

# ***In vitro* antimalarial activity of tigecycline against *Plasmodium falciparum* culture-adapted reference strains and clinical isolates from the Brazilian Amazon**

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## **ABSTRACT**

**Introduction:** We evaluated the *in vitro* antimalarial activity of tigecycline as an alternative drug for the treatment of severe malaria. **Methods:** A chloroquine-sensitive *Plasmodium falciparum* reference strain, a chloroquine-resistant reference strain, and three clinical isolates were tested for *in vitro* susceptibility to tigecycline. A histidine-rich protein *in vitro* assay was used to evaluate antimalarial activity. **Results:** The geometric-mean 50% effective concentration (EC50%) of tigecycline was 535.5 nM (confidence interval (CI): 344.3-726.8). No significant correlation was found between the EC50% of tigecycline and that of any other tested antimalarial drug. **Conclusions:** Tigecycline may represent an alternative drug for the treatment of patients with severe malaria.

**Keywords:** *Plasmodium falciparum*. *In vitro* susceptibility. Tigecycline.

In combination with traditional antimalarial drugs, antibiotics with antimalarial activity, such as tetracyclines and clindamycin, are recognized options for the treatment of multidrug-resistant *Plasmodium falciparum* malaria<sup>1-3</sup>. In recent decades, doxycycline has been included in the World Health Organization Model List of Essential Medicines as an antibacterial agent for the prevention and treatment of malaria<sup>4</sup>. The development of additional antimicrobial drugs has led to the discovery of glycylicyclines, which are tetracycline derivatives containing a glycydamido substitution at position nine<sup>5</sup>. Tigecycline, the first marketed compound of the glycylicycline class, is a semisynthetic derivative of minocycline that is specifically designed to overcome two common mechanisms of tetracycline resistance, including resistance mediated by acquired efflux pumps and ribosomal protection<sup>6</sup>. Pharmacological functional assays have shown that tigecycline inhibits bacterial protein synthesis with a potency that is 3- and 20-fold greater than that of minocycline and tetracycline, respectively<sup>5</sup>. This drug is currently registered only for intravenous use, and a twice-daily dosing regimen is relatively easy to administer and is generally well tolerated<sup>7</sup>. Clinical studies have shown that tigecycline possesses an expanded spectrum of *in vitro* and *in vivo* activity against Gram-positive, Gram-negative, atypical, anaerobic, and

other difficult-to-treat pathogens<sup>8</sup>. However, there is insufficient information on the drug's clinical efficacy for malaria treatment, as only two previous studies have demonstrated *in vitro* anti-*P. falciparum* activity for this drug<sup>9,10</sup>. Because tigecycline is an exclusively injectable antibiotic, its use in malaria treatment should be reserved for critically ill patients. Moreover, this drug should always be used in combination with fast-acting antimalarials, given its moderate-to-slow *P. falciparum* schizonticide effect<sup>10</sup>. To determine its suitability as an alternative drug for the treatment of severe malaria, we evaluated the *in vitro* antimalarial activity of tigecycline against chloroquine-sensitive and chloroquine-resistant reference strains of *P. falciparum* and clinical isolates from the Brazilian Amazon.

*Plasmodium falciparum* clinical isolates were obtained from patients with uncomplicated malaria presenting at the Malaria Research Unit of the Julio Muller University Hospital in Cuiabá, State of Mato Grosso, Brazil between March and December 2011. After informed consent was obtained, a blood sample was collected in a vacuum tube containing heparin and was properly processed within 30min of collection to adapt the parasites to a continuous culture system. The study protocol was approved by the Ethical Research Committee of the Julio Muller University Hospital, Cuiabá (Mato Grosso), Brazil. Patients were included if they demonstrated *P. falciparum* mono-infection; parasitemia above 250 parasites/ $\mu$ L blood, as assessed by Giemsa-stained thick blood film; and no history of antimalarial drug treatment for at least 30 days.

Chloroquine-sensitive (3D7) and chloroquine-resistant (DW2) reference strains and the *P. falciparum* clinical isolates were kept in complete culture medium (RPMI 1640, 25mM HEPES, 2mM L-glutamine, 40 $\mu$ g/mL gentamicin, 0.36mM hypoxanthine, 10mM glucose, and 10% v/v inactivated human

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TABLE 1 - *In vitro* susceptibilities of culture-adapted reference strains and clinical isolates of *Plasmodium falciparum* from the Brazilian Amazon to tigeocycline.

<i>Plasmodium falciparum</i> strains/isolates	Antimalarial activity*	
	(nM)	
	EC50%	EC99%
DW2	568.9	13,095.3
3D7	332.0	19,459.5
HUJM1	674.6	22,426.2
HUJM3	678.6	18,675.3
HUJM2	423.4	18,605.2
Mean (CI95%)	535.5 (344.3; 726.8)	18,452.3 (14,261.0; 22,643.6)

\*Determined using HRP2 in an immune-enzymatic assay; EC50%: geometric-mean 50% effective concentration; EC99%: geometric-mean 99% effective concentration. 95% CI: 95% confidence interval.

serum at 37°C, 5% oxygen, and 5% hematocrit. All parasitized cultures were synchronized by sorbitol treatment and seeded as ring stages before susceptibility assays were performed.

A histidine-rich protein 2 (HRP2) *in vitro* assay was performed to evaluate anti-*P. falciparum* activity. The HRP2 assays were performed according to standard procedures. Briefly, the culture-adapted *P. falciparum* isolates and the reference strains were cultured in the presence of 3-fold serial dilutions of the antimalarial drugs tigeocycline (332-21,250 nM), chloroquine (25-1,600nM), quinine (50-3,200nM), mefloquine (2.5-160nM), lumefantrine (1.25-80nM), artemisinin (0.25-16nM), and artesunate (0.25-16nM). After 72h of culture, the plates were frozen and stored at -20°C. Chloroquine was dissolved in double-distilled water; quinine, mefloquine, artemisinin, and artesunate were dissolved in methanol; lumefantrine was dissolved in ethanol-linoleic acid-Tween 80 mixture (v/v/v 1:1:1); and tigeocycline was obtained from the commercial product Tygacil® (Wyeth, MO, USA) and dissolved in phosphate-buffered saline. The plates were then thawed, and parasite growth inhibition was quantified using a highly sensitive HRP2 ELISA. The optical density was measured at 450nm using a standard Enzyme linked immunosorbent assay (ELISA) plate reader (iMark™, Bio-Rad, USA). The optical density readings were used to estimate the inhibitory concentrations by nonlinear regression analysis<sup>11</sup>.

Of 10 patients, only 3 were successfully tested. Overall, the geometric-mean 50% effective concentration (EC50%) of tigeocycline for the 3 culture-adapted isolates and the 2 reference strains was 535.5nM (95% confidence interval (CI): 344.3-726.8). The EC50% and 99% effective concentration (EC99%) values for all tested drugs are listed in **Table 1**. The individual effective concentrations were calculated for all tested drugs in parallel and were correlated using nonparametric correlation analysis to determine potential cross-sensitivity and/or cross-resistance patterns between the drugs at their EC50% levels<sup>12</sup>. The EC50% of tigeocycline exhibited no significant correlation with any other tested antimalarial, suggesting different modes of action and the absence of cross-resistance/sensitivity among the drugs<sup>1</sup>. However, the high value of Spearman's correlation

coefficient ( $r=0.831$ ;  $p=0.080$ ) between tigeocycline and chloroquine was of concern, as this coefficient may indicate the potential for cross-resistance between these drugs (**Table 2**).

Our findings demonstrate that tigeocycline possesses *in vitro* antimalarial activity against chloroquine-sensitive and chloroquine-resistant culture-adapted isolates of *P. falciparum*, which is consistent with the findings of previous studies<sup>9,10</sup>. In general, patients with severe and complicated malaria are treated with artemether or artesunate by parenteral administration. To potentiate the schizonticide effect of these artemisinin derivatives, the co-administration of an injectable antibiotic, such as clindamycin<sup>13,14</sup>, is also recommended. As an exclusively parenterally administered antibiotic, tigeocycline may represent an alternative drug for treating patients with severe and complicated *P. falciparum* malaria. However, *in vivo* assays and randomized clinical trials are needed to establish the true efficacy and clinical applicability of tigeocycline.

TABLE 2 - Correlation between the EC50% of tigeocycline and standard antimalarial drugs against culture-adapted reference strains and clinical isolates of *Plasmodium falciparum* from the Brazilian Amazon.

Drugs	Tigeocycline (nM)	
	EC50%	EC99%
	r (p value)	r (p value)
Chloroquine	0.831 (0.080)	-0.776 (0.122)
Mefloquine	0.035 (0.954)	-0.977 (0.004)
Quinine	0.567 (0.318)	-0.338 (0.577)
Lumefantrine	0.354 (0.558)	-0.980 (0.003)
Artemisinin	0.163 (0.792)	-0.527 (0.361)
Artesunate	-0.212 (0.731)	-0.422 (0.479)

r: Spearman's correlation coefficient; EC50%: geometric-mean 50% effective concentration; EC99%: geometric-mean 99% effective concentration.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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