Desferrioxamine Suppresses *Plasmodium falciparum* in Aotus Monkeys

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Abstract. Clinical observation has suggested that iron deficiency may be protective in malaria, and we have found that desferrioxamine (DF), an iron-specific chelating agent, inhibited *Plasmodium falciparum* growth *in vitro*. It was difficult to be confident that DF would be effective in an intact animal, however, because continuous exposure to DF was required *in vitro* and, *in vivo*, DF is rapidly excreted. Also, the *in vitro* effect of DF was overcome by addition of iron to the culture and *in vivo* there are potentially high local iron concentrations when iron is absorbed from the diet or released from reticuloendothelial cells. We now show that DF given by constant subcutaneous infusion does suppress parasitemia in *P. falciparum*-infected Aotus monkeys. © 1987 Society for Experimental Biology and Medicine.

Virtually all organisms need iron to survive. The iron supports electron transport (in cytochromes and iron–sulfur protein), the Krebs cycle (in aconitase), and even DNA synthesis (in ribonucleotide reductase). Endogenous host defenses include several which, viewed teleologically, withdraw iron from pathogens: serum iron decreases with inflammation and lactoferrin, an iron-sequestering protein, is released at sites where white cells are disrupted (1). Siderophores, organic molecules secreted by microorganisms which bind iron with great affinity and specificity, have been used in an effort to deprive pathogens of iron. Some organisms are susceptible, and others are not, probably because they can utilize the siderophore-bound iron (2–4). It did not seem likely that an intraerythrocytic parasite, such as *P. falciparum*, would be among the sensitive because it lives in an exceedingly iron-rich environment. But experimentation proved that supposition incorrect.

*Plasmodium falciparum*, growing in red cells *in vitro*, is inhibited by 15 μM desferrioxamine (DF), a siderophore which is iron specific and which can be used to treat iron overload in humans (5). Since that concentration of DF would be tolerated *in vivo*, it raised the hope that DF would also be effective *in vivo*. However, continuous exposure of the parasite to DF was required *in vitro* (pulses of DF as long as half the parasite’s life cycle in the asynchronous culture had a limited impact on growth) and constant blood levels of DF are difficult to obtain *in vivo* because the drug is so rapidly excreted. To address this problem, DF was given by continuous subcutaneous infusion to *P. falciparum*-infected Aotus monkeys.

Methods. Aotus monkeys, ranging in weight from 667 to 901 g, were maintained as described (6). They were inoculated intravenously with either 5 or 30 × 10⁹ parasitized erythrocytes from donor Aotus infected with the Uganda Palo Alto strain of *P. falciparum*. At designated times, infected monkeys were given subcutaneous implants of Alzet pumps (Alza Corporation, Palo Alto, CA) which had been loaded with 2 ml of desferrioxamine (200 mg/ml) dissolved in deionized water. These pumps deliver their contents at a nominal rate of 10 μl/hr for 1 week. In some experiments, infected control animals were implanted with
pumps which had been loaded with normal saline. Blood parasite counts were performed by the method of Earle and Perez (7). Desferrioxamine was a gift from Ciba-Geigy (Summit, NJ). Data were analyzed using a two-tailed $t$ test where

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t = \frac{\bar{Y} - \bar{Y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \tag{8}
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**Results.** In an initial experiment (Fig. 1), parasitemia was suppressed in both monkeys receiving DF at the nominal rate of 800 mg/7 days (with two pumps implanted). Both of these treated monkeys died during the experiment, one of peritonitis, and the other with a fatty liver. Nevertheless, there was statistically significant suppression of parasitemia on Days 5, 6.5, and 7. In a second experiment, DF given at nominal dose of 400 mg/7 days (with one pump implanted) suppressed parasitemia in two out of three infected monkeys. In a third experiment, DF given at a nominal dose of 400 mg/7 days was supplemented with twice daily subcutaneous doses (30 mg/kg). Parasitemia was significantly suppressed on Days 5, 6, 7, and 9; there was also a large difference in parasitemia on Day 8, but the variance in the control on that day overwhelmed this difference (Fig. 2). Two other monkeys given twice daily doses of DF 30 mg/kg had no suppression of parasitemia, supporting the conclusion from the in vitro experiments that sustained exposure to the drug is needed for effect.

**Discussion.** Iron deprivation for the treatment of an intraerythrocytic parasite would seem unpromising because of the huge excess of heme iron in the immediate environment. But, this notwithstanding, epidemiologic evidence has suggested that iron deficiency might be partially protective against malaria (9, 10), and in the present in vivo experiments we have shown that DF inhibits the growth of the malaria parasite *P. falciparum*.

DF is probably effective because *P. falciparum* is dependent on an intraerythrocytic
chelatable pool of iron (11) and utilizes neither heme nor transferrin iron directly. Direct utilization of heme iron is unlikely because free heme is toxic to the parasite (12) and because the concentration of DF which inhibits is 1000-fold less than that of the surrounding heme (5). It is unlikely that *P. falciparum* utilizes transferrin iron directly because DF does not readily remove iron from transferrin (13). But both catabolized heme and transferrin deliver iron to a chelatable intracellular pool (14), the iron in which can be removed by DF.

DF is too expensive and inconvenient to use to be considered for the routine treatment of malaria. Moreover, it suppresses parasitemia rather than being cidal; thus much more effective drugs are available. But when the malaria is drug resistant or the patient cannot tolerate the needed medicine, DF could prove useful in suppressing parasitemia until the patient’s endogenous immune response became effective. DF’s safety is buttressed by more than two decades of use in the treatment of siderosis in Cooley’s anemia (15). It is possible that other iron-chelating compounds also have antimalarial activity and that the ongoing effort to develop agents which remove iron with greater efficiency and which can be given orally may prove to have a double benefit (16).
