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In Vitro Activity of Mirincamycin (U24729A) against *Plasmodium falciparum* Isolates from Gabon▽

Jana Held,[1,2] Richard Westerman,[3] Peter G. Kremsner,[1,2] and Benjamin Mordmüller[1,2]*

Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany[1]; Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon[2]; and Maldevco LTT, 1603 Evanston Ave., Kalamazoo, Michigan 49008[3]

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We assessed the in vitro activity of mirincamycin, a lincosamide antibiotic, against *Plasmodium falciparum* clinical isolates from Gabon. Growth was determined by HRP2 enzyme-linked immunosorbent assay using an adapted protocol with a prolonged incubation time (6 days) to account for antibiotic-induced delayed death. Mirincamycin's *cis* and *trans* isomers are more active (median 50% inhibitory concentrations [IC_{50}s], 3.2 nM and 2.6 nM) than the comparator drugs clindamycin (IC_{50}, 12 nM) and doxycycline (IC_{50}, 720 nM), and therefore, further clinical development is promising.

Drug resistance in *Plasmodium falciparum* populations around the world and the short half-life of artemisinins have led to a resurgence of interest in combination therapies for the treatment of malaria (9, 14, 16). It is hypothesized that the rate of resistance development is reduced when antimalarial combinations are given. Most commonly, fast-acting antimalarials with short half-lives are combined with slow-acting and slowly eliminated drugs. A drawback of this approach is the exposure of persisting parasites to subinhibitory levels of the slowly eliminated partner. Due to their short half-life but delayed action, antibiotics are particularly attractive combination partners for fast-acting drugs such as quinine, artemisinins, or other drugs (1, 10, 23, 27). Antibiotics act mainly on plasmodial organelles of prokaryotic origin: the mitochondrium and the apicoplast (8). These compounds inhibit parasite growth in the second cycle after exposure. This so-called "delayed-death" phenomenon is due to apicoplast dysfunction, which explains the seeming paradox of parasite death after a short exposure to the drug in the preceding cycle. Molecular details of this process are not known (4, 5). We report our results on the antimalarial properties of mirincamycin (Fig. 1), a lincosamide antibiotic similar to clindamycin which is synthetically produced (13). Mirincamycin has been evaluated as an antimalarial drug in animal models before (18, 19, 25, 26), but investigations were not continued, mainly because there was no perceived need for combination therapy at that time. Interest in mirincamycin has reemerged, and the first clinical trials are expected to start in 2010.

We tested the inhibitory activities of doxycycline, clindamycin, and mirincamycin with *P. falciparum* isolates from patients with malaria in Lambaréné, Gabon, between February and May 2009. Parasites were from patients of ages 15 month to 18 years who presented with *P. falciparum* monoinfection (parasitemia between 10^3 and 6×10^5 parasites/μl blood, assessed by thick blood smear [17]) and had no reported intake of antimalarial drugs for at least 1 month. Informed consent and assent were obtained from the legal representative and the participating child, respectively. The investigation was approved by the ethics committee of the International Foundation for the Albert Schweitzer Hospital in Lambaréné. Doxycycline hyclate (molecular weight [MW], 512.94) and clindamycin hydrochloride (MW, 461.44) were obtained from Sigma Aldrich and dissolved in H_2O at stock solutions of 86 mM and 100 mM, respectively; 4′-*trans*-mirincamycin hydrochloride (MW, 475.47) and 4′-*cis*-mirincamycin hydrochloride (MW, 475.47) were provided by Richard Westerman (Maldevco) and dissolved in dimethyl sulfoxide at stock solutions of 57 mM and 78 mM, respectively. Drugs were predosed in 96-well plates in threefold serial dilution. Parasites were added in complete culture medium (RPMI 1640, 25 mM HEPES, 2.4 mM L-glutamine, 50 μg/ml gentamicin, and 0.5% [wt/vol] Albumax) at a hematocrit of 1.5% with an adjusted parasitemia of 0.05% and incubated at 37°C in a candle jar. Pilot experiments to determine the best cultivation period and medium change strategy for slow-acting antibiotics showed best results when parasites were kept in culture for 6 days with medium changes on days 2 and 4 without drug replacement. After 6 days, plates were stored at −20°C until the histidine-rich protein 2 (HRP2) concentration was measured according to standard procedures (15). Only samples positive for growth in microscopy (assessed by thick blood smear on days 4, 5, and 6) and with an at least twofold increase in the HRP2 concentration after 6 days of incubation were included in the analysis.

Individual inhibitory concentrations were determined by nonlinear regression analysis of log-concentration-response curves using the drc package, v0.9.0, of R v2.6.1 (22, 24). The laboratory strain 3D7A was used to determine the range of activities of the antibiotics tested after 3 and 6 days. The median 50% inhibitory concentrations (IC_{50}s) for *cis*- and *trans*-mirincamycin were 1.5 nM and 3.6 nM in the 6-day assay; after 3 days, mirincamycin's IC_{50} was more than 10,000-fold higher. This finding was expected, since the difference in activity between the first and second cycles is similar for clindamycin.

Twenty-three of 27 collected *P. falciparum* isolates fulfilled the criteria for successful culture for clindamycin and *cis*-mirincamycin, 22 for doxycycline, and 21 for *trans*-mirincamycin.

* Corresponding author. Mailing address: Eberhard Karls Universität Tübingen, Institut für Tropenmedizin, Wilhelmstraße 27, D-72074 Tübingen, Germany. Phone: 49 7071 2982187. Fax: 49 7071 295189. E-mail: benjamin.mordmueller@uni-tuebingen.de.

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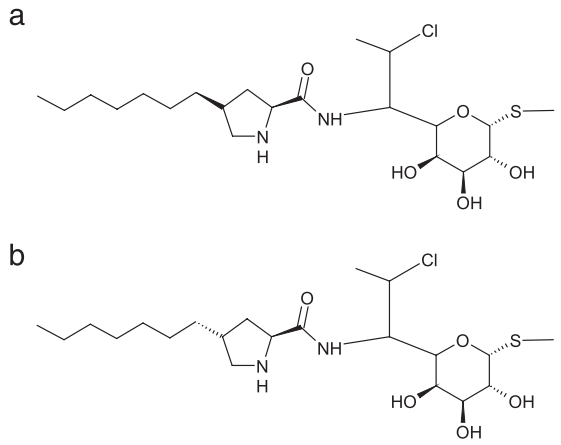

FIG. 1. Chemical structure of the *cis* (a) or *trans* (b) isomer of mirincamycin.

No significant difference was observed between the *trans* and *cis* isomers of mirincamycin. Both showed a low median IC_{50}, with 2.6 nM and 3.2 nM, respectively, and a narrow range between isolates (0.6 to 7.7 nM and 0.5 to 8.2 nM, respectively). The median IC_{50} of clindamycin was 11.6 nM, with a range between 2.4 and 29.1 nM. Doxycycline's IC_{50} was considerably higher (720 nM), with a wide range of activities (104 nM to 12 μM). Notably, two isolates had unexpectedly high IC_{50}s for doxycycline (6 and 12 μM) but not for the lincosamides. The IC_{50}s of *trans*- and *cis*-mirincamycin are significantly correlated to each other ($P < 0.001$) and to that of the other lincosamide drug, clindamycin ($P < 0.001$). The correlation of doxycyline with clindamycin and *cis*- and *trans*-mirincamycin was significant (for all comparisons, $P < 0.001$), although not as strong as those for the lincosamide antibiotics among each other (Fig. 2).

This work shows for the first time that mirincamycin has a high in vitro activity against clinical *P. falciparum* isolates. IC_{50}s of both isomers are substantially lower than those for any other antibiotic tested so far (5, 8), including the lincosamide comparator clindamycin (3, 5). The range of activity in individual isolates is narrow, and no outliers that would indicate frequent naturally occurring variants with decreased sensitivity were identified.

Preclinical studies of mice and monkeys have shown in vivo activity of mirincamycin against plasmodia. In *Plasmodium cynomolgi* infections of rhesus monkeys, mirincamycin was curative as a monotherapeutic regimen (18) and showed an additive effect when given together with primaquine (25). In *Plasmodium berghei*-infected mice, mirincamycin showed activity after subcutaneous and oral administration (12). Interestingly, a hypnozoitocidal effect was observed in monkeys in another study (26). If the same effect is seen in human malarias, then mirincamycin could become an urgently needed alternative to primaquine. Phase I and ADMET studies of mirincamycin were performed in the late 1960s (R. Westerman, personal communication). Toxicity was reported to be similar to that of clindamycin. In contrast to clindamycin, mirincamycin tends to partition into erythrocytes. Normal adults given repeated doses of mirincamycin exhibit both a dose-dependent and accumulative increase in concentrations in erythrocytes that is greater

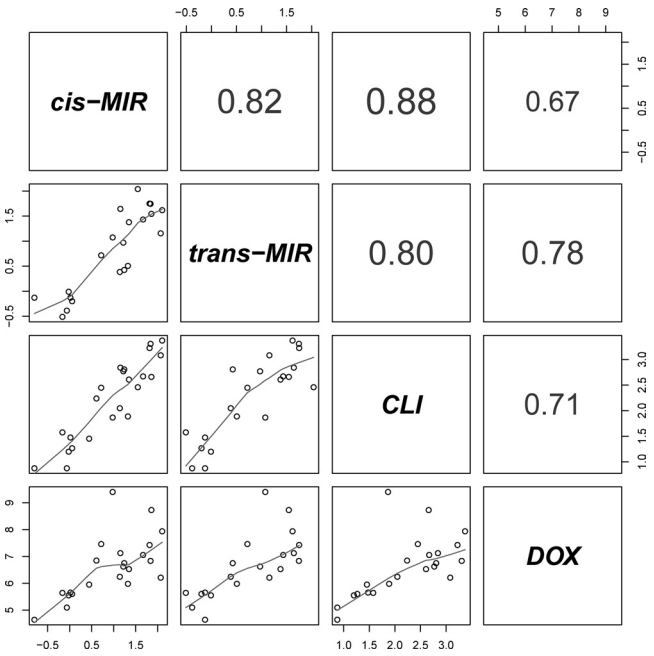

FIG. 2. Correlation matrix of doxycycline (DOX), clindamycin (CLI), and the two isomers of mirincamycin (*cis*-MIR and *trans*-MIR). The leftmost column shows the log-transformed data for the association of *cis*-MIR with *trans*-MIR, CLI, and DOX activities, respectively. The lower two boxes of the second column show the association of *trans*-MIR with CLI and DOX, and the lowest box of the third column shows that for for CLI and DOX. A smoothed line is drawn to visualize the associations. Mirrored through the diagonal, Spearman's rho for the different correlations is shown. The font size is proportional to the degree of correlation.

than the plasma concentration. Similar repeated dosing of clindamycin leads to a plateauing of serum and erythrocyte levels with no changes in relative drug concentrations. The pharmacokinetics of mirincamycin in parasitized human erythrocytes and its impact on antiparasitic activity have not been investigated. Their study should be incorporated into anticipated clinical trials.

We observed lower IC_{50}s for doxycycline and clindamycin than in previous publications using clinical isolates tested with standard protocols (2, 11, 21). This is most likely due to our longer incubation time, because for drugs with delayed action, a 10- to 10,000-fold increase in activity between the first and second cycles is present. This is especially true for clindamycin (5). Our results are similar to results for laboratory strains incubated for two cycles (5–7, 20).

In conclusion, in vitro activity of mirincamycin in clinical isolates from Gabon is higher than those for doxycycline and clindamycin. Further clinical development of this drug is worth pursuing, since it would add a very interesting combination partner to fast-acting antimalarials.

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