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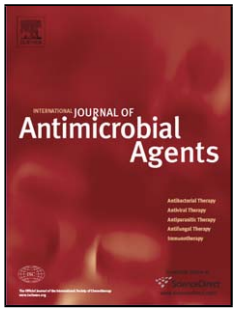

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In vitro activity of tigecycline in *Plasmodium falciparum* culture-adapted strains and clinical isolates from Gabon

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ABSTRACT

Emerging drug resistance in *Plasmodium falciparum* and its rapid spread in endemic countries have made the quest for new antimalarials a research priority. Tetracycline and its derivatives are well established compounds for combination with faster-acting drugs in the current practice of malaria treatment. Tigecycline is the first marketed derivative of a new class of tetracycline antibiotics. Its altered structure leads to enhanced activity against bacteria and may also be associated with improved antimalarial activity. Using the histidine-rich protein 2 (HRP2) drug sensitivity assay, we determined the geometric mean 50% inhibitory concentrations (IC_{50}) of tigecycline in culture-adapted strains as well as in 23 clinical *P. falciparum* isolates from Lambaréné, Gabon. These values were compared with other tetracyclines as well as with clindamycin. Assays with 3 days and 6 days of incubation were evaluated to explore the impact of delayed parasite death on drug activity. IC_{50} values in clinical isolates after 6 days of incubation were 160.0 nM [95% confidence interval (CI) 114.6–223.4 nM] for tigecycline, 739.4 nM (445.9–1226.1 nM) for doxycycline and 9.2 nM (95% CI 6.6–12.9 nM) for clindamycin. Tigecycline was found to act faster against plasmodia than any of the other antibiotics tested. This study demonstrates the potential of tetracycline derivatives in the development of improved antimalarials.

1. Introduction

Emerging resistance in *Plasmodium falciparum* against standard treatment regimens has made the quest for new antimalarials a research priority [1]. A successful strategy for identifying new therapeutic agents is to exploit drugs that were primarily developed and marketed against bacteria, a commercially more attractive target [2]. A major advantage of this approach is that candidate compounds can be rapidly evaluated in clinical trials once their antiplasmodial activity has been demonstrated. Clindamycin and tetracycline derivatives are notable examples for the great potential of this strategy. Both have become valuable compounds for combination with faster-acting drugs in the current practice of malaria treatment [3,4].

The mechanisms underlying the antimalarial activity of antibiotics have been elucidated only recently. The parasite's apicoplast and mitochondria are of prokaryotic descent and are therefore susceptible to certain antibiotics [5]. It has been found that apicoplast function is lost in the parasite generations following antibiotic exposure, which explains the phenomenon of parasite death one cycle *after* exposure, the substantial difference in activity between the first and second cycle, and the slow antimalarial effect of clindamycin and tetracyclines in vivo [5–7].

Glycylcyclines are tetracycline derivatives containing a glycyl amido substitution at position 9. Tigecycline, the 9-t-butylglycylamido derivative of minocycline, is the first marketed compound of this new class of antibiotics. Its increased affinity for the ribosome yields a more potent inhibition of protein synthesis. This is thought to be the

basis for tigecycline's improved antibacterial spectrum, including activity against multidrug-resistant species [8,9].

We studied the in vitro activity of tigecycline in comparison with clindamycin and doxycycline in clinical isolates of *P. falciparum* from Lambaréné, Gabon. Additionally, we evaluated the presence of a delayed death phenomenon in plasmodia after treatment with tigecycline using culture-adapted strains.

2. Methods

Doxycycline hyclate [molecular weight (MW) 512.94] and clindamycin hydrochloride (MW 461.44) were dissolved in H_2O at a stock solution of 86 mM and 100 mM, respectively; tetracycline hydrochloride (MW 480.90) was dissolved in methanol at 20 mM; and tigecycline (MW 585.65) and minocycline hydrochloride (MW 493.94) were dissolved in dimethyl sulphoxide (DMSO) at 50 mM. Further dilutions were made in complete medium. Drugs were pre-coated on 96-well plates in two-fold (laboratory strains) or three-fold (clinical isolates) dilutions.

A chloroquine-sensitive (3D7) and a chloroquine-resistant (Dd2) *P. falciparum* strain were kept in complete culture medium (RPMI 1640, 25 mM HEPES, 2 mM L-glutamine, 50 μg/mL gentamicin and 0.5% w/v AlbuMAX™) at 37 °C, 5% oxygen and 5% haematocrit. Laboratory isolates were synchronised by sorbitol treatment and seeded as ring stages. The histidine-rich protein 2 (HRP2) drug sensitivity assay was performed

according to standard procedures [10]. However, in those assays incubated for 6 days, 0.16 mL of medium was changed on Days 2 and 4 without replacement of drug.

Clinical isolates were from patients with mild malaria presenting at the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné, Gabon, between February 2009 and May 2009. Inclusion criteria were: (i) *P. falciparum* monoinfection and parasitaemia between 10^3 and 6×10^5 parasites/μL blood, assessed by Giemsa-stained thick blood film; (ii) aged 1–18 years; and (iii) no history of intake of antimalarial drugs for at least 1 month. Immediately before standard medical care was initiated, venous blood was collected in lithium heparin tubes. Parasitised blood was processed within 4 h and added to complete culture medium at a haematocrit of 1.5% and an adjusted parasitaemia of 0.05%. Ninety-six-well plates were incubated for 6 days at 37 °C in a candle jar. Samples were only included in the analysis if the drug-free control showed at least twice the HRP2 concentration of the sample with the highest drug concentration. Beyond that, identical methods as for culture-adapted strains were used.

Individual inhibitory concentrations were determined by non-linear regression analysis of log concentration–response curves using the drc-package v0.9.0 of R v2.6.1 (http://www.r-project.org). For each drug assayed, the geometric means of the 50%, 90% and 99% inhibitory concentrations (IC_{50}, IC_{90} and IC_{99}, respectively) were calculated with 95% confidence intervals (CIs). Two-sided paired *t*-tests were used for comparison of geometric mean IC_{50} values. Correlation of activity between drugs was assessed by Spearman's (non-parametric) test in Stata v10.0 (StataCorp LP, College Station, TX).

Informed consent and assent were obtained from the legal representative and the participating child.

3. Results and discussion

3.1. In vitro activity in culture-adapted strains

The presence of a delayed death phenomenon was assessed by comparing inhibitory concentrations after 3 days and 6 days of incubation. Compared with antiplasmodial activity after 3 days of incubation, activity after 6 days was remarkably higher for all antibiotics. Clindamycin in particular showed very little activity after 3 days but was the most active antibiotic after 6 days of incubation. Among the tetracyclines, tigecycline was the most active compound at both time points, followed by minocycline, doxycycline and tetracycline. Moreover, tigecycline showed a less pronounced delayed death effect than its sister compounds. Comparing the results between strains, 3D7 was significantly less susceptible than Dd2 against clindamycin and tetracycline when incubated for 3 days. However, this difference was not present following prolonged incubation. Tables 1 and 2 summarise the geometric mean inhibitory concentrations in culture-adapted strains.

3.2. In vitro activity in clinical isolates

Patients aged between 15 months and 18 years of age were included in the study. Geometric mean parasite density at enrolment was 45 174 parasites/μL (95% CI 21

750–93 827/μL). Of 27 *P. falciparum* clinical isolates, 22 fulfilled the criteria of successful culture for doxycycline and 23 for both clindamycin and tigecycline. IC_{50} values after 6 days of incubation are summarised in Table 3. Tigecycline exhibited approximately five times the activity of doxycycline (IC_{50} = 160.0 nM vs. 739.4 nM; $P < 0.0001$), whilst clindamycin in turn was more than 16-fold more active than tigecycline (9.2 nM vs. 160.0 nM; $P < 0.0001$). Compared with its activity against culture-adapted strains, the activity of doxycycline in clinical isolates appeared to be reduced (739.4 nM vs. 354.3 nM for 3D7 and 256.0 nM for Dd2). This was due to a decreased sensitivity of certain strains as indicated by an increased range (103.9–12 146.0 nM) and a markedly right-skewed distribution of inhibitory concentrations. Correlation analysis of IC_{50} values showed a positive correlation between doxycycline and clindamycin ($r = 0.71$, $P = 0.0002$), but no correlation between tigecycline and any of the other drugs.

4. Discussion

This study demonstrates a substantial in vitro activity of tigecycline in culture-adapted strains and clinical isolates of *P. falciparum*. This new compound exhibits approximately five times the activity of doxycycline in field isolates. Moreover, it appears to act faster than any of the other tetracyclines, exhibiting the highest activity at Day 3. The IC_{50} values of classic tetracyclines and clindamycin in this study are consistent with those from previous studies using a similar approach [6,11–13]. This underlines that prolonged incubation is required to assess adequately the antimalarial potential of most antibiotics in vitro.

While preparing this manuscript, Starzengruber et al. [14]presented their results from an independent study using clinical isolates from Bangladesh. They found an increased in vitro antimalarial activity of tigecycline compared with classic tetracycline derivatives such as doxycycline, similar to our results. However, owing to their methodologically different approach using 3 days of incubation only, activities for tigecycline and doxycycline were four and six times lower, respectively, compared with our study [14]. Nevertheless, the ratio expressing the activity of tigecycline as a multiple of the IC_{50} for doxycycline was similar: 6.1 in the report from Bangladesh and 4.6 in the present study [14]. We could not reproduce the weak correlation between the activities of doxycycline and tigecycline as reported by Starzengruber et al. [14]. This might be due to the wide scatter of doxycycline activities and the heterogeneous parasite population in Central Africa.

Despite its improved activity, clinical use of tigecycline in the treatment of malaria may be limited due to its pharmacokinetic properties. Its rapid distribution into tissues [15] may expose parasite populations to a prolonged period of subtherapeutic concentrations, thus increasing the risk of resistance. In addition, the need for intravenous administration restricts the use of tigecycline to hospitalised patients. Furthermore, as for any tetracycline derivative, it is not recommended in pregnant women and small children. On the other hand, it might be interesting to investigate this class of compounds with respect to their use in combination with artemisinins for the treatment of severe multiresistant malaria in adults.

In summary, the structural modifications of tigecycline compared with standard tetracyclines appear to confer an increased and accelerated antimalarial activity. Tigecycline itself is unlikely to replace existing drug combination partners on a large scale owing to its pharmacokinetic and safety characteristics. However, its enhanced activity against *P. falciparum* demonstrates the potential of new tetracycline derivatives in the development of improved antimalarials and calls for further research in this highly promising field.

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Competing interests

None declared.

Ethical approval

The investigation was approved by the Ethics Committee of the International Foundation for the Albert Schweitzer Hospital in Lambaréné, Gabon (Ref.: 1/90).

References

[1] Mordmüller B, Kremsner PG. Malarial parasites vs. antimalarials: never-ending rumble in the jungle. Curr Mol Med 2006;6:247–51.

[2] Rosenthal PJ. Antimalarial drug discovery: old and new approaches. J Exp Biol 2003;206:3735–44.

[3] Lell B, Kremsner PG. Clindamycin as an antimalarial drug: review of clinical trials. Antimicrob Agents Chemother 2002;46:2315–20.

[4] Kremsner PG, Radloff P, Metzger W, Wildling E, Mordmüller B, Philipps J, et al. Quinine plus clindamycin improves chemotherapy of severe malaria in children. Antimicrob Agents Chemother 1995;39:1603–5.

[5] Goodman CD, Su V, McFadden GI. The effects of anti-bacterials on the malaria parasite *Plasmodium falciparum*. Mol Biochem Parasitol 2007;152:181–91.

[6] Dahl EL, Rosenthal PJ. Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. Antimicrob Agents Chemother 2007;51:3485–90.

[7] Dahl EL, Shock JL, Shenai BR, Gut J, DeRisi JL, Rosenthal PJ. Tetracyclines specifically target the apicoplast of the malaria parasite *Plasmodium falciparum*. Antimicrob Agents Chemother 2006;50:3124–31.

[8] Olson MW, Ruzin A, Feyfant E, Rush TS 3rd, O'Connell J, Bradford PA. Functional, biophysical, and structural bases for antibacterial activity of tigecycline. Antimicrob Agents Chemother 2006;50:2156–66.

[9] Bauer G, Berens C, Projan SJ, Hillen W. Comparison of tetracycline and tigecycline binding to ribosomes mapped by dimethylsulphate and drug-directed Fe^{2+} cleavage of 16S rRNA. J Antimicrob Chemother 2004;53:592–9.

[10] Noedl H, Bronnert J, Yingyuen K, Attlmayr B, Kollaritsch H, Fukuda M. Simple histidine-rich protein 2 double-site sandwich enzyme-linked immunosorbent assay for use in malaria drug sensitivity testing. Antimicrob Agents Chemother 2005;49:3575–7.

[11] Divo AA, Geary TG, Jensen JB. Oxygen- and time-dependent effects of antibiotics and selected mitochondrial inhibitors on *Plasmodium falciparum* in culture. Antimicrob Agents Chemother 1985;27:21–7.

[12] Pradines B, Rogier C, Fusai T, Mosnier J, Daries W, Barret E, et al. In vitro activities of antibiotics against *Plasmodium falciparum* are inhibited by iron. Antimicrob Agents Chemother 2001;45:1746–50.

[13] Pradines B, Spiegel A, Rogier C, Tall A, Mosnier J, Fusai T, et al. Antibiotics for prophylaxis of *Plasmodium falciparum* infections: in vitro activity of doxycycline against Senegalese isolates. Am J Trop Med Hyg 2000;62:82–5.

[14] Starzengruber P, Thriemer K, Haque R, Khan WA, Fuehrer HP, Siedl A, et al. Antimalarial activity of tigecycline, a novel glycylcycline antibiotic. Antimicrob Agents Chemother 2009;53:4040–2.

[15] Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother 2006;58:256–65.

Table 1

Geometric mean of 50%, 90% and 99% inhibitory concentrations (IC_{50}, IC_{90} and IC_{99}, respectively) (95% confidence interval) of selected antibiotics against the culture-adapted strain 3D7 of *Plasmodium falciparum* after 3 days and 6 days of incubation [a]

Drug	3 days (μM) [b]			6 days (nM) [b]		
	IC_{50}	IC_{90}	IC_{99}	IC_{50}	IC_{90}	IC_{99}
Tigecycline	2.3 (2.0–2.8)	6.9 (5.3–8.9)	22.6 (13.4–38.2)	220.2 (174.0–278.6)	256.7 (203.4–323.8)	303.4 (240.1–383.4)
Minocycline	5.6 (4.6–6.8)	8.8 (6.3–12.3)	14.3 (8.3–24.9)	263.6 (253.3–274.3)	325.3 (289.2–365.8)	409.2 (327.5–511.3)
Doxycycline	14.6 (11.6–18.4)	22.2 (16.7–29.6)	35.2 (20.6–60.2)	354.3 (297.3–422.2)	548.2 (425.0–706.9)	882.6 (584.7–1332.3)
Tetracycline	134.1 (58.3–308.4)	712.9 (101.6–5003.5)	>1000	359.7 (200.5–645.1)	459.0 (245.7–857.4)	598.8 (304.1–1179.2)
Clindamycin	445.4 (184.9–1072.5)	>1000	>1000	12.2 (10.0–15.0)	18.8 (15.1–23.3)	30.1 (21.0–43.2)

[a] Pooled data from six assays.

[b] Note that concentrations after 3 days are given in μM and after 6 days in nM.

Table 2

Geometric mean of 50%, 90% and 99% inhibitory concentrations (IC_{50}, IC_{90} and IC_{99}, respectively) (95% confidence interval) of selected antibiotics against the culture-adapted strain Dd2 of *Plasmodium falciparum* after 3 days and 6 days of incubation [a]

Drug	3 days (μM) [b]			6 days (nM) [b]		
	IC_{50}	IC_{90}	IC_{99}	IC_{50}	IC_{90}	IC_{99}
Tigecycline	2.8 (1.9–4.1)	12.5 (6.4–24.2)	63.3 (10.8–370.1)	173.1 (107.2–279.6)	225.1 (131.5–385.3)	299.7 (158.7–565.9)
Minocycline	3.8 (2.5–6.0)	12.8 (5.2–31.8)	47.7 (10.5–217.7)	202.7 (136.7–300.6)	249.9 (189.1–330.2)	314.0 (263.0–374.9)
Doxycycline	10.7 (8.6–13.3)	16.7 (12.1–23.1)	27.0 (15.1–48.4)	256.0 (193.5–338.8)	395.7 (241.1–649.6)	636.5 (287.5–1408.9)
Tetracycline	23.6 (11.1–50.1)	168.6 (53.9–527.5)	>1000	408.3 (224.7–741.9)	539.3 (293.8–989.7)	823.1 (499.2–1357.3)
Clindamycin	37.3 (17.2–81.0)	>1000	>1000	11.5 (9.6–13.7)	15.1 (10.8–21.3)	20.5 (11.3–37.2)

[a] Pooled data from six assays.

[b] Note that concentrations after 3 days are given in μM and after 6 days in nM.

Table 3

Geometric mean of 50%, 90% and 99% inhibitory concentrations (IC_{50}, IC_{90} and IC_{99}, respectively) (95% confidence interval) for tested antibiotics against 23 clinical isolates of *Plasmodium falciparum* from Gabon after 6 days of incubation

Drug	IC_{50} (nM)	IC_{90} (nM)	IC_{99} (nM)
Tigecycline	160.0 (114.6–223.4)	367.3 (275.0–490.6)	929.4 (688.1–1255.5)
Doxycycline [a]	739.4 (445.9–1226.1)	6884.4 (3959.0–11 971.4)	66 709.1 (35 442.4–125 558.7)
Clindamycin	9.2 (6.6–12.9)	79.1 (46.2–135.3)	856.3 (373.7–1962.2)

[a] *n* = 22 due to one missing value.