Antimicrobial Agents and Chemotherapy, Aug. 1995, p. 1902–1905 Vol. 39, No. 8
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Effects of Zinc-Desferrioxamine on *Plasmodium falciparum* in Culture

MORDECHAI CHEVION,[1] LI CHUANG,[2] AND JACOB GOLENSER[2]*

Departments of Cellular Biochemistry[1] and Parasitology,[2] Hebrew University—Hadassah Medical School, Jerusalem 91120, Israel

Received 10 February 1995/Returned for modification 13 April 1995/Accepted 23 May 1995

The zinc-desferrioxamine (Zn-DFO) complex is considered to be more permeative into parasitized erythrocytes than is the metal-free DFO. The former may penetrate the cell and exchange its bound zinc for ferric ions, rendering the iron unavailable for vital parasite functions. The effects of these compounds on the in vitro development of *Plasmodium falciparum* are compared. The results indicate that Zn-DFO is superior to DFO, especially at concentrations below 20 μM, as shown by decreased levels of hypoxanthine incorporation, lower levels of parasitemia, and interference with the life cycle of the parasite. At low concentrations, DFO even enhanced parasite growth. Such an enhancement was not observed following exposure to Zn-DFO. Experiments in which the compounds were removed from the cultures indicated that parasites treated with Zn-DFO are less likely to recover at a later stage. Since DFO has already been used in humans for the treatment of malaria, its complex with zinc, which is more effective in vitro, should also be examined in vivo.

The resistance of *Plasmodium falciparum* to commonly used antimalarial drugs has created an urgent need for new compounds which could effectively act against the resistant strains (17). Iron-chelating agents have demonstrated antimalarial activity and seem to represent bona fide candidates for such novel drugs (7, 25). Most of the experiments relating to iron chelators were performed with desferrioxamine (DFO). DFO has been shown to suppress malarial parasites in culture (19), in infected mice (5), and in aotus monkeys (18). Iron chelation has been recently suggested as a therapeutic means to cure human malaria (2, 7, 8, 12, 24). However, most of the treated patients suffered recrudescences 7 to 10 days following the termination of chelation therapy (2). It should be noted that previous studies showed that DFO slowly penetrates into infected cells, is removed relatively quickly from body fluids, and can induce deleterious side effects (12).

Zinc has been known to play an antioxidant role, protecting cells against free radical-mediated damage (27). The deregulation of zinc metabolism (i.e., its excess or deficiency) also interferes with iron metabolism (4, 21, 22). The zinc(II)-DFO complex (Zn-DFO) is a well-defined and organized globular complex which is more permeative into cells than is the metal-free DFO (3). DFO chelates predominantly ferric iron (Fe^{3+}). The affinity of DFO for trivalent ions, in general, is much higher (complex stability constant for Fe^{3+}, 10^{31}) than that for the corresponding divalent ions (complex stability constant for Zn^{2+}, 10^{11}) (14). Consequently, Zn-DFO should readily exchange its bound zinc for available ferric ions, rendering the iron unavailable for vital parasite functions (3).

The goals of the present study were to determine whether Zn-DFO demonstrates antimalarial activity and to compare its activity to that of the metal-free DFO. The significance of the data in regard to antimalarial chemotherapy is discussed.

P. falciparum (strain FCR-3) was cultured according to the method of Trager and Jensen (23). The cultures were synchronized by sorbitol treatment (15) at the ring stage, every other day. Normal erythrocytes were obtained from healthy humans. The experiments were initiated within 14 days after the collection of the blood. The parasites were treated with DFO (Desferal; Ciba Geigy, Basel, Switzerland) or the complex Zn^{2+}-DFO (a patented agent effective against free radical- and iron-mediated injurious processes [Israeli patent 91047, 1989]). Parasitized erythrocytes at the ring or trophozoite stage were adjusted to 1 to 2% levels of parasitemia in an erythrocyte suspension (5% hematocrit in RPMI 1640 containing 10% plasma and the compounds under examination). The cells were dispensed into 96-well microplates and incubated with or without the drugs under study at 37°C by the candle jar method (23). The supernatant was replaced every day with identical medium, unless otherwise stated. The incorporation of radioactive hypoxanthine (Hx) was measured as a parameter for parasite development according to the method of Golenser et al. (6) as follows. [^{3}H]Hx (specific activity, 18.5 kBq per well; New England Nuclear) was added in 25 μl of medium to the culture. The cells were collected by filtration on glass microfiber filters, and radioactivity was counted with a Minaxi Tri-Carb 4000 (Packard). The incorporation of Hx by uninfected normal erythrocytes did not exceed 3% of the lowest level of uptake by untreated parasitized erythrocytes. Levels of parasitemia were determined with Giemsa-stained blood smears. The total number of erythrocytes counted per smear was 10,000.

Parasitized erythrocytes adjusted to 1% levels of parasitemia at the ring stage were exposed to five concentrations of DFO or Zn-DFO for 24 or 48 h. Growth inhibition was assessed by monitoring the incorporation of Hx in treated and control plasmodial cultures, and the inhibition or enhancement of the growth was calculated. Our results indicate that Zn-DFO is more efficient than the metal-free DFO in inhibiting the development of *P. falciparum* in vitro. Inhibition was observed at a DFO concentration of 12 μM after 24 h of continuous exposure to the drug. The inhibitory effect was dose dependent and increased monotonically with the DFO concentration. Zn-DFO demonstrated a similar but more pronounced effect: inhibition was noticed already at 3.75 μM (Fig. 1). The 50% inhibitory doses of DFO which were evaluated from dose-response curves for 24 and 48 h were about 25 and 15 μM,

* Corresponding author. Mailing address: Department of Parasitology, Hebrew University, Jerusalem 91904, Israel. Phone: 972 2 758090. Fax: 972 2 432527.

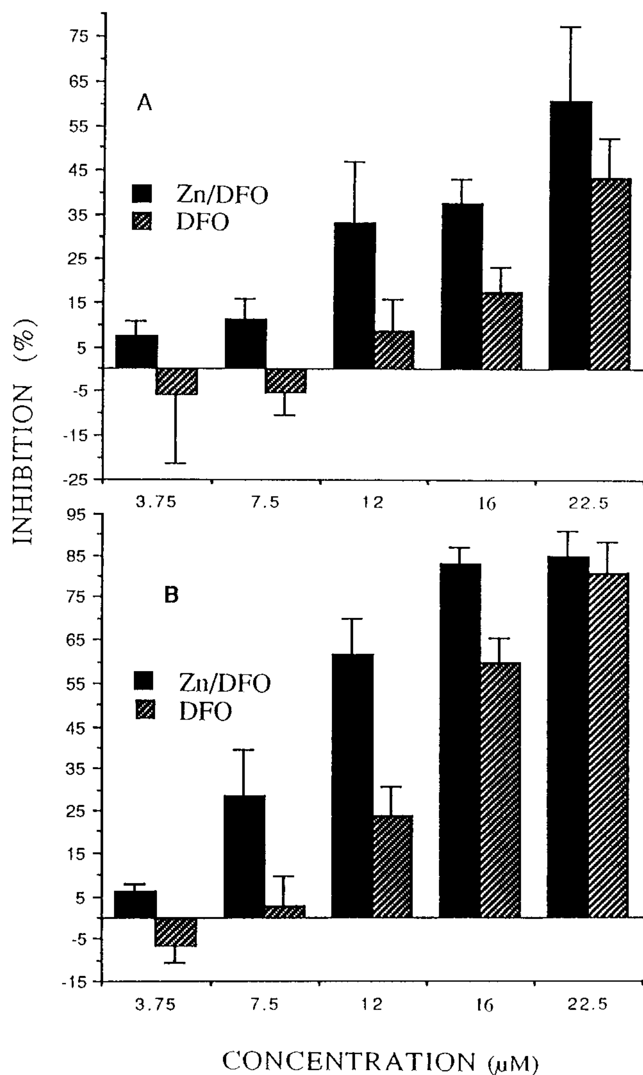

FIG. 1. Inhibition of hypoxanthine incorporation by *P. falciparum*. Cells were treated with various concentrations of Zn-DFO or DFO for 24 (A) or 48 h (B). Data represent the means of five determinations. Bars show standard errors.

respectively. Similar inhibitions by Zn-DFO occurred at 24 and 48 h at concentrations of 18 and 10 μM, respectively. The uptake of Hx by the parasites exposed to DFO at concentrations of 7.5 μM or lower was even greater than that by the control parasites. At DFO and Zn-DFO concentrations of 22.5 μM and more and after 48 h of exposure, the Hx incorporation by both DFO- and Zn-DFO-treated cells approached complete inhibition and no significant difference could be observed. The difference in the activities of the two compounds is also reflected in the level of parasitemia (Fig. 2) and the stage distribution of the plasmodia (Fig. 3). The lower concentrations of Zn-DFO were more efficient at reducing levels of parasitemia than were the corresponding concentrations of DFO; i.e., 3.75 μM Zn-DFO reduced the level of parasitemia by about 50%, while 3.75 μM DFO had no inhibitory effect but slightly increased the level of parasitemia (Fig. 2). In addition, 98% of the remaining parasites were arrested at the trophozoite stage (the experiment started with ring forms). It is not clear what proportion of the parasites was arrested at the trophozoite stage and what proportion was dying. However, these parasites

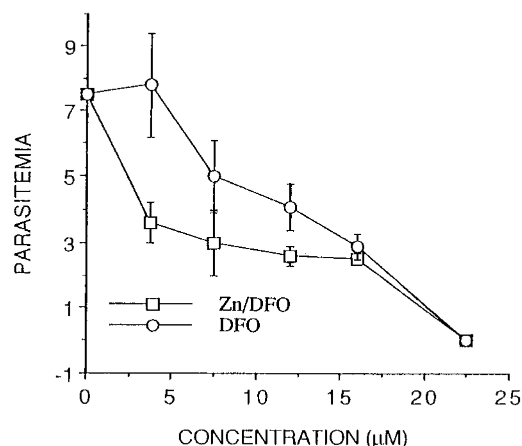

FIG. 2. Effects of Zn-DFO and DFO on *P. falciparum* parasitemia after 48 h of treatment. Data represent the means of three experiments. Bars show standard errors.

are partially capable of nucleic acid synthesis. An increased in vivo effect of Zn-DFO, yielding trophozoites which are either dying or just arrested, may allow the immune system to overcome the infection. Identical concentrations of DFO reduced only 24% of Hx incorporation, reduced the level of parasitemia by 45%, and arrested 35% of the parasites at the trophozoite stage, while slowing down the development of an additional 30% (Fig. 3).

The cumulative effects of each drug at 12 and 22.5 μM were evaluated by a series of 6-h pulses of Hx incorporation into the parasites. This demonstrated that the antimalarial effects of both DFO and Zn-DFO are dose and time dependent. However, a pronounced inhibitory effect on Hx incorporation occurred after 12 h in culture, when the parasites were still in their early stage of development, with Zn-DFO only. Zn-DFO remained more effective at a concentration of 12 μM than did DFO, even after 48 h. At 22.5 μM, after 48 h, the effects of the two compounds reached similar levels of inhibition (more than 80%) (data not shown).

The residual inhibitory effects were determined by the extent of inhibition of Hx incorporation during a 4-h pulse, which was performed following incubation with the drug and washing away of the remainder of the drug. The cultures containing 2% young trophozoites were exposed to DFO or Zn-DFO (30 μM) for 10 h. The agents were then removed by washing three times with RPMI 1640, and the parasites were retained in culture without drugs. The results (Fig. 4) demonstrate that the parasites treated with DFO or Zn-DFO could recover. However, there was a delay of 4 h in the recovery of parasites treated with Zn-DFO. Even 12 h after removal of the drugs, the level of parasite growth in Zn-DFO-treated cultures was only two-thirds of that of the cultures treated with DFO alone.

The mechanism of suppression of parasite growth by DFO is still not fully understood, although most investigators agree that the activity of most chelators is based on the specific chelation of transient iron pools (11, 20). Alternatively, DFO may act as an inhibitor of ribonucleotide reductase, possibly by extracting the enzyme-bound iron which is essential for the enzymatic activity (13). The fact that the maximal efficiency of DFO coincides with the period of maximal DNA synthesis by the parasite indirectly supports this hypothesis (26). Iron chelators could also act by redistributing available forms of low-molecular-weight iron, leading to site-specific oxidative stress (1, 3, 9, 10). The speed of antimalarial action of an iron che-

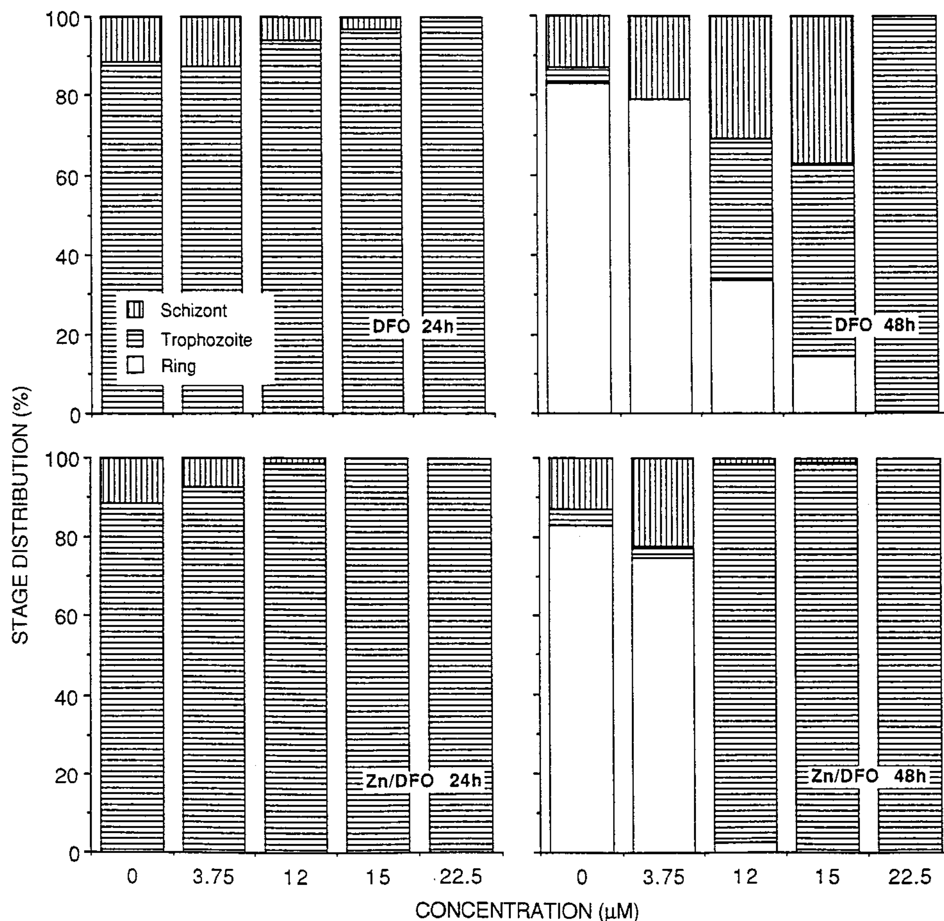

FIG. 3. Stage distribution of *P. falciparum* after treatment with Zn-DFO or DFO for 24 or 48 h. The effects of these compounds on parasitemia are presented in Fig. 2). Data represent the means of three determinations.

lator and the stage specificity of its effect are largely the results of its membrane permeation properties and iron-binding capacity (16).

The advantage of Zn-DFO over DFO was more pronounced when the effects of low concentrations were compared. This might be also important for the treatment of malaria: high concentrations of DFO were needed for the treatment of human malaria, and these could be obtained only by infusion for 3 days (2). At low concentrations, DFO even enhanced the development of the parasites, perhaps by preventing the production of free radicals. Such an enhancement was not observed following incubation with low concentrations of Zn-DFO. A preliminary in vivo experiment revealed that Zn-DFO was more efficient than DFO against rodent cerebral malaria (4a).

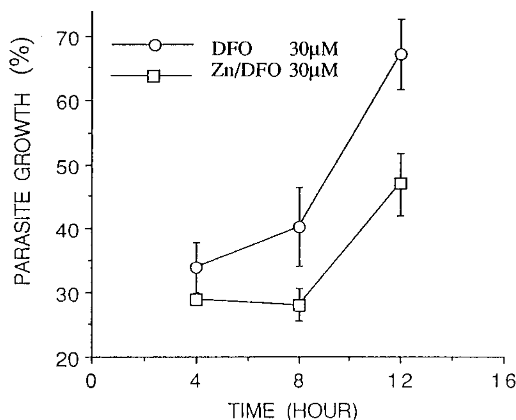

FIG. 4. Sustained effects of Zn-DFO and DFO treatments on growth of *P. falciparum*. The compounds were removed from the culture after 10 h of exposure, and parasite development was estimated by Hx incorporation in successive pulses of 4 h. The results are expressed as percent incorporation by untreated cultures. Each datum point represent the mean of four determinations. Bars show standard errors.

Zn-DFO is a complex of zinc, which is a redox-inactive metal ion and acts as an antioxidant agent in a variety of biological systems, and DFO, which is a randomly oriented and disorganized molecule. Within the complex, DFO assumes a well-organized structure which facilitates the permeation of the complex into cells and allows the exchange of transient iron for the zinc (26). By means of this exchange, the redox-active iron ions are rendered inactive and their oxidative potential is neutralized. Thus, the special role of Zn-DFO in plasmodial culture, and possibly also in vivo, is to scavenge the transient iron which is necessary for the parasite's vital biosynthetic processes.

Mordechai Chevion is the incumbent of the Dr. William Ganz Chair of Heart Studies, which was established in 1990 at the Hebrew University of Jerusalem. This work was supported by NIH grant AI20342.

Li Chuang was a scholar under the auspices of the Israel-China Scientific Exchange Program.

REFERENCES

1. **Aust, S. D., L. A. Morehouse, and C. E. Thomas.** 1985. Role of metals in oxygen radical reactions. Free Radical Biol. Med. **1:**3–25.
2. **Bunnag, D., A. A. Poltera, C. Viravan, S. Looareesuwan, and K. T. Harinasuta.** 1992. Plasmodicidal effect of desferrioxamine B in human vivax or falciparum malaria from Thailand. Acta Trop. **52:**59–67.
3. **Chevion, M.** 1991. Protection against free radical-induced and transition metal-mediated damage: the use of "pull" and "push" mechanisms. Free Radical Res. Commun. **12–13:**691–696.
4. **Cox, D. H., and D. L. Haris.** 1960. Effect of excess dietary zinc on iron and copper in the rat. J. Nutr **70:**514–520.
4a. **Eling, et al.** Unpublished data.
5. **Fritsch, G., and A. Jung.** 1986. ^{14}C-desferrioxamine B: uptake into erythrocytes infected with *Plasmodium falciparum*. Z. Parisitenkd. **72:**709–714.
6. **Golenser, J., D. Casutu, and Y. Pollack.** 1981. P. falciparum: in vitro induction of resistance to aminopterin. Exp. Parasitol. **52:**371–377.
7. **Gordeuk, V. R., G. M. Brittenham, and P. E. Thuma.** 1994. Iron chelation as a therapeutic strategy in the treatment of malaria, p. 11–13. *In* R. J. Bergeron and G. M. Brittenham (ed.), The development of iron chelators for clinical use. Boca Raton, Fla.
8. **Gordeuk, V. R., P. E. Thuma, G. M. Brittenham, C. McLaren, D. Parry, A. Backenstose, G. Biemba, R. Msiska, L. Holmes, E. McKinley, et al.** 1992. Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. N. Engl. J. Med. **372:**1473–1477.
9. **Halliwell, B., and J. M. C. Gutteridge.** 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. **219:**1–14.
10. **Halliwell, B., and J. M. C. Gutteridge.** 1989. Free radicals in biology and medicine. Clarendon Press, Oxford.
11. **Heppner, D. G., P. E. Hallaway, G. J. Kontoghiorghes, and J. W. Eaton.** 1988. Antimalarial activity of orally active iron chelator. Blood **72:**358–361.
12. **Hershko, C., and D. J. Wheatherall.** Iron chelating therapy. 1988. Crit. Rev. Clin. Lab. Sci. **26:**303–345.
13. **Hoffbrand, A. V., K. Ganeshagureu, J. W. L. Hooton, and M. H. N. Tattersall.** 1976. Effect of iron deficiency and desferrioxamine on DNA synthesis in human cells. Br. J. Haematol. **33:**517–526.
14. **Keberle, H.** 1964. The biochemistry of desferrioxamine and its relation to iron metabolism. Ann. N. Y. Acad. Sci. **119:**758–768.
15. **Lambros, C., and J. P. Wanderberg.** 1979. Synchronization of *P. falciparum* erythrocytic stage in culture. J. Parasitol. **65:**418–420.
16. **Loyevsky, M., S. D. Lytton, B. Mester, J. Libman, A. Shanzer, and Z. I. Cabantchik.** 1993. The antimalarial action of Desferal involves a direct access route to erythrocytic (*Plasmodium falciparum*) parasites. J. Clin. Invest. **91:**218–224.
17. **Peters, W.** The problem of drug resistance in malaria. 1985. Parasitology **90:**705–715.
18. **Pollack, S., R. N. Rosan, D. E. Davidson, and A. Escajadillo.** 1987. Desferrioxamine suppresses *P. falciparum* in aotus monkeys. Proc. Soc. Exp. Biol. Med. **184:**162–164.
19. **Raventos-Suarez, C., S. Pollack, and R. L. Nagel.** 1982. *P. falciparum* inhibition of in vitro growth by desferrioxamine. Am. J. Trop. Med. Hyg. **31:**919–922.
20. **Scott, M. D., A. Ranz, F. A. Kuipers, B. H. Lubin, and S. R. Meshnick.** 1990. Parasite uptake of desferrioxamine: a prerequisite for antimalarial activity. Br. J. Haematol. **75:**598–602.
21. **Seeling, M. S.** 1973. Proposed role of copper-molybdenum interaction in iron-deficiency and iron storage diseases. Am. J. Clin. Nutr. **26:**657–672.
22. **Solomons, N. W., and R. A. Jacob.** 1981. Studies on the availability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. Am. J. Clin. Nutr. **34:**475–482.
23. **Trager, W., and J. B. Jensen.** 1976. Continuous culture of human malaria parasites. Science **193:**673–675.
24. **Traore, O., P. Carnevale, L. Kaptue-Noche, J. M. Bede, M. Desfontaine, J. Elion, D. Labie, and R. L. Nagel.** 1991. Preliminary report on the use of desferrioxamine in the treatment of *Plasmodium falciparum* malaria. Am. J. Hematol. **37:**206–208.
25. **Van Zyl, R. L., I. Havlik, and F. Monteagudo.** 1992. The combined effect of iron chelators and classical antimalarials on the in vitro growth of *P. falciparum*. J. Antimicrob. Chemother. **30:**273–278.
26. **Whitehead, S., and T. E. A. Peto.** 1990. Stage-dependent effect of desferrioxamine on growth of *P. falciparum* in vitro. Blood **76:**1250–1255.
27. **Willson, R. L.** 1989. Zinc and iron in free radical pathology and cellular control, p. 147–166. *In* C. F. Mills (ed.), Zinc in human biology. Springer-Verlag, Berlin.