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Antimalarial efficacy and drug interactions of the novel semi-synthetic endoperoxide artemisone *in vitro* and *in vivo*

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***Objectives*: The *in vitro* and *in vivo* efficacy and drug–drug interactions of the novel semi-synthetic endoperoxide artemisone with standard antimalarials were investigated in order to provide the basis for the selection of the best partner drug.**

***Methods*: Antimalarial activity and drug interactions were evaluated *in vitro* against *Plasmodium falciparum* by the incorporation of [^{3}H]hypoxanthine. *In vivo* efficacy and drug interactions were assessed using the standard 4-day Peters' test.**

***Results*: Artemisone was 10 times more potent than artesunate *in vitro* against a panel of 12 *P. falciparum* strains, independent of their susceptibility profile to antimalarial drugs, and consistently 4 to 10 times more potent than artesunate in rodent models against drug-susceptible and primaquine- or sulfadoxine/pyrimethamine-resistant *Plasmodium berghei* lines and chloroquine- or artemisinin-resistant lines of *Plasmodium yoelii*. Slight antagonistic trends were found between artemisone and chloroquine, amodiaquine, tafenoquine, atovaquone or pyrimethamine and additive to slight synergistic trends with artemisone and mefloquine, lumefantrine or quinine. Various degrees of synergy were observed *in vivo* between artemisone and mefloquine, chloroquine or clindamycin.**

***Conclusions*: These results confirm the increased efficacy of artemisone over artesunate against multidrug-resistant *P. falciparum* and provide the basis for the selection of potential partner drugs for future deployment in areas of multidrug-resistant malaria. Artemisone represents an important addition to the repertoire of artemisinin combination therapies currently in use, as it has enhanced antimalarial activity, improved bioavailability and stability over current endoperoxides.**

Keywords: *P. falciparum*, artemisinins, drug combination

Introduction

Artemisinin derivatives are the most potent and rapidly acting drugs against multidrug-resistant *Plasmodium falciparum* malaria and are currently being used in combination with other antimalarials. Artemisinin-based combination therapies are the WHO-recommended treatment policy for uncomplicated malaria[1] in countries where standard antimalarials are already ineffective due to drug resistance.[2] Artemisinins are increasingly being used in combination with standard antimalarials such as amodiaquine, sulfadoxine/pyrimethamine, mefloquine and lumefantrine, considerably reducing treatment times, the dose of artemisinins and the potential risk for selection and transmission of drug-resistant parasites.[2–5] The superior advantage of artemisinin combination therapy has been demonstrated in Thailand, where the combination of mefloquine with artesunate is effective in areas of established mefloquine resistance, considerably delaying its progression and augmenting cure rates when

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compared with mefloquine alone.[6] Efficacy trials have also shown the superiority of artemisinin combination therapy when compared with monotherapy in areas of drug-resistant malaria in Africa.[7,8] Furthermore, artesunate monotherapy therapeutic failures in non-immune individuals from Central Africa were associated with reduced *in vitro* susceptibility.[9,10] In another study in French Guiana, in which artemether-lumefantrine is the recommended policy, a reduction in the level of *in vitro* susceptibility was associated with point mutations in the sarco/endoplasmic reticulum calcium-dependent ATPase (SERCA), possibly representing the first step towards clinical resistance.[11,12] Therefore, there is a pressing need for the discovery and development of novel, cheaper alternatives, including new artemisinin derivatives with efficacy against artemisinin-resistant parasites.

Artemisone is a new semi-synthetic 10-alkylaminoartemisinin that can be synthesized from dihydroartemisinin in a one-step process.[13] Artemisone shows increased antiplasmodial activity, improved *in vivo* half-life, improved oral bioavailability and metabolic stability, no neurotoxicity[14,15] and is well tolerated in humans,[16] with a curative effect at dose levels at least half those of artesunate in Phase IIa trials in comparison with artesunate.[17] Thus, it is of particular urgency to assess the effect of combination of artemisone with other antimalarials.

As cheaper and more effective artemisinin derivatives are potentially incorporated into artemisinin combination therapy for the treatment of uncomplicated malaria, the aim of this study was to confirm that artemisone showed comparable or better activity than other members of its class and to provide the basis for the selection of the best partner drug for use as part of artemisinin combination therapy.

Materials and methods

Drugs

The following drugs were used: chloroquine diphosphate, quinine-HCl, pyrimethamine-HCl, amodiaquine, clindamycin and mefloquine-HCl (Sigma, Dorset, UK); tafenoquine and atovaquone (GSK, UK); lumefantrine (Novartis Pharma, Basel, Switzerland); artemisone (Bayer AG, Leverkusen, Germany) and artesunate (WHO/TDR, Geneva, Switzerland).

P. falciparum in vitro culture

All parasite clones, isolates and strains were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, VA, USA). Strains/isolates used in this study were: the drug-susceptible 3D7 clone of the NF54 isolate (unknown origin); the drug-susceptible strains FCR-8 (Gambia) and FCC2 (China); the chloroquine-resistant, pyrimethamine-resistant and cycloguanil-resistant K1 strain (Thailand); the chloroquine-resistant and pyrimethamine-resistant FCR3 strain (Gambia); the chloroquine-resistant FCB strain (Colombia); and the chloroquine-resistant, pyrimethamine-resistant and cycloguanil-resistant isolate Tm90C2A (Thailand). *In vitro* culture of *P. falciparum* was carried out following standard methods[18] with modifications as described previously.[19]

In vitro parasite growth inhibition assays and in vitro drug–drug interactions

In vitro parasite growth inhibition was assessed by the incorporation of [^{3}H]hypoxanthine based on the method used by Desjardins *et al.*[20] and modified as described previously.[19] *In vitro* interactions were examined by a modified fixed ratio method as described previously.[21] Stock drug solutions were prepared in 100% dimethylsulphoxide (DMSO) (Sigma) except for chloroquine which was dissolved at 10 mg/mL in distilled water. Amodiaquine, mefloquine, tafenoquine, quinine and pyrimethamine stocks were prepared at 10 mg/mL, artesunate and artemisone stocks freshly prepared at 10 mg/mL and atovaquone and lumefantrine stocks at 1 mg/mL. Artemisone (Drug A) plus second test drug (Drug B) solutions were prepared in assay medium at ratios of 5:0, 4:1, 3:2, 2:3, 1:4 and 0:5 followed by 2-fold serial dilutions in assay medium of each ratio, allowing the IC_{50} to fall approximately at the mid-point of the serial dilution of each drug alone. Fifty microlitres of *P. falciparum* (65–75% ring stage) culture at 0.5% parasitaemia or uninfected red blood cells (URBCs) was added to each well, reaching a final volume of 100 μL per well, a final haematocrit of 2.5% and final DMSO concentrations between 0.01 and 0.1%. Plates were incubated at 37°C in a 5% CO_2/95% air mixture for 24 h, at which point 20 μL (0.1 μCi/well) of [^{3}H]hypoxanthine (Perkin Elmer, Hounslow, UK) was added to each well and returned to the incubator for an additional 24 h incubation period, at which point the experiment was terminated by placing the plates in a −80°C freezer. Plates were thawed and harvested onto glass fibre filter mats using a 96-well cell harvester (Harvester 96^{TM}; Tomtec, Oxon, UK) and left to dry. After the addition of MeltiLex^{TM} solid scintillant (Perkin Elmer), the incorporated radioactivity was counted using a Wallac® 1450 Betalux scintillation counter (Wallac®). Data acquired by the Wallac® BetaLux scintillation counter were exported into a MICROSOFT® EXCEL spreadsheet (Microsoft Corp.), and the IC_{50}/IC_{90} values of each drug were calculated by using XLFit® (ID Business Solutions Ltd, UK) line fitting software. A 30–50-fold difference in [^{3}H]hypoxanthine uptake between drug-untreated infected red blood cells and URBCs was considered sufficient to construct dose–response curves and to determine IC_{50} values for each drug. Activity correlations between artesunate and artemisone were analysed by Pearson correlation (r) using STATA^{TM} (StataCorp LP, USA). Statistical significance was defined as $P < 0.05$.

Data analysis of in vitro drug–drug interactions

IC_{50} values were used to calculate $FIC_{50/90}$s for each drug ratio fractional inhibitory concentration (FIC), as described previously,[21–24] $\sum FIC_{50/90}$s of artemisone or $\sum FIC_{50/90}$s of artesunate with standard antimalarials were calculated by the following equation and represented as isobolograms:

$$\sum \mathrm{FIC}_{50/90} = \left(\frac{\mathrm{IC}_{50/90} \text{ of Drug A in combination}}{\mathrm{IC}_{50/90} \text{ of Drug A alone}}\right) + \left(\frac{\mathrm{IC}_{50/90} \text{ of Drug B in combination}}{\mathrm{IC}_{50/90} \text{ of Drug B alone}}\right)$$

An overall mean $\sum FIC_{50}$ or $\sum FIC_{90}$ value for each combination was determined and synergy or antagonism defined

as a mean $\sum$FIC $<$ or $>$ 1, respectively, as reviewed by Bell.[25] Lack of interaction or 'additivity' was defined as $\sum$FICs = 1.

Full suppressive 4-day Peters' test

In vivo tests were performed under the Home Office Animals (Scientific Procedures) Act 1986. The rodent malaria lines used were *Plasmodium berghei* NY (drug-susceptible), *P. berghei* P (primaquine-resistant), *P. berghei* KFY (sulfadoxine/pyrimethamine-resistant), *Plasmodium yoelii* NS (chloroquine-resistant), *P. yoelii* ART (artemisinin-resistant) and *Plasmodium chabaudi* AS (drug-susceptible). Swiss outbred 20 g male Tuck Farmed White (TFW) albino mice (A. Tuck and Son, Rayleigh, Essex, UK) were kept in specific pathogen-free conditions and fed *ad libitum* with SDS RM3 expanded diet (supplied by Special Diet Services, Witham, Essex, UK). For subcutaneous administration, artemisone and artesunate were dissolved in 10% DMSO/0.05% Tween 80 (Sigma) in distilled water. For oral administration, compounds were dissolved in standard suspending formula [0.5% sodium carboxymethylcellulose/0.5% benzyl alcohol/0.4% Tween 80/0.9% NaCl (all Sigma)]. Mice were infected intravenously with 2×10^6 infected red cells and treated subcutaneously or orally with 0.2 mL of a solution of the test compounds 2 h (day 0) and on days 1, 2 and 3 post-infection. Parasitaemia was determined by microscopic examination of Giemsa-stained blood films taken on day 4. Microscopic counts of blood films from each mouse were processed using GraphPad Prism 4 (GraphPad Software, Inc., CA, USA) and expressed as percentages of inhibition from the arithmetic mean parasitaemias of each group in relation to the untreated group. Dose–response curves were obtained and ED_{50} and ED_{90} values calculated. The degree of cross-resistance was determined by comparing the activity in the parent and resistant lines using the following formula:

$$\text{Index of cross-resistance}(I_{50/90}) = \frac{(ED_{50}/ED_{90}\text{ resistant line})}{(ED_{50}/ED_{90}\text{ parent line})}$$

Differences in ED_{50}/ED_{90} values between treatment groups were analysed by a paired Student's *t*-test using GraphPad Prism 4 (GraphPad Software, Inc.) and differences considered significant if $P < 0.05$. Activity correlations between artesunate and artemisone at the ED_{50} and ED_{90} levels were analysed by Pearson correlation (*r*) using STATA™ (StataCorp LP). Statistical significance was defined as $P < 0.05$.

In vivo drug–drug interaction study

Drug interactions of artemisone with mefloquine, chloroquine and clindamycin were investigated in *P. berghei* NY (drug-susceptible), *P. berghei* N1100 (mefloquine-resistant) and *P. yoelii* NS (chloroquine-resistant) lines. Interactions were analysed by the chequerboard method and the dose range for each compound was selected to give a range from an inactive dose to the ED_{90}. The ED_{90} values obtained with the combination were compared with those of the individual compounds to obtain an isobolar equivalent (IE):

$$\text{IE} = \frac{ED_{90}\text{ drug combination}}{ED_{90}\text{ drug alone}}$$

IEs were plotted in isobolograms to visualize the presence of synergism, antagonism or no interaction. Synergy or antagonism was defined when the value of the IE was below or above the line of additivity, respectively.

Results

In vitro anti-P. falciparum activity

Artemisone was found to be approximately 10 times more active than artesunate against all the *P. falciparum* strains tested (Figure 1 and Table 1). The IC_{50} values of artemisone were comparable across the 12 *P. falciparum* strains, independently of their drug-susceptibility profile, showing mean IC_{50} values of 0.83 nM (95% CI: 0.62–1.04). Activity correlations between the IC_{50}s of artesunate and artemisone showed no significant relationship across the 12 strains tested ($r^2 = 0.13$, $P = 0.25$).

In vitro drug–drug interactions

The isobolograms obtained with the susceptible 3D7 clone and drug-resistant K1 strain at the IC_{50} level are shown in Figure 2. There were slight antagonistic trends between artemisone and chloroquine, amodiaquine, tafenoquine, atovaquone or pyrimethamine. Additive to slight synergistic interactions were seen with artemisone and mefloquine, lumefantrine or quinine. Isobolograms at the IC_{90} levels are shown as Supplementary data [Figure S1, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)] and confirm the trends seen at the IC_{50} level. In general, a similar profile of interactions was observed between artemisone and artesunate with the antimalarial drugs tested.

In vivo blood schizontocidal activity

Artemisone was tested subcutaneously and orally using the 4-day Peters' test against the drug-susceptible (NY), the primaquine-resistant (P) and sulfadoxine/pyrimethamine-resistant (KFY) lines of *P. berghei*, the chloroquine-resistant (NS) and artemisinin-resistant (ART) lines of *P. yoelii* NS and the drug-susceptible *P. chabaudi* AS. The ED_{50} and ED_{90} values are summarized in Table 2, where the resistance factor (I_{90}) is included to compare the ED_{90} values of the compounds against resistant strains with those found against the parent strain. When compared with artesunate, artemisone was around 4- and 10-fold more effective at suppressing the parasitaemia in the *P. berghei* NY susceptible strain by the subcutaneous route (artemisone $ED_{90} = 9.62$ and artesunate $ED_{90} = 41.21$ mg/kg; $P < 0.001$) and oral route (artemisone $ED_{90} = 11.67$ and artesunate $ED_{90} = 111.94$ mg/kg; $P < 0.001$), respectively. Similarly, artemisone was 9- and 6-fold more effective than artesunate against *P. berghei* P (artemisone $ED_{90} = 1.92$ and artesunate $ED_{90} = 18.20$ mg/kg; $P = 0.043$) and *P. berghei* KFY (artemisone $ED_{90} = 0.83$ and artesunate $ED_{90} = 5.38$ mg/kg; $P < 0.001$), respectively. In *P. yoelii* NS, artemisone was 4- and 6-fold more effective than artesunate by the subcutaneous route (artemisone $ED_{90} = 11.3$ and artesunate $ED_{90} = 49.54$ mg/kg; $P = 0.001$) and oral route (artemisone $ED_{90} = 27.99$ and artesunate $ED_{90} = 179.47$ mg/kg); $P < 0.001$), respectively. In *P. chabaudi* AS, artemisone was 14-fold more effective than

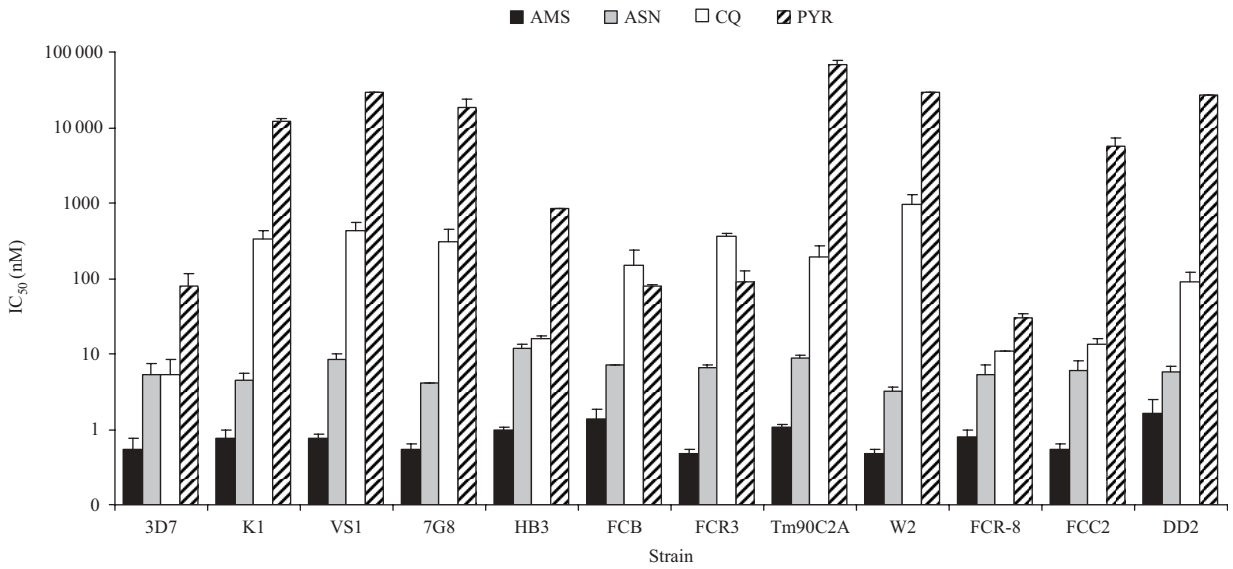

Figure 1. Antimalarial activity of artemisone (AMS) *in vitro* against 12 *P. falciparum* strains. The mean IC_{50}s values from three independent experiments are shown. Chloroquine (CQ), artesunate (ASN) and pyrimethamine (PYR) are included as controls. 3D7, drug-susceptible clone of the NF54 isolate (unknown origin); FCR-8 (Gambia) and FCC2 (China), drug-susceptible; K1 (Thailand), CQ-resistant/PYR-resistant; VS1 strain (Vietnam), CQ-resistant/PYR-resistant/cycloguanil-resistant; 7G8 (Brazil), CQ-resistant/PYR-resistant; HB3-2 (Honduras), CQ-susceptible/PYR-resistant; FCB (Colombia), CQ-resistant; FCR3 (Gambia), CQ-resistant/PYR-resistant; Tm90C2A (Thailand), CQ-resistant/PYR-resistant/mefloquine-resistant; W2 (Indochina), CQ-resistant/PYR-resistant; DD2 (clone from W2), CQ-resistant/PYR-resistant. Values represent the mean $IC_{50} \pm$ SD (nM) from two to three independent experiments performed in triplicate.

artesunate (artemisone $ED_{90} = 1.38$ and artesunate $ED_{90} = 19.68$ mg/kg; $P < 0.001$). Most importantly, artemisone showed 7-fold greater activity than artesunate (artemisone $ED_{90} = 12.13$ and artesunate $ED_{90} = 87.50$ mg/kg; $P = 0.004$) against the *P. yoelii* artemisinin-resistant line. Activity correlations between the ED_{90}s obtained after subcutaneous administration of artesunate and artemisone across the rodent lines tested showed some degree of cross-susceptibility ($r^2 = 0.8$, $P = 0.015$) not seen at the ED_{50} level ($r^2 < 0.001$, $P = 0.99$). However, artemisone showed consistent superior efficacy against drug-resistant lines when compared with artesunate.

Table 1. *In vitro* anti-*P. falciparum* activity of artemisone and standard antimalarial drugs

	P. falciparum mean $IC_{50} \pm$ SD (nM)[a]	
Drug	3D7	K1
CQ	9.73 ± 5.11	255.86 ± 65.58
AMQ	20.3 ± 2.18	40.89 ± 10.27
TFQ	399.45 ± 51.16	266.45 ± 7.63
MFQ	6.11 ± 3.89	1.41 ± 0.69
QN	102.34 ± 31.56	262.50 ± 65.47
LFT	7.46 ± 1.75	2.56 ± 0.01
PYR[b]	78.4 ± 35.47	$11.92 \pm 1.16 \times 10^3$
ATQ	1.11 ± 0.79	2.32 ± 1.35
ASN	9.44 ± 5.81	5.18 ± 2.49
AMS	0.88 ± 0.59	1.23 ± 0.64

CQ, chloroquine; AMQ, amodiaquine; TFQ, tafenoquine; MFQ, mefloquine; QN, quinine; LFT, lumefantrine; PYR, pyrimethamine; ATQ, atovaquone; ASN, artesunate; AMS, artemisone.
[a]Results shown are the mean IC_{50} values and SD from at least three independent experiments.
[b]Pyrimethamine was tested in standard assay medium containing 1 mg/L folic acid and 1 mg/L *para*-amino benzoic acid.

In vivo drug–drug interactions

In order to complement the information obtained *in vitro*, drug interactions of artemisone with some standard antimalarials were examined *in vivo.* Artemisone in combination with mefloquine against the drug-susceptible *P. berghei* NY and the mefloquine-resistant *P. berghei* N1100 lines showed a synergistic effect against both resistant and susceptible parasites (Figure 3a and b). When combined with chloroquine, no interaction against the drug-susceptible *P. berghei* NY was observed, but a synergistic effect against the chloroquine-resistant line *P. yoelii* NS was observed (Figure 4a and b). Artemisone in combination with clindamycin showed an additive to weak synergistic effect against *P. berghei* NY (Figure 5).

Discussion

In this study, we have demonstrated the superior efficacy of artemisone over artesunate against a broad range of *P. falciparum* strains with different antimalarial drug-susceptibility profiles and geographical origins. Although some degree of cross-susceptibility was observed between artesunate and artemisone *in vivo*, the efficacy profile against drug-resistant rodent malaria line highlights the potential of artemisone for use in areas of multidrug-resistant *P. falciparum* malaria. The enhanced potency of artemisone over artesunate against *P. berghei* NY (between 4- and 10-fold) correlates with the findings obtained in

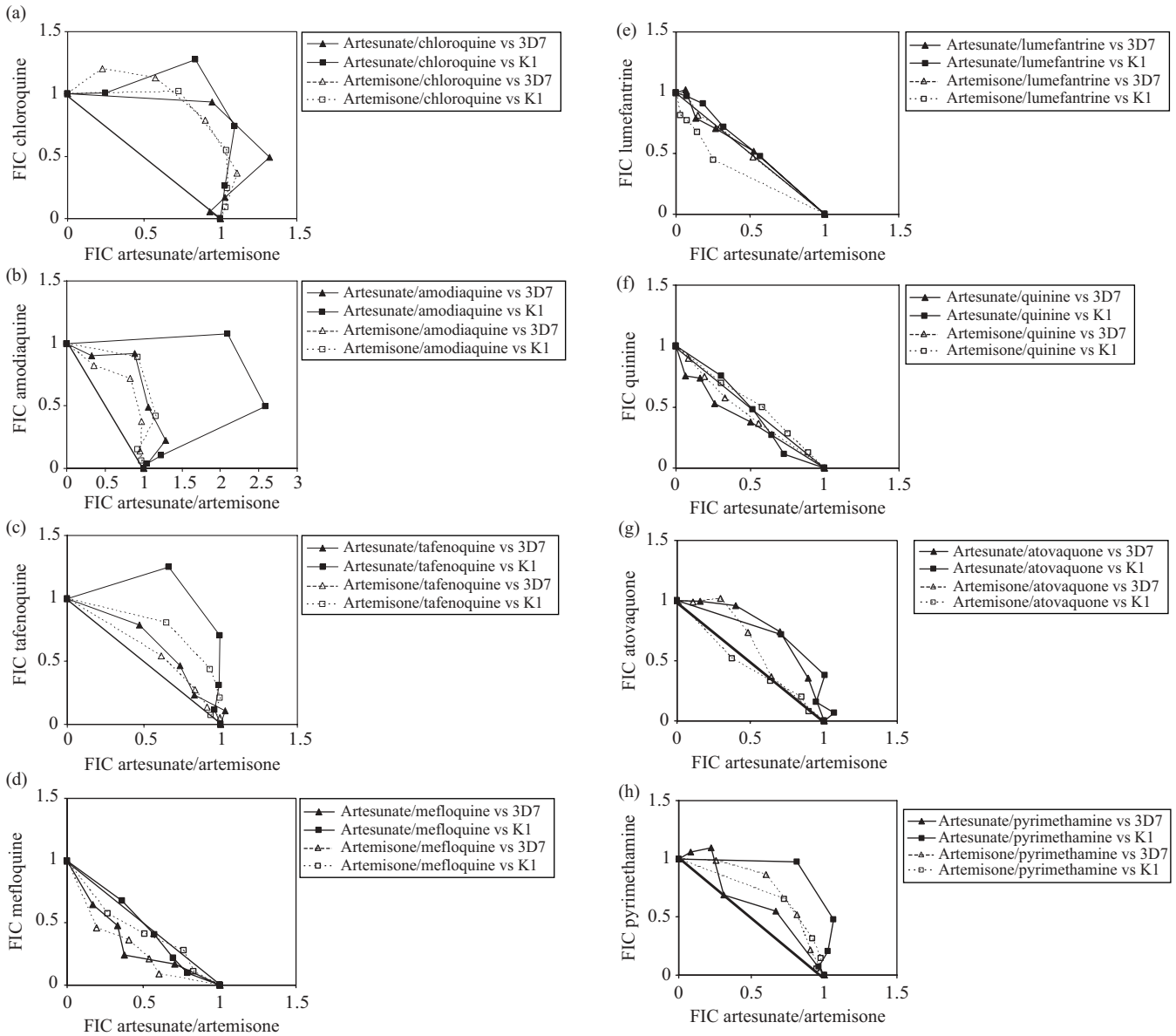

Figure 2. Isobolograms showing *in vitro* interactions at the IC_{50} level between artesunate or artemisone with chloroquine, amodiaquine, tafenoquine, mefloquine, lumefantrine, quinine, atovaquone and pyrimethamine against drug-susceptible 3D7 and drug-resistant K1 strain.

an *in vitro Xenopus laevis* oocyte expression system, in which *P. berghei* SERCA, a primary target for artemisinins, was 36-fold less susceptible to artemisinin than to artemisone.[26]

Artemisone and artesunate showed a similar pattern of interactions with standard antimalarials. The slightly antagonistic trend between artemisone and chloroquine, atovaquone or pyrimethamine, and the synergism between artemisone and mefloquine or lumefantrine *in vitro* concur with previous data obtained with other artemisinins.[21,23,27–29] In our studies, the interaction of artemisone with lumefantrine was slightly synergistic against the drug-resistant K1 strain. We observed slight antagonism between artemisone or artesunate and tafenoquine against 3D7 and K1 strains, which contrasts with the synergistic effect between tafenoquine and artemisinin against multidrug-resistant isolates reported previously.[30] Similar contrasting results were found with artemisone and artesunate plus amodiaquine, in which antagonism was observed, despite a synergistic interaction between artemisinin and amodiaquine reported previously.[31] These differences may be related to different drug ratios used or varying degrees of drug susceptibility of *P. falciparum* strains or isolates used in each study.

The synergistic effects observed *in vitro* with artemisone and mefloquine were reproduced *in vivo* and were similar to those reported previously for artesunate in combination with mefloquine.[32] The combination of artemisone with chloroquine and with clindamycin had a synergistic effect *in vivo*, particularly with chloroquine against the chloroquine-resistant *P. yoelii* NS and less apparent with clindamycin in *P. berghei* NY. It is likely that the differences observed between the nature of the *in vitro* and *in vivo* interactions are related to the pharmacokinetic and metabolic components of the *in vivo* systems. Mild antagonism *in vitro* does not justify the rejection of a drug combination, particularly where one of the drugs is rapidly eliminated *in vivo*.[33]

Table 2. Summary of *in vivo* activity of artemisone (AMS) and artesunate (ASN) against drug-susceptible and drug-resistant rodent malaria lines in a 4-day Peters' test

		AMS			ASN		
Line	Route	ED_{50} (95% CI) mg/kg	ED_{90} (95% CI) mg/kg	I_{90}	ED_{50} (95% CI) mg/kg	ED_{90} (95% CI) mg/kg	I_{90}
P. berghei NY	sc	1.24 (0.92–1.66)	9.62 (4.81–19.23)	1.0	7.80 (5.35–11.35)	41.21 (17.66–96.16)	1.0
	po	2.12 (1.71–2.61)	11.67 (7.11–19.10)	1.0	12.66 (9.25–17.32)	111.94 (52.36–238.78)	1.0
P. berghei P	sc	0.63 (0.54–0.72)	1.92 (1.46–2.53)	0.2	21.65 (1.19–2.31)	18.20 (8.44–39.26)	0.4
P. berghei KFY	sc	0.22 (0.18–0.28)	0.83 (0.55–1.26)	0.1	0.70 (0.40–1.23)	5.38 (2.27–12.76)	0.1
P. yoelii NS	sc	1.28 (1.02–1.60)	11.30 (6.56–19.50)	1.0	2.21 (1.31–3.73)	49.54 (13.18–186.21)	1.0
	po	2.34 (1.76–3.12)	27.99 (13.96–56.23)	1.0	12.97 (9.02–18.65)	179.47 (69.66–461.32)	1.0
P. yoelii ART	sc	1.14 (0.81–1.61)	12.13 (5.16–28.64)	1.1	2.14 (1.53–3.00)	87.50 (35.48–271.64)	1.8
P. chabaudi AS	sc	0.42 (0.38–0.47)	1.38 (1.06–1.78)	1.0	0.69 (0.41–1.17)	19.68 (8.58–44.88)	1.0

sc, subcutaneous route; po, oral route. *P. berghei* NY, drug-susceptible; *P. berghei* P, primaquine-resistant; *P. berghei* KFY, sulfadoxine/pyrimethamine-resistant; *P. yoelii* NS, chloroquine-resistant; *P. yoelii* ART, artemisinin-resistant; *P. chabaudi* AS, drug-susceptible. $I_{90} = ED_{90}$ resistant line/ED_{90} parent line.

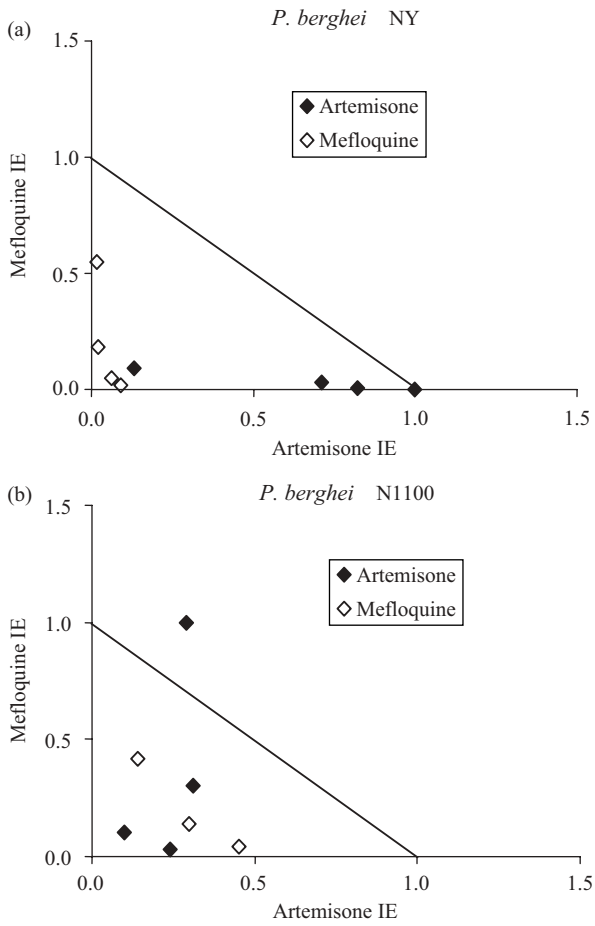

Figure 3. Isobologram illustrating the interaction between artemisone (subcutaneously) and mefloquine (orally) against (a) *P. berghei* NY drug-susceptible strain and (b) *P. berghei* N1100 mefloquine-resistant line. Points shown represent the IE for each drug. The range of doses used for artemisone and mefloquine was 3, 1, 0.3 and 0.1 mg/kg.

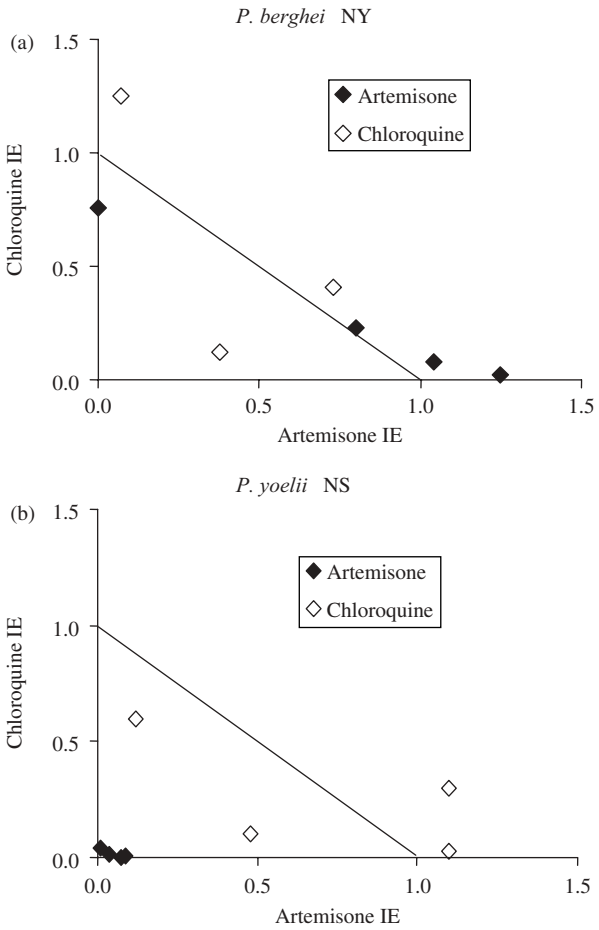

Figure 4. Isobologram illustrating the interaction between artemisone (subcutaneously) and chloroquine (subcutaneously) against (a) *P. berghei* NY drug-susceptible strain and (b) *P. yoelii* NS chloroquine-resistant strain. Points shown represent the IE for each drug. The range of doses used for artemisone was 3, 1, 0.3 and 0.1 mg/kg and that for chloroquine was 60, 30, 10 and 3 mg/kg.

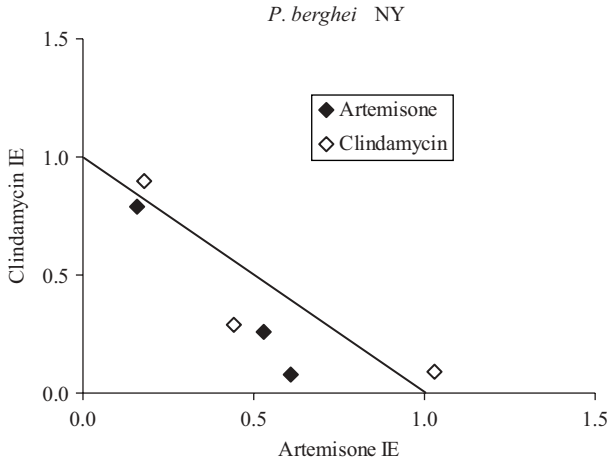

Figure 5. *In vivo* interactions against *P. berghei* NY drug-susceptible strain of artemisone and clindamycin (subcutaneously). Points shown represent the IE for each drug. The range of doses used for artemisone was 3, 1, 0.3 and 0.1 mg/kg and that for clindamycin was 100, 30, 10 and 3 mg/kg.

In summary, these results suggest that artemisone may prove useful in areas of multidrug-resistant *P. falciparum* malaria and will be an important addition to the repertoire of artemisinin derivatives already in use or in development, such as the new trioxolanes.[34] The enhanced antimalarial activity of artemisone will result in low-dose treatment regimens, a reduction in costs and requirement for artemisinin as a natural source with the possibility of using a long-acting blood schizontocide such as mefloquine as partner drug. Artemisone, unlike the carboxylic acid artesunate, should also be compatible with amodiaquine and other basic quinoline antimalarials in fixed formulation combinations. In the light of recently reported reduced *in vitro* susceptibility of human isolates to artemisinins,[11] the superior efficacy of artemisone when compared with artesunate against a *P. yoelii* artemisinin-resistant line underlines the potential that artemisone may have to combat artemisinin-resistant *P. falciparum* in the future.

Further investigation of the effect of artemisone against *P. falciparum* sexual and liver stages, and other *Plasmodium* species is relevant to assess its potential in transmission reduction, prophylaxis and efficacy in areas with a high prevalence of mixed infections.

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Transparency declarations

The authors declare no conflict of interest. All the authors have no interest in the form of stocks and shares in a company, which might be financially affected by the conclusions of this article. The development of artemisone has been supported by Bayer AG under a non-profit initiative.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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