

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/25091>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

The Treatment of Animal Models of Malaria with Iron Chelators by Use of a Novel Polymeric Device for Slow Drug Release¹

JACOB GOLENSER, ABRAHAM DOMB, DORON TEOMIM, APPOLINAIRE TSAFACK, ORNA NISIM, PREM PONKA, WIJNAND ELING and Z. IOAV CABANTCHIK

Departments of Parasitology (J.G., O.N.), Pharmaceutical Chemistry (A.D., D.T.) and Biological Chemistry (A.T., Z.I.C.), The Hebrew University, Jerusalem, Israel; Lady Davis Institute of Research (P.P.), Jewish General Hospital, McGill University, Montreal, Canada; Department of Microbiology (W.E.), University of Nijmegen, Nijmegen, The Netherlands

Accepted for publication February 4, 1997

ABSTRACT

The hydrophilic desferrioxamine (DFO) and the lipophilic salicylaldehyde isonicotinoyl hydrazone (SIH) are iron chelators which inhibit *in vitro* proliferation of *Plasmodium falciparum* with similar potency ($IC_{50} \sim 20 \mu M$ in 24- to 48-h tests). The *in vivo* assessment of these drugs was performed on Swiss mice infected with *Plasmodium vinckei petteri* with novel modes of drug administration and release. The drugs were delivered postpatently either by multiple i.p. injections or by a single i.p. or s.c. insertion of a drug-containing polymeric device which released most of the drug within 7 days at apparently first-order rates. A regimen of three daily i.p. injections of 5 mg DFO for 3 consecutive days or a 70-mg dose of the drug given as an i.p. or s.c. polymer implant evoked similar delay and reduction in peak parasitemias and reduced mortality with no apparent signs of toxicity. Relatively faster, but otherwise similar results

were obtained with the less hydrophilic SIH. In combination, the two drugs apparently potentiated each other. The polymeric devices were particularly useful for treating *Plasmodium berghei* K173-infected C57Bl mice, a suggested model of cerebral malaria, in which classical methods of DFO delivery were ineffective. The insertion of a 140-mg DFO-containing device on day 6 postinfection (parasitemia $\sim 1\%$) led to a marked reduction in parasite proliferation, appearance of neurological sequelae and mortality of mice. Our studies indicate that polymeric devices for slow drug release might be highly advantageous for both hydrophilic and lipophilic drugs whose antimalarial efficacy might depend on the maintenance of sustained blood levels. The results obtained with slow-release devices have implications for malaria chemotherapy as well as for iron chelation therapy in iron overload conditions.

Resistance of *Plasmodium falciparum* to chloroquine, quinine, sulfadoxine, pyrimethamine and mefloquine has created an urgent need for new drugs which are effective against multidrug-resistant strains of malaria (Peters, 1990). Strategies to overcome resistance have relied on the application of drugs of different chemical character and mode of action (Vennerstrom *et al.*, 1991), including iron-chelating agents (reviewed in Cabantchik *et al.*, 1996; Gordeuk *et al.*, 1994). At present the only iron-chelating drug approved for human treatment is DFO, of which the parenteral administration demands hospitalization. Synthetic iron chelators which can be administered orally are not suitable for chemotherapy, either because of human toxicity or lack of antimalarial efficacy (Mabeza *et al.*, 1996). In general, various types of drugs

that inhibit parasite growth in culture were found to be only partially effective in *in vivo* studies because of inadequate speed of action on parasite development, reversibility of inhibition or pharmacokinetic factors. DFO is a hydrophilic agent that permeates rather slowly into parasitized cells and only at advanced stages of parasite growth (Fritsch and Jung 1986; Scott *et al.*, 1990; Loyevsky *et al.*, 1993). Therefore, the time window of action of such an agent is relatively limited and the antimalarial activity is slow to develop, even after continuous *in vitro* or *in vivo* exposure to the drug (Lytton *et al.*, 1994; Cabantchik *et al.*, 1996). The same has been observed in DFO-treated malaria patients, most of whom had recrudescence 7 to 10 days after cessation of drug treatment (Bunnag *et al.*, 1992; Gordeuk *et al.*, 1993).

We have recently shown that the antimalarial potential of various iron chelators could be markedly improved by 1) increasing drug lipophilicity leading to increased access of drug to intracellular parasites and to faster speed of action (Loyevsky *et al.*, 1993; Lytton *et al.*, 1994) and 2) incorpora-

Received for publication August 13, 1996.

¹This work was supported in part by the National Institutes of Health, grant AI20342 (Z.I.C.) and by the Authority for Research and Development of the Hebrew University (J.G.). *Plasmodium vinckei petteri* was obtained from Prof. I. Landau from Pasteur Institute, Paris.

ABBREVIATIONS: CM, cerebral malaria; DFO, desferrioxamine; DMSO, dimethyl sulfoxide; poly(FAD-SA), poly (dimer erucic acid-co-sebacic acid, 22:78); PSA, poly sebacic anhydride; SIH, salicylaldehyde isonicotinoyl hydrazone; PBS, phosphate-buffered saline.

tion of cleavable groups for augmenting intracellular drug retention and attaining more persistent cytotoxic effects (Cabantchik *et al.*, 1996; Tsafack *et al.*, 1996a). Although fast speed of action and persistent inhibition are conferred to drugs by apparently opposite chemical properties, they can be accomplished by use of permeant lipophilic prodrugs which produce intracellular impermeant hydrophilic drugs (Tsafack *et al.*, 1996a). However, a major drawback of lipophilic drugs is their poor water solubility, which might limit their *in vivo* application. An alternative and versatile means of drug administration to animals is based on polymers into which lipophilic or hydrophilic drugs can be encapsulated and released in a controlled fashion (Chasin *et al.*, 1990). Those polymers whose degradation products are both nontoxic and can be completely eliminated from the circulation are particularly useful because they serve both as a safe medium for drug administration and as a vehicle for slow drug delivery (Domb *et al.*, 1993). A polymer implant of nondegradable properties has recently been used as a slow-release device for the antifolate pyrimethamine in rodent malaria (Vandamme and Heller, 1995).

The two iron chelators chosen for this study, the hydrophilic DFO and the lipophilic SIH, were previously shown to act synergistically on *in vitro* cultures of *P. falciparum* (Tsafack *et al.*, 1996b). In this work they were assessed in two rodent models of malaria: Swiss mice infected with *Plasmodium vinckei petteri* and the C57Bl mice infected with *Plasmodium berghei*, a suggested model of CM. In this CM model, the petechial hemorrhages that are characteristic of CM were attributed to sequestration of mononuclear white cells which interact with, and infiltrate across, the endothelium and postcapillary venules (Grau *et al.*, 1986; Curfs *et al.*, 1989; Eling and Sauerwein, 1995). However, the hallmark of CM in human malaria is parasite sequestration (MacPherson *et al.*, 1985), a property which has also been described recently in a rodent model (Kaul *et al.*, 1994). The chelators were assessed as therapeutic agents *via* two modes of administration: by multiple i.p. injections and by s.c. or i.p. insertion of a single dose of drug (or combination of drugs) which was encapsulated into biodegradable polymers. The latter were found to be convenient, safe and efficient vehicles for antimalarial drug delivery.

Materials and Methods

Iron Chelators

SIH was prepared as described elsewhere (Baker *et al.*, 1992) and DFO was purchased from Ciba Geigy (Basel, Switzerland) as Desferal.

Animal Models of Malaria

Two plasmodial strains that cause malaria in mice were used: 1) *P. vinckei petteri* in Swiss mice (females, 35–45 g, 10–12 weeks old) and 2) *P. berghei* K 173 in C57Bl mice (same sex and age) which display neurological disorders. The mice were infected with plasmodia by i.p. injection of infected blood (diluted with saline) from donor animals in which parasites were maintained by weekly transfer of infected blood. The parasitemias and body temperatures were measured. A correlation was found between decrease in body temperature (below 30°C) within approximately 24 h before death in the second week and the presence of lesions in the brain as determined *post mortem* (Polder *et al.*, 1992; Eling and Sauerwein, 1995).

Parasite Development

Parasitemias were assessed by use of Giemsa-stained blood smears.

Drug Delivery Systems

Devices containing DFO. We used matrices of a bioerodible polymer, poly(FAD-SA), that releases DFO *in vitro* for 7 days with first-order kinetics. The matrices were designed to obtain a similar *in vitro* release profiles for all screened drugs. The matrices used undergo *in vivo* degradation and elimination within 6 weeks after release of the drugs (Domb *et al.*, 1995). They were prepared first by melting the polymer on a hot plate at 70°C, removing it from the plate and mixing quickly with the drug powder (30–40% by weight). The mixture was then pressed between two metal plates to obtain a film of the appropriate thickness (2 mm) and the polymer was cut to obtain the desired dose. *In vitro* slow-release tests of the drugs were performed by placing the polymer tablet in 20 ml PBS (0.1 M, pH 7.4) and incubating it at 37°C. The solution, which was replaced daily, was used for drug determination by UV-visible absorption of the iron-hydroxamate complex (at 420 nm) after addition of excess FeCl₃. The polymer itself was estimated according to its dry weight.

Devices containing SIH. SIH (160 mg) was dissolved in 2.5 ml of DMSO and added to the polymer solution (114 mg polyethylene glycol 4000, 320 mg PSA and 22.8 mg Tween 80 in 8 ml chloroform). The mixture was stirred thoroughly, the solvents were evaporated with a rotavaporator and traces of DMSO were vaporized with high vacuum. Another 160 mg of PSA was added, the mixture was melted at 60–70°C for complete mixing and was allowed to stand overnight to form tablets of 97.5 mg (about 2 × 3 × 5 mm). Devices loaded with different amounts of SIH were produced similarly by use of proportional amounts of the ingredients. SIH released from the polymer was monitored by placing each polymer sample in 20 ml PBS at 37°C

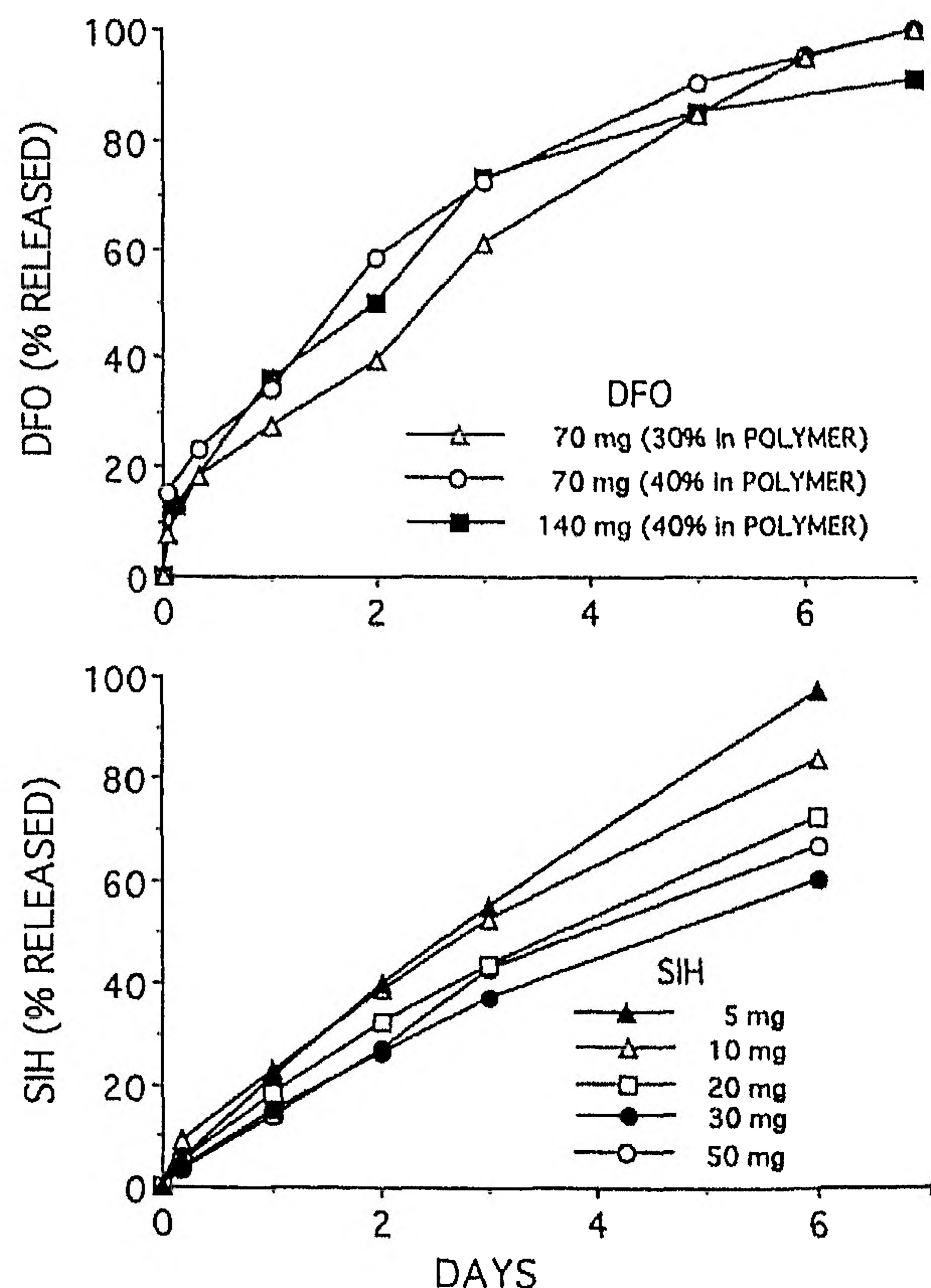


Fig. 1. Cumulative release of DFO (upper) or SIH (lower) from polymers into salt solutions. The polymers [poly(FAD-SA)] contained 30 or 40% DFO or 30% SIH and the indicated amounts of the drugs.

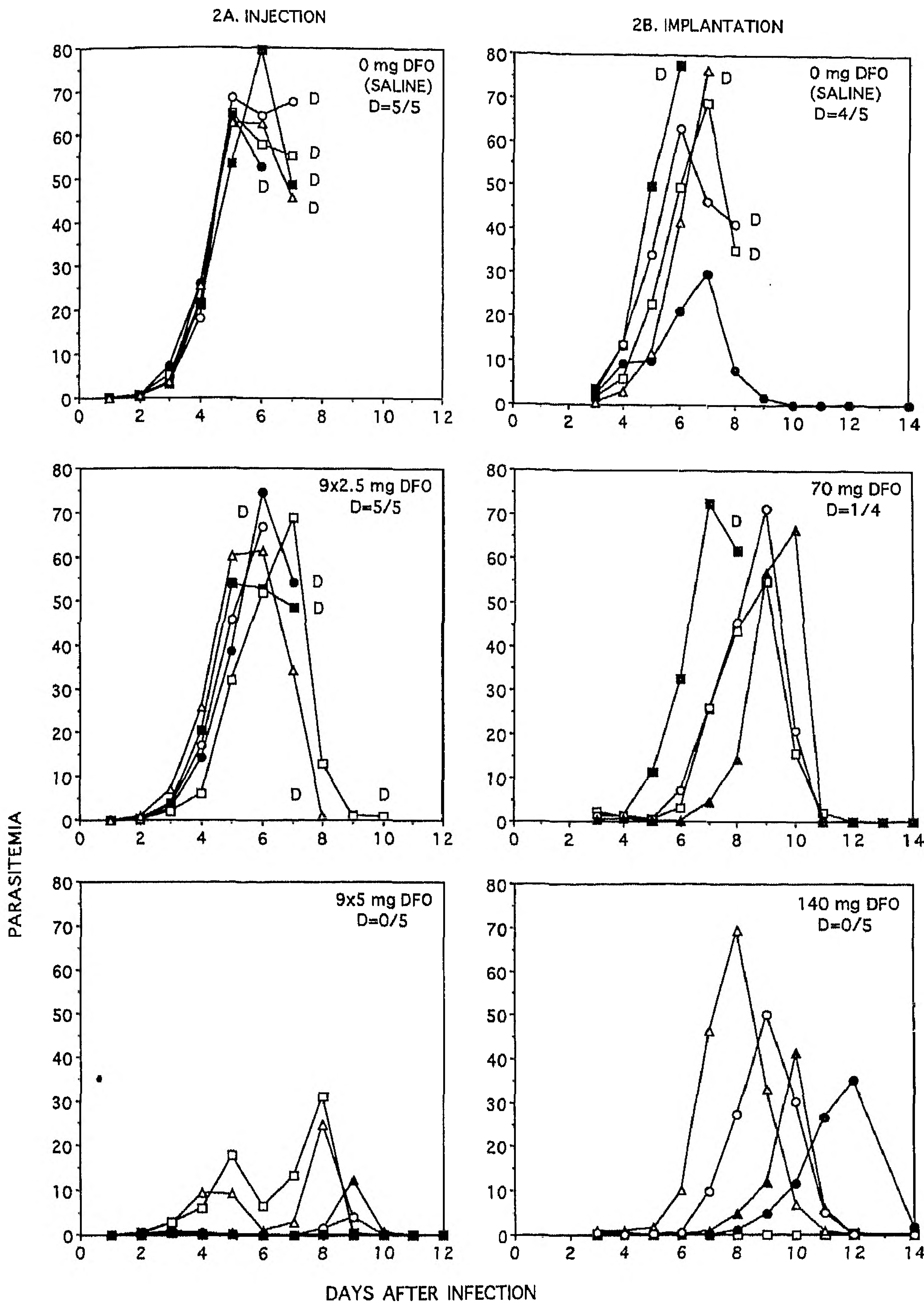


Fig. 2. Comparative efficacy of modes of DFO administration to Swiss mice infected with *P. vinckei petteri*. DFO was either injected i.p. at the indicated dose, starting 2 days postinfection at a frequency of three times/day for 3 days or administered as a 40% drug-polymeric s.c. implant. Each line represents the percent parasitemia of a single mouse. D = death.

and determining the amount released into the PBS by UV absorption at 333 nm.

The matrices containing given amounts of drug were implanted s.c. or i.p. in infected mice. The devices were dipped in 70% ethanol for sterilization and inserted through an incision in the lower abdominal region of ether-anesthetized mice. The implanted devices were retrieved from the implant site of some of the treated animals, and the drug content was quantified to determine total drug release (Domb, 1994).

Experimental Design

Chelators were either injected at a frequency of three times a day for 2 or 3 consecutive days, or administered as polymeric devices on day 2, 3 or 4 after infection. Each experiment was performed at least

three times (usually with 5–6 mice/group). Statistical analysis was conducted for each individual experiment. This approach was adapted because of variability between results obtained in different experiments caused by variations inherent in the biological systems. These include mice and experimental factors, such as the stage in the life cycle of the plasmodia in the infective inoculum. However, all individual experiments lead to the same qualitative conclusions. Consequently, we depicted a graphically representative set of results for each type of experiment and conducted statistical evaluation of two parameters: the parasitemia 2 days after drug administration (decreased values indicate a delay in parasite development) and the highest value of parasitemia (peak parasitemia). Significance is defined in terms of $\alpha(2)$, the two paired probability according to Mann-Whitney's test (with values above .05 indicating no statistically significant difference between experimental and control groups).

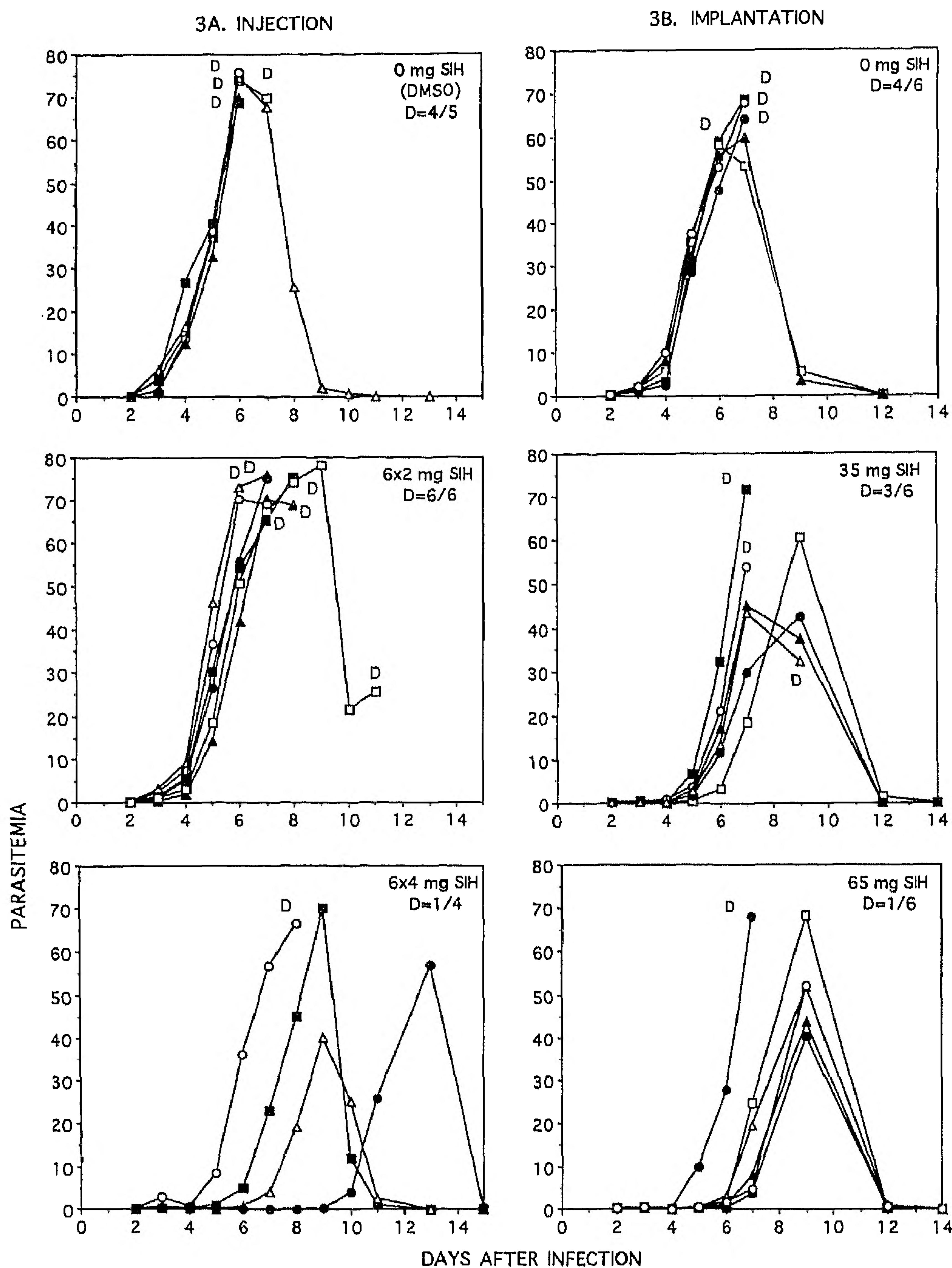


Fig. 3. Comparative efficacy of modes of SIH administration to Swiss mice infected with *P. vinckei petteri*. SIH was either injected i.p. at the indicated dose, starting 2 days postinfection at a frequency of three times/day for 2 days or administered as a 40% drug-polymeric s.c. implant. Each line represents the percent parasitemia of a single mouse. D = death.

Mice that recovered from malaria were followed for an additional month to monitor possible recurrence of parasitemia. In some experiments, mice were challenged after this period with viable plasmodia to assess the acquisition of immunity.

Results

In vitro release of DFO and SIH. The iron chelators used for these studies were selected on the basis of their performance as growth inhibitors in *in vitro* cultures (Tsafack *et al.*, 1996b). A series of preliminary experiments were conducted to evaluate the *in vitro* release of the iron chelators from drug-loaded polymers of poly(FAD-SA). The two factors considered were drug dose and the weight ratio of drug/polymer which determine the rate of drug release. The higher the ratio of the hydrophilic drug to the polymer, the

faster the release of drug, and *vice versa*, for lipophilic drugs (Domb, 1994). Different doses of drug were obtained by weighing a given preparation of the drug-containing polymer. Figure 1 displays the cumulative drug-release profiles for various drug/polymer formulations. Although a 30% DFO-containing polymer (wt/wt) released the 70-mg dose continuously during a 7-day period, a 40% polymer containing the same DFO dose released the drug at about 25% faster speed during the first 2 days (fig. 1A). We selected the 40% polymers for all the reported studies with animals. The release of the lipophilic SIH from 40% polymers was linear for the first 3 days for the various doses used (5–50 mg). In a 6-day period, 65 to 95% of the drug was released from the polymers which were selected for further experiments (above 20 mg, fig. 1B). The experiments were performed in triplicates and

repeated at least three times. For each individual experiment the standard deviation exceeded no more than 10% of the mean value.

In vivo effects of DFO. The effects of DFO on an animal model of malaria were assessed in relation to the mode of administration: i.p. injections of DFO (fig. 2A) and s.c. or i.p. implants of the polymer containing DFO (fig. 2B). Swiss mice infected with *P. vinckei petteri* (3×10^6 parasitized erythrocytes) by i.p. injection of infected blood were treated starting from day 2 postinfection, at which point the first parasites were detected in peripheral blood (parasitemia = 0.01%, herewith defined as onset of patency). The treatment comprised either i.p. injections of DFO (2.5- or 5-mg DFO doses in 50 μ l saline, three times per day for 3 days) or a single s.c. insertion of the drug-containing device. Analysis of a dose-response profile of the injected DFO revealed that although 2.5 mg doses were ineffective, 5-mg doses led to marked reduction in parasitemias and total elimination of mortality. A peculiar feature displayed by some animals treated with 2.5 mg DFO was an unexplained death at 3 to 5 days after cessation of treatment and after a demonstrable clearance of parasites from the peripheral blood (fig. 2A). The s.c. insertion of a single polymeric tablet containing 70 or 140 mg DFO also conferred protection to infected mice (fig. 2B). This was manifested as a reduction in parasitemia, a delay in peak parasitemia and a reduction in mortality. After peak parasitemia, there was an abrupt clearance of parasites in DFO-treated animals, as manifested by the parasitemias. The presence of the polymer, with or without drug, was apparently well tolerated by the mice and there were no indications of adverse reactions or toxicity. The antimalarial effect of the DFO tablet was essentially identical whether the polymer was inserted s.c. or i.p. (data not shown).

In vivo effects of SIH. A dose-response effect was obtained after SIH i.p. injections three times per day for only 2 days. Additional i.p. injections of SIH were generally avoided, particularly with relatively high doses of drug, because of toxic and, occasional lethal effects of prolonged treatments. Although 2-mg SIH injections (in DMSO, 50 μ l) had virtually no effect on the course of the infection, 4-mg injections (in DMSO, 50 μ l) produced a fast attenuation of the infection and prevented death in most of the animals (fig. 3A). It should be noted that although SIH was initially dissolved in DMSO for injection into animals, the SIH-containing polymer was rendered mostly free of DMSO. A result analogous to that produced by DFO was obtained with polymeric inserts of SIH. The drug released from a single 35-mg SIH-polymer insert significantly delayed the parasite infection, and most of the animals survived at a dose of 65 mg (fig. 3B). The data shown in figures 2 and 3 revealed similar performances of DFO and SIH. This includes also an abrupt decrease in parasite survival after reaching peak parasitemia values. However, a closer inspection of the data obtained during the first 2 days of treatment which started at patency (when parasitemia was about 0.01%) reveals a marked difference in the onset of inhibition of parasite proliferation by both drugs (table 1). The treatment, which commenced 2 days after infection, included multiple injections of either SIH (3×2 mg/mouse/day) or DFO (3×2.5 mg/mouse/day) or implants of SIH (35 mg) or DFO (70 mg). Although the dose of SIH was relatively lower or equal to that of DFO, at day 3 of infection

(day 1 of treatment), the inhibition of parasite proliferation was already 60% and 72% for SIH administered by injection and by polymer implants, respectively, whereas for DFO the values were 15 and 29%, respectively. At day 4, SIH was still more effective than DFO when given by injection, but equally effective when given by polymer. It should be stressed that the speed of action of SIH was faster despite the fact that it was used at relatively lower iron-chelating doses than DFO, because DFO binds iron at 1:1 stoichiometry whereas SIH binds it at 2:1 stoichiometry (Baker *et al.*, 1992).

To further assess the efficacy of the antimalarial treatment in controlling progression of the disease, we examined the polymer-drug implants on animals that had reached fulminant stages of infection, that is at 1% and 9.5% parasitemia. The results shown in figure 4 indicate that both DFO (70 mg) and SIH (35 mg) were demonstrably effective in attenuating parasite proliferation and reducing mortality of infected animals even at a late stage of the infection. At the relatively lower dose (35 mg SIH *vs.* 70 mg DFO), SIH gave better protection than DFO, particularly when applied at high (9.5%) parasitemia. However, it should be emphasized that the two agents could not be assessed at the same concentration, either because DFO was inefficient at doses <50 mg/implant, or because of SIH apparent toxicity at doses >50 mg/implant.

Combined in vivo action of DFO and SIH. A combination of DFO and SIH added in separate polymers was examined and compared with the individual effects of the two drugs. A 50-mg DFO dose had only a minor effect on the course of infection, whereas a 20-mg SIH dose significantly attenuated it and reduced mortality. However, the combination of the two drugs had a more pronounced effect, particularly in preventing mortality (fig. 5).

Effect of DFO on a rodent model of CM. The effect of a DFO-containing polymer was also examined on *C57Bl* mice infected with *P. berghei k173*. The treatment commenced at day 6 postinfection, when parasitemias reached about 1%. Preliminary studies indicated that administration of DFO by injection (up to 12 mg \times 3/day/5 days) was ineffective in changing the course of the disease (not shown). However,

TABLE 1

The effects of DFO and SIH on in vivo parasite development at the initial stages of treatment

Mice infected with *P. vinckei petteri* (five mice per group) were treated with either DFO or SIH starting at day 2 postinfection ($P \sim 0.01\%$) as follows: DFO injections were given i.p. in saline at a dose of 2.5 mg/mouse for 3 days, three times daily (8 h apart) (control = saline only). SIH injections were given in DMSO for 2 days (2 mg/mouse, same regimen as DFO) (control = DMSO only). Polymers (40%) containing either 70 mg DFO or 35 mg SIH were implanted s.c. on day 2 postinfection. Each group of treatment consisted of 10 infected mice, 5 control and 5 treated. P = average % parasitemias (S.E. values were <10%). The inhibition of growth was significantly higher ($P < .05$) for SIH- relative to DFO-treated mice on day 3 (both by injection and by polymer implant: 72% *vs.* 29% and 60% *vs.* 15%, respectively) and on day 4 (only by injection: 73% *vs.* 14%).

	Drug Treatment of Parasitemia			
	i.p. Injection		Polymer implantation	
	day 3	day 4	day 3	day 4
Control P	4.8	20	2.1	9.1
DFO P	4.1	17	1.5	1.1
% inhibition	15	14	29	87
Control P	5.3	17.5	1.5	8.9
SIH P	2.2	4.8	0.4	0.9
% inhibition	60	73	72	91

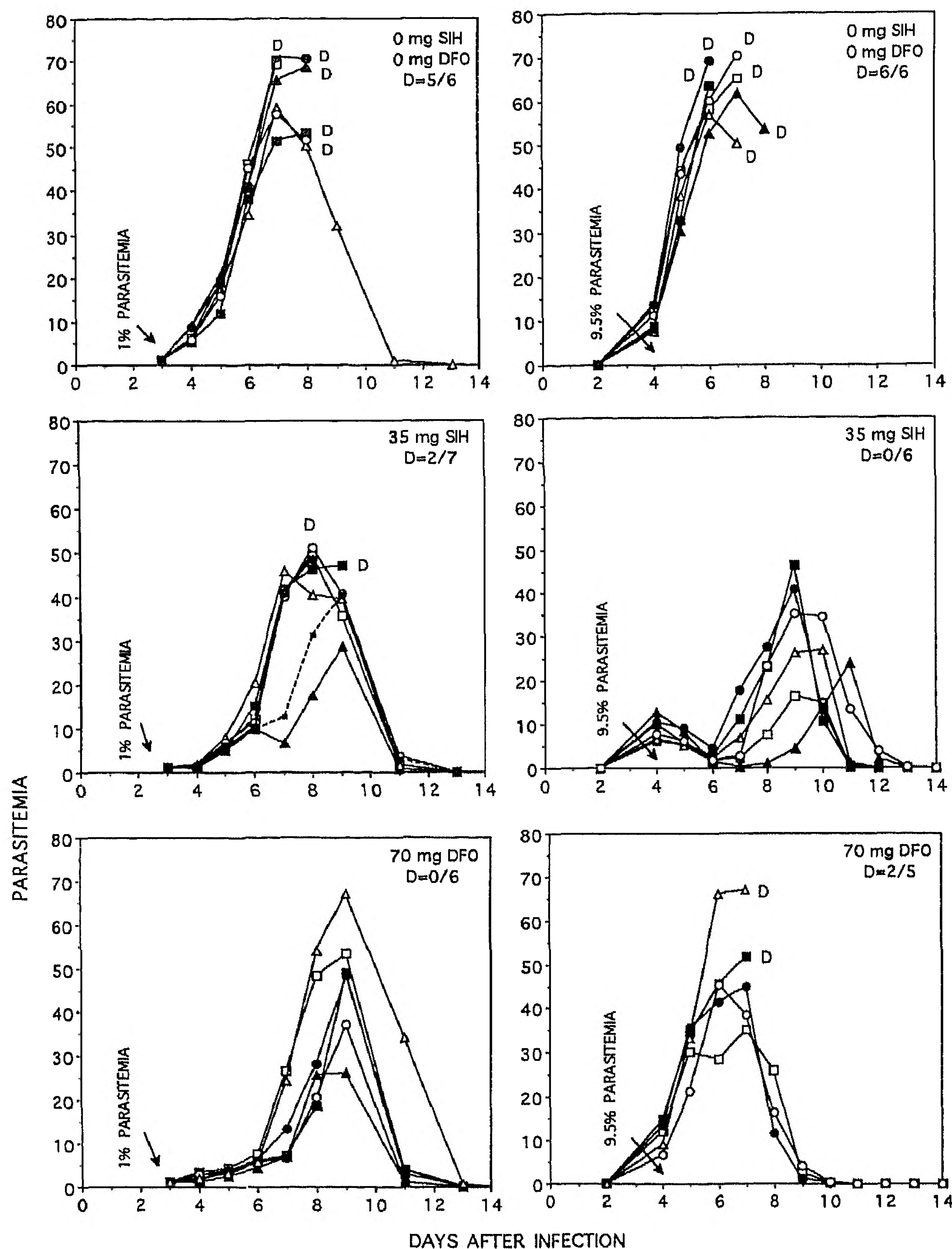


Fig. 4. The effects of DFO and SIH administered as s.c. polymer implants to Swiss mice at various stages of infection with *P. vinckei petteri*. Polymers were inserted 3 or 4 days after infection, after reaching the indicated parasitemias (1 or 9.5%, respectively). Each line represents parasitemia of a single mouse. D = death.

animals treated with s.c. inserts of polymers containing 140 mg DFO had a significant reduction in parasitemia and relief from CM symptoms (table 2).

Recurrence of parasitemias in polymer-treated mice. Mice which recovered after treatment (about 2 weeks after infection, figs. 2–5) were observed for an additional month to monitor possible recurrence of disease. Blood smear examinations (twice a week) revealed no parasites. These mice were challenged after this period with 20×10^6 parasitized erythrocytes and did not show any parasitemias over a further 2 weeks. This experiment was repeated twice with mice from all the experimental groups, including convalescent mice from control groups. Typical high parasitemias and mortality were demonstrated in control mice which had not been previously exposed to malaria.

Discussion

The demand for new chemotherapeutic strategies for the treatment of malaria has increased with the widening spread of parasite drug resistance. This is demonstrated in the case of mefloquine, which is widely used for the treatment of malaria and is the prophylactic drug of choice (Wallace *et al.*, 1996). Strategies to overcome resistance have relied on the application of single or pairs of drugs of either different chemical character and mode of action (Vennerstrom *et al.*, 1991) or similar mode of action but different cytotoxicity for the various stages of parasite growth. The latter strategy was used primarily with iron chelators in combination with DFO (Golenser *et al.*, 1995; Tsafack *et al.*, 1995; Cabantchik *et al.*, 1996), a clinically approved agent that can only be given

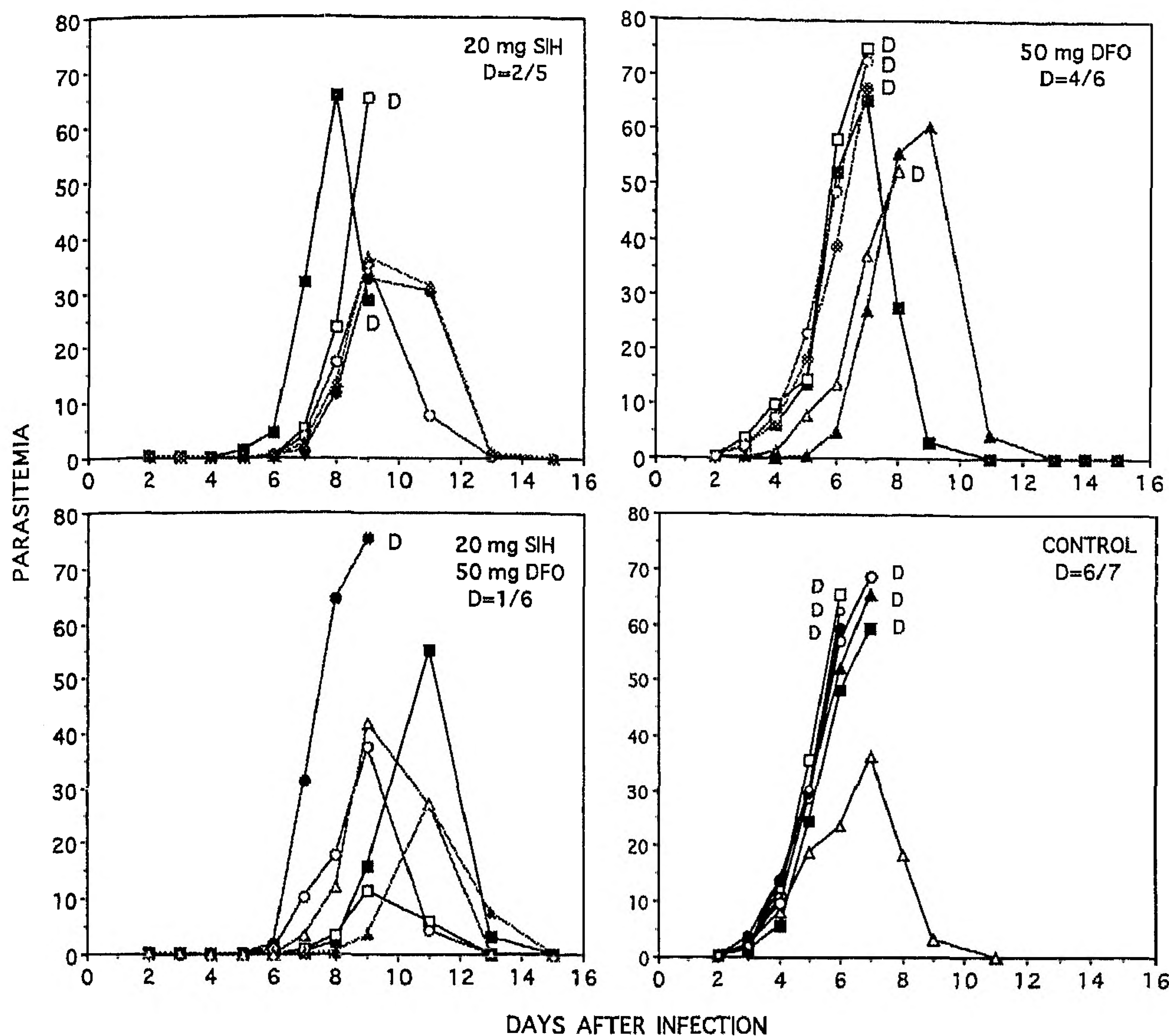


Fig. 5. The effects of SIH, DFO and their combination, administered as polymer implants on *P. vinckei petteri* infections in Swiss mice. Polymers were inserted 2 days after infection, at which point parasitemia was 0.01%. Each line represents parasitemia of a single mouse.

parenterally, thus demanding hospitalization. Oral chelators of the hydroxypyridinone family, which are highly efficient in the treatment of iron overload (Olivieri *et al.*, 1995), were virtually ineffective in human clinical trials of malaria (Mabeza *et al.*, 1996), despite the fact that some of them showed antimalarial activity in infected mice (Hershko *et al.*, 1991).

For animal chemotherapy of malaria based on iron chelators, we considered four factors which might affect the efficacy of the treatment: 1) the proven *in vitro* record of the drug, such as speed of action and wide action profiles on all blood stages of parasite development; 2) the possibility of parenteral drug treatment based on a single drug application; 3) the possibility of attaining sustained blood levels of the drug by slow-drug release devices; and 4) possible synergistic effects of combination of drugs. The most effective antimalarial iron chelators we have tested *in vitro* were reversed siderophores of high lipophilic character (Lytton *et al.*, 1994), particularly when used in combination with the hydrophilic DFO (Golenser *et al.*, 1995; Tsafack *et al.*, 1996b). However, the poor aqueous solubility of lipophilic agents limited their animal usefulness because of the need for re-

peated injections and the mode of action of both types of agents which demanded extended exposure of the parasites. Both limitations could be overcome by use of polymers as possible carriers of lipophilic and hydrophilic drugs and as slow drug release devices.

The polymers we considered were (Domb, 1994; Domb *et al.*, 1995): 1) biodegradable, whose products are eliminated from the organism; 2) implantable as drug carriers (e.g., polysilicones, ethylene-vinyl acetate copolymers, various acrylate-based hydrogels and segmented polyurethane) or as cleavable drug-polymer conjugates; 3) nontoxic, both locally and systemically. Other considerations given were drug loading and uniformity, duration and rate of drug release, pharmacokinetics of polymer degradation products, route of administration, drug stability in the polymer, storage stability and a possibility of terminal sterilization (by γ -irradiation). The selected polymers used were composed of sebacic acid and a dimer of erucic acid, linked by hydrolyzable anhydride bonds. They were produced as s.c. or i.p. implantable tablets, although they can be formulated as injectable microspheres or small pellet inserts (Domb and Maniar, 1993). The test

TABLE 2

Effect of DFO-polymer implants on parasitemia and CM symptoms in a rodent CM model of malaria

Day refers to time after infection (treatment was initiated on day 6: a s.c. implant of DFO (140 mg drug, 40% polymer). P = percent parasitemia; T = body temperature; S = mice survival; ND = not determined.

	Day 8			Day 9			Day 10			Day 11
	P	T	S	P	T	S	P	T	S	S
Control	34	31.2	11/11	36	27.0	8/11	ND	27.7	2/11	0/11
DFO	7	35.4	5/5	5	31.7	4/5	ND	36.6	3/5	3/5

TABLES 3-6

Statistical analysis of results from Figs. 2 to 5, respectively.

Treatment	Parasitemia at Day 4		Peak Parasitemia	
Control	22.4 ± 1.5		68.0 ± 3.0	
9 × 2.5 mg DFO	16.9 ± 3.3	$\alpha(2) = 0.1$	65.0 ± 3.5	$\alpha(2) \geq 0.2$
9 × 5 mg DFO	3.3 ± 1.9	$\alpha(2) = 0.01$	14.6 ± 5.9	$\alpha(2) = 0.01$
Control	9.1 ± 2.1		63.2 ± 8.8	
70 mg DFO	1.1 ± 0.2	$\alpha(2) = 0.02$	66.2 ± 3.9	$\alpha(2) \geq 0.2$
140 mg DFO	0.3 ± 0.1	$\alpha(2) = 0.005$	32.8 ± 11.3	$\alpha(2) \geq 0.2$

TABLE 4

Treatment	Parasitemia at Day 4		Peak Parasitemia	
Control	16.8 ± 2.6		72.6 ± 1.4	
6 × 2 mg SIH	5.5 ± 1.1	$\alpha(2) = 0.005$	74.1 ± 1.3	$\alpha(2) \geq 0.2$
6 × 4 mg SIH	0.3 ± 0.11	$\alpha(2) = 0.02$	58.5 ± 6.7	$\alpha(2) = 0.1$
Control	5.8 ± 1.1		62.4 ± 2.1	
35 mg SIH	0.3 ± 0.1	$\alpha(2) = 0.002$	52.8 ± 4.7	$\alpha(2) = 0.2$
65 mg SIH	0.1 ± 0.1	$\alpha(2) = 0.002$	54.0 ± 4.8	$\alpha(2) \geq 0.2$

TABLE 5

Treatment	Parasitemia at Day 5		Peak Parasitemia	
Control	17.3 ± 1.2		63.1 ± 3.0	
35 mg SIH	6.0 ± 0.4	$\alpha(2) = 0.002$	43.6 ± 2.9	$\alpha(2) = 0.002$
70 mg DFO	3.4 ± 0.3	$\alpha(2) = 0.002$	46.8 ± 5.7	$\alpha(2) = 0.03$
Parasitemia at day 6				
Control	59.9 ± 2.4		64.3 ± 2.0	
35 mg SIH	2.5 ± 0.5	$\alpha(2) = 0.002$	31.6 ± 4.6	$\alpha(2) = 0.002$
70 mg DFO	45.4 ± 6.0	$\alpha(2) = 0.05$	48.8 ± 5.3	$\alpha(2) = 0.075$

TABLE 6

Treatment	Parasitemia at Day 4		Peak Parasitemia	
Control	10.6 ± 1.1		59.8 ± 4.1	
20 mg SIH	0.1 ± 0.01	$\alpha(2) = 0.035$	47.1 ± 7.5	$\alpha(2) = 0.2$
50 mg DFO	5.2 ± 1.6	$\alpha(2) = 0.035$	65.6 ± 3.4	$\alpha(2) \geq 0.2$
20 mg SIH + 50 mg DFO	0.1 ± 0.01	$\alpha(2) = 0.005$	41.5 ± 9.1	$\alpha(2) = 0.1$

iron chelators selected for this study were the lipophilic SIH and the hydrophilic DFO. The results indicated that the drug-containing tablets fulfilled the above-stated requirements in terms of sterility, lack of toxicity and slow-release properties, which were apparently dictated by the relative amount of drug in the polymer (Domb, 1994). With the hydrophilic DFO, the higher its relative content in the polymer, the higher the overall hydrophilicity of the device and the speed of drug release (fig. 1A). The converse was apparently the case with the lipophilic SIH, as shown in figure 1B.

In tests performed in Swiss mice infected with *P. vinckei petteri*, the polymers containing 70 mg DFO or 65 mg SIH gave results similar to those of three i.p. injections/day/3 days of 5 mg DFO (a total of 45 mg DFO) or three injections/day/2 days of 4 mg SIH (a total of 24 mg SIH) (figs. 2 and 3). These were manifested in delay of peak parasitemia and lower mortality. Although at comparable polymeric doses, SIH outperformed DFO in terms of speed of action (table 1), the overall performance of both drugs in reducing parasite

load and mortality was similar (figs. 2 and 3). Both SIH and DFO polymer insertions were effective even when the initial parasitemias were as high as 9.5% (fig. 4). Combinations of the iron chelators should be considered because they were more efficient than the individual polymers in reducing mortality (fig. 5).

The effect of slow release of iron chelators was also examined on a CM model in mice infected with *P. berghei* highly resistant to DFO. Six days postinfection, when parasitemias were about 1%, the animals were treated by s.c. insertion of tablets containing 140 mg DFO. The treatments reduced parasitemias and also prevented CM symptoms in most of the mice, whereas repeated injections of DFO were ineffective in this model.

The rapid clearance of parasites from chelator-treated animals that have reached relatively high parasitemia might be indicative of an improved clearance of parasites by the reticuloendothelial system. Such action might be the result of chelator-mediated reduction of toxic iron load which is generated by phagocytosis of infected cells and which might hamper macrophage activities (Schwarzer *et al.*, 1992). Thus, this mode of chelator action should be added to the proposed modes of action of iron chelators as antimalarials. These have been focused either on chelator action on intraerythrocytic parasites (reviewed in Gordeuk, 1994; Cabantchik 1994; Cabantchik *et al.*, 1996) or in the prevention of pathological iron-dependent processes (Hunt *et al.*, 1992; Golenser and Chevion, 1994; Mabeza *et al.*, 1996). Drug treatment may affect the course of the disease directly by killing the plasmodia and indirectly by altering the immune responses (*e.g.*, by changing cytokine response). Recent retrospective clinical studies support the notion of a possible iron chelator action on immune cell responses (Thuma *et al.*, 1996). This also strengthens the suggestion that chemotherapy should be complementary to vaccination (Tanner and Evans, 1994).

Taking *in toto* the results of this study and the clinical record of DFO (Gordeuk *et al.*, 1994; Mabeza *et al.*, 1996), we propose that iron chelators delivered by slow-release polymeric devices can be advantageous for malaria therapy. They can be particularly useful when used as combinations of drugs that display different speeds of action. Moreover, similar parenteral treatments might be also applicable to iron-overloaded individuals that require frequent iron chelation therapy (Olivieri *et al.*, 1995; Porter, 1996).

Acknowledgments

We acknowledge the technical assistance of C.C. Hermsen from the University of Nijmegen.

References

- BAKER, E., RICHARDSON, D. R., GROSS, S. AND PONKA, P.: Evaluation of the iron chelation potential of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde using the hepatocyte in culture. *Hepatology* 15: 492-501, 1992.
- BUNNAG, D., POLTERA, A. A., VIRAVAN, C., LOOAREESUWAN, S., HARINASUTA, K. T. AND SCHINDLERY, C.: Plasmodicidal effect of DFO in human vivax or falciparum malaria from Thailand. *Acta Trop.* 52: 59-67, 1992.
- CABANTCHIK, Z. I.: The mode of action of iron (III) chelators as anti-malarials. The biochemical basis of selective cytotoxicity. *Parasitol. Today* 11: 74-78, 1994.
- CABANTCHIK, Z. I., GLICKSTEIN, H., GOLENSER, J., LOYEVSKY, M. AND TSAFACK, A.: Iron chelators: Mode of action as antimalarials. *Acta Haematol.* 95: 70-77, 1996.
- CHASIN, M., DOMB, A. J., RON, E., MATHIOWITZ, K., LEONG, K., LAURENCIN, H., BREM, H. AND LANGER, R.: Polyanhydrides as drug delivery systems. In *Degradable Polymers as Drug Delivery Systems*, ed. by R. Langer and M. Chasin, pp. 43-71, Marcel Dekker, New York, NY, 1990.
- CURFS, J., SCHERRERS, T., HERMSEN, C., JERUSALEM, C., VAN ZON, A. AND ELING, W.:

- Immunological aspects of cerebral lesions in murine malaria. *Clin. Exp. Immunol.* **75**: 136-140, 1989.
- DOMB, A. J.: Implantable biodegradable polymers for site specific drug delivery. In *Polymeric Site-Specific Pharmacotherapy*, ed. by A. J. Domb, pp. 1-26, Wiley, Chichester, England, 1994.
- DOMB, A. J., AMSELEM, S., LANGER, R. AND MANIAR, M.: Polyanhydrides as carriers of drugs. In: *Designed to Degrade Biomedical Polymers*, ed. by S. Shalaby, pp. 69-96, Carl Hauser Verlag, 1993.
- DOMB, A. J. AND MANIAR, M.: Absorbable biopolymers derived from dimer fatty acids. *J. Polym. Sci.* **31**: 1275-1285, 1993.
- DOMB, A. J., ROCK, M., PERKIN, C., YIPCHUCK, G., BROXAP, B. AND VILLEMURE, J. G.: Excretion of a radiolabelled anticancer biodegradable polymeric implant from the rabbit brain. *Biomaterials* **16**: 319-323, 1995.
- ELING, W. M. C. AND SAUERWEIN, R. W.: Severe and cerebral malaria: Common or distinct pathophysiology? *Rev. Med. Microbiol.* **5**: 1-9, 1995.
- FRITSCH, G. AND JUNG, A.: ^{14}C -DFO: Uptake into erythrocytes infected with *P. falciparum*. *Z. Parasitenkd.* **72**: 709-713, 1986.
- GOLENSER, J. AND CHEVION, M.: Implications of oxidant stress and malaria. In *Tropical Medicine, Implications of Oxygen Free Radicals*, ed. by O. I. Aruoma, pp. 53-79, Harwood Academic Publishers, Chur, Switzerland, 1994.
- GOLENSER, J., TSAFACK, A., LIBMAN, J., SHANZER, A. AND CABANTCHIK, Z. I.: The antimalarial action of hydroxamate-based iron chelators. Effects of drug combinations. *Antimicrob. Agents Chemother.* **39**: 61-69, 1995.
- GORDEUK, V. R., BRITTENHAM, G. M. AND THUMA, P. E.: Iron chelation as a chemotherapeutic strategy in the treatment of malaria. In *The Development of Iron Chelators for Clinical Use*, ed. by R. J. Bergeron and G. M. Brittenham, pp. 111-130, CRC Press Inc., Boca Raton, FL, 1994.
- GORDEUK, V. R., THUMA, P. E. AND BRITTENHAM, G. M.: Iron chelation therapy in malaria. In *Progress in Iron Research*, ed. by C. Hershko, pp. 371-383, Plenum Press, New York, 1994.
- GORDEUK, V. R., THUMA, P. E., BRITTENHAM, G. M., BIEMBA, G., ZULU, S., SIMWANZA, G., KALENSE, P., M'HANGO, A., PARRY, D., POLTERA, A. A. AND AIKAWA, M.: Iron chelation as a chemotherapeutic strategy for falciparum malaria. *Am. J. Trop. Med. Hyg.* **48**: 193-197, 1993.
- GRAU, G. E., PIGUET, P. F., ENGERS, H. D., LOUIS, J. A., VASSALLI, P. AND LAMBERT, P. H.: L3T4+ T lymphocytes play a major role in the pathogenesis of murine cerebral malaria. *J. Immunol.* **137**: 2348-2354, 1986.
- HERSIKO, C., IHEANACHO, E. N., SPIRA, D. T., PETER, H. H., DOBBIN, P. AND HIDER, R. C.: The effect of N-alkyl modification on the antimalarial activity of 3-hydroxypyridin-4-one oral iron chelators. *Blood* **77**: 637-643, 1991.
- HUNT, A. N., KOPP, M. AND STOCKER, R.: Free radicals and antioxidants in malaria. In: *Lipid Soluble Antioxidants: Biochemistry and Clinical Application*, ed. by O. N. G. Ash and L. Packer, pp. 337-354, Birkhauser Verlag, Basel, Switzerland, 1992.
- KAUL, D. K., NAGEL, R. L., LLENA, J. F. AND SHEAR, H. L.: Cerebral malaria in mice: demonstration of cytoadherence of infected red blood cells and micro-rheologic correlates. *Am. J. Trop. Med. Hyg.* **50**: 512-521, 1994.
- LOVEVSKY, M., LYTTON, S. D., MESTER, B., LIBMAN, J., SHANZER, A. AND CABANTCHIK, Z. I.: The antimalarial action of Desferal involves a direct access route to erythrocytic parasites (*P. falciparum*). *J. Clin. Invest.* **91**: 218-224, 1993.
- LYTTON S. D., MESTER, B., LIBMAN, J., SHANZER, A. AND CABANTCHIK, Z. I.: Mode of action of iron (III) chelators as antimalarials: II. Evidence for differential effects on parasite iron-dependent nucleic acid synthesis. *Blood*. **84**: 910-915, 1994.
- MABEZA, G. E., BIEMBA, G. AND GORDEUK, V. R.: Clinical studies of iron chelators in malaria. *Acta Haematol.* **95**: 78-86, 1996.
- MACPHERSON, G. G., WARRELL, M. J. AND WHITE, N. J.: Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am. J. Pathol.* **119**: 385-401, 1985.
- OLIVIERI, N. F., BRITTENHAM, G. M., MATSUI, D., BERKOVITCH, M. AND OTHERS. IRON- CHELATION THERAPY WITH ORAL DEFERIPRONEIN PATIENTS WITH THALASSEMIA MAJOR. *N. ENGL. J. MED.* **332**: 918-922, 1995.
- PETERS, W.: Drug resistance in malaria. *Recent Progr. Med.* **81**: 749-753, 1990.
- POLDER, T., ELING, W., CURFS, J., JERUSALEM, C. AND WIJERS-ROUW, M.: Ultrastructural changes in the blood brain barrier of mice infected with *P. berghei*. *Acta Leiden.* **60**: 31-46, 1992.
- PORTER, J. B.: Evaluation of new iron chelators for clinical use. *Acta Haematol.* **95**: 13-25, 1996.
- SCHWARZER, E., TURRINI, F., ULLIERS, D., GIRIBALDI, G., GINSBURG, H. AND ARESE, P.: Impairment of macrophage functions after ingestion of *Plasmodium falciparum* infected erythrocytes or isolated malarial parasites. *J. Exp. Med.* **176**: 1033-1041, 1992.
- SCOTT, M. D., RANZ, A., KUYPERS, F. A., LUBIN, B. H. AND MESHNICK, S. R.: Parasite uptake of deferoxamine: a prerequisite for antimalarial activity. *Br. J. Haematol.* **75**: 598-602, 1990.
- TANNER, M. AND EVANS, D.: Vaccines or drugs: Complementarity is crucial. *Parasitol. Today* **10**: 406-407, 1994.
- THUMA, T. E., WEISS, G., HEROLD, M. AND GORDEUK, V. R.: Serum neopterin, interleukin 4 and interleukin 6 concentrations in cerebral malaria patients and the effect of iron chelation therapy. *Am. J. Trop. Med. Hyg.* **54**: 164-168, 1996.
- TSAFACK, A., GOLENSER, J., LIBMAN, J., SHANZER, A. AND CABANTCHIK Z. I.: Mode of action of iron (III) chelators as antimalarials. III. Overadditive effects in the combined action of hydroxamate-based agents on *in vitro* growth of *P. falciparum*. *Mol. Pharmacol.* **47**: 403-409, 1995.
- TSAFACK, A., GOLENSER, J., LIBMAN, J., SHANZER, A. AND CABANTCHIK, Z. I.: Chemical determinants of iron chelators as antimalarials. *Antimicrob. Agents Chemother.*, in press, 1996a.
- TSAFACK, A., LOVEVSKY, M., PONKA, P. AND CABANTCHIK Z. I.: Mode of action of iron (III) chelators as antimalarials. IV. Potentiation of Desferal action by benzoyl and isonicotinoyl hydrazone derivatives. *J. Lab. Clin. Med.* **127**: 574-582, 1996b.
- VANDAMME, T. F. AND HELLER, J.: Poly (ortho esters) as bioerodible matrices for the controlled delivery of pyrimethamine in chemoprophylaxis of malaria. *J. Controlled Release* **36**: 209-214, 1995.
- VENNERSTROM, J. L., W. Y. ELIS, W. K. MILHOUS AND J. W. EATON. ANTIMALARIAL SYNERGISM AND ANTAGONISM. In *SYNERGISM AND ANTAGONISM IN CHEMOTHERAPY*, ED. BY T. C. CHOU AND D. C. RIDEOUT, PP.183-222, ACADEMIC PRESS, SAN DIEGO, 1991.
- WALLACE, M. R., SHARP, T. W., SMOAK B., IRIYE, C., ROZMAJZL, P., THORNTON, S. A., BATCHELOR, R., MACILL, A. J., LOBEL, H. O., LONGER, C. F. AND BURANS, J. P.: Malaria among US troops in Somalia. *Am. J. Med.* **100**: 49-55, 1996.

Send reprint requests to: Prof. J. Golenser, Department of Parasitology, Hadassah Medical School, The Hebrew University, Jerusalem 91010, Israel.
