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# ACTIVITY OF AZITHROMYCIN AS A BLOOD SCHIZONTICIDE AGAINST RODENT AND HUMAN PLASMODIA IN VIVO

S. L. ANDERSEN, A. AGER, P. McGREEVY, B. G. SCHUSTER, D. WESCHE, R. KUSCHNER, C. OHRT, W. ELLIS, R. ROSSAN, AND J. BERMAN

*Division of Experimental Therapeutics and Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, District of Columbia; Center for Tropical Parasitic Diseases, Department of Microbiology and Immunology, Miami, Florida; Gorgas Memorial Laboratory, Panama, Panama*

*Abstract.* We compared the efficacy of azithromycin to the clinical antimalarial doxycycline in *Plasmodium berghei*–infected mice and in *P. falciparum*-infected *Aotus* monkeys. When mice were administered drug orally twice a day for three days, the minimum total dose of azithromycin that cured all mice was 768 mg/kg. Doxycycline at a dose of 1,536 mg/kg cured no mice. The efficacy of fast-acting blood schizonticides (quinine, halofantrine, artemisinin) against *P. berghei* was augmented by azithromycin. In monkey experiments in which there were two animals per experimental group, azithromycin (100 mg/kg/day for seven days) eliminated parasitemia; azithromycin (30 mg/kg/day) initially cleared 99.8–100% of the parasites with recrudescence in the one completely cleared case. Doxycycline (30 mg/kg/day) cleared 100% of the parasites with recrudescence in both cleared cases. Since azithromycin can be clinically administered at a somewhat higher daily dosage than doxycycline, the data suggest that it may be possible to replace drugs of the tetracycline class with azithromycin in combination with fast-acting blood schizonticides for the treatment of *P. falciparum* infection.

*Plasmodium falciparum* can be clinically resistant to all monotherapy with current antimalarial drugs. In Southeast Asia, the combination of quinine (650 mg every 8 hr) and the antibacterial antibiotic tetracycline (250 mg every 6 hr) is the treatment of choice for multidrug-resistant *P. falciparum* infections.[1] This use of drugs of the tetracycline class derives from the work of Rieckmann and others, who administered tetracycline to partially immune volunteers experimentally infected with chloroquine-resistant or chloroquine-sensitive *P. falciparum*.[2] Seven days of treatment cured 13 of 14 persons with chloroquine-resistant infections and six of six persons with chloroquine-sensitive infections. Because initial clearance of asexual parasites was slow (mean = 4.5 days), Rieckmann and others recommended that for acute malaria, tetracycline be used as an adjunct to a fast-acting schizonticide. Although tetracycline is used for adjunctive treatment of malaria and doxycycline, which has a longer half-life, is used for prophylaxis, the tetracycline class of compounds have the side effects of diarrhea and interference with bone development, the latter of which leads to these drugs being contraindicated for children and pregnant women.

In contrast, another anti-ribosomal antibiotic, erythromycin, was inactive against chloroquine/quinine–resistant malaria in Thailand.[3] Analogs of erythromycin such as azithromycin have now been marketed for bacterial and chlamydial infections. Gingras and Jensen reported that the in vitro efficacy of azithromycin against *P. falciparum* (the dose expected to kill 50% of the organisms [$ED_{50}$] = 3–6 μM) was 2–10 times lower than that of erythromycin.[4] We determined the preclinical in vivo schizonticidal efficacy of azithromycin and other antibacterial agents to evaluate whether azithromycin might reasonably be substituted for drugs of the tetracycline class in clinical antimalarial regimens.

## MATERIALS AND METHODS

**Rodent experiments.** Rodent experiments were conducted according to the protocols of Thompson and others.[5] Blood-stage parasites of the drug-sensitive parasite *P. berghei* (KBG 173 strain) within red blood cells were obtained from donor Swiss mice, and 500,000 parasites were inoculated intraperitoneally into naive mice on day 0. Drug suspended in carrier was administered either orally or via subcutaneous injection twice a day on days 3, 4, and 5 after parasite inoculation. Survival of the mice on day 60 after parasite inoculation was determined. Control mice (animals administered only the carrier) typically die of malaria between days 7 and 30 after inoculation. Mice in which drug causes death typically die on days 3–6 (Andersen S, unpublished data).

**Simian experiments.** Simian experiments were performed according to the protocols of Rossan and others.[6] Red blood cells infected with the chloroquine-resistant *P. falciparum* Vietnam Smith/RE strain were obtained from donor *Aotus l. lemurinis* monkeys and were injected into malaria-naive monkeys. When the animals had parasitemias of approximately 5,000/$mm^3$, drug was administered orally once each day for the next seven days.

Animals were evaluated for parasitemia daily until parasites cleared and then twice a week until day 100 to determine both the initial clearance of parasites and parasite recrudescence.

**Drugs.** All drugs were obtained from the Walter Reed Army Institute of Research Drug Repository. For oral administration, drug was suspended in 0.5% hydroxymethylcellulose/0.1% Tween 80. For subcutaneous administration, drug was suspended in peanut oil.

## RESULTS

**Rodent experiments.** When azithromycin was administered as a single agent (Table 1), a total dose of 384 mg/kg cured 71% (orally administered drug) to 86–100% of the mice (subcutaneously administered drug).

Azithromycin was more active than the other tested macrolides. Roxithromycin was not curative while clarithromycin was 43% curative at a total subcutaneous dose of

TABLE 1

*Efficacy of azithromycin, other macrolides and doxycycline against* Plasmodium berghei *infection in mice**

| Drug | Route | Total dose (mg/kg) | Mice surviving on day 60 | |
|---|---|---|---|---|
| | | | No. | (%) |
| Azithromycin | SC | 1,536 | 7/7 | (100) |
| | | 768 | 6/7 | (86) |
| | | 384 | 6/7–7/7† | (86–100) |
| | | 192 | 4/7 | (57) |
| | | 96 | 0/7–3/7† | (0–43) |
| | | 24 | 0/7–1/7† | (0–14) |
| | PO | 768 | 7/7 | (100) |
| | | 384 | 5/7 | (71) |
| | | 192 | 4/7 | (57) |
| | | 96 | 0/7 | (0) |
| Clarithromycin | SC | 1,536 | 3/7 | (43) |
| | | 384 | 0/7 | (0) |
| | PO | 1,536 | 0/7 | (0) |
| Roxithromycin | SC | 1,536 | 0/7 | (0) |
| Erythromycin | SC | 6,144 | 0/7 | (0) |
| | PO | 6,144 | 0/7 | (0) |
| Doxycycline | SC | 768 | 6/6 | (100) |
| | | 384 | 2/6 | (33) |
| | | 192 | 0/6 | (0) |
| | PO | 1,536 | 0/7 | (0) |
| | | 768 | 0/7 | (0) |

* Drugs were administered subcutaneously (SC) or orally (PO) twice a day for three days to 6–7 animals in each cohort. The total dose of drug, the number of surviving animals, and the percent of animals that survived are shown. For drugs other than azithromycin, only data from high doses are listed.

† Results from two independent experiments

1,536 mg/kg. Erythromycin was not curative at a dose of 6,144 mg/kg. Azithromycin was also more active than doxycycline, which was used in these experiments, because it is routinely administered at a dosage of 100 mg once or twice a day in humans, whereas tetracycline is routinely administered four times a day. In contrast to azithromycin, neither subcutaneous (384 mg/kg) nor oral doses (768 mg/kg) of doxycycline cured more than 50% of the mice.

Azithromycin was also administered orally in combination with quinine, halofantrine, and artemisinin. Neither quinine at a dose of 1,536 mg/kg nor azithromycin at a dose of 96 mg/kg were curative in this model when administered alone, but the combination cured five of seven animals. Halofantrine at a dose of 12 mg/kg cured four of seven mice; the combination of halofantrine (12 mg/kg) and azithromycin (48 mg/kg) cured seven of seven mice. Artemisinin at a dose of 48 mg/kg cured two of seven mice; the combination of artemisinin (48 mg/kg) and azithromycin (48 mg/kg) cured five of seven mice. Rough isobolograms indicated that the activity of azithromycin was additive, rather than synergistic or antagonistic, with fast-acting schizonticides.

**Simian experiments.** Azithromycin at a dose of 30 mg/kg/day was slightly less active than doxycycline at the same dose (Table 2). One of the azithromycin-treated animals had a parasitemia of 6,000/mm$^3$ prior to therapy, reached a level of 394,000 parasites/mm$^3$ on day 3 of treatment, and had < 10 parasites/mm$^3$ on day 10, but never completely cleared all parasites. The other monkey cleared the parasitemia on day 10 but recrudesced five days later. Both animals treated with doxycycline cleared their parasitemias on days 10–12 but recrudesced 7–11 days later. Azithromycin at a dose of 100 mg/kg/day was more active; parasitemia cleared on day 10 in one monkey and on day 14 in the other and had not recrudesced up to 100 days later. In previously performed experiments, the curative dose of a fast-acting blood schizonticide (mefloquine) typically clears parasitemia by four days of therapy.

## DISCUSSION

The treatment of choice for malaria due to multidrug-resistant *P. falciparum* in Thailand and the United States is seven days of the combination of quinine plus tetracycline. Although this combination is presently effective,[1] drug toxicity and possible decreased efficacy in the future suggest that other regimens should be developed.

Azithromycin is a newly marketed erythromycin analog used at doses of 1,000 mg (14 mg/kg) for chlamydial urethritis,[2] 500 mg on day 1 followed by 250 mg/day for four days for outpatient pneumonia,[3] and 500 mg/day (7 mg/kg/day) for 20–30 days for treatment of *Mycobacterium avium* disease.[4] The major side effect of azithromycin is mild abdominal pain and diarrhea that occurs in 3–5% of patients administered 500 mg on day 1 followed by 250 mg/day for four days, and in approximately 16% of patients administered 500 mg/day for 20–30 days.[2,4]

The schizonticidal activity of azithromycin was determined in the *P. berghei*/mouse model and the *P. falciparum*/*Aotus* monkey model, the standards in which the efficacy of fast-acting blood schizonticidal agents are evaluated. We are not aware that the efficacy of slower-acting agents has been reported in these models. Doxycycline was used as an internal control because of the possibility that for slower-acting antiribosomal agents, effective doses in these models

TABLE 2

*Efficacy of azithromycin compared with doxycycline against* Plasmodium falciparum *in* Aotus *monkeys**

| Drug | Dose (mg/kg/day) | Parasitemia (× 1,000) on day | | | | | | | | Recrudesce | Final result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | −1 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | | |
| Azithromycin | 100 | 3 | 62 | 121 | 66 | 5 | 0.3 | <0.01 | 0 | No | Cure |
| | 100 | 2 | 124 | 13 | 0.7 | 0.03 | 0 | 0 | 0 | No | Cure |
| Azithromycin | 30 | 10 | 122 | 401 | 6 | 0.1 | 0 | 0 | 0 | Day 15 | Clear/recrudesce |
| | 30 | 6 | 99 | 145 | 2 | 0.1 | <0.01 | <0.01 | <0.01 | (Not clear) | Not clear |
| Doxycycline | 30 | 5 | 140 | 148 | 3 | 0.04 | 0 | 0 | 0 | Day 21 | Clear/recrudesce |
| | 30 | 4 | 93 | 112 | 55 | 4 | <0.01 | 0 | 0 | Day 12 | Clear/recrudesce |

* Monkeys were infected with blood-stage *P. falciparum*. Treatment was administered to two monkeys in each treatment group on days 1–7. Parasitemia denotes the number of organisms (× 1,000/mm$^3$) of blood on day −1 to day 14 with respect to drug administration.

might not correlate in absolute value with effective values in humans.

Against *P. berghei* in mice, the 100% orally effective dose of azithromycin was 768 mg/kg, whereas orally administered doxycycline was inactive even at a dose of 1,536 mg/kg.

Combinations of azithromycin and fast-acting blood schizonticides were evaluated in the mouse model. The activity of azithromycin was additive with that of quinine, halofantrine, and artemisinin. These results support the conclusion of previous work by Gingras and Jensen,[9] who showed additivity of azithromycin and chloroquine against *P. berghei* strain N.

For experiments with *P. falciparum* in *Aotus*, standard procedure is to use two animals per therapeutic group. For chloroquine-resistant *P. falciparum*, oral azithromycin at a dose of 100 mg/kg/day for seven days completely cured the infection in both treated animals. A lower dose, 30 mg/kg/day for seven days, initially suppressed 99.8–100% of the parasites, and was slightly less active than an equal dose of doxycycline, which suppressed 100% of parasites in both treated monkeys. However, no monkeys treated with 30 mg/kg/day of either drug were cured. The results obtained in simians show that azithromycin was more effective than doxycycline when the drugs were administered at the approximate ratio (3:1) tolerated by humans.

The absolute values of the 100% curative doses of azithromycin in the mouse and monkey models (768 mg/kg and 100 mg/kg/day) were 55 and 14 times higher, respectively, than clinically tolerated doses of 14 mg/kg and 7 mg/kg/day. However, the absolute values of the < 100% curative doses of doxycycline were > 1,536 mg/kg and 20 mg/kg/day, values that are > 500 and 10–20 times higher than clinically tolerated doxycycline doses of 1.5–3 mg/kg/day. In these models, effective doses of anti-ribosomal agents are much greater than effective clinical doses, and the absolute value of the clinically effective azithromycin dose can not be predicted from the absolute values needed for efficacy in the models.

The comparability or superiority of azithromycin efficacy to that of doxycycline in *Aotus* at an azithromycin/doxycycline ratio of 3:1 makes it possible to suggest clinical schizonticidal regimens in which doxycycline/tetracycline would be replaced by azithromycin. Since azithromycin, like the tetracyclines, slowly clears *P. falciparum* parasitemia, azithromycin should only be used in combination with a fast-acting blood schizonticide. Because the function of the fast-acting agent is to quickly kill most of the parasites and the function of azithromycin would be, as for the tetracyclines, to more slowly eliminate the remaining parasites, the fast-acting agent should show RI or low-level RII resistance, but not high-level RII or RIII resistance, in the endemic region in question. With respect to the daily dose of azithromycin, the dose should be approximately three times the 100–200 mg/day dose of doxycycline. Thus, regimens ranging from the clinically approved regimen, 500 mg on day 1 followed by 250 mg on days 2–5, to a regimen using somewhat more drug, 500 mg/day for seven days, might be tried. Although we propose that these azithromycin regimens might be superior to regimens using doxycycline/tetracycline on the basis of efficacy and subjective toxicity, a further advantage is that there is no contraindication to administration of azithromycin to pregnant women or young children.

Authors' addresses: S. L. Andersen, P. McGreevy, B. G. Schuster, D. Weseke, C. Ohrt, W. Ellis, and J. Berman, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20307-5100. A. Ager, Center for Tropical Parasitic Diseases, Department of Microbiology and Immunology, 12500 SW 152 Street, Miami, FL 33177. R. Kuschner, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100. E. Rossan, Gorgas Memorial Laboratory, Panama, Panama.

Reprint requests: S. L. Andersen, Walter Reed Project, Kenyan Medical Research Institute, Mbigathi Road, Nairobi, Kenya.

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