CONCISE REPORT

Antimalarial Properties of Orally Active Iron Chelators

By D. Gray Heppner, Philip E. Hallaway, George J. Kontoghiorghes, and John W. Eaton

The appearance of widespread multiple drug resistance in human malaria has intensified the search for new antimalarial compounds. Metal chelators, especially those with high affinity for iron, represent one presently unexploited class of antimalarials. Unfortunately the use of previously identified chelators as antimalarials has been precluded by their toxicity and, in the case of desferrioxamine, the necessity for parenteral administration. The investigators now report that a new class of orally active iron chelators, namely the derivatives of α-ketohydroxypyridines (KHPs), are potent antimalarials against cultured Plasmodium falciparum. The KHPs evidently exert this effect by sequestering iron because a preformed chelator:iron complex has no antimalarial action. The pool(s) of iron being sequestered by the chelators have not been identified but may not include serum transferrin. Prerunecubation of human serum with KHPs followed by removal of the drug results in the removal of >97% of total serum iron. Nonetheless, this serum effectively supports the growth of P falciparum cultures. Therefore the KHPs may exert antimalarial effect through chelation of erythrocytic rather than serum iron pool(s). The investigators conclude that these powerful, orally active iron chelators may form the basis of a new class of antimalarial drugs.

Although the in vivo use of these chelators against P falciparum in humans may be precluded by toxicity or by the nonabsorbable nature of agents such as desferrioxamine, the latter has been shown effective in vivo against murine malaria (although repeated and frequent injections of the drug were required to show such an effect).10

As mentioned above, none of the previously tested chelators fulfills the criteria of being relatively nontoxic as well as orally absorbable. For this reason the investigators have tested the antimalarial effects of a new class of iron chelators, the α-ketohydroxypyridines (KHPs). These iron chelators are orally absorbable, inexpensive to produce, powerful, and, evidently, devoid of serious toxicity.11,12 The following experiments represent a preliminary test of the in vitro efficacy of these iron chelators and an attempt to define the mechanism by which they exert their potent antimalarial action.

MATERIALS AND METHODS

Plasmodial cultures. P falciparum (FCR-3) was cultured in candle jars by the method of Jensen and Trager.13 For estimation of the antimalarial potency of the iron chelators tested, the investigators cultured unsynchronized parasites in 24-well Costar plates (1-cm diameter wells). Parasites were routinely grown in blood group A/rh(+) erythrocytes, previously washed free of leukocytes and resuspended to hematocrit 5 vol% in RPMI (GIBCO, Grand Island, NY) containing 10% heat-inactivated human serum, 100 μg/mL gentamycin, 25 mmol/L n-2-HEPES and 2 g/L NaHCO3.

Chelators. Desferrioxamine mesylate was obtained from Ciba-Geigy (Basel, Switzerland), and was pharmaceutical-grade material. The KHPs were synthesized as previously reported.14,15 These drugs are designated according to the substituents on the 3-hydroxy-pyrid-4-one ring: L1, 1,2-dimethyl; L1NET, 1-ethyl-2-methyl; L1NP, 1-propyl-2-methyl; EL1, 1-methyl-2-ethyl; EL1E, 1,2-diethyl; EL1Pr, 1-propyl-2-ethyl. Unless otherwise noted, stock solutions of these chelators were prepared at a concentration of 1 mmol/L and filter sterilized before addition to the malarial cultures.

Additional procedures. The six KHP chelators, their two chemical precursors (maltol and ethylmaltol), and desferrioxamine mesylate were all tested at final concentrations of 10 and 100 μmol/L. Parasitized cells were diluted with uninfected erythrocytes to yield a starting parasitemia of roughly 0.5% to 1.0%. Growth of the parasites over the following 48 hours was then assessed. Except for
The investigators find that all of the KHP derivatives tested have potent antimalarial activity against cultured *P. falciparum* (Fig 1). In every case these drugs are active at a final concentration of 100 μmol/L, and L1 appears to be malarialstatic even at 10 μmol/L. The KHP chelators are almost as potent as desferrioxamine. This is interesting in view of the fact that the preferred drug:iron binding ratio is 3:1 for the KHPs and 1:1 for desferrioxamine.

The basis for the antimalarial effect of the KHPs, like that of desferrioxamine, appears to be sequestration of iron required for parasite growth and replication. Thus, as shown in Fig 2, the simultaneous addition of an equimolar concentration of ferric iron (as a freshly prepared solution of ferric ammonium sulfate) completely obliterates any antimalarial effect of either desferrioxamine or the KHPs.

In an effort to determine the important pool(s) of iron being chelated by these drugs, the investigators carried out incubations of human serum with L1 (the most effective of the KHP chelators tested; Fig 1). As reported earlier, these very potent iron chelators will actually strip bound iron from human transferrin during prolonged incubations (3 to 6 hours). Following incubation the free drug was removed from the serum by rapid filtration on Sephadex G-25 columns. The amounts of treated serum added to the culture medium were adjusted accordingly. As above, all culture experiments were conducted in duplicate. The iron content of the treated and chromatographed serum was determined by the very sensitive ferene assay, as described previously.

**RESULTS**

The experiment depicted in Fig 1, duplicate wells were set up for each drug concentration, and duplicate slides were made of each well. A minimum of 2,000 erythrocytes was counted for each treatment.

The possibility that the KHPs exerted antimalarial effect through depletion of serum iron was tested by incubation of heat-inactivated human serum with 10 μmol/L chelator L1 at 37°C for 72 hours. Control incubations included serum incubated 72 hours without added L1, serum incubated with drug at room temperature for only ten minutes, and a fourth aliquot of serum held at ~70°C under usual conditions of storage. Following incubation the free drug was removed from the serum by rapid filtration on Sephadex G-25 columns. The protein content of the high molecular weight (mol wt) fraction of the filtrate was measured (using the technique of Smith et al) and was found to be roughly 65% that of whole serum. The amounts of treated serum added to the culture medium were adjusted accordingly. As above, all culture experiments were conducted in duplicate. The iron content of the treated and chromatographed serum was determined by the very sensitive ferene assay, as described previously.

The investigators find that all of the KHP derivatives tested have potent antimalarial activity against cultured *P. falciparum* (Fig 1). In every case these drugs are active at a final concentration of 100 μmol/L, and L1 appears to be malarialstatic even at 10 μmol/L. The KHP chelators are almost as potent as desferrioxamine. This is interesting in view of the fact that the preferred drug:iron binding ratio is 3:1 for the KHPs and 1:1 for desferrioxamine.

The basis for the antimalarial effect of the KHPs, like that of desferrioxamine, appears to be sequestration of iron required for parasite growth and replication. Thus, as shown in Fig 2, the simultaneous addition of an equimolar concentration of ferric iron (as a freshly prepared solution of ferric ammonium sulfate) completely obliterates any antimalarial effect of either desferrioxamine or the KHPs.

In an effort to determine the important pool(s) of iron being chelated by these drugs, the investigators carried out incubations of heat-inactivated human serum with L1 (the most effective of the KHP chelators tested; Fig 1). As reported earlier, these very potent iron chelators will actually strip bound iron from human transferrin during prolonged incubations (3 to 6 hours). Following rapid chromatographic removal of the drug, the investigators found that serum iron concentration had been reduced to less than 3% of the starting concentration (ie, 72 μmol/L to ~3.6 μmol/L). Interestingly, this almost iron-free serum was very effective in supporting the growth of *P. falciparum* (Fig 3). This result indicates that all
of the components of human serum necessary for replication of *P. falciparum* fall within the high mol wt fraction and that transferrin-bound iron is not essential.

**DISCUSSION**

Overall, the investigators' results clearly establish the potential of KHPs as antimalarials. These potent iron chelators evidently exert antimalarial action through chelation of one or more pools of iron essential to growth of the parasite. Preformation of a drug:iron chelate completely obliterates the depressive effect of the drug on parasite growth and multiplication. Therefore the important principle of drug action evidently resides in iron deprivation of the parasite. However, the critical pool of iron that is being chelated has yet to be defined. The investigators find that human serum can be >97% depleted of bound iron through prolonged incubation with 1,2-dimethyl-3-hydroxypyrid-4-one. However, after removal of the free drug, the almost iron-free high mol wt components of serum are fully capable of supporting parasite growth. This result argues against—but does not fully disprove—earlier suggestions that the malaria parasite may obtain iron through binding and internalization of host transferrin. This finding also indicates that those elements in human serum that enable parasite replication reside within the high mol wt fractions and that no easily dissociable low mol wt substances (aside from those in RPMI) are necessary for normal growth.

Although the authors have not yet identified the iron pools of critical importance to the parasite, it is now clear that several different chelators are effective against cultured *P. falciparum*. In the past this pharmacologic principle has not been useful in design of new antimalarial agents because the previously tested chelators are either too toxic or cannot be administered orally. In the present instance the authors find that the KHPs, which are potent, relatively nontoxic, orally effective ferric iron chelators, are quite active against *P. falciparum* in culture. In view of the present need for additional effective antimalarial drugs, these preliminary observations should certainly be pursued.

**REFERENCES**

ANTIMALARIAL IRON CHELATORS

Antimalarial properties of orally active iron chelators

DG Heppner, PE Hallaway, GJ Kontoghiorghes and JW Eaton

Updated information and services can be found at:
http://www.bloodjournal.org/content/72/1/358.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml