Antimalarial activity of artefenomel (OZ439), a novel synthetic antimalarial endoperoxide, in patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria: an open-label phase 2 trial

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Summary

Background Artefenomel (OZ439) is a novel synthetic trioxolane with improved pharmacokinetic properties compared with other antimalarial drugs with the artemisinin pharmacophore. Artefenomel has been generally well tolerated in volunteers at doses up to 1600 mg and is being developed as a partner drug in an antimalarial combination treatment. We investigated the efficacy, tolerability, and pharmacokinetics of artefenomel at different doses in patients with *Plasmodium falciparum* or *Plasmodium vivax* malaria.

Methods This phase 2a exploratory, open-label trial was done at the Hospital for Tropical Diseases, Bangkok, and the Shoklo Malaria Research Unit in Thailand. Adult patients with acute, uncomplicated *P falciparum* or *P vivax* malaria received artefenomel in a single oral dose (200 mg, 400 mg, 800 mg, or 1200 mg). The first cohort received 800 mg. Testing of a new dose of artefenomel in a patient cohort was decided on after safety and efficacy assessment of the preceding cohort. The primary endpoint was the natural log parasite reduction per 24 h. Definitive oral treatment was given at 36 h. This trial is registered with ClinicalTrials.gov, number NCT01213966.

Findings Between Oct 24, 2010, and May 25, 2012, 82 patients were enrolled (20 in each of the 200 mg, 400 mg, and 800 mg cohorts, and 21 in the 1200 mg cohort). One patient withdrew consent (before the administration of artefenomel) but there were no further dropouts. The parasite reduction rates per 24 h ranged from 0·90 to 1·88 for *P falciparum*, and 2·09 to 2·53 for *P vivax*. All doses were equally effective in both *P falciparum* and *P vivax* malaria, with median parasite clearance half-lives of 4·1 h (range 1·3–6·7) to 5·6 h (2·0–8·5) for *P falciparum* and 2·3 h (1·2–3·9) to 3·2 h (0·9–15·0) for *P vivax*. Maximum plasma concentrations, dose-proportional to 800 mg, occurred at 4 h (median). The estimated elimination half-life was 46–62 h. No serious drug-related adverse effects were reported; other adverse effects were generally mild and reversible, with the highest number in the 1200 mg cohort (17 [81%] patients with at least one adverse event). The most frequently reported adverse effect was an asymptomatic increase in plasma creatine phosphokinase concentration (200 mg, n=5; 400 mg, n=3; 800 mg, n=1; 1200 mg, n=3).

Interpretation Artefenomel is a new synthetic antimalarial peroxide with a good safety profile that clears parasitaemia rapidly in both *P falciparum* and *P vivax* malaria. Its long half-life suggests a possible use in a single-dose treatment in combination with other drugs.

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Introduction

Malaria is the most important parasitic disease in people, and a major cause of morbidity and mortality in tropical regions. WHO has declared malaria control a global development priority and has changed its focus from containment and control to elimination.[1] Drug resistance in *Plasmodium* species poses a major obstacle. Resistance in *Plasmodium falciparum*, the main cause of malarial death, has rendered several first-line antimalarial drugs (first chloroquine, then sulfadoxine-pyrimethamine, and in some areas amodiaquine) largely ineffective. Since 2005, WHO has recommended artemisinin-based combination therapy as the first-line treatment for falciparum malaria.[2] Artemisinin and its derivatives are the most potent and rapidly acting antimalarial drugs available.[3] The peroxidic pharmacophore is essential for their activity.[4] The antimalarial activities of artemisinin derivatives are characterised by high parasite killing rates and broad-stage specificity of antimalarial action. These antimalarial drugs produce more rapid clinical and parasitological responses than other classes of available drugs.[5] However, they are eliminated rapidly (half-lives <1 h), which necessitated a treatment course of 7 days when they were given alone for falciparum malaria. Combination with a more slowly eliminated partner drug has allowed for 3-day courses of artemisinin-based

Research in context

Evidence before this study

Despite progress made during the past few decades, WHO reported in 2014 that malaria kills more than half a million patients per year (2013 data), and parasite resistance against the latest class of antimalarial drugs, the artemisinins, is spreading. New antimalarial drugs are needed to drive the elimination of malaria.

We searched MEDLINE, the Medicines for Malaria Venture website (http://www.mmv.org), the US National Institutes of Health and Australian/New Zealand (ANZCTR) trial registries, and the Cortellis (Thomson Reuters) database for earlier work on artefenomel (OZ439) and similar compounds, with the keywords "artefenomel", "OZ277", "arterolane", "Synriam", "ozonides", "trioxolane", and "synthetic endoperoxides" to search for publications, trial registrations, and other activities by mid-July 2015.

Artefenomel is a new, fast-acting inhibitor of all asexual erythrocytic *Plasmodium falciparum* stages associated with malaria, and has an in-vitro potency that is similar to clinically used artemisinin derivatives. Its intrinsically unstable peroxide pharmacophore was optimised, resulting in slower elimination compared with artemisinin derivatives and the first-generation ozonide arterolane (OZ277). Two phase 1 human volunteer studies have shown that oral doses of 50 mg, 100 mg, 200 mg, 400 mg, 800 mg, 1200 mg, and 1600 mg artefenomel are well tolerated, and suggest a good safety profile for the compound.

Added value of this study

To our knowledge, this study is the first to assess the antimalarial activity of artefenomel in patients with malaria. Our study assessed the safety and efficacy of artefenomel before administration of the definitive oral antimalarial treatment. Our findings show that this synthetic peroxide provides rapid parasite clearance in both falciparum and vivax malaria. We have established the pharmacokinetic parameters that will guide optimum dosing and the choice of appropriate partner drugs for antimalarial combination therapy. A retrospective genetic analysis of the malaria parasites in this study suggests that artefenomel-mediated clearance is not substantially affected by mutations known to confer partial resistance against artemisinins.

Implications of all the available evidence

Our data show that artefenomel has a good pharmacokinetic and safety profile, with antimalarial efficacy against both *P falciparum* and *Plasmodium vivax*, clearing the path for further (ongoing) clinical studies. Artefenomel has a longer half-life than other antimalarial endoperoxides, with potential for a single-dose malaria cure when used with a partner drug. Clinical trials in which artefenomel is combined with ferroquine (SSR97193), piperaquine, or DSM265 are being planned or in progress.

combination therapy, improved treatment outcomes, and enhanced patient adherence.[6,7]

WHO recommends that new antimalarial drugs should be deployed as combination treatments to provide mutual protection against resistance.[2,8] Unfortunately, artemisinin resistance in *P falciparum* has emerged, and has now spread in southeast Asia, slowing therapeutic responses, increasing treatment failure rates, and so jeopardising regional control and elimination efforts.[9–11] This situation emphasises the urgent need for new antimalarial drugs if current control and elimination initiatives are to be sustained.[12]

Trioxolanes are synthetic antimalarial drugs with a similar peroxide pharmacophore to the artemisinins.[13] The first trioxolane, arterolane (RBx11160/OZ277),[14,15] proved to be well tolerated in people.[16–19] Arterolane has been developed and registered in India in combination with piperaquine and was recently approved in seven African countries. The combination has proved highly effective in a limited number of clinical trials.[20] Artefenomel (OZ439) is the second synthetic trioxolane to advance to clinical candidate selection (appendix p 14). In a phase 1 study, oral artefenomel (given at doses of 50, 100, 200, 400, 800, 1200, and 1600 mg) was generally well tolerated.[21] The good oral bioavailability, slow clearance, and increased metabolic stability of artefenomel might allow for single-dose cure in combination with a suitable partner drug.[22] A separate phase 1 study found that piperaquine, a potential partner drug, but not artefenomel, prolonged the QTc interval in volunteers.[23] We undertook a dose evaluation of artefenomel in acute malaria, which assessed parasite clearance rate as a measure of antimalarial activity.

See Online for appendix

Methods

Study design and participants

This phase 2a exploratory, open-label assessment of single-dose artefenomel in adult patients with acute uncomplicated *P falciparum* or *Plasmodium vivax* malaria was done at the Hospital for Tropical Diseases, Bangkok, Thailand, and the Shoklo Malaria Research Unit (SMRU) on the northwestern border of Thailand. Five single oral doses of artefenomel were planned over the range 100 mg to 1600 mg in cohorts of 20 patients each: two parallel groups of ten patients with *P falciparum* and ten patients with *P vivax* malaria (figure 1). Cohort sizes allowed for adequate measurement of treatment responses.

Adult male or non-pregnant female febrile patients aged 18–60 years and weighing 40–90 kg who presented with symptomatic malaria (*P falciparum* or *P vivax* infections) and 5000–50 000 parasites per μL of blood (microscopy confirmed) were eligible, provided that they gave fully

informed written consent. Exclusion criteria were clinical or laboratory signs of severe malaria,[2] inability to tolerate oral drugs, or having received any other antimalarial treatment within 14 days before admission.

The protocol, protocol amendments, written study patient information, informed consent forms, and other appropriate study-related information were reviewed and approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The study was done in accordance with Good Clinical Practice as required by the International Conference on Harmonisation guidelines and in accordance with country-specific laws and regulations governing clinical studies of investigational products. Written, informed consent was obtained from all participants.

Procedures

On admission, patients were examined fully, blood samples were taken for full blood count and routine biochemistry, and the diagnosis of malaria was confirmed by microscopy. The patients were treated with a single oral dose of artefenomel after a small full-fat milk drink, because food has been reported to affect exposure.[21] Patients were monitored closely for the next 36 h. Definitive oral antimalarial treatment was given at 36 h, or earlier if parasitaemia showed no reduction after 12 h, or if the reduction after 24 h was lower than 75% (compared with baseline). Definitive treatment for patients with *P falciparum* malaria was mefloquine 8 mg/kg (base) plus 4 mg/kg artesunate given once daily for 3 days. Patients with *P vivax* malaria received as definitive treatment 25 mg base/kg chloroquine; primaquine (0·5 mg base/kg per day for 14 days) was given for radical cure of vivax malaria, apart from in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, who instead received 0·75 mg primaquine once weekly for 8 weeks.[2] Drugs for definitive treatment were sourced locally by the study sites. Patients were considered to have completed the study at day 7, but were reviewed again on day 30 (±2 days) to ensure cure without complications, and in case any adverse event had not resolved.

Patients were recruited sequentially to each dose cohort.[21] The first cohort received a dose of 800 mg. The decision to decrease or increase the dose (within the 100 mg to 1600 mg range) for sequential cohorts was made after a review of the safety data, drug exposure levels, and parasite clearance data for the current cohort. Parasite genotyping for *P falciparum* kelch mutations was done as described elsewhere.[24] G6PD deficiency was assessed with the fluorescent spot test.[25]

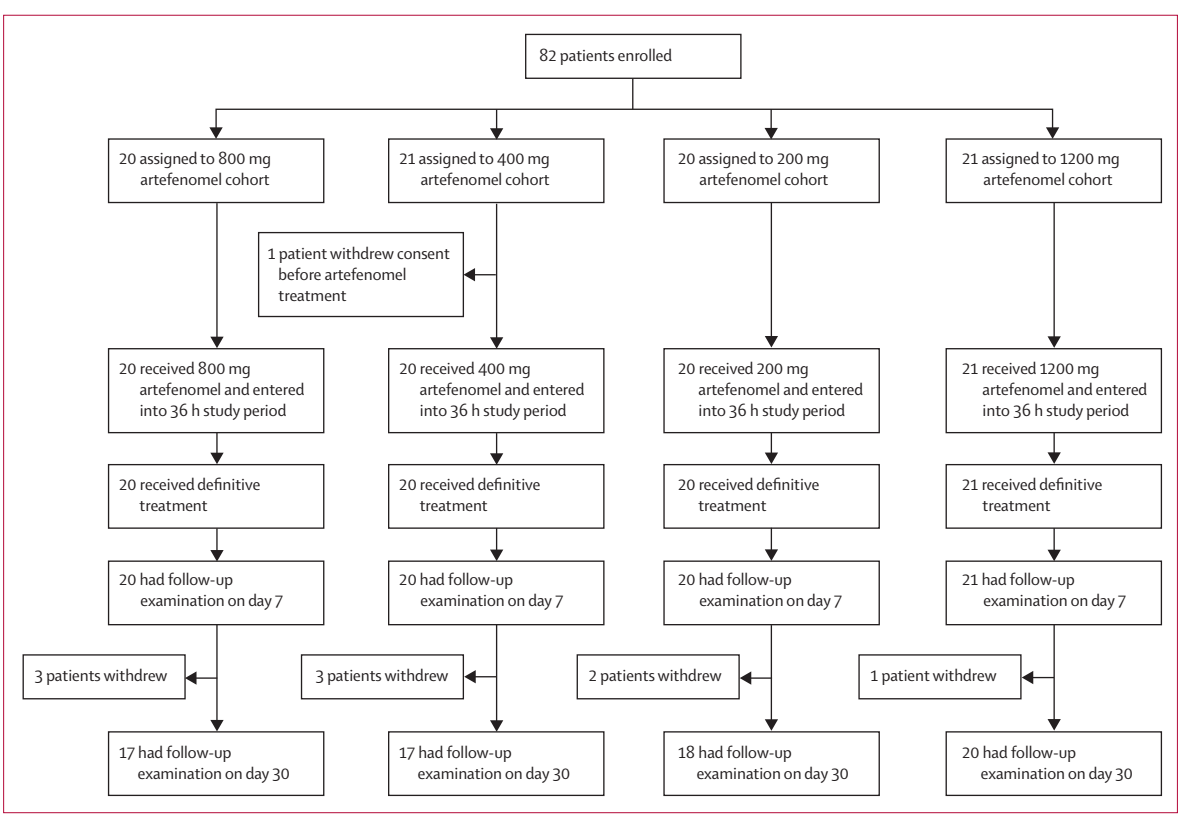

Figure 1: **Study flow diagram**
A new dose (cohort) was initiated after review of the findings from the preceding cohort. During the 36 h study period, parasitaemia, artefenomel exposure, and other variables were assessed. Definitive treatment to cure malaria involved standard drugs (mefloquine plus artesunate or chloroquine plus primaquine) and was given to avoid recrudescence. Patients infected with *Plasmodium falciparum* and *Plasmodium vivax* followed the same clinical protocols.

Artefenomel was manufactured by Unimark, India, and supplied by Penn Pharmaceuticals in glass vials containing 200 mg or 100 mg artefenomel mesylate salt stored at 2–8°C. It was administered as aqueous dispersion.

Blood samples for the assay of artefenomel and its metabolites were obtained before dosing and at 0·5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 96, and 168 h after dosing. An additional blood sample was withdrawn 4 h after starting definitive antimalarial treatment (ie, typically 40 h or 76 h after artefenomel administration). Blood samples (3 mL) were collected into glass tubes containing EDTA (edetic acid) as anticoagulant. Plasma was separated within 30 min of sample collection by centrifugation at 4°C at 1500 rpm for 15 min, and transferred into four polypropylene tubes (three replicates of about 500 mL each). The tubes were stored frozen at –70°C to –80°C until shipment to the laboratory where the analysis was done for the content of artefenomel and its metabolites by liquid chromatography–mass spectrometry.[21] The limit of quantification was 1 ng/mL for all analytes.

For **WWARN analytical procedures** see http://www.wwarn.org

	200 mg (n=20)	400 mg (n=21)	800 mg (n=20)	1200 mg (n=21)	Total (n=82)
Sex (male)	19 (95%)	17 (81%)	18 (90%)	17 (81%)	71 (87%)
Age (years)	26·7 (9·8)	29·1 (9·8)	27·2 (8·4)	29·3 (8·2)	28·1 (9·0)
Height (cm)	161·3 (5·6)	164 (8·5)	162·4 (7·4)	159·8 (6·8)	161·9 (7·2)
Bodyweight (kg)	52·4 (5·3)	53·6 (6·2)	57·1 (10·9)	51 (4·4)	53·5 (7·4)
Body-mass index (kg/m²)	20·1 (1·4)	19·9 (2·2)	21·5 (2·9)	20 (1·2)	20·4 (2·1)
Body temperature (°C)	38·1	37·9	37·6	37·6	37·8 (0·2)
Haemoglobin (g/L)	121 (15)	125 (17)	123 (14)	170 (13)	124 (89–151)
P falciparum parasites per µL (range)	15 425	16 868	42 857	20 914	24 016 (384–243 270)
P vivax parasites per µL (range)	17 369	15 455	20 675	10 118	15 905 (5010–53 400)

Data are n (%) or geometric means (SD or range). See appendix (pp 3–5) for characteristics of individual patients. All patients were of Asian origin.

Table 1: **Baseline characteristics**

	Parasite reduction rate per 24 h	Clearance rate (per h)	Slope half-life (h)	Duration of lag phase (h); number of profiles with lag	Number of valid profiles
P falciparum					
200 mg	0·90 (1·7)	0·16 (0·08–0·20)	4·24 (3·50–8·70)	6·0 (4·0–8·40); 4	6
400 mg	1·88 (0·6)	0·13 (0·08–0·35)	5·27 (1·99–9·20)	4·0 (3·90–4·0); 5	10
800 mg	1·55 (0·7)	0·17 (0·10–0·54)	4·05 (1·29–6·70)	6·0 (4·0–8·0); 2	7
1200 mg	1·85 (0·8)	0·12 (0·08–0·34)	5·59 (2·02–8·49)	6·0 (4·0–8·0); 2	11
P vivax					
200 mg	2·09 (0·4)	0·22 (0·05–0·81)	3·22 (0·86–15·0)	4·0 (4·0–8·0); 5	10
400 mg	2·20 (0·9)	0·24 (0·10–0·35)	3·10 (2·00–7·23)	8·0 (4·0–8·0); 3	10
800 mg	2·53 (0·7)	0·24 (0·17–0·34)	2·92 (2·01–4·15)	4·0 (4·0–4·0); 1	10
1200 mg	2·22 (0·5)	0·30 (0·18–0·56)	2·34 (1·24–3·88)	4·0 (4·0–4·0); 3	7

Clearance parameters, expressed as median (range), were obtained with the online WWARN Parasite Clearance Estimator calculator[26] using default settings (40 parasites per µL detection cutoff). Parasite clearance profiles were scored as valid from criteria used by the online WWARN calculator that allow meaningful parameter estimations.[26] The 24 h parasite reduction rates (SD) were calculated separately (see Methods).

Table 2: **Parasite reduction rate per 24 h and parasite clearance, by parasite and artefenomel dose cohort**

Outcomes

The primary endpoint was the natural log parasite reduction rate per 24 h. Secondary endpoints were the first-order parasite clearance rate constant and derived half-life (estimated with the online Worldwide Antimalarial Resistance Network [WWARN] toolkit), parasitaemia reduction times (50%, 80%, 90%, and 99%), parasite clearance time,[26–28] gametocytaemia reduction, and fever clearance time. Safety endpoints were incidence, severity, drug-relatedness, and seriousness of adverse events and of laboratory abnormalities. Additionally, clinically significant electrocardiogram (ECG) abnormalities were assessed including prolonged QTcF (>450 ms).

The parasite reduction per 24 h was estimated separately for each patient from a regression model. The relation between parasite counts and time was analysed by fitting a variable lag phase then a linear decline to the natural logarithm of parasite count versus time relation. The slope of this log-linear relation was the primary endpoint, and all secondary variables related to parasite reduction were derived from this best fit. The WWARN analytical procedures have been described previously.[26]

Fever clearance time was defined as the time from artefenomel administration to the first of at least two consecutive normal body temperature measurements (by axillary temperature ≤37·5°C or oral, rectal, or tympanic temperature ≤38°C) obtained within an interval of 6–24 h after artefenomel administration. Incidence of all adverse events was scored with the MedRA (latest version) primary system organ class and preferred term.[29] 12-lead ECGs were performed on day 0 pre-dose and after dosing, 2–4 h post-dose, and around 24 h after dosing, and were repeated if clinically indicated.

Statistical analysis

Efficacy and safety analyses were done by Datamap GmbH (Freiburg, Germany) for all patients who received the study drug. The primary efficacy endpoint (parasite reduction rate per 24 h) was derived from linear regression fitting $\log_{10}$-transformed parasitaemia data against their observed times (hours). Only data up to 36 h after dosing and before or equal to the start of definitive treatment were used. The timepoints chosen for the regression were those that yield the highest degree of significance when assessing the regression when the number of timepoints are greater than or equal to three. Parasite reduction rate per 24 h and slope were reported with 95% CIs and summarised when the regression fit had a p value less than or equal to 0·01 and a corresponding R^2 greater than or equal 0·85. Graphical displays of the Kaplan-Meier estimates for parasite clearance time and fever clearance

time by treatment group were provided. The relation between artefenomel exposure (area under the plasma concentration-time curve $[AUC]_{0-36}$) and selected efficacy endpoints was explored graphically; for each group the endpoint was plotted versus AUC_{0-36}, additionally showing a LOESS (local regression) fit, if feasible. This analysis was done for parasite reduction rate per 24 h, the slope, and 36 h ratio, all with linear and log-linear exposure. The WWARN Parasite Clearance Estimator calculator is described elsewhere.[26]

Pharmacokinetic analysis was done by Swiss BioQuant AG (Reinach, Switzerland). All pharmacokinetic variables (normally and log-normally distributed) were summarised by arithmetic and geometric means, minimum, median, maximum, SD, and coefficient of variation of arithmetic and geometric means. A non-linear power model was used to assess dose proportionality based on $AUC_{0-\infty}$ (defined as area under the concentration-time curve from 0 h to infinity) and C_{max} (defined as maximum or peak plasma concentration) values for artefenomel. AUCs were calculated by the linear trapezoidal method.

The assessment of dose proportionality was done with SAS/STAT software in a UNIX environment. Mean plots were generated with GraphPad Prism version 5.00. Individual plasma concentration-time profiles and non-compartmental pharmacokinetic analysis were done with Professional WinNonlin version 5.2.1. This trial is registered with ClinicalTrials.gov, number NCT01213966.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

82 patients of Thai, Karen, or Burman origin with acute uncomplicated malaria were enrolled sequentially: 20 in the 200 mg and 800 mg cohorts, and 21 in the 400 and 1200 mg cohorts (figure 1). The study took place between Oct 24, 2010 (first patient enrolled), and May 25, 2012. The first cohort was given a dose of 800 mg. The decisions on dose escalation or dose reduction for the subsequent cohorts were taken after a review of the parasite reduction, adverse event, and clinical laboratory data after each dosing cohort. The order that doses were given to cohorts was 800 mg, 400 mg, 200 mg, and finally 1200 mg. The proportions of patients with *P falciparum* or *P vivax* malaria differed between the two study sites; 15% of patients (four of 26) presenting in Bangkok were infected with *P falciparum* compared with 67% of patients (37 of 55) at the SMRU site; another SMRU patient carried a mixed infection (*P falciparum* plus *P vivax*).

One patient (400 mg cohort) withdrew consent and was not treated with artefenomel. Thus, there were

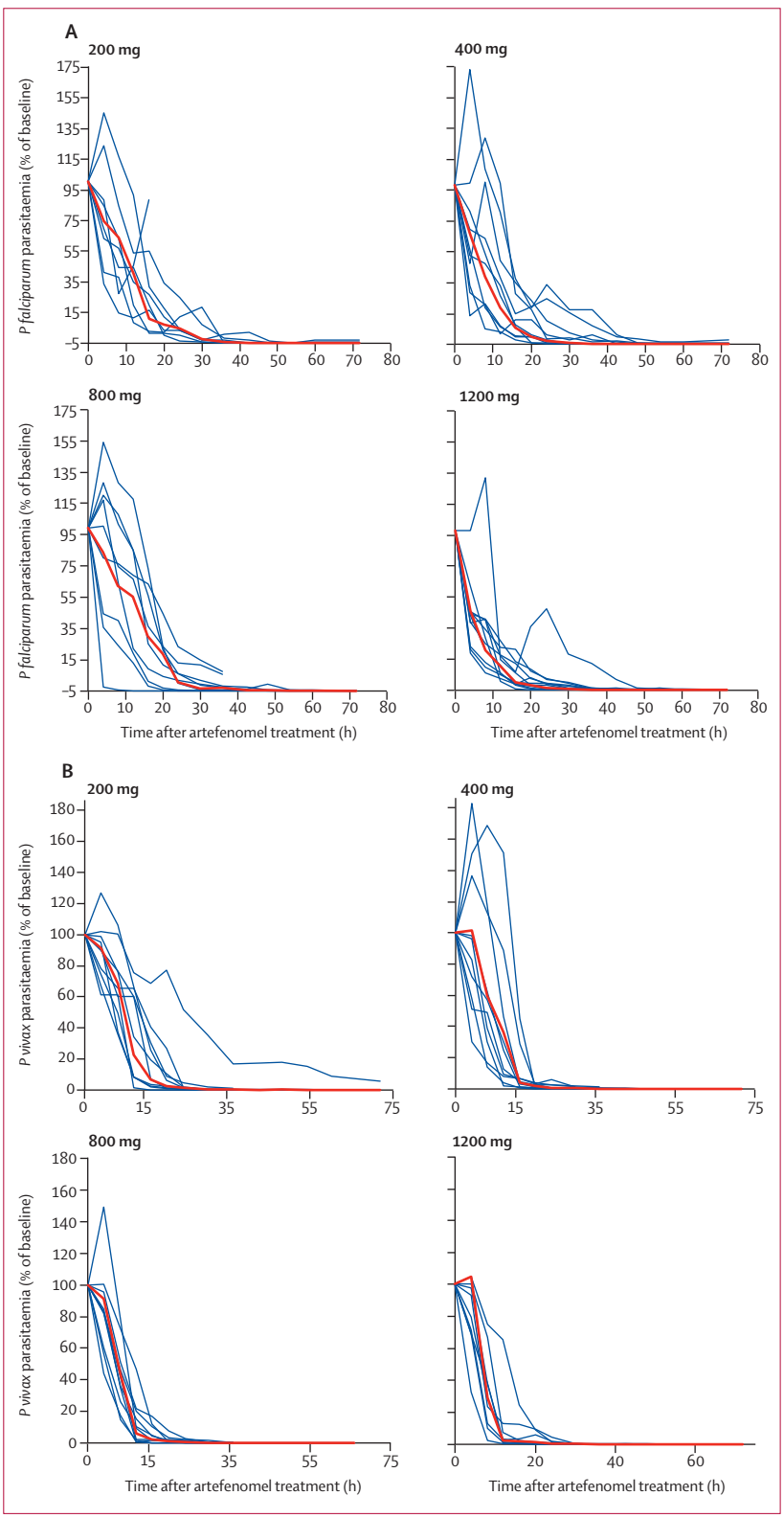

Figure 2: **Parasite counts after treatment start per protocol set**
Patients with (A) *Plasmodium falciparum* and (B) *Plasmodium vivax*. Blue lines show individual patient curves before rescue treatment (one patient in 200 mg cohort). The red line represents the median.

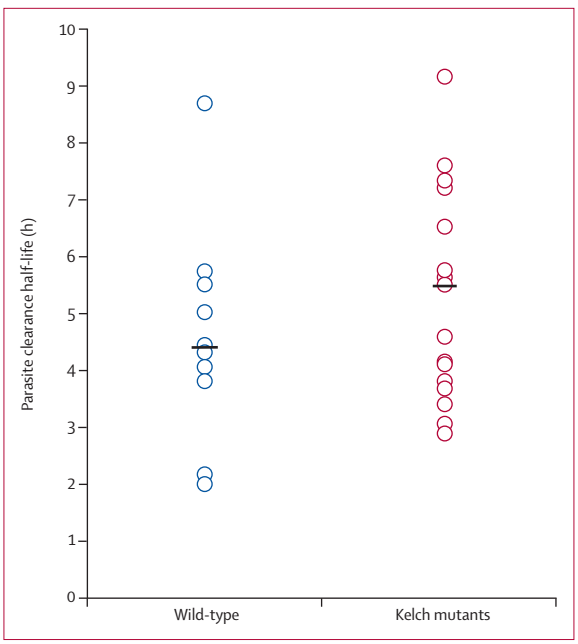

Figure 3: **Parasite clearance and kelch mutations**
Clearance times for patients infected with parasites with kelch mutations or wild-type. The horizontal line represents the median.

	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)*	$t_{1/2}$ (h)*
Artefenomel					
200 mg	339 (90)	4·0 (2–8)	2490 (101)	3180 (37)	46·3 (54)
400 mg	732 (31)	3·0 (1–6)	6140 (33)	6450 (34)	62·3 (33)
800 mg	1710 (38)	4·0 (2–18)	19 000 (50)	19 700 (50)	58·0 (34)
1200 mg	1500 (90)	4·1 (0·5–12)	21 700 (105)	25 100 (85)	57·0 (47)
OZ567					
200 mg	15·6 (59)	4·0 (2–8)	137 (76)	NC	NC
400 mg	33·2 (39)	4·0 (2–8)	370 (37)	NC	NC
800 mg	60·1 (57)	4·0 (2–18)	828 (67)	NC	NC
1200 mg	69·2 (85)	4·0 (3–12)	1140 (94)	NC	NC
OZ579					
200 mg	30·9 (75)	4·0 (2–8)	306 (96)	NC	NC
400 mg	63·4 (46)	4·0 (2–8)	813 (44)	915 (19)	43·2 (31)
800 mg	105 (73)	4·0 (2–18)	1720 (72)	2100 (54)	41·1 (46)
1200 mg	122 (83)	4·0 (3–24)	2380 (93)	3300 (56)	40·0 (39)
OZ580					
200 mg	37·7 (109)	6·0 (4–8)	452 (133)	NC	NC
400 mg	72·2 (73)	6·0 (3–8)	1150 (76)	NC	NC
800 mg	83·0 (138)	4·0 (2–12)	1730 (107)	NC	NC
1200 mg	97·5 (105)	4·0 (3–12)	2200 (117)	NC	NC

Data are geometric means (coefficient of variation) or for t_{max}, median (range). Number of patients: 20 patients each in the 200 mg, 400 mg, and 800 mg cohorts; 21 patients in the 1200 mg cohort. Patients with no assessment at 36 h (n=16) were not taken into account. C_{max}=maximum or peak plasma concentration. t_{max}=timepoint at which the maximal plasma concentration is reached. AUC_{0-t}=area under the concentration-time curve from 0 h to the last pharmacokinetic sample (96 h). $AUC_{0-\infty}$=area under the concentration-time curve from 0 h to infinity. $t_{1/2}$=estimated terminal phase half-life. *The number of patients may differ for the variables $AUC_{0-\infty}$ and $t_{1/2}$. AUC and half-lives were not calculated (NC) when fewer than 70% of patients had valid data.

Table 3: **Plasma pharmacokinetic variables of artefenomel (OZ439) and its metabolites OZ567, OZ579, and OZ580, by artefenomel dose cohort**

41 evaluable patients with *P falciparum* (four in Bangkok and 37 in SMRU) and 40 evaluable patients with *P vivax* (22 in Bangkok and 18 in SMRU, plus the one SMRU patient with mixed infection). No patients required definitive antimalarial treatment before the end of the 36 h study period and all recovered uneventfully. Nine patients did not return for the follow-up phase (two in the 200 mg, three in the 400 mg, three in the 800 mg, and one in the 1200 mg cohort). Six (15%) patients with *P falciparum* and none of the patients with *P vivax* had G6PD deficiency. Individual baseline characteristics are shown in table 1 and in the appendix (pp 3–5).

The 24 h parasite reduction rates are listed in table 2. These varied from 0·90 to 1·88 for patients with *P falciparum*, and from 2·09 to 2·53 for those with *P vivax*, with no clear dose effect for either.

All patients responded rapidly to treatment with a mean fever clearance time of 8–30 h (appendix p 6). All investigated doses of artefenomel resulted in similar rates of parasite clearance (figure 2, table 2). The median clearance rate constant for *P falciparum* ranged from 0·12 to 0·17 per hour leading to a 98% reduction in parasite densities by 36 h (appendix p 6 and p 17). The median clearance rate constant for *P vivax* ranged from 0·22 to 0·30 per hour leading to a 99·6% reduction in parasite densities by 36 h. Estimated median parasite clearance half-life estimates ranged from 4·1 h to 5·6 h for *P falciparum* and 2·3 h to 3·2 h for *P vivax* (table 2). In all cohorts, parasites were undetectable in blood smears at 30–36 h after artefenomel administration in patients with *P falciparum* and after 18–24 h in patients with *P vivax*.

In mainland southeast Asia, slow rates of *P falciparum* parasite clearance after treatment with artesunate are associated with mutations in the kelch propeller domain.[9,30] The median parasite clearance half-life in the 19 patients with *P falciparum* parasites with resistance-associated mutations in the kelch 13 propeller region (*P441L*, *N458Y*, *F446I*, *P527H*, *G538V*, *C580Y*, *A675A/V*, or *P667T*) was 5·5 h (SD 1·8) compared with 4·4 h (1·8) in patients with parasites with no mutations in the propeller region (figure 3; p=0·34; appendix p 13).

There was substantial variability in gametocytaemia over time and between cohorts. The low number of patients with *P falciparum* malaria with gametocytaemia precluded accurate estimation of gametocyte clearance (appendix p 14). For the patients with *P vivax*, the median proportional reduction in gametocytaemia was 97·9% in the 800 mg cohort and 90·4% in the 1200 mg cohort at 24 h, and 100% in the 1200 mg cohort at 48 h.

Maximum plasma concentrations of artefenomel were reached about 4 h after administration (table 3, appendix p 15), followed by a multiphasic decline. The C_{max} values were 339 ng/mL, 732 ng/mL, 1710 ng/mL, and 1500 ng/mL for the 200 mg, 400 mg, 800 mg, and 1200 mg doses, respectively. The estimated geometric mean terminal phase $t_{1/2}$ for the parent compound ranged from

46·3 h to 62·3 h. The plasma concentration-time profiles for the artefenomel metabolites[21] followed those of the parent compound with the highest exposures for OZ580 and the lowest for OZ567 (table 3). These metabolites are not thought to contribute significant antimalarial activity.[21] Exposure to all metabolites was lower than for the parent drug. Inter-patient variability was generally high for exposures to artefenomel and its metabolites (appendix p 15). Exposures to artefenomel were similar between patients with *P falciparum* and *P vivax* infection and between male and female patients, and there were no apparent effects of bodyweight and age.

All patients recovered uneventfully. No patients withdrew because of adverse effects (see appendix pp 1–2 and pp 7–9 for a more detailed safety assessment). Two serious adverse effects were reported, although neither was deemed drug related.

Most of the adverse effects reported in this study were deemed mild in severity by the investigators and no particular pattern was evident. The proportion of patients with at least one moderate, drug-related event was similar between those with *P falciparum* (43%, 17 of 40 patients) and *P vivax* mono-infection (33%, 13 of 40 patients). Most changes in laboratory findings and adverse effects were mild, reversible, not dose-related, and compatible with acute malaria (appendix pp 7–9). Mild, asymptomatic increases in hepatic transaminases (alanine aminotransferase and aspartate aminotransferase) were noted, but were not dose dependent and were never accompanied by hyperbilirubinaemia. Two patients in the 200 mg cohort had a transient alanine aminotransferase reading of 83 U/L (normal range 7–40 U/L for alanine aminotransferase and aspartate aminotransferase) on days 2 and 7. The highest concentrations for aspartate aminotransferase were 82 U/L, 75 U/L, and 71 U/L for different patients in the 200 mg (2 h and 48 h) and 400 mg (24 h) cohorts, respectively. At least one adverse event was reported for 13 (65%) patients in the 200 mg cohort, for 11 (55%) patients in the 400 mg cohort, for ten (50%) patients in the 800 mg cohort, and for 17 (81%) patients in the 1200 mg cohort. The highest proportion of patients with at least one adverse event and the highest number of events were reported in the 1200 mg cohort for both *P falciparum* and *P vivax*. 12 (15%) patients had an asymptomatic rise in plasma creatine phosphokinase (maximum rise 4·9-fold), which did not correlate with dosing (appendix p 8).

Two patients had electrocardiograph QTcF intervals of more than 450 ms (one patient at 2 h and 4 h, and one patient at 24 h). The longest value recorded was 506 ms (pre-dose 423 ms). Both patients were in the 1200 mg cohort. In 18 patients, there were 26 instances of QTcF prolongation of more than 30 ms compared with baseline and three patients had one instance each of QTcF more than 60 ms. There was no significant correlation between QTcF prolongation and concentrations of artefenomel or any of its metabolites (appendix p 11). There were also three instances of reversible right bundle branch block, one in a patient with accompanying T-wave changes compatible with pericarditis evident at baseline, and minor non-specific T-wave changes were noted. The cardiac changes did not seem to be dose-related (one in each of the 400 mg, 800 mg, and 1200 mg cohorts) nor were they associated with cardiac-related clinical symptoms or adverse events.

Discussion

The synthetic peroxide artefenomel is being developed as a potential partner drug in an antimalarial combination treatment. The main goal of this first study of antimalarial activity in vivo was to assess the efficacy of artefenomel in clearing parasitaemia in uncomplicated malaria. The artemisinin derivatives accelerate ring-form parasite clearance providing a readily measurable pharmacodynamic endpoint for dose-finding, and this was investigated for artefenomel. Doses as low as 200 mg proved effective at eliminating ring-stage parasites rapidly and providing rapid and reliable resolution of symptoms and parasitaemia.[2] Artefenomel is structurally dissimilar to the artemisinins and is eliminated much more slowly, yet it retains their key pharmacodynamic advantages.

The elimination half-life of artefenomel in patients with malaria ranged from 46 h to 62 h, greatly exceeding that of other peroxide antimalarial drugs (arterolane 2–4 h,[19] artesunate 0·8–1·3 h,[31] and dihydroartemisinin 0·9–2 h.[32,33] The pharmacokinetic properties of artefenomel in acute malaria differed substantially from those reported earlier in healthy volunteers,[21] with higher concentrations of parent drug and lower concentrations of metabolites, suggesting that acute illness affects both distribution and metabolic clearance. The fairly slow elimination and good tolerability up to single doses of 1200 mg suggest that a single-dose combination with one or more other slowly eliminated antimalarial drugs might be curative, particularly against blood-stage vivax malaria. The substantially slower elimination of artefenomel would provide much longer protection against the emergence of partner drug resistance than the protection provided by artemisinin derivatives in the artemisinin-based combination therapies currently in use.

Artemisinin resistance is regarded as the main threat to our attempts to control and eliminate malaria. Artemisinin resistance in *P falciparum* manifests as slow parasite clearance, delaying clinical recovery, and increased gametocyte carriage. Resistance increases transmissibility and contributes to increased failure rates with artemisinin combination treatments.[9,26,34–38] Artesunate produces clearance rates in falciparum malaria of between 0·23 and 0·39 per hour in sensitive infections and 0·10 per hour in resistant infections.[9,26] These values correspond to parasite clearance half-lives of about 2–3 h and 6 h, respectively. By comparison, artefenomel provided median parasite clearance rates of 0·12 to 0·17 per hour for *P falciparum*

malaria, corresponding to half-lives of 4·1–5·6 h. Thus, artefenomel provides parasite clearance rates that are slower than those of artesunate on artemisinin-sensitive parasites, and slightly faster than those of artesunate on artemisinin-resistant parasites (see appendix p 12 for a full comparison).

Despite having a similar pharmacophore, the antimalarial activity of artefenomel in infections carrying the kelch 13 propeller mutations strongly linked with artemisinin resistance was not substantially different to its activity in infections that did not have these mutations (median parasite clearance half-life 5·5 h *vs* 4·4 h). This finding suggests that artefenomel might be less affected by the mechanisms causing artemisinin resistance and so might have an important therapeutic role as artemisinin resistance worsens. However, this important potential advantage will need to be assessed in larger patient cohorts.

In general, artefenomel was well tolerated. This study included only 81 patients and was not powered to detect risks for wider use of the drug. There were no drug-related serious adverse effects and no significant trends in nausea and vomiting. Although, overall, drug tolerability was good, higher frequencies of adverse events and drug-related adverse events were reported in the 1200 mg cohort than at the lower doses. The most frequently reported adverse effect was an asymptomatic increase in plasma creatine phosphokinase concentration. The highest increase (4·9-fold) was seen in a patient in the 200 mg cohort, and there was no dose-correlation in the 12 readings of increased creatine phosphokinase (appendix p 10). Three instances of reversible right bundle branch block were reported, one in a patient with accompanying T-wave changes compatible with pericarditis evident at baseline. Other minor non-specific T-wave changes were reported. The observed cardiac changes did not seem to be dose-related and were not associated with cardiac-related clinical symptoms or adverse effects. Such changes may be seen in healthy individuals,[39] as well as in patients with malaria,[40] so the significance of these findings is unclear. Electrocardiograms showed QTcF prolongation compared with baseline, but this finding was expected because malaria is associated with QTcF shortening,[41] and so there is predictable lengthening of the QT interval with recovery. There was no relation between QTcF prolongation and plasma concentrations of either parent compound or metabolites (appendix p 11). Taken together with the absence of QTc prolongation in preclinical studies in dogs, these results suggest that artefenomel does not substantially affect the QT interval in patients.

Our preliminary findings suggest that artefenomel has the potential to become an important new antimalarial compound. To prevent or delay the development of parasite resistance against newly introduced drugs, it is common practice to give antimalarial drugs in combination. Potential complementary partner drugs for artefenomel are ferroquine (SSR97193),[42,43] piperaquine,[32,44,45] and DSM265.[46] More information on efficacy and safety will be needed from additional phase 2 and phase 3 evaluation of artefenomel combination studies.

Contributors

APP (co-investigator) contributed to study design, data collection, data analysis, data interpretation, and writing of the report. PJ (co-investigator) contributed to patient recruitment, data collection, and data processing. FHN (co-investigator) supervised the study and assisted in writing and reviewing the manuscript. SP contributed to case recruitment, study design, and data collection. MI processed the samples for quantitative PCR and performed molecular analysis. NJW (co-investigator) contributed to the design, conduct, and interpretation of the study and to writing of the manuscript. SD contributed to the study design and data interpretation, and reviewed and validated the manuscript. FM was study Project Director and involved in overseeing the trial's scientific aspects: data analysis, the statistical analysis plan, and reviewing the clinical study report. FM wrote sections of, and reviewed the manuscript; MB performed and reviewed the pharmacokinetic and pharmacodynamic analyses and wrote the relevant sections for the manuscript. JJM contributed to the study design, wrote the study protocol, conducted project management of the study, and contributed to the study analysis, results review, and writing of the clinical study report and manuscript.

Declaration of interests

We declare no competing interests.

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