Efficacy of DB289 in Thai Patients with *Plasmodium vivax* or Acute, Uncomplicated *Plasmodium falciparum* Infections

Patrick Yeramian,[1] Steven R. Meshnick,[2] Srivicha Krudsood,[4] Kobsiri Chalermrut,[4] Udomsak Silachamroon,[4] Noppadon Tangpukdee,[4] James Allen,[1] Reto Brun,[5] Jesse J. Kwiek,[2] Richard Tidwell,[3] and Sornchai Looareesuwan[4]

[1]Immtech International, Vernon Hills, Illinois; [2]Department of Epidemiology, School of Public Health, and [3]Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill; [4]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [5]Swiss Tropical Institute, Basel, Switzerland

Background. DB289 is the orally active prodrug of the diamidine DB75, which was developed for the treatment of human African trypanosomiasis.

Methods. We tested the safety and efficacy of DB289 for the treatment of *Plasmodium vivax* and acute, uncomplicated *P. falciparum* infections in an open-label pilot study at the Hospital for Tropical Diseases in Bangkok. Nine patients with *P. vivax* infections and 23 patients with *P. falciparum* infections were admitted and treated with 100 mg of DB289 given orally twice a day for 5 days and were followed for 28 days. Patients with *P. vivax* infections were also treated with primaquine on days 10–23.

Results. All patients cleared parasites by day 7, with a mean ± SD clearance time of 43 ± 41 h. One patient with a *P. vivax* infection had a recurrence of parasitemia on day 9. Of the 23 patients with *P. falciparum* infections, 3 had recurrences of parasitemia caused by *P. vivax* and 2 had recurrences of parasitemia caused by *P. falciparum*. In only 1 of 2 recurrences of parasitemia caused by *P. falciparum* were the parasites genotypically distinct from the infecting parasites the patient had at enrollment, which means there was a 96% cure rate.

Conclusions. DB289 is a promising new antimalarial compound that could become an important component of new antimalarial combinations.

Cross-resistance represents a major impediment in the development of new antimalarial compounds. Many antimalarial compounds under development are structurally related to existing ones and are likely to encounter cross-resistance. Thus, new classes of antimalarial compounds are needed. One such novel class is the diamidines. Although results of preliminary studies have shown that diamidines such as pentamidine have in vitro activity against *Plasmodium falciparum* [1], they have never been used to treat malaria. Pentamidine and other older diamidines are toxic and very poorly absorbed after oral administration [2]. Newer diamidines that are far less toxic and are orally bioavailable have been synthesized [3].

The 2,5-bis(4-amidinophenyl)furan DB75 is a new diamidine with excellent broad-spectrum antimicrobial activity—against *Plasmodium* species, *Trypanosoma brucei, Pneumocystis jiroveci, Cryptosporidium parvum,* and other pathogens—when administered systemically. DB75 is active in vitro against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. DB75 given subcutaneously or intravenously for 4 days is active against *Plasmodium berghei* and *Plasmodium chabaudi* infections (R.B., unpublished data).

DB289, the diamidoxime derivative of DB75, acts as an orally bioavailable prodrug that is readily metabolized to the active aromatic dication [4]. Phase 1 clinical studies demonstrated that DB289 is rapidly metabolized to DB75 in humans and that a twice daily regimen of oral DB289 will sustain DB75 plasma levels above the target antimicrobial level [5]. In phase 2 clinical trials of DB289 against *P. jiroveci* pneumonia and human African trypanosomiasis, no serious adverse events were noted [3].

Patients, materials, and methods. Patients with *Plasmodium vivax* infections and acute, uncomplicated *P. falciparum* infections were enrolled between August and November 2003, in an open-label, phase 2 trial to evaluate the efficacy, safety, tolerance, and pharmacokinetics of DB289 at the Hospital for Tropical Diseases, Mahidol University, Bangkok. Patients came to the hospital from nearby rural regions of Thailand that had low levels of transmission of malaria [6]. The study was approved by the Mahidol University Institutional Ethics Committee and the Ethics Committee of the Thai Ministry of Public Health.

Written, informed consent was obtained from each patient. We enrolled male and female patients, ⩾18 years old, who had a minimum body weight of 35 kg. Patients were included in the study if they had <25,000 parasites/μL of blood and had either a single-species infection with an asexual form of *P. vivax* that

Received 29 October 2004; accepted 14 February 2005; electronically published 7 June 2005.

Reprints or correspondence: Dr. Steven R. Meshnick, Dept. of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC 27599-7435 (meshnick@unc.edu).

The Journal of Infectious Diseases 2005; 192:319–22

0022-1899/2005/19202-0016$15.00

Potential conflicts of interest: J.A. is vice president for regulatory affairs at Immtech International; S.R.M., R.T., and P.Y. are or have been Immtech consultants; J.A., R.T., and P.Y. own stock in Immtech.

Financial support: Medicines for Malaria Venture; Immtech International.

was confirmed by peripheral blood smear or an acute, uncomplicated, single-species infection with an asexual form of *P. falciparum* that was confirmed by peripheral blood smear. Women of childbearing potential were included if they were not lactating, had a negative result for a urine test for pregnancy within 24 h before treatment with DB289, and agreed to use a valid method of contraception from the day of consent until 7 days after the completion of treatment with DB289 (study day 12). Not all patients were febrile at enrollment. Patients who required parenteral treatment, had severe organ dysfunction, or had been exposed to antimalarial compounds within 3 days (or to mefloquine within 5 days) before enrollment were excluded. Patients with severe malaria (according to the 2000 World Health Organization criteria), a history of drug hypersensitivity or allergies, chronic heart disease or clinically relevant abnormalities on an electrocardiogram, psychiatric disorders, a glucose-6-phosphate dehydrogenase deficiency, or other laboratory test results that deviated from baseline levels were also excluded.

All patients were treated with 100 mg of DB289 given orally twice a day from day 1 to day 5. DB289 gelcaps (100 mg) were provided by Immtech International. Primaquine (15 mg/kg/day for 14 days; Government Pharmaceutical Organization of Thailand) was administered to patients on days 10–23 for the treatment of hypnozoite-stage *P. vivax* infections. No other drugs that have antimalarial activity were given to patients until completion of the study or relapse. Patients were maintained in the hospital for observation during treatment and for no less than 5 days after the completion of treatment. Blood smears were taken every 12 h until patients were free of parasites, daily to day 7, and on days 10, 14, 21, and 28. Parasite counts were done from Giemsa-stained thick and thin films. Any patient who had a treatment failure after treatment with DB289 received the standard of care at the site: artesunate with mefloquine or chloroquine with primaquine, as appropriate.

The following analyses were performed on blood or serum from all patients on days 0, 3, 7, 10, 21, and 28: complete and differential blood count, hemoglobin concentration, measurement of prothrombin and activated partial thromboplastin time, and measurement of levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, glucose, total bilirubin, total protein, albumin, sodium, potassium, chloride, creatinine, blood urea nitrogen, and amylase. Urine tests to measure the pH level, glucose level, and ketone levels and to detect the presence of protein, red blood cells, and white blood cells were also performed on the same days.

For the determination of DB289 and DB75 plasma concentrations, blood samples were collected immediately before the administration of the morning dose on days 1–5 (trough concentrations). Plasma concentrations were determined by liquid chromatography/tandem mass spectrometry (LC/MS/MS) [7]. Briefly, DB289 and DB75 were extracted by a solid-phase extraction procedure and reconstituted with 0.05% tetrafluoro acetic acid in water and acetonitrile (90%/10% vol/vol). The sample extracts were analyzed for DB289 and DB75 using LC/MS/MS in the positive-ion multiple reaction monitoring mode. DB2897-d8 and DB75-d8 were used as internal standards.

To distinguish between recrudescence and reinfection, parasites in enrollment and study blood samples were genotyped by heteroduplex tracking assay using probes AF42 and AF63 designed to hybridize with the merozoite surface protein 1 gene [8]. These probes distinguished 6 and 8 different genotypes, respectively, in 14 blood samples obtained at enrollment and in 2 blood samples obtained during the study (data not shown).

Results. A total of 32 patients were enrolled into the study during a 3-month period. Nine patients (1 woman and 8 men), with a mean age of 24.2 years (range, 18–30 years), had *P. vivax* infections, and 23 patients (2 women and 21 men), with a mean age of 26.9 years (range, 18–44 years), had *P. falciparum* infections. The mean pretreatment parasite count was 2484 parasites/μL of blood (range, 1–16,350 parasites/μL of blood) for patients with *P. vivax* infections and 1830 parasites/μL of blood (range, 1–6610 parasites/μL of blood) for patients with *P. falciparum* infections.

Mean trough plasma concentrations of DB289 and DB75 are shown in figure 1. Plasma concentrations of DB75 increased rapidly, and mean trough plasma concentrations appear to have reached a maximum as early as day 2 of treatment (third dose of DB289). DB75 plasma concentrations were well in excess of DB289 plasma concentrations in all patients, and, on average, the DB75:DB289 plasma concentration ratio was >40. DB75 plasma concentrations exceeded the target concentration of 5.85 μg/L of blood (i.e., the IC_{50} for the K1 [Thai] strain of *P. falciparum* [9]) by the third dose of DB289 (morning of day 2), and trough plasma concentrations remained 4–5-fold higher than the target concentration for >3 days.

DB289 was very well tolerated. All patients completed treatment without interruptions in or adjustments to the dosing. All adverse events noted were mild and self-resolved: 2 patients had elevated ALT/AST levels (1 patient's levels increased from 53/45 U/L to 97/209 U/L on day 14; 1 patient's levels increased from 19/19 U/L to 94/129 on day 28). One patient had an elevation in amylase levels from 183 U/L at baseline to 575 U/L on day 14. All 3 measurements returned to reference levels without further treatment. No other adverse events that were deemed to be related to treatment were noted.

All patients cleared parasites within 7 days of the initiation of treatment with DB289. Overall, the mean ± SD parasite clearance time (PCT) was 43 ± 41 h from the initiation of treatment, and the mean ± SD fever clearance time (FCT) was 18 ± 22 h from the initiation of treatment.

All 9 patients with *P. vivax* infections cleared parasites within 7 days of the initiation of treatment with DB289 (table 1). The

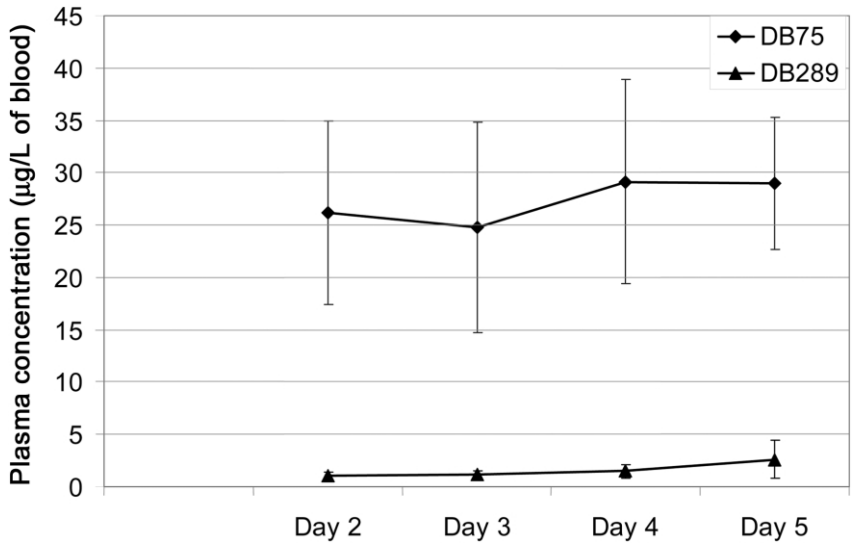

Figure 1. Mean *(symbols)* trough plasma concentrations of DB289 and DB75 and 95% confidence intervals (error bars) ($n = 32$)

mean ± SD PCT was 67 ± 55 h from the initiation of treatment. The mean ± SD FCT was 18 ± 29 h from the initiation of treatment. One patient had a recurrence of parasitemia caused by *P. vivax* on day 9, before he was scheduled to receive primaquine. This was presumed to be a relapse.

All 23 patients with *P. falciparum* infections cleared parasites within 7 days of the initiation of treatment with DB289 (table 1). The mean ± SD PCT was 34 ± 31 h from the initiation of treatment. The mean ± SD FCT was 18 ± 20 h from the initiation of treatment. Three of 23 patients had parasitemia caused by *P. vivax* during the 28 days of follow-up, probably because of a relapse from an undetected mixed infection. Two of 23 patients with *P. falciparum* infections had recurrences of parasitemia caused by *P. falciparum:* 1 on day 23 and 1 on day 28. Genotype analysis was performed by a heteroduplex tracking assay [8]. In the patient whose recurrence occurred on day 23, genetic identity was found in 2 of 2 probes, which suggested that this patient had a recrudescence, and the patient was conservatively classified as having a treatment failure. In the patient whose recurrence occurred on day 28, new bands were seen in 2 of 2 probes, which suggested that this patient had a reinfection. The latter patient could have become reinfected, because he had returned home for 14 days. Thus, 22 of 23 patients with *P. falciparum* infections were cured, for a cure rate of 96%.

Discussion. Most new antimalarial compounds that are in development are derivatives of currently available drugs, and their eventual deployment may be hampered by preexisting cross-resistance. Thus, the in vivo efficacy of DB289 is important, because this antimalarial compound is unrelated to any existing one and could represent the prototype for an entirely new class of antimalarial compounds.

Diamidines may have both novel transport pathways and novel targets. These compounds appear to be transported into *P. falciparum*–infected erythrocytes via a parasite-induced permeability pathway in the host cell membrane [10]. Possible intracellular targets of diamidines include mitochondrial respiration [11], hemoglobin degradation [12], and DNA replication [13].

DB289 is also being developed for the treatment of human African trypanosomiasis and *P. jiroveci* pneumonia under an investigational new drug application from the US Food and Drug Administration. Because the present study was the first trial of DB289 in humans for the treatment of infections with *Plasmodium* species, DB289 was given in the same regimen that

Table 1. Clearance of *Plasmodium* species, relapses, recrudescence, and reinfection after treatment with DB289.

		Patients, no.				
Parasite	Treatment	Total	Parasite clearance in ≤7 days	*P. vivax* relapse ≤28 days	*P. falciparum* recrudescence ≤28 days	Reinfection ≤28 days
P. vivax	DB289, days 1–5; primaquine, days 10–23	9	9	1	0	0
P. falciparum	DB289, days 1–5	23	23	3	1	1

had previously been administered to patients with human African trypanosomiasis (2 doses per day for 5 days; P.Y., unpublished data). To avoid the development of drug resistance [14], combination regimens, such as a 3-day combination regimen of DB289 and artesunate, are being developed for use in future studies.

DB289 demonstrated clear efficacy against *P. falciparum,* with a 96% cure rate. Although all patients with *P. vivax* infections cleared parasites, 1 patient who had this infection at enrollment and 3 patients who had *P. falciparum* infections at enrollment had recurrences. These events were likely the result of relapses from patent or subpatent infections, but they could have been the result of reinfections or drug failure. To distinguish between these possibilities, further studies are needed.

The PCT for DB289 in patients with *P. falciparum* infections appears to be quite low (mean, 34 h). Because some patients who had extremely low levels of parasites were enrolled into the study, a more precise determination of the PCT could be obtained if it were calculated for patients who had higher levels of parasites at enrollment. In the group of 11 patients who had $\geqslant$100 parasites/μL of blood at enrollment, the mean $\pm$ SD PCT was 46.5 $\pm$ 30.5 h. In the group of 8 patients who had $\geqslant$1000 parasites/μL of blood at enrollment, the mean $\pm$ SD PCT was similar: 49.0 $\pm$ 28.1 h. This finding is consistent with the observation that DB75 plasma concentrations exceeded the in vitro IC_{50} for *P. falciparum* by the third dose of DB289 (morning of day 2). Thus, the PCT may be longer than that found for oral artesunate monotherapy (28–40 h) and possibly shorter than that found for mefloquine monotherapy (63–82 h) in Thai studies (reviewed in [15]).

The distribution and elimination of DB75 from patients were characterized in a phase 2 trial of DB289 for the treatment of early stage human African trypanosomiasis (P.Y., unpublished data). Despite its relatively long terminal half-life, peak plasma concentrations of DB75 on day 2 reached 75%–80% of steady-state values. These data are consistent with the pharmacokinetic and pharmacodyamic findings in the present study.

In summary, DB289 is a promising new antimalarial compound that is orally active, safe, effective, and structurally novel. Either DB289 or another one of the many second-generation diamidine prodrugs currently being developed might become an important addition to our antimalarial armamentarium.

References

1. Bell CA, Hall JE, Kyle DE, et al. Structure-activity relationships of analogs of pentamidine against *Plasmodium falciparum* and *Leishmania mexicana amazonensis.* Antimicrob Agents Chemother **1990**; 34:1381–6.
2. Peters BS, Carlin E, Weston RJ, et al. Adverse effects of drugs used in the management of opportunistic infections associated with HIV infection. Drug Saf **1994**; 10:439–54.
3. Yeates C. DB-289 Immtech International. IDrugs **2003**; 6:1086–93.
4. Boykin DW, Hall JE, Bender BC, Tidwell RR. Anti-pneumocystis activity of bis-amidoximes and bis-O-alkylamidoxime prodrugs. Biorg Med Chem Lett **1996**; 6:3017–20.
5. Yeramian P, Kruze MS, Keczkes S, et al. Clinical pharmacokinetics of DB289, a new orally bioavailable dicationic drug [abstract 1561]. In: Program and abstracts of the 41st Annual International Congress of Antimicrobial Agents and Chemotherapy (Chicago, 2001). Herndon, VA: AMS Press, **2001**:255.
6. Singhasivanon P. Mekong malaria: malaria, multi-drug resistance and economic development in the greater Mekong subregion of Southeast Asia. Southeast Asian J Trop Med Public Health **1999**; 30(Suppl 4):i–iv, 1–101.
7. Trendler K, Allen J, Hall JE, et al. Quantification of the prodrug DB289 and an active metabolite, DB75, in rat and monkey plasma using SPE, liquid chromatography and electrospray ionization tandem mass spectrometry. AAPS PharmSci **2000**; 2:abstract 2212.
8. Ngrenngarmlert W, Kwiek JJ, Kamwendo DD, et al. Measuring allelic heterogeneity in *Plasmodium falciparum* by heteroduplex tracking assay. Am J Trop Med Hyg **2005**; 72:694–701.
9. Ismail MA, Brun R, Easterbrook JD, Tanious FA, Wilson WD, Boykin DW. Synthesis and antiprotozoal activity of aza-analogues of furamidine. J Med Chem **2003**; 46:4761–9.
10. Bray PG, Barrett MP, Ward SA, de Koning HP. Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. Trends Parasitol **2003**; 19:232–9.
11. Lanteri CA, Trumpower BL, Tidwell RR, Meshnick SR. DB75, a novel trypanocidal agent, disrupts mitochondrial function in *Saccharomyces cerevisiae.* Antimicrob Agents Chemother **2004**; 48:3968–74.
12. Stead AM, Bray PG, Edwards IG, et al. Diamidine compounds: selective uptake and targeting in *Plasmodium falciparum.* Mol Pharmacol **2001**; 59:1298–306.
13. Nguyen B, Hamelberg D, Bailly C, et al. Characterization of a novel DNA minor-groove complex. Biophys J **2004**; 86:1028–41.
14. Yeung S, Pongtavornpinyo W, Hastings IM, Mills AJ, White NJ. Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices. Am J Trop Med Hyg **2004**; 71:179–86.
15. Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev **1996**; 60:301–15.