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

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Received 2 August 2009/Returned for modification 15 September 2009/Accepted 12 October 2009

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 [IC50s], 3.2 nM and 2.6 nM) than the comparator drugs clindamycin (IC50, 12 nM) and doxycycline (IC50, 720 nM), and therefore, further clinical development is promising.

Drug resistance in Plasmodium falciparum populations around the world and the short half-life of artemisinin has 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 mitochondrion 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 months to 18 years with 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 (IC50s) for cis- and trans-mirincamycin were 1.5 nM and 3.6 nM in the 6-day assay; after 3 days, mirincamycin’s IC50 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.
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 isolates (0.6 to 7.7 nM and 0.5 to 8.2 nM, respectively, and a narrow range of 2.6 nM and 3.2 nM, respectively, and a narrow 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$_{50}$ for doxycycline (6 and 12 µM) but not for the lincosamides. The IC$_{50}$ 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 doxycycline 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$_{50}$ 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 malaria, 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 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$_{50}$ 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.

We thank all participants and the teams in Lambaréné and Tübingen for their enthusiasm and continuous support.

REFERENCES


