ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1994, p. 1862–1863
0066-4804/94/$04.00+0

Vol. 38, No. 8

Efficacy of Azithromycin as a Causal Prophylactic Agent against Murine Malaria

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Received 1 March 1994/Returned for modification 19 April 1994/Accepted 14 May 1994

The efficacy of the newly marketed azalide azithromycin was compared with that of the clinical agent doxycycline in a murine model of sporozoite-induced malaria. Drug was administered once; *Plasmodium yoelii* sporozoites were administered 2 h later; survival at day 60 was determined. For parenterally administered drug, 160 mg of azithromycin or doxycycline per kg of body weight was 100% effective; 40 mg of azithromycin per kg was 80% effective, but 40 mg of doxycycline per kg was 40% effective. Orally administered azithromycin was somewhat less effective than parenterally administered drug, consistent with the 37% clinical oral bioavailability of this agent. For orally administered azithromycin, 160 mg/kg was 100% effective and 40 mg/kg was 40% effective. The efficacy of azithromycin in comparison with that of doxycycline and the known prolonged levels of azithromycin in the livers of humans suggest that azithromycin has potential as a clinical causal prophylactic agent for malaria.

Malaria, particularly that caused by *Plasmodium falciparum*, is a leading cause of morbidity and mortality in many developing countries, with an estimated 200 million infections and 2 million deaths worldwide (7).

After *P. falciparum* sporozoites are inoculated via the bite of the female mosquito, the organisms invade the liver, where they undergo asexual division (exoerythrocytic schizogony). From the liver, merozoites are released into the blood beginning approximately 7 days after sporozoite inoculation and invade the erythrocytes, where asexual division (blood stage schizogony) again occurs. The prepatent period—the time between the mosquito bite and the first detection of plasmodia in the peripheral blood—for *P. falciparum* in humans normally ranges between 9 and 12 days.

Malaria prophylaxis can be viewed as affecting the liver stage (causal prophylaxis) or the subsequent stage of blood infection (suppressive prophylaxis). Causal prophylaxis is particularly attractive because drug treatment could be terminated shortly after exposure. Despite the attractiveness of causal prophylaxis, there is not an accepted causal prophylactic agent for malaria at present. Primaquine, which has activity against exoerythrocytic stages, is too toxic to be used chronically. Dihydrofolate reductase inhibitors such as pyrimethamine and proguanil are used, but there is considerable resistance, at least of blood stage forms, to these agents (3). Chloroquine, mefloquine, and other blood schizonticides are suppressive prophylactic drugs. Doxycycline is an antibacterial antibiotic that is also active against plasmodia. Because doxycycline is not sufficiently effective when used solely as a causal prophylactic agent (8), it is used as a combined causal and suppressive prophylactic agent.

Azithromycin is a semisynthetic derivative of erythromycin that differs from erythromycin by possessing a methyl-substituted nitrogen in the macrolide ring. This chemical substitution confers pharmacological characteristics that give the drug high and prolonged levels in liver tissue (5) and that make the drug potentially attractive as a causal prophylactic antimalarial agent.

We compared azithromycin with doxycycline in a murine model of antimalarial causal prophylaxis (4), with modifications. On day 0, drug was administered once orally (p.o.) or subcutaneously (s.c.) to 6- to 7-week-old CD-1 mice. Two hours later, 250,000 *Plasmodium yoelii* sporozoites were inoculated intraperitoneally (i.p.) into the animals. The sporozoites were obtained by mascerating in saline female *Anopheles stephensi* mosquitoes that had fed for 17 days previously on donor mice harboring a 5 to 20% parasitemia. The survival of animals up to day 60 was determined. All drugs were obtained from the Walter Reed Army Institute of Research drug inventory. Drug to be administered p.o. was suspended in 0.5% hydroxymethylcellulose–0.1% Tween 80; drug to be injected s.c. was suspended in peanut oil.

In the mouse-*P. yoelii* sporozoite model, the prepatent period is 2 to 3 days. It is possible that drug administered prior to sporozoite inoculation might kill a portion of the parasites in the liver and also persist for 2 days and kill the blood stage forms resulting from viable liver parasites. In this case, an experiment designed only to investigate causal prophylactic activity would in fact be quantitating causal plus suppressive prophylactic activity. Therefore, in each causal prophylactic experiment, further experiments were performed in which suppressive prophylactic activity was determined separately. Animals were administered drug on day 0, inoculated i.p. with 50,000 *P. yoelii* blood stage parasites (obtained from donor mice) 2 days later, and observed for survival by day 60 of the experiment. Survival of animals in the suppressive prophylactic experiments signifies that sufficient drug was present 2 days after dosing to kill blood stage forms when they emerged from the liver and invaded the erythrocytes. The reason that 250,000 sporozoites but only 50,000 blood stage forms are used is that it has been empirically determined that with these numbers of parasites, non-drug-treated animals die at the same time: day 10 after sporozoite inoculation and day 8 after blood stage form inoculation.

The survival of animals who underwent prophylaxis against *P. yoelii* is given in Table 1.

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TABLE 1. Efficacy of drugs for the prophylaxis of *P. yoelii* infection in mice[a]

Expt and drug	Route	Dose (mg/kg)	Survival at day 60 (no. of mice alive/total no. tested)	
			Causal + suppressive mode	Suppressive mode
Expt 1				
Azithromycin	p.o.	640	5/5	0/5
		160	5/5	0/5
		40	2/5	0/5
		0	0/5	0/4
Azithromycin	s.c.	640	5/5	4/5
		160	5/5	1/5
		40	4/5	0/5
		10	2/5	0/5
		0	0/5	0/5
Doxycycline	s.c.	640	5/5	1/5
		160	5/5	1/5
		40	2/5	0/5
		10	0/5	0/5
		0	0/5	0/5
Expt 2				
Doxycycline	p.o.	512	4/5	0/5
		128	3/5	0/5
		32	1/5	0/5
		0	0/5	0/5

[a] Animals were administered one dose of drug either per p.o. or s.c. and were then administered *P. yoelii* sporozoites 2 h later (causal plus suppressive prophylactic mode) or *P. yoelii* blood stage parasites 2 days later (suppressive prophylactic mode). The survival of animals at day 60 of the experiment was determined. All animals who died did so between days 10 and 41. Initially, there were five animals per experimental group.

When azithromycin was administered p.o. at 160 to 640 mg/kg of body weight prior to inoculation with sporozoites, all animals survived. Two of five animals (40%) that received 40 mg/kg survived. When azithromycin was administered p.o. prior to inoculation with blood stage forms, no mouse survived. The prophylactic efficacy of azithromycin administered p.o. was therefore entirely due to causal activity against liver stage forms and not to the activity of residual drug against blood stage forms.

Parenterally administered azithromycin was more active than orally administered azithromycin. All mice that received 160 to 640 mg/kg survived. In addition, four of five mice (80%) that received 40 mg/kg survived, and two of five mice (40%) administered 10 mg/kg survived. The higher level of activity of parenterally administered azithromycin in comparison with that of orally administered azithromycin is consistent with the 37% oral bioavailability of the drug (in humans) (2).

Parenterally administered azithromycin had residual suppressive prophylactic activity at the high dose of 640 mg/kg: four of five mice, challenged 2 days later with blood stage forms, survived. At dosages of 10 to 160 mg/kg, however, virtually all prophylactic activity could be attributed to causal efficacy.

Parenterally administered doxycycline was somewhat less active than parenterally administered azithromycin. All animals administered doxycycline at 160 to 640 mg/kg survived, but only two of five (40%) animals receiving 40 mg/kg survived and 10 mg/kg was unprotective. Similarly, doxycycline administered p.o. appeared to be somewhat less active than azithromycin administered p.o., with the caveat that doxycycline was dosed p.o. in a separate experiment.

Small clinical studies with azithromycin and doxycycline as causal prophylactic agents have been completed. In the azithromycin study, drug was administered at a dose of 500 mg 2 days prior to inoculation with *P. falciparum* sporozoites and then at a dose of 250 mg/day from 1 day prior through 5 days after sporozoite challenge. This regimen spans most of the period of the exoerythrocytic stage for *P. falciparum* and is similar to the U.S. Food and Drug Administration-approved regimen for pneumonia (500 mg on day 1 and then 250 mg on days 2 to 5). Three of four human volunteers were protected against malaria (6). In the doxycycline study, drug was administered at its clinical dose of 100 mg/day from 3 days before to 6 days after challenge with *P. falciparum* sporozoites, and 8 of 12 human volunteers were protected against malaria (8).

In mice and in small clinical trials, the causal prophylactic activity of azithromycin appears to be equal to or somewhat better than that of the clinical agent doxycycline. Drugs of the tetracycline class are contraindicated for young children and women with reproductive potential because of interference with skeletal and tooth development. Thus, an additional advantage of azithromycin is that it can be used in a more general patient population than that which can receive tetracyclines. On the other hand, azithromycin is licensed for short-term use, and side effects in humans because of chronic dosing are as yet unreported. Nevertheless, the activity of azithromycin in comparison with that of doxycycline suggests that azithromycin may have a clinical role in causal prophylaxis against malaria. If causal activity is insufficient, the suppressive prophylactic activity evidenced here and the blood schizonticidal activity demonstrated in other experiments (1) suggest that azithromycin, like the tetracyclines, may have potential as a combined causal and suppressive prophylactic agent.

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