Activity of Two Chlorinated Lincomycin Analogues Against Chloroquine-Resistant Falciparum Malaria in Owl Monkeys

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The chloroquine-resistant Oak Knoll strain of Plasmodium falciparum, recently adapted to the owl monkey (Aotus trivirgatus), was insensitive to chloroquine therapy. Two chlorinated lincomycin analogues tested in this host-parasite system cured blood-induced infections. Acute infections were treated orally for 7 consecutive days with either 15 or 75 mg of clindamycin hydrochloride (U-21) per kg per day, 10 or 50 mg of N-demethyl-4'-pentyl clindamycin hydrochloride (U-24) per kg per day, or 20 mg of chloroquine base per kg per day. These lincomycin analogues cleared trophozoites from the peripheral blood by the end of the 7-day treatment period. The speed of clearance of parasites was not dose-related, but curative activity appeared dependent upon the amount of drug given as well as the number of daily treatments. The efficacy of U-21 and U-24 is of particular interest since they represent major structural departures from compounds commonly used in the treatment of malaria.

Since the appearance of chloroquine-resistant falciparum malaria in man (5), there has been a world-wide interest in the discovery of effective compounds for the treatment of this disease. Particular emphasis has been placed on identifying drugs which differ chemically and in mode of action from commonly used antimalarials. Promising compounds in this regard are two chlorinated lincomycin analogues which are active against Plasmodium cynomolgi in rhesus monkeys (7, 9) as well as chloroquine-resistant or normally sensitive strains of P. berghei in mice (3, 7). These compounds, designated U-21 (clindamycin hydrochloride) and U-24 (N-demethyl-4'-pentyl clindamycin hydrochloride), are prepared by chlorination of lincomycin hydrochloride at the 7 position and substitution of methyl and N-propyl in the amino-sugar moiety (Fig. 1) (4). The parent compound does not possess antiplasmodial activity (3). U-21 is commercially available and is relatively nontoxic, whereas tests for human toxicity of U-24 are still incomplete and the drug is not yet approved for human use. We now report the curative activity of these compounds against a strain of P. falciparum which is highly resistant to chloroquine.

MATERIALS AND METHODS

Healthy owl monkeys (Aotus trivirgatus) weighing 0.8 to 1.0 kg were injected intravenously with 5 million erythrocytes parasitized with the chloroquine-resistant Oak Knoll strain of P. falciparum. This strain was isolated from man and adapted to the owl monkey in Q. M. Geiman's laboratory (W. A. Siddiqui et al., Amer. J. Trop. Med. Hyg., in press).

Two experiments were conducted to determine the activity of the lincomycin analogues. In all instances, treatment was begun 7 days after infection when rising parasitemias ranged from 50 to 400 parasites per 10,000 erythrocytes. All doses were administered by stomach tube each morning for 7 consecutive days. In the first experiment, three monkeys received 50 mg of the U-24 hydrochloride salt per kg per day, suspended in 0.25% Methocel; two received 20 mg of chloroquine base per kg per day as an aqueous solution of the diphasate salt; and two controls were given only the Methocel carrier. In the second experiment, five groups of three infected monkeys each were used. Monkeys received either 15 or 75 mg of U-21 per kg per day, 10 mg of U-24 per kg per day, 20 mg of chloroquine base per kg per day, or the Methocel carrier. Infections were monitored daily by thick and thin blood smears stained by the Giemsa method. Splenectomy was performed on all monkeys 30 days after termination of treatment. If recrudescence of parasites had not occurred within 30 days after splenectomy, the monkeys were considered cured. All monkeys were subsequently challenged with the

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homologous strain of *P. falciparum* to determine their susceptibility to infection.

**RESULTS**

Experiment 1. Parasitemias in monkeys treated with U-24 continued to rise during the first 3 days of treatment (Fig. 2). Thereafter, a sharp decline occurred and all asexual parasites were cleared by the end of the 7-day treatment period. Gametocytes persisted in some monkeys for 2 or 3 additional days. Since no recrudescence of parasitemias was observed in any of these animals throughout a 60-day observation period, they were considered cured of the infection. In contrast, parasites were not eliminated in either of the monkeys treated with chloroquine, and they died 11 and 25 days after exposure. Normally pigmented parasites were seen throughout the study including the period of chloroquine treatment. One of two control monkeys treated with Methocel experienced a fulminating infection and died 17 days after exposure. Parasitemias in the other control paralleled those in the previous monkey through day 10 when it was inadvertently treated with a single 50 mg/kg dose of U-24. The single dose apparently affected the course of infection as the monkey became negative 5 days later. Although the animal remained negative for 30 days, recrudescence occurred shortly after splenectomy, indicating that a single 50 mg/kg dose was not curative (Fig. 2).

Surviving monkeys were challenged 31 days after splenectomy with the homologous strain which had been stored for 70 days in liquid nitrogen. All three monkeys in the U-24-treated group became patent 8 days after challenge and developed a prolonged infection which persisted for about 25 days with maximal parasitemias ranging from 70 to 200 parasites per 10,000 erythrocytes. The fact that these animals developed little if any immunity and were susceptible to challenge is further evidence that the course of treatment effectively cured the initial infection. In contrast, a barely detectable parasitemia of short duration appeared 3 days after challenge in the monkey which was not cured with the single dose of U-24 and was probably due to recrudescence rather than to the challenge dose.

Experiment 2. Both levels of U-21 cleared the blood of trophozoites by the end of the 7-day treatment period (Fig. 3). Fifteen milligrams per kilogram per day cured only one of three monkeys, whereas all monkeys were cured with the 75 mg/kg dose. The 10 mg/kg daily dose of U-24 had therapeutic and curative activities similar to those of the 50 mg/kg/day regimen of U-24 in experiment 1 (Fig. 2, 3). Chloroquine was again ineffective in clearing parasites, and the course of infection was similar to that seen in monkeys treated with the Methocel carrier. One chloroquine-treated monkey which became negative 25 days after exposure apparently cured itself of the infection, since no parasites were subsequently detected. None of the animals died as a result of the infection.

Monkeys were challenged with frozen parasitized blood of the homologous strain 72 days after splenectomy. All of the cured monkeys became patent 9 days after challenge. Parasitemias, often going above 200 parasites per 10,000 erythrocytes, persisted in these animals during a 30-day observation period. None of the monkeys treated
Fig. 2. Course of asexual parasitemia in owl monkeys infected with Plasmodium falciparum (experiment 1). Seven daily treatments with either U-24, chloroquine, or Methocel carrier were given orally on days 7 through 13 after infection. T, One monkey treated with a single dose of U-24 on day 10 after infection. S, Splenectomy on day 46.

Fig. 3. Course of asexual parasitemia in owl monkeys infected with Plasmodium falciparum (experiment 2). Seven daily treatments with either U-21, U-24, chloroquine, or Methocel carrier were given orally on days 7 through 13 after infection. S, Splenectomy on day 42.
with chloroquine or Methocel, nor the two monkeys not cured with 15 mg/kg doses of U-21, became patent as a result of challenge. However, intermittent low-grade parasitemias, probably due to recrudescences, were seen in some of the animals during a 90-day postchallenge period.

DISCUSSION

The above results demonstrate that U-21 and U-24 have curative activity against a strain of falciparum malaria which is highly resistant to chloroquine. Although the antimalarial activity of these compounds against P. cynomolgi (7, 9) and chloroquine-resistant or normally sensitive strains of P. berghei (3) has been established, this is the first report of efficacy against a human malarial parasite and, more importantly, against chloroquine-resistant falciparum malaria. Such activity is of particular interest since only a few of the antibiotics evaluated against malaria in man and laboratory animals have been active and none of these has shown promise as an antimalarial agent (10). The efficacy of U-21 and U-24 against chloroquine-resistant strains of malaria coupled with the structural differences between these compounds and the 4-aminoquinolines suggest that the mode of action may be different from conventional antimalarials. Although little information is available on the mode of action of chlorinated lincomycin analogues, the parent compound is reported to inhibit synthesis of protein in bacterial systems (2).

Previous reports indicated that U-21 and U-24 were relatively slow in clearing P. cynomolgi in monkeys and suggested that this slowness of action might be a problem with fulminating infections such as P. falciparum (7, 9). Evidently these compounds affected parasites in the present study shortly after the first dose since changes in parasite morphology were seen within 24 hr. However, an actual decrease in population was not apparent until after the 48-hr generation period. The speed of clearance of trophozoites from the peripheral blood of owl monkeys was not dose-related, but curative activity appeared dependent upon the amount of drug given and the length of the treatment period. Although these compounds controlled falciparum infections in owl monkeys, their slowness of action would be an important consideration in the therapy of this infection in man. Combination with a more rapidly acting compound such as quinine or chloroquine to control the initial parasitemia and one of the lincomycin analogues to cure the infection might be indicated. In man, chloroquine-resistant P. falciparum usually responds rapidly to chloroquine or quinine, but a low rate of cure is achieved with these compounds (1).

The persistence of gametocytes after clearance of asexual forms in owl monkeys suggests that U-21 and U-24 may have little effect against sexual forms of the parasite. We have no information on the infectivity of these persisting gametocytes. Contrary to observations on chloroquine-resistant strains of rodent malaria which are characterized by their lack of pigment (6, 8), we saw normally pigmented parasites throughout this study including the periods under chloroquine pressure.

It is difficult to explain why the parasite was less virulent in the second experiment. The only obvious difference in the protocol of the two studies was that monkeys in experiment 1 were infected from a donor which had received fresh, infected blood maintained by monkey passage, whereas the donor in experiment 2 received infected blood which had been stored for 54 days in liquid nitrogen. Apparent reduction in virulence or pathogenicity as a result of freezing has been observed for P. cynomolgi by L. H. Schmidt and for P. falciparum in A. tririgratus by W. A. Siddiqui (personal communications).

The efficacy of U-21 and U-24 is of particular interest since they represent major structural departures from compounds commonly used in the treatment of malaria. Although our results demonstrate the effectiveness of these analogues against chloroquine-resistant falciparum malaria in owl monkeys, their ultimate usefulness can only be determined by studies in man.

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