings could be explained by differences in the isotopic method used; Wrong and Davis used a modified microdilution isotopic method with 50% plasma (versus 10% in commonly used *in vitro* test). In addition, their IC_{50} of DHA alone was 12.5 nM, while the commonly accepted IC_{50} on 3D7 was <4 nM. We also illustrated that this synergy occurred at AVA plasma concentrations achievable in patients taking 80 mg of AVA daily (0.1 to 0.5 μM) (2). Furthermore, the doses of AVA administered to humans could be increased to 120 mg daily with only limited additional side effects (9). A dose of 120

TABLE 1. *In vitro* susceptibilities of 13 *P. falciparum* strains to AVA alone, DHA alone, and combinations of DHA and different concentrations of AVA

Strain or parameter	IC_{50}				
	AVA alone (μM)	DHA alone (nM)	DHA + 0.1 μM AVA (nM)	DHA + 0.5 μM AVA (nM)	DHA + 1.0 μM AVA (nM)
3D7	9.5	2.50	1.97	1.55	1.42
PA	8.1	1.30	1.25	1.10	1.04
FCR3	8.9	1.62	1.23	1.13	0.97
FCM29	6.7	1.15	1.05	0.89	0.74
W2	5.5	2.83	2.75	2.52	2.38
IMT K2	9.4	1.05	0.95	0.88	0.78
IMT K14	7.5	2.07	1.65	1.58	1.41
IMT L1	8.2	0.92	0.92	0.85	0.78
IMT Vol	8.4	1.10	1.07	0.90	0.74
IMT 31	6.1	1.70	1.46	1.36	1.21
IMT 9881	7.4	3.45	3.31	3.21	3.07
IMT 10500	6.5	3.33	3.26	2.98	2.53
IMT 16332	6.0	0.94	0.82	0.85	0.65
Geometric mean IC_{50} (95% CI)	7.45 (6.74–8.22)	1.65 (1.24–2.21)	1.49 (1.11–1.99)	1.35 (1.01–1.81)	1.19 (0.87–1.63)
Avg % IC_{50} diminution (95%CI)			10 (5–15)	18 (14–22)	28 (24–32)
P value (vs DHA alone)			<0.001	<0.001	<0.001

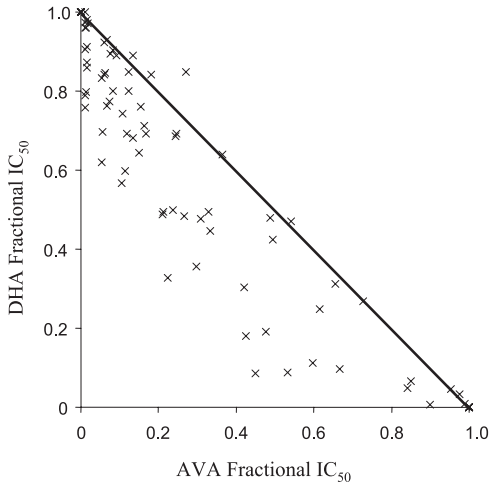

FIG. 1. *In vitro* synergy of AVA with DHA against 13 strains of *P. falciparum*.

mg of AVA increased the maximal plasma concentration (C_{max}) after oral administration by 4- to 10-fold. Cerebral malaria has pathophysiological features in common with sepsis, especially with regard to the pathology of the endothelium (4), and critically ill patients with sepsis had a significantly higher C_{max} than healthy volunteers (110.5 versus 5.9 ng/ml) (7).

Artemisinin-based combination therapy is beginning to fail in southern Cambodia and Thailand, particularly the mefloquine-artesunate drug combination (3, 11, 12). AVA could improve the activity of artemisinin derivatives.

All of these observations support calls for both an *in vivo* evaluation with a pharmacokinetic component and clinical trials of AVA as an antimalarial therapy.

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Hélène Savini
Service de Maladies Infectieuses
Hôpital d'Instruction des Armées Laveran
Unité de Recherche en Biologie et Epidémiologie Parasitaires
Unité mixte de recherche 6236
Institut de Recherche Biomédicale des Armées—Antenne de Marseille
Marseille, France

Jean Baptiste Souraud
Service d'Anatomie et Cytologie Pathologiques
Hôpital d'Instruction des Armées Saint-Anne Toulon
Institut de Recherche Biomédicale des Armées—Antenne de Marseille
Marseille, France

Sébastien Briolant
Eric Baret
Rémy Amalvict
Christophe Rogier
Bruno Pradines*
Unité de Recherche en Biologie et Epidémiologie Parasitaires
Unité mixte de recherche 6236
Institut de Recherche Biomédicale des Armées—Antenne de Marseille
Marseille, France

*Phone: 33 4 91 15 01 10
Fax: 33 4 91 15 01 64
E-mail: bruno.pradines@free.fr

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