Antimalarial Activity of Tigecycline, a Novel Glycylcycline Antibiotic[▽]

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Tigecycline is a novel glycylcycline antibiotic with a broad antibacterial spectrum. Tigecycline was tested with 66 clinical isolates of *Plasmodium falciparum* from Bangladesh using the histidine-rich protein 2 in vitro drug susceptibility assay. The 50% and 90% inhibitory concentrations of tigecycline were 699 (95% confidence interval, 496 to 986) and 5,905 nM (4,344 to 8,028). Tigecycline shows no activity correlation with traditional antimalarials and has substantial antimalarial activity on its own.

The spreading resistance of *Plasmodium falciparum* to existing drugs (15) and first reports of artemisinin resistance (7) call for the search for novel antimalarial drugs. Antibiotics with antimalarial activity, such as azithromycin, doxycycline, and clindamycin, in combination with traditional antimalarial drugs are an interesting option for treating multidrug-resistant falciparum malaria (11, 13, 14). Particularly, tetracyclines, azithromycin, or clindamycin in combination with quinine or artesunate is considered to be a potential second-line therapy for the treatment of uncomplicated falciparum malaria (16).

Tigecycline is the first member of a new class of antimicrobials, the glycylcyclines, and is currently registered only for intravenous therapy. It is a semisynthetic derivative of minocycline with a unique and novel mechanism of action in bacteria. These tetracycline analogues are specifically designed to overcome two common mechanisms of tetracycline resistance, namely, resistance mediated by acquired efflux pumps and/or ribosomal protection (4). Clinical studies have shown that intravenously administered tigecycline has an expanded spectrum of in vitro and in vivo activity against gram-positive, gram-negative, atypical, anaerobic, and other difficult-to-treat pathogens. With a twice-daily dosing regimen, tigecycline is relatively easy to administer and generally well tolerated (6).

The project was carried out at the MARIB (Malaria Research Initiative Bandarban) field site in Bandarban in south-eastern Bangladesh. Written informed consent was obtained from all study participants or their legal representatives, and the study protocol was approved by the Ethical Review Committees of the Medical University of Vienna and the International Centre for Diarrhoeal Disease Research, Bangladesh. Blood samples were taken from male and nonpregnant female patients of ages 8 to 65 years who presented with microscopically confirmed *P. falciparum* monoinfections with a parasite density of 100 to 100,000 asexual parasites per μ l. Parasite samples with more than 1% parasitemia were diluted with

uninfected red blood cells. Pregnant or breastfeeding women and patients with malaria drug therapy in the preceding 30 days were excluded from the study. All samples were tested in the histidine-rich protein 2 (HRP2) in vitro drug susceptibility assay. The culture and enzyme-linked immunosorbent assay (ELISA) were carried out as described previously (8, 9). Fresh *P. falciparum* isolates were cultured in the presence of three-fold serial dilutions of the antimalarial drugs tigecycline (34.3 to 25,000 nM), doxycycline (68.6 to 50,000 nM), azithromycin (68.6 to 50,000 nM), dihydroartemisinin (0.014 to 10.0 nM), chloroquine (3.4 to 2,500 nM), quinine (3.4 to 2,500 nM), and mefloquine (0.3 to 250 nM). Culture was carried out at a 1.5% hematocrit value in complete RPMI 1640 medium with 0.5% Albumax (Albumax I; Gibco Bangkok, Thailand) and 25 mg/liter of gentamicin without freezing, washing, dilution, the addition of serum, or preculture. After 72 h of culture, the plates were frozen and stored at -20°C . The plates were then thawed, and parasite growth inhibition was quantified by using a highly sensitive histidine-rich protein 2 ELISA. The optical density was measured at 450 nm using a standard ELISA plate reader (Tecan Sunrise; Tecan Austria). The optical density readings were used for estimating inhibitory concentrations by nonlinear regression analysis (10).

From a total of 94 patients, 66 samples (70.2%; 95% confidence interval [CI], 59.8 to 79.0) with a geometric mean parasite density of 10,684 per μ l (95% CI, 8,311 to 13,735) were successfully tested. The geometric mean 50% inhibitory concentration (IC_{50}) for tigecycline was 699.11 nM ($n = 66$; 95% CI, 495.74 to 985.91), and the corresponding value for doxycycline was 4,276.01 nM ($n = 33$; 95% CI, 3,084.28 to 5,928.21). IC_{50} s, 90% inhibitory concentrations (IC_{90} s), and 99% inhibitory concentrations (IC_{99} s) with 95% confidence intervals for all drugs tested are shown in Table 1. No correlation was found between parasite density and IC_{50} s of tigecycline ($r = -0.001$; $P = -0.994$) and doxycycline ($r = 0.23$; $P = 0.188$), suggesting little influence of the inoculum size on the outcome of in vitro assays (2). Individual inhibitory concentrations were calculated for all drugs tested in parallel and correlated by nonparametric correlation analysis to determine potential cross-sensitivity and/or cross-resistance patterns between the drugs at IC_{90} levels. Tigecycline showed a significant

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TABLE 1. Geometric mean inhibitory concentrations for various drugs against fresh *P. falciparum* isolates from Bangladesh

Drug (<i>n</i> ^a) ^b	Antibacterial activity [nm] (95% CI)		
	IC ₅₀	IC ₉₀	IC ₉₉
TGC (66)	699.11 (495.74–985.91)	5,905.83 (4,344.41–8,028.45)	12,416.87 (9,481.71–16,260.65)
DOX (33)	4,276.01 (3,084.28–5,928.21)	19,434.92 (12,737.79–29,653.17)	27,201.30 (17,411.38–42,495.81)
AZM (59)	4,314.35 (3,265.58–5,699.94)	18,420.58 (15,060.37–22,530.51)	26,745.20 (21,616.20–33,091.19)
DHA (59)	0.77 (0.59–1.00)	2.76 (2.13–3.57)	4.00 (3.06–5.24)
QNN (62)	105.68 (78.80–141.73)	440.92 (345.63–562.49)	673.42 (522.36–868.15)
MEF (62)	14.38 (10.95–18.89)	64.05 (51.19–80.15)	105.92 (85.67–130.94)
CHL (62)	73.97 (56.40–97.02)	302.68 (222.82–411.15)	436.30 (307.72–618.60)

^a *n*, no. of isolates tested.^b TGC, tigecycline; DOX, doxycycline; AZM, azithromycin; DHA, dihydroartemisinin; CHL, chloroquine; QNN, quinine; MEF, mefloquine.

activity correlation with doxycycline ($r = 0.51$; $P = 0.003$; $n = 32$) but no evidence of a correlation with any of the other tested antimalarials (for dihydroartemisinin, $r = 0.038$; $P = 0.775$; and $n = 58$; for mefloquine, $r = -0.159$; $P = 0.234$; and $n = 58$; for quinine, $r = 0.210$; $P = 0.114$; and $n = 58$; for chloroquine, $r = 0.198$; $P = 0.135$; and $n = 58$; and for azithromycin, $r = 0.252$; $P = 0.057$; and $n = 58$).

In a subset of samples, doxycycline and tigecycline were also tested after 24 h of incubation using the WHO microtest. IC₅₀s were 13 and 49 times higher than those after 72 h of incubation. This confirms earlier findings by Pradines et al. (12) and suggests that tigecycline (just like doxycycline) may have a slow mode of action in malaria parasites. These findings are also consistent with a study by Goodman et al. (3) which suggests increasing (up to 10-fold) in vitro drug activity of tetracyclines in test systems when the culture is extended from 48 to 96 h due to the so-called “delayed-death phenomenon,” when apparently normal cell division and segregation of organelles under initial drug exposure are followed by severe defects in the apicoplast growth and failure to complete cytokinesis during the second cycle (3).

With an IC₅₀ in the nanomolar range and a relatively steep dose-response curve, tigecycline shows one of the highest activities of all antibiotics against *P. falciparum*. In our study, tigecycline was up to six times more active ($P < 0.0001$) against *P. falciparum* than doxycycline. Against gram-positive and gram-negative aerobe and anaerobe bacteria, Zahnel et al. (17) reported an in vitro activity relation generally in a similar range (but up to 600 times higher in selected organisms). The lack of any activity correlation with traditional antimalarial drugs suggests a different mode of action and the absence of cross-sensitivity. However, the correlation with doxycycline, a tetracycline antibiotic that also shows clinical and in vitro activity against malaria parasites, may indicate similar modes of action in *P. falciparum* (11). Our data with 24- and 72-h incubations confirm earlier data from Pradines et al. (12) and Goodman et al. (3) and suggest that tigecycline potentially induces a delayed-death response. With 37 to 67 h, tigecycline has a relatively long half-life (5), potentially making it simpler to administer (6) than tetracycline (6 to 10 h) and doxycycline (12 to 25 h) (1). We conclude that tigecycline has substantial antimalarial activity on its own and may be a potential candidate for exploring its clinical efficacy in combination with faster-acting antimalarials (e.g., artemisinins or quinine) in the

parenteral treatment of multidrug-resistant falciparum malaria in seriously ill patients.

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