Antimalarial Activity of Tulathromycin in a Murine Model of Malaria

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There is an urgent need for new antimalarial agents and strategies to treat and control malaria. This study shows an antiplasmodium effect of tulathromycin in mice infected with Plasmodium yoelii. The administration of tulathromycin around the time of infection prevented the progression of disease in 100% of the animals. In addition, highly parasitized mice treated with tulathromycin showed a decreased parasite burden and cleared the parasite faster than did untreated infected mice.

Malaria is one of the most significant global medical and scientific burdens, affecting nearly 40% to 50% of the world’s population (available in the World Health Organization [WHO] database at http://www.who.int/malaria/media/world_malaria_report_2013/en/index.htm). Despite advances in the development of malaria vaccines, no vaccine has shown high efficacy in protecting humans from Plasmodium infection or clinical malaria (1). Consequently, the use of antimalarial drugs will continue to be necessary for the management of this disease. Furthermore, morbidity and mortality due to malaria are exacerbated by undiagnosed bacterial coinfections and severe pneumonia (2, 3). Thus, the coadministration of drugs with both antimalarial and antibiotic effects may be beneficial in certain field circumstances.

Malaria chemotherapy has relied on a limited number of drugs that include chloroquine, sulfas and pyrimethamine, and artemisinin-derived drugs, among others (4). Unfortunately, Plasmodium has developed mechanisms of resistance against these drugs, limiting their efficacy in controlling the disease (5, 6; also available in the WHO database [http://www.who.int/malaria/publications/atoz/9789241500838/en/]). The lack of an effective malaria vaccine and the continued development of drug-resistant parasites highlight the need for new antimalarial agents and strategies to treat and control the disease.

Novel, effective, and safe antimalarial agents are urgently needed, especially drugs that could be used in combination with existing medicines to prevent the development of drug resistance. Macrolides are of particular interest as antimalarial drugs because, in contrast to others, they can be used safely in children and pregnant women (7–11).

The objective of this study was to evaluate the anti-Plasmodium activity of tulathromycin during both early and advanced blood stages of infection in a murine model of malaria. Here, we show the antimalarial effect of tulathromycin, a semisynthetic antimicrobial macrolide with a large margin of safety approved by the United States Food and Drug Administration and the European Medicines Agency for use in veterinary species (12).

The novelty of tulathromycin lies in its unique structure, three nitrogen/amine functional groups, representing the first member of a subclass of macrolides known as triamilides (13). In addition, tulathromycin could be formulated for different routes of delivery, since it is readily bioavailable when administered orally (14), subcutaneously (15), and intramuscularly (16). Furthermore, the antibacterial activity of tulathromycin has the potential to target bacterial coinfections, making it ideal as a combinatorial therapy.

We evaluated the efficacy of tulathromycin during the early blood stage of infection using an infective inoculum of Plasmodium yoelii. For this, C57BL/6N female mice (n = 23) (6 to 8 weeks old, 18 to 20 g in body weight) from the National Cancer Institute (Frederick, MD) were infected with 10^5 P. yoelii 17XNL-parasitized red blood cells (RBCs) intravenously at time zero. Mice were treated subcutaneously in the interscapular region with tulathromycin (n = 8) (25 mg/kg of body weight) (Draxxin injectable solution [100 mg/ml], lot A201905; Zoetis, Kalamazoo, MI, USA) or azithromycin (n = 8) (25 mg/kg of body weight) (diluted in propylene glycol at 100 mg/ml, lot P500088; Sigma-Aldrich, St. Louis, MO, USA) at −1 h and 12, 24, 36, and 48 h post- P. yoelii infection. The selection of the dose and route of administration of tulathromycin and azithromycin used in this study was based on information available in the literature (17). Azithromycin was used as a positive-control antimalarial macrolide (18). In this study, all mice tolerated the treatments. The rest of the mice (n = 7) remained untreated, representing the control group. Parasitemia (percentage of red blood cells infected with P. yoelii) was assessed by evaluation of Giemsa-stained monolayer RBC smears. A sample was taken from each mouse every 2 days from 5 to 30 days postinfection by snipping the tail and collecting a drop of blood. We assessed at least 10 high-power (1,000×) fields (the detection limit was ~1 parasite per 5,000 RBCs). The number (mean ± standard deviation [SD]) of RBCs evaluated for each infected mouse/time point was 427 ± 131. One-way analysis of variance (ANOVA) and Tukey’s multiple-comparison test were used to compare statistically the parasitemia and area under the parasitemia-time curve (AUC) between groups. The AUC was estimated for each group according to the trapezoidal rule (19, 20).

Following infection with P. yoelii, untreated mice exhibited peak parasitemia reaching about 50% 17 days postinfection followed by gradual clearance (Fig. 1). In striking contrast, treatment with tulathromycin before and shortly after infection with P. yoelii

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of the mean) are cumulative data from two independent experiments. More, they demonstrate that tulathromycin could be used in combination with drug combinations increase the rates of clinical cures and thereby overcome the selection pressure for the emergence of antimalarial resistance (21).

Due to its pharmacokinetic features, antimicrobial activity, and very promising anti-Plasmodium effect, tulathromycin warrants future research as a candidate for treatment of human malaria. Alternatively, its unique chemical structure may serve as a template to design new macrolide entities with antimalarial effects.

In this study, we discovered that tulathromycin is active against P. yoelii and is able to effectively eliminate the infection when used as a monotherapy. Whereas tulathromycin exhibits strong antimalarial activity as a monotherapy, this approach is strongly discouraged since it increases the likelihood that Plasmodium will develop drug resistance. Importantly, we also showed that tulathromycin could be combined with artemether without this combinational treatment interfering with the antimalarial activity of either. Since tulathromycin is in the same chemical family as azithromycin, it is possible that these drugs will exhibit the same mechanism of action and pharmacokinetics/pharmacodynamics, which may negate the need to develop tulathromycin as an antimalarial drug. However, given the key differences in the chemical structure between tulathromycin and other macrolides, it is equally likely that tulathromycin functions as an antimalarial drug through a novel mechanism and may exhibit important differences in pharmacokinetics/pharmacodynamics. Consequently, the novel findings on the antiplasmodium activity of tulathromycin certainly deserve further research. These could include an analysis of the causal prophylactic effect and pharmacokinetics/pharmacodynamics using different human Plasmodium species to overcome the selection pressure for the emergence of antimalarial resistance (21).

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