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Activities of Pefloxacin and Ciprofloxacin against Experimental Malaria in Mice

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We investigated the in vivo antimalarial activities of pefloxacin and ciprofloxacin in Swiss albino mice infected intravenously with 5×10^6 *Plasmodium yoelii* N67 parasites 1 h before treatment. Groups of 20 mice received a subcutaneous injection of 40, 80, or 160 mg of ciprofloxacin or pefloxacin per kg of body weight every 8 h for 3 days. Parasitologic activity was assessed on day 4, and survival was assessed on day 21. Control mice had a fulminant course with a parasitemia of 61.3% ± 12.1% on day 4, and 90% of the mice were dead on day 21. The lower dosages of pefloxacin and ciprofloxacin (40 and 80 mg/kg) were not efficient. With 160 mg/kg, ciprofloxacin achieved an 85.8% reduction in parasitemia and 17 of 20 mice survived. Pefloxacin achieved a 92.8% reduction in parasitemia, and all mice survived. All treated, noninfected control mice survived. With ciprofloxacin, the antimalarial activity was similar with injections of 240 mg/kg every 12 h but was strongly diminished with injections of 160 mg/kg every 12 h. With pefloxacin, similar activities were observed with injections of 160 mg/kg every 8 h or injections of 160 or 240 mg/kg every 12 h. With both drugs, this activity was highly reduced when the treatment was delayed by 24 h. This underlines the need to provide treatment within the first hours after infection to achieve an optimal effect in this rapidly lethal experimental model of malaria. Pefloxacin and, to a lesser extent, ciprofloxacin are potent antimalarial drugs which might prove useful in the treatment of less rapidly aggressive human malaria.

Resistance of *Plasmodium falciparum* to standard antimalarial agents, mainly chloroquine and pyrimethamine-sulfadoxine, is increasing rapidly in most areas where malaria is endemic. New antimalarial drugs that differ in their mechanism of action against *P. falciparum* are urgently needed. Nalidixic acid, a quinolone antibiotic that inhibits DNA gyrase of circular DNA, is slightly active in vitro against *P. falciparum* at concentrations of 10^{-5} to 10^{-4} M (3). Structurally related agents, such as fluoroquinolones, achieve higher concentrations in serum and also inhibit the growth of *P. falciparum* in vitro. This was recently demonstrated for several fluoroquinolones: ciprofloxacin exhibited the highest antimalarial activity in vitro against both chloroquine-susceptible and -resistant strains of *P. falciparum* (4, 8, 9; P. Deloron, M. Mahltora, C. Gaudin, and D. Salmon, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1326, 1989). While less effective than ciprofloxacin against *P. falciparum* in vitro, pefloxacin was also active and is known to possess pharmacokinetic properties that allow better tissue diffusion. After 96 h of exposure to the drug, the 50% inhibitory concentrations (IC_{50}s) were shown to reach 0.54 μg/ml for ciprofloxacin and 2.7 μg/ml for pefloxacin (4). For these fluoroquinolones, the in vitro antimalarial effect occurred at concentrations comparable to those achieved in the sera of humans with bacterial infections during therapeutic regimens (the peak level in serum was between 3 and 4 μg/ml after a usual oral therapeutic dose of either drug) (6, 10).

Using a model recognized to be important in the screening of antimalarial drugs to be used in humans, we compared the in vivo antimalarial properties of ciprofloxacin and pefloxacin in mice experimentally infected with *Plasmodium yoelii*, a parasite strain resistant to chloroquine.

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MATERIALS AND METHODS

The drugs used in this study were pefloxacin mesylate (Roger Bellon Laboratories, Neuilly sur Seine, France), which was used as an intravenous injectable solution for animal experiments, and ciprofloxacin chlorhydrate, which was supplied in standard powder form (Bayer Pharma, Puteaux, France). Ciprofloxacin was dissolved in 1 N NaOH and was diluted in distilled water, and the pH was adjusted to between 8 and 9 with 1 N HCl. Antibiotic suspensions (ciprofloxacin) or solutions (pefloxacin) were prepared each day and stored at 4°C between administrations.

For in vivo assay, the Nigerian N67 strain of *P. yoelii* was used. This line is fully resistant to chloroquine and is lethal to infected mice in 6 days (11).

Randomly bred female Swiss albino mice (NMRI line) were used. The mice received a standard pellet diet (A04; UAR 91390; Villemaison) and tap water ad libitum. They were held at a temperature of 22 ± 3°C and in about 50% relative humidity. Animals were inoculated intravenously with 5×10^6 parasitized erythrocytes in 0.2 ml. These parasitized erythrocytes were obtained from the blood of highly infected mice (average, 38% rising parasitemia); blood was diluted in 0.9% NaCl to give 25×10^6 parasites per ml. For each experiment, mice were randomly assigned to a given treatment group (10 mice in each group). Within 1 h after infection, drugs were administered subcutaneously in 0.2-ml aliquots.

For the first series of experiments, injections were administered every 8 h and a 3-day treatment duration was chosen

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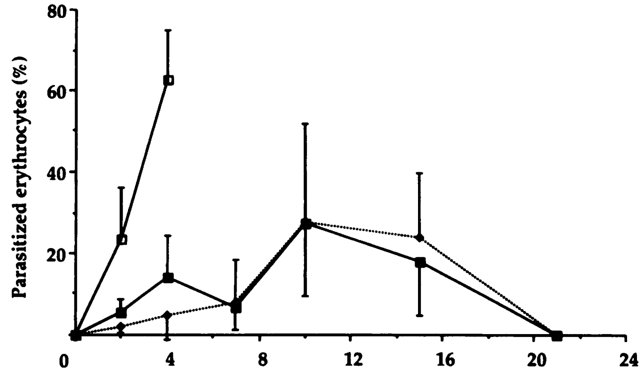

FIG. 1. Evolution of parasitemia in mice parasitized by *P. yoelii* and receiving fluoroquinolones (160 mg/kg three times daily over 3 days). Each point represents the mean parasitemia ± standard deviation for 20 mice treated with either pefloxacin (♦), or ciprofloxacin (■) or for control parasitized mice (□). On day 7, 6 of 20 control mice were still alive. The extent of hemolysis resulting from the prolonged high parasitemia did not allow us to calculate accurate parasitemias. This was also the case on day 10, day 14 (4 of 20 mice were alive), and day 21 (2 mice were alive).

(in accordance with World Health Organization recommendations for chloroquine therapy in humans suffering from malaria). Two series of experiments that included 10 mice in each group were conducted. Increasing drug concentrations were used, starting from 40 mg/kg of body weight three times daily (this dosage is often used for antibacterial experiments) up to 160 mg/kg three times daily.

Subsequently, alternative drug regimens were tested: injections of 160 or 240 mg/kg every 12 h for 3 days. Lastly, the activities of injections of 240 mg/kg every 12 h initiated 24 h after infection and lasting for 1, 2, or 3 days were determined.

For all therapeutic regimens, the parasiticidic activity was assessed on day 4 on Giemsa-stained thin blood smears. The clinical efficacy was assessed on day 11 according to the weight of the mice and was assessed on day 21 according to the number of mice that were alive.

For statistical analysis we used Student's *t* test for comparison of means. The level of significance was ≤5%.

RESULTS

During the first series of experiments with injections of drugs every 8 h for 3 days, parasitemia in drug-free parasitized control mice peaked to 61.3% ± 12.1% at day 4 and 18 of 20 control mice were dead before day 21. The lower dosages of pefloxacin and ciprofloxacin (40 and 80 mg/kg for 3 days) were poorly active, with less than 10% reduction in parasitemia compared with that in controls and with 60% mortality at day 21. With the regimen of 160 mg/kg given every 8 h for 3 days, all mice were parasitized at day 4, but a significant decrease in parasitemia compared with that in drug-free parasitized controls was observed in mice given either pefloxacin (4.5% ± 5.9%) or ciprofloxacin (14.1% ± 10.6%) ($P < 0.001$ between controls and both drugs). Pefloxacin was more active than ciprofloxacin ($P < 0.01$ between both drugs) (Fig. 1). These results correspond to a reduction of parasitemia of 92.8% for pefloxacin and 85.8% for ciprofloxacin (Table 1). Parasitemia was monitored until day 21.

TABLE 1. Effect of treating mice within 1 h of infection with *P. yoelii*[a]

Treatment regimen and drug concn (mg/kg)	% Reduction in parasite density[b]	Mortality on day 21 (no. of dead mice/total no. tested)	Wt (g) on day 11
Controls[c]	0	18/20	19.6
Pefloxacin			
80 (3)[d]	5.7	6/10	
160 (2)	90.3	0/10	26.2
160 (3)	92.8	0/20	27.4
240 (2)	99.4	0/10	27.6
Ciprofloxacin			
80 (3)	0.5	6/20	
160 (2)	53.7	0/10	26.2
160 (3)	85.8	3/20	29
240 (2)	76.1	0/10	26.6

[a] Treatment lasted for 3 days.
[b] Percent reduction of parasitemia at day 4 was calculated as follows: [1−(mean parasitemia of treated mice/mean parasitemia of control mice)] × 100.
[c] Mean parasitemia was 61.3% ± 12.1% in drug-free parasitized controls at day 4.
[d] Values in parentheses are numbers of daily injections.

After a recrudescence of parasitemia at day 15, parasites were eradicated in mice treated with both pefloxacin and ciprofloxacin. On day 21, all mice treated with pefloxacin survived, and 17 of 20 of the mice treated with ciprofloxacin survived (Fig. 2). None of the noninfected control mice receiving one of these two drug regimens died. Following infection, parasitized control mice lost weight, reaching a minimum mean value of 19.6 g on day 11. The weight of treated mice did not follow a similar evolution. On day 11, the mean weights of pefloxacin- and ciprofloxacin-treated mice were 27.4 and 29 g, respectively.

Drugs were then administered every 12 h for 3 days (Table 1). When the total daily dose was reduced to 160 mg/kg given every 12 h instead of 160 mg/kg given every 8 h, pefloxacin was equally active but ciprofloxacin was not ($P < 0.01$). When the total daily dose was divided into two injections of 240 mg/kg instead of three injections of 160 mg/kg, the antimalarial activity was not significantly different for pefloxacin or ciprofloxacin.

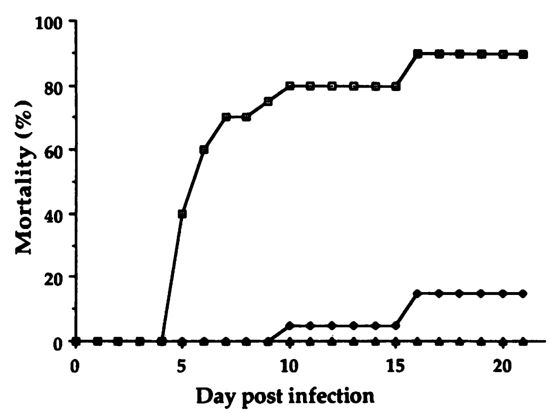

FIG. 2. Mortality of mice parasitized by *P. yoelii* receiving fluoroquinolones (160 mg/kg three times over 3 days). Each point represents the percentage of dead mice (n = 20 in each group) treated with pefloxacin (▲) or ciprofloxacin (♦) or control infected mice (□).

TABLE 2. Effect of treatment delay for 24 h after infection of mice with *P. yoelii* and effect of treatment duration[a]

Treatment and duration	% Reduction of parasite density at day 4[b]	Mortality at day 21 (no. of dead mice/total no. tested)
Controls[c]	0	18/20
Pefloxacin		
1 day	64.2	9/20
2 days	73.1	6/20
3 days	76.7	7/20
Ciprofloxacin		
1 day	20.3	13/20
2 days	29.1	7/20
3 days	21.9	5/20

[a] Treatment was with 240 mg of drug per kg of body weight every 12 h.
[b] See footnote *b* of Table 1.
[c] Mean parasitemia was 71.0% ± 19.4% in drug-free parasitized controls at day 4.

In another treatment regimen, administration of drugs at 240 mg/kg given every 12 h was then delayed by 24 h after infection and lasted 1, 2, or 3 days (Table 2). Parasitemias at day 4 were higher than those after the early initiation of treatment. Parasitemias of treated mice were still significantly lower than those of controls at day 4 ($P < 0.001$ between pefloxacin and controls, and $P < 0.05$ between ciprofloxacin and controls). In the first experiment, the activities of the drugs in each regimen did not vary with the duration for treatment (1, 2, or 3 days). In a second experiment, in which controls were highly parasitized (85% ± 12.9% at day 4 instead of the average of 60% parasitemia obtained in all the previous experiments), the activities of pefloxacin and ciprofloxacin were weaker with 1 day of treatment than they were with treatments of 2 or 3 days.

In one experiment, seven surviving mice previously infected and treated with fluoroquinolones were reinoculated at month 2 and were not subsequently treated. The parasitemia was initially negative in all mice, and after infection it either remained negative or became transiently positive (mean parasitemia, 0.45% at day 4); but in all cases, parasitemia was definitively negative after day 7.

DISCUSSION

Our results demonstrate that ciprofloxacin and pefloxacin, potent inhibitors of the in vitro growth of *P. falciparum*, are also active in treating plasmodial infections in mice. Converse to its in vitro activity, pefloxacin is more active than ciprofloxacin in vivo. The interest of this experimental model of blood-induced *P. yoelii* infections in Swiss albino mice lies in the fact that it leads to a fulminant infection that peaks to 60 to 80% parasitemia in 4 to 6 days. Most animals die within 5 to 8 days, and the efficacy of an antimalarial drug can be assessed in the first days after infection. However, after the beginning of the second week after infection, surviving animals develop a very effective cellular immune response that contributes to control of the infection and clears the parasitemia. This makes the animals unsusceptible to further relevant infections with this plasmodial strain (and, potentially, with other related strains or species).

Recent studies have investigated the in vitro activity of various fluoroquinolones against *P. falciparum*. Ciprofloxacin was among the most active, with IC_{50}s of between 5.2 and 14 μg/ml in a 48-h experiment, depending on the in vitro model and strain used (4, 8, 9), whereas the IC_{50} for pefloxacin was 39 μg/ml for a chloroquine-resistant strain of *P. falciparum* (4). We had similar results and established that the IC_{50}s of pefloxacin and ciprofloxacin against a chloroquine-resistant strain of *P. falciparum* were 17.8 and 4.3 μg/ml, respectively (Deloron et al., 29th ICAAC). As demonstrated by Divo et al. (4), when exposure to antibiotics lasted 96 h instead of 48 h, the IC_{50}s decreased to 0.54 μg/ml for ciprofloxacin and to 2.7 μg/ml for pefloxacin. Pharmacokinetic studies have shown that peak levels in serum reached 3.77 to 3.84 μg/ml after an oral dose of 400 mg of pefloxacin was given to human volunteers (10) and reached 3.41 to 4.21 μg/ml after an oral dose of 750 mg of ciprofloxacin (6). Thus, concentrations of drug in serum achieved after a single therapeutic dose by the oral route in humans correspond to concentrations that are active against *P. falciparum* in vitro, and for a few hours, they stay above the IC_{50}s for *P. falciparum* found after 96 h of exposure, since the terminal half-life of the fluoroquinolones is relatively long, ranging from 3 to 4 h for ciprofloxacin and 10 to 11 h for pefloxacin (7).

Following intravenously induced infection with a chloroquine-resistant strain of *P. yoelii*, the administration of 160 mg of pefloxacin or ciprofloxacin per kg of body weight three times a day for 3 days resulted in reductions of parasitemia of 92.8 and 85.8%, respectively, compared with that in untreated mice. Moreover, mortality was significantly decreased and weight loss was prevented. Those parameters followed similar variations as parasitemia did.

Lower unit doses decreased the antimalarial activities of both drugs. When the same daily doses of both drugs were administered every 12 h (240 mg/kg two times instead of 160 mg/kg three times), pefloxacin and ciprofloxacin were equally active. When treatment was initiated 24 h after infection, the activities of both drugs were reduced, but to a lesser extent with pefloxacin than with ciprofloxacin. Various explanations might account for those results: (i) an inoculum effect between quinolones and plasmodia, as has been described by some investigators (2) for gram-negative bacteria, could exist, and (ii) quinolones are relatively slow-acting antimalarial agents (as demonstrated by the differences in the IC_{50}s for durations of contact with *P. falciparum* in vitro of between 48 and 96 h), but *P. yoelii* multiplies rapidly (its asexual cycle is 24 h). It is therefore possible that, when the drug begins to be effective, it is too late to reverse completely the physical damage undergone by the host and to block the growth of so many parasites.

The wide distribution and concentration of fluoroquinolones were demonstrated in human tissues (7, 10, 14) and were demonstrated for ciprofloxacin in human neutrophils (5), but the extent of the intraerythrocytic concentration is still unknown. The higher in vivo activity of pefloxacin compared with that of ciprofloxacin might be explained by pharmacokinetic differences between the two drugs.

The fluoroquinolones are known to inhibit the A subunit of DNA gyrase (15), an enzyme which catalyzes several reactions, including the production of negative superhelical twists within circular double-stranded DNA. This enzyme is present in bacteria and organelles such as mitochondria in superior organisms (1). *P. falciparum* contains a functional mitochondrion, and Riou et al. (12) have characterized DNA gyrase from the murine malaria species *Plasmodium berghei*. Thus, it is likely that fluoroquinolones act upon similar enzyme systems in *P. falciparum* species. Other mechanisms of action may, however, be involved since dosage regimens that were effective in our experiments probably give concentrations in blood higher than those usually clinically achievable in humans.

The therapeutic activities that we observed and the in vitro efficacies of ciprofloxacin and pefloxacin suggest that these two drugs might prove useful in the treatment of *P. falciparum* infections in humans. Indeed, a preliminary report (13) stated the efficacy of using norfloxacin at the usual antibacterial concentration of 400 mg twice daily for 3 days in treating *P. falciparum* malaria in India. This new family of antimalarial agents may be a valuable tool in the treatment of infections caused by multi-drug-resistant *P. falciparum*.

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