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Haem polymerase as a novel target of antimalarial action of cyproheptadine

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Abstract

An antihistaminic drug, cyproheptadine (20–25 mg/kg × 4 days), showed significant schizontocidal activity in the blood against a lethal multidrug-resistant (MDR) strain of *Plasmodium yoelii nigeriensis* (highly resistant to chloroquine, mefloquine, and quinine); the protection of mice ranged between 75 and 100%. A combination of cyproheptadine (15 mg/kg) and chloroquine improved antimalarial activity compared to treatment with either drug alone, whereas a combination of cyproheptadine with quinine or mefloquine did not improve its antimalarial activity. Chloroquine and cyproheptadine inhibited haem polymerization activity in cell-free extracts and in *in vivo* experiments with MDR *P. yoelii*, but the combination did not cause a more significant inhibition than found with either drug alone. Cyproheptadine has been shown to produce dose-dependent inhibition of haem polymerization activity both *in vitro* and *in vivo*. The mechanism of the antimalarial action of cyproheptadine and its enhanced antimalarial activity with chloroquine could be due, in part, to their inhibitory effect on haem polymerization.

Keywords: *Plasmodium yoelii nigeriensis*; Chloroquine; Cyproheptadine; Haemozoin; Haem polymerase; Malaria

1. Introduction

Malaria is a protozoan infection that threatens millions of people throughout the world. The transmission of malaria is rising, multidrug-resistant strains of *Plasmodium falciparum* are spreading, and the degree of resistance to conventional antimalarials is gradually increasing [1]. The control of drug-resistant cases of malaria may be achieved either by developing alternative chemotherapeutic agents active against resistant strains of the parasite or by identification of agents that can modulate the drug-resistant phenotype of the parasite and that may be used in adjunct therapy with conventional antimalarials. Several classes of compounds have been identified as resistance-reversal agents.

The calcium channel blocker verapamil [2], the antidepressant desipramine [3], and the antihistaminic drug cyproheptadine, have been shown to reverse the resistance of *P. falciparum* isolates to chloroquine in an *in vitro* culture system [4]. Among these, the antihistamine cyproheptadine, which bears some structural resemblance to desipramine, has also been shown to possess inherent antimalarial action *in vitro* against resistant and sensitive culture-adapted strains of *P. falciparum* [5]. Isobologram analysis showed that cyproheptadine exerts a marked synergistic action with chloroquine against chloroquine-resistant parasites, but does not modify the activity of chloroquine in sensitive strains of the parasite [6,7]. The same type of study has also been carried out against the chloroquine-resistant strain of *P. berghei in vivo*. Cyproheptadine was found to be more active than other antihistaminics when used in combination with chloroquine [4], but was unable to reverse mefloquine resistance activity in the NS/1100 line of *P. yoelii* (a mefloquine-resistant strain) [8]. In the present paper, we report on the *in vivo* antimalarial activity of cyproheptadine against a lethal,

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Abbreviations: MDR, multidrug-resistant (resistance); and MST, mean survival time.

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multidrug-resistant strain of *P. yoelii nigeriensis* infection in mice and also its slight potentiating action when combined with chloroquine. The biochemical mechanism of the antimalarial action of cyproheptadine was also investigated by evaluating the effect of the drug both *in vitro* and *in vivo* on the formation of haemozoin by MDR *P. yoelii*, which is a known potential target of the antimalarial action of several schizontocidal drugs in the blood [9,10].

2. Materials and methods

2.1. Parasite and host

A lethal, multidrug-resistant strain of *P. yoelii nigeriensis*, originally obtained from Professor P.C.C. Garnham in 1978, was employed in the present studies. This strain of *P. yoelii* has developed resistance to chloroquine (128 mg/kg), mefloquine (128 mg/kg), and quinine (400 mg/kg). The infection is maintained *in vivo* in Swiss mice (18–20 g) of either sex by serial passage with infected erythrocytes. The parasitaemia in infected mice was monitored by preparing thin blood smears from the tail vein. Smears were fixed in methanol, stained with Giemsa, and examined under a microscope for assessing the degree of parasitaemia.

2.2. In vivo drug sensitivity assays

The mice were divided into different groups, each group consisting of at least 7–8 animals. All of the mice of each group were inoculated intraperitoneally with 1×10^6 or 1×10^7 *P. yoelii* (MDR)-infected erythrocytes suspended in 0.5 mL of sterile citrate saline. Drug sensitivity tests were carried out using a 4-day treatment schedule as described previously by Peters [11]. Treatment with chloroquine, quinine, mefloquine, or cyproheptadine alone or chloroquine/quinine/mefloquine plus cyproheptadine was initiated from day 0 (the day of infection) and continued until day 3 or until day 6 in the case of quinine. The doses and combination of the drugs used are mentioned in Section 3. One group of *P. yoelii*-infected mice that received no treatment served as the controls. The drugs were administered orally to the mice in a volume of 0.5 mL/20 g body weight. Parasitaemia in the drug-treated and untreated mice was monitored on days 4, 7, 10, 14, 18, 21, 24, and 28 post-inoculation. The MST of mice belonging to each group was computed by monitoring the survival rate of mice from day 0 to the end of the experiment, i.e. day 28, and these results were analyzed statistically using Student's *t*-test.

2.3. Haem polymerization assay and estimation of haemozoin content

Haem polymerization activity in the extract of *P. yoelii*-infected erythrocytes was assayed by a method described by Pandey *et al.* [12]. Blood from the infected mice was collected in a sterile solution of citric acid and dextrose (0.73 g citric acid, 2.20 g sodium citrate, and 2.45 g dextrose in 100 mL of distilled water). Erythrocytes were washed twice with PBS and suspended in 4 vol. of PBS containing 0.9% glucose (PSG). The erythrocytes were lysed by the freeze–thaw process in liquid nitrogen. The pellet was thawed and centrifuged at 10,000 *g* for 10 min at 4°. The pellet was washed twice with PSG and finally suspended in acetate buffer (100 mM, pH 5). To assay for haem polymerase activity, a reaction mixture was prepared, in a total volume of 1 mL, consisting of the following components: acetate buffer, 100 mM (pH 5.0), haemin (100 μM), the drug, and 50 μL of the parasite lysate (approximately 1–2 nmol of preformed haemozoin). Controls without the substrate (haemin) or parasite extract were run simultaneously. The reaction mixture was incubated at 37° for 4 hr in a metabolic shaker, and the reaction was stopped by centrifuging the tubes at 10,000 *g* (5 min, 4°). The pellets were washed twice with Tris–HCl (100 mM, pH 8.0) containing SDS (2.5%, w/v) and finally with bicarbonate buffer (100 mM, pH 9.0). For quantitation of haemozoin, the pellets were dissolved in 50 μL of 2 N NaOH and made up to 1 mL with 2.5% SDS. Haemozoin when dissolved in NaOH is converted back to the monomer haem and gives maximum absorbance at 400 nm. Haemozoin haem was quantitated using an extinction coefficient of 91 mM^{-1} cm^{-1} at 400 nm.

To quantify the haemozoin content of *P. yoelii*-infected erythrocytes (40–50% parasitaemia), the erythrocytes were collected, washed with PBS, and resuspended in 1 mL of acetate buffer (100 mM, pH 5). Then 200-μL aliquots of the erythrocyte suspension were incubated for 1, 2, 3, and 4 hr at 37° with the drugs. The erythrocytes were subsequently centrifuged and suspended directly into Tris–HCl (100 mM, pH 8) containing 2.5% (w/v) SDS. The suspensions were centrifuged at 10,000 *g* for 10 min, and the pellets were washed twice with Tris/SDS and once with bicarbonate buffer (100 mM, pH 9). The haemozoin pellets were dissolved in 50 μL of 2 N NaOH and quantitated as haemozoin haem as described above.The IC_{50} values presented are the average of at least two separate determinations of full dose–response curves, each performed in triplicate.

3. Results

3.1. Effect of chloroquine and/or cyproheptadine treatment on P. yoelii infection

P. yoelii nigeriensis infection was lethal to the mice as all of them died within 6–7 days post-infection. Treatment of the infected mice with chloroquine alone at doses of 4 and 8 mg/kg body weight did not affect the increasing parasitaemia caused by the multidrug-resistant strain of

Table 1
Effect of *in vivo* chloroquine and/or cyproheptadine treatment of *P. yoelii nigeriensis* infection in mice

Serial no.	Drug(s)	Dose (mg/kg)	MST (days)	% Parasitaemia						Cure rate (%)
				4	7	10	14	21	28	
1	Control	–	6.5 ± 0.5	8.0 ± 11.0	–	–	–	–	–	Nil
2	Chloroquine	4	8.4 ± 2.1	Nil (8)	28.3 ± 2.16 (7)	19.5 (1)	–	–	–	Nil
3	Chloroquine	8	10.4 ± 1.2	Nil (8)	5.1 ± 8.1 (8)	7.2 ± 8.5 (8)	Nil (3)	–	–	Nil
4	Cyproheptadine	10	16.5 ± 7.7	Nil (8)	0.001 ± 0.003 (8)	6.9 ± 8.1 (7)	Nil (3)	Nil (2)	Nil (2)	25.0
5	Cyproheptadine	15	22.3 ± 8.0	Nil (8)	Nil (8)	4.0 ± 1.3 (8)	4.7 ± 9.2 (6)	Nil (5)	Nil (5)	62.5
6	Cyproheptadine	20	23.0 ± 10.0	Nil (8)	Nil (8)	0.04 ± 0.11 (8)	4.6 ± 12.1 (7)	Nil (6)	Nil (6)	75.0
7	Cyprophepatadine	25	>28.0 ± 0.0	Nil (8)	Nil (8)	Nil (8)	Nil (8)	Nil (8)	Nil (8)	100.0
8	Cyprophepatadine + chloroquine	10 + 4	19.6 ± 9.0	Nil (8)	0.2 ± 0.3 (8)	7.9 ± 13.2 (7)	Nil (4)	Nil (4)	Nil (4)	50.0
9	Cyprophepatadine + chloroquine	10 + 8	18.0 ± 8.4	Nil (8)	Nil (8)	Nil (7)	9.3 ± 18.5 (4)	Nil (3)	Nil (3)	37.5
10	Cyprophepatadine + chloroquine	15 + 4	26.0 ± 5.7	Nil (8)	Nil (8)	Nil (8)	Nil[a] (7)	Nil (7)	Nil (7)	87.5
11	Cyprophepatadine + chloroquine	15 + 8	26.4 ± 4.6	Nil (8)	Nil (8)	Nil (8)	4.4 ± 10.8 (7)	Nil (7)	Nil (7)	87.5

Values are means ± SD of the number of observations from the surviving animals (indicated in parentheses). Statistical significance between groups 2 vs 4 < 0.02; 2 vs 5 < 0.001; 2 vs 6 < 0.002; 2 vs 7 < 0.001; 3 vs 4 < 0.05; 3 vs 5 < 0.001; 3 vs 6 < 0.005; 3 vs 7 < 0.001; 2 vs 8 < 0.005; 2 vs 10 < 0.001; 3 vs 9 < 0.05; 3 vs 11 < 0.001; 4 vs 8, 9, 10, 11–not significant. Calculated from the MST column.

[a] In Serial no. 10, one mouse died due to the development of parasitaemia.

P. yoelii. A marginal increase in mean survival time (MST), however, was noticeable with chloroquine treatment (Table 1). Treatment of mice with the antihistaminic drug cyproheptadine alone provided significant protection against *P. yoelii* infection. The effect was found to be dose-dependent and was clearly noticeable even at a dose of 10 mg/kg body weight. The *P. yoelii*-infected mice treated with 25 mg cyproheptadine/kg body weight were cured of the infection. No parasitaemia appeared in the treated mice 28 days post-inoculation, and all the treated animals survived. However, in the mice treated with the lower doses of cyproheptadine (10, 15, and 20 mg/kg body weight), parasitaemia appeared, but it was suppressed from 12–14 days post-infection and the animals that survived were free of parasitaemia until 28 days post-infection (Table 1).

A combination treatment of chloroquine (4 or 8 mg/kg) with cyproheptadine (10 or 15 mg/kg) produced a significant increase in MST compared to chloroquine treatment alone. It also potentiated the cure rate in comparison to chloroquine alone (Table 1). When the combined effect was compared to cyproheptadine alone, the increase in MST/cure rate seemed to be additive although not significant (Table 1).

3.2. Effect of quinine and/or cyproheptadine treatment

Different doses of quinine, viz. 200, 300, and 400 mg/kg, were administered to three different groups of mice, and their MST values were 11.0, 9.16, and 15.25 days, respectively. Cyproheptadine was tested at doses of 5 and 10 mg/kg × 4, producing MST values of 8.5 and 12.5 days. When quinine and cyproheptadine were given together, there was no enhancement of their individual antimalarial effects. There was an increase in survival time of only 1–2 days when the doses of quinine and cyproheptadine were 300 + 5 and 10 and 200 + 5 and 10 mg/kg; however, the 400 mg/kg dose of quinine in combination with cyproheptadine produced some toxic effects, and the MST was decreased in comparison to lower doses of quinine (Table 2).

3.3. Effect of mefloquine and/or cyproheptadine treatment

Treatment of animals with 2, 4, and 8 mg/kg doses of mefloquine produced only a marginal (non-significant) increase in MST, and the level of parasitaemia was also high. When both drugs (mefloquine and cyproheptadine) were used in combination, the combined drug response was not potentiated; only a marginal difference was seen in the last group (cyp 15 + mq 8 mg/kg) (Table 3).

3.4. Effect of cyproheptadine in vitro on haemozoin content and haem polymerization activity

The erythrocytes isolated from *P. yoelii*-infected mice (parasitaemia 40–50%) were washed twice with PBS and suspended in PSG. Intact erythrocytes suspended in PSG were incubated *in vitro* at 37° with different drugs, and the intraerythrocytic level of haemozoin was evaluated (Fig. 1). The infected erythrocytes incubated without any drug showed an increase from 2.33 to 2.82 nmol/mL (17%) in the level of haemozoin over the 4-hr incubation period (Fig. 1). The *P. yoelii*-infected erythrocytes incubated with chloroquine (100 μM) alone did not register a significant

Table 2
Effect of *in vivo* quinine and/or cyproheptadine treatment on *P. yoelii nigeriensis* (1×10^7) infected mice

Serial no.	Drug(s)	Dose (mg/kg)	MST (days)	% Parasitaemia							
				4	7	9	10	11	12	15	21
1	Control	–	6.28 ± 1.30	45.0 (3)	–	–	–	–	–	–	–
2	Quinine	200	11.0 ± 3.80	Nil (5)[a]	5.75 ± 7.05 (4)	23.66 ± 33.38 (3)	1.0, 21.0 (2)	3.0, 76.0 (2)	4.5 (1)	ND (1)	–
3	Quinine	300	9.16 ± 4.70	Nil (4)[a]	0.55 ± 0.96 (4)	0.5, 4.0 (2)	1.5, 8.0 (2)	2.5, 13.2 (2)	18.0, 34.0 (2)	–	–
4	Quinine	400	15.25 ± 11.15	Nil (3)[b]	0.02, 0.05 (2)	2.5, 7.0 (2)	13.0, 24.0 (2)	23.5, 26.0 (2)	32.0 (1)	15.0 (1)	Nil (1)
5	Cyproheptadine	5	8.50 ± 3.57	53.4 ± 10.7 (5)	35.0 (1)	25.0 (1)	30.0 (1)	35.0 (1)	38.0 (1)	–	–
6	Cyproheptadine	10	12.5 ± 7.60	0.38 ± 0.56 (6)	22.4 ± 6.80 (6)	36.5 ± 36.45 (3)	3.5 (1)	4.2 (1)	14.0 (1)	12.0 (1)	ND (1)
7	Cyproheptadine + quinine	5 + 200	12.0 ± 3.89	0.30 ± 0.60 (6)	8.25 ± 11.12 (6)	14.65 ± 18.84 (4)	0.0, 0.1 (2)	ND (2)	ND (2)	ND (2)	–
8	Cyproheptadine + quinine	5 + 300	16.0 ± 7.17	Nil (4)[a]	2.45 ± 1.56 (4)	14.8 ± 16.19 (4)	23.2 ± 36.2 (3)	0.0, 21.0 (2)	0.0, 91.0 (2)	Nil (1)	Nil (1)
9	Cyproheptadine + quinine	5 + 400	12.3 ± 2.38	Nil (4)[b]	0.45 ± 0.34 (4)	9.0 ± 2.64 (4)	45.0 ± 15.7 (3)	35.0, 78.0 (2)	45.0 (1)	ND (1)	–
10	Cyproheptadine + quinine	10 + 200	11.14 ± 2.91	Nil (6)	1.72 ± 2.26 (5)	12.45 ± 16.88 (5)	32.5 ± 45.6 (3)	19.0, 35.0 (2)	72.0 (1)	ND (1)	–
11	Cyproheptadine + quinine	10 + 300	14.0 ± 9.87	Nil (5)[a]	0.03 ± 0.05 (4)	0.03 ± 0.05 (3)	0.5 ± 0.86 (3)	1.83 ± 3.17 (3)	7.53 ± 12.5 (3)	15.0 ± 25.98 (3)	Nil (1)
12	Cyproheptadine + quinine	10 + 400	9.8 ± 3.20	Nil (4)[b]	Nil (2)	0.0, 0.1 (2)	0.2, 1.5 (2)	4.2, 28.0 (2)	61.5 (1)	–	–

Values are means ± SD where N ≥ 3. Where N = 2, individual values are given.
[a] Non-specific deaths.
[b] Early deaths due to toxicity of the drug.

Table 3
Effect of *in vivo* mefloquine and/or cyproheptadine treatment on *P. yoelii nigeriensis* infected mice

Serial no.	Drug(s)	Dose (mg/kg)	MST (days)	% Parasitaemia						Cure rate (%)
				4	7	10	14	21	28	
1	Control	–	7.14 ± 1.07	5.27 ± 5.00 (7)	10.0, 60.0 (2)	–	–	–	–	Nil
2	Mefloquine	2	8.00 ± 1.15	0.72 ± 0.59 (7)	30.0 ± 14.14 (4)	–	–	–	–	Nil
3	Mefloquine	4	11.42 ± 7.39	0.15 ± 0.33 (7)	37.37 ± 29.60 (7)	Nil (1)	Nil (1)	Nil (1)	Nil (1)	14.30
4	Mefloquine	8	12.29 ± 6.97	Nil (7)	6.14 ± 7.18 (7)	Nil (1)	Nil (1)	Nil (1)	Nil (1)	14.30
5	Cyproheptadine	10	12.86 ± 8.01	0.07 ± 0.19 (7)	18.0 ± 22.18 (6)	0.0, 11.0 (2)	0.0 18.0 (2)	Nil (1)	Nil (1)	14.30
6	Cyproheptadine	15	11.14 ± 1.77	Nil (7)	3.84 ± 6.13 (7)	39.0, 45.0 (2)	–	–	–	Nil
7	Cyproheptadine + mefloquine	10 + 2	8.29 ± 1.50	0.35 ± 0.50 (7)	28.93 ± 19.82 (4)	–	–	–	–	Nil
8	Cyproheptadine + mefloquine	10 + 4	10.71 ± 5.47	Nil (6)	6.62 ± 8.76 (6)	6.0 (1)	10.0 (1)	26.0 (1)	–	Nil
9	Cyproheptadine + mefloquine	10 + 8	13.00 ± 6.66	Nil (7)	1.50 ± 3.97 (7)	0.0, 30.0 (2)	Nil (1)	Nil (1)	Nil (1)	14.30
10	Cyproheptadine + melfoquine	15 + 2	10.57 ± 0.79	Nil (7)	0.91 ± 1.25 (7)	–	–	–	–	Nil
11	Cyproheptadine + melfoquine	15 + 4	10.43 ± 0.79	Nil (7)	1.26 ± 2.21 (7)	–	–	–	–	Nil
12	Cyproheptadine + melfoquine	15 + 8	17.33 ± 8.29	Nil (6)	Nil (5)	16.20 ± 20.27 (5)	Nil (2)	Nil (2)	Nil (2)	33.33

Values are means ± SD of the number of observations from the surviving animals (indicated in parentheses). Where N = 2, individual values are given.

change in haemozoin content over control for up to 3 hr. The cells incubated with cyproheptadine (100 μM) alone or in combination with chloroquine (100 μM) caused a significant decrease in the intraerythrocytic haemozoin content of *P. yoelii*-infected cells at 4 hr. The decrease in haemozoin in the cyproheptadine and chloroquine combination was much more prominent and was evident even 1 hr post-incubation. The effects of the drugs were also evaluated on the haem polymerization activity of a cell-free extract of *P. yoelii in vitro* (Fig. 2). No polymerization of haem was detected at 37° for 4 hr in acetate buffer. The addition of cyproheptadine to the lysate caused a marked inhibition of haem polymerization activity. Decreased haem polymerization activity was concentration-dependent, i.e. a 1000, 100, and 50 μM concentration of cyproheptadine caused 56, 15.53, and 4.26% inhibition, respectively (Fig. 2). For haem polymerization, the IC_{50} values of verapamil, desipramine, chloroquine, and cyproheptadine were 10,000, 2800, 35, and 1000 μM, respectively.

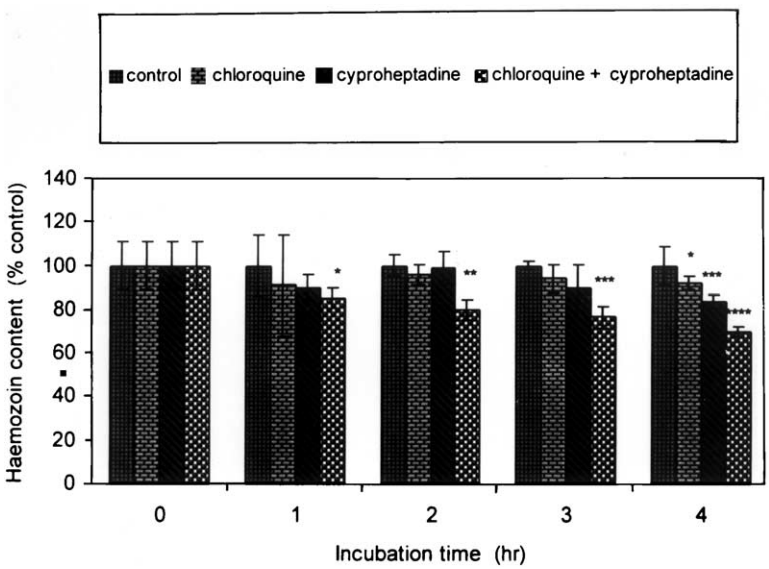

Fig. 1. Effect of cyproheptadine and/or chloroquine *in vitro* on the haemozoin content of intact mouse erythrocytes infected with a multidrug-resistant strain of *P. yoelii*. Each bar shows the mean ± SD of 3 observations from the same experiment. The concentration of each drug was 100 μM. Control values of haemozoin (nmol haem/hr): 0 hr, 2.33 ± 0.11; 1 hr, 2.51 ± 0.14; 2 hr, 2.56 ± 0.05; 3 hr, 2.17 ± 0.02; and 4 hr, 2.82 ± 0.09. Key: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.005$; and (****) $P < 0.001$ vs control.

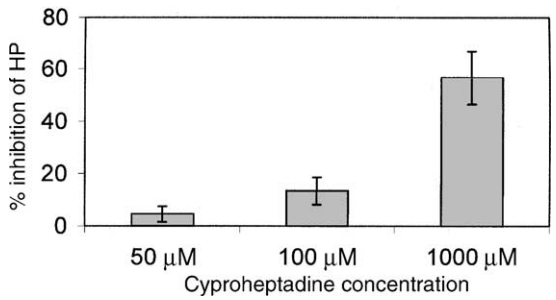

Fig. 2. Concentration-dependent effect of cyproheptadine *in vitro* on haem polymerization (HP) inhibition by *P. yoelii* extracts. Each bar represents the mean ± SD of at least 3 observations from the same experiment. The control value of haem polymerization activity was 26.49 ± 0.58 nmol haem/hr/mg protein (considered as 100% to calculate experimental values).

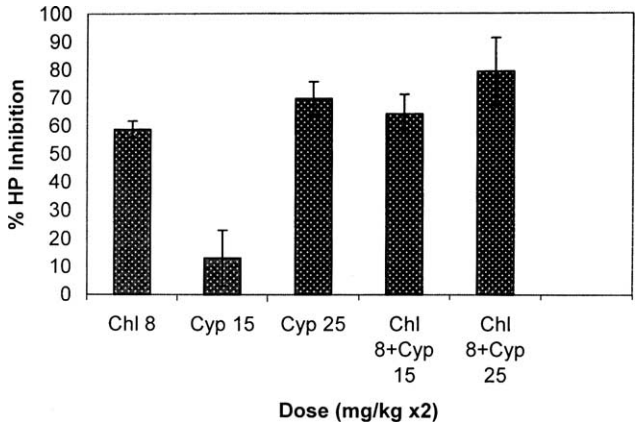

Fig. 3. Effect of chloroquine (chl), cyproheptadine (cyp), and the chl–cyp combination on the haem polymerization (HP) activity of *P. yoelii nigeriensis*-infected erythrocytes, *in vivo*. Each bar represents the mean ± SD of at least four observations from six separate animals. The control value of haem polymerization activity was 0.92 ± 0.07 nmol haem/hr/mg protein.

3.5. *In vivo effect of chloroquine and cyproheptadine treatment on haemozoin content and haem polymerase activity*

For testing the effects of drugs on haem polymerization *in vivo*, *P. yoeli*-infected mice with parasitaemia levels of 3–15% were treated with two doses of the drugs alone or in combination. These treatments did not have noticeable effects on the parasitaemia levels in mice treated with chloroquine alone; rather the parasitaemia level had increased marginally (12–15%) when the animals were killed to harvest the parasite (data not shown). However, when treated with cyproheptadine alone or in combination with chloroquine, a marginal (5–10%) reduction in the level of parasitaemia occurred (data not shown). After 24 hr of treatment the infected erythrocytes were isolated from control and treated animals, and then haem polymerization activity was determined (Fig. 3). A decrease in haem polymerization activity to the extent of 58.70% was found in cells treated with chloroquine (8 mg/kg), while the inhibition by cyproheptadine (25 mg/kg) was 69.57%; the chloroquine plus cyproheptadine combination (8 + 25 mg/kg) produced the maximum inhibition of enzyme activity (79%), which seemed to be additive in nature (Fig. 3). Similar drug effects were found on the haemozoin content (Fig. 4). The combination of the highest dose of cyproheptadine with chloroquine showed a 60% decrease in the haemozoin content.

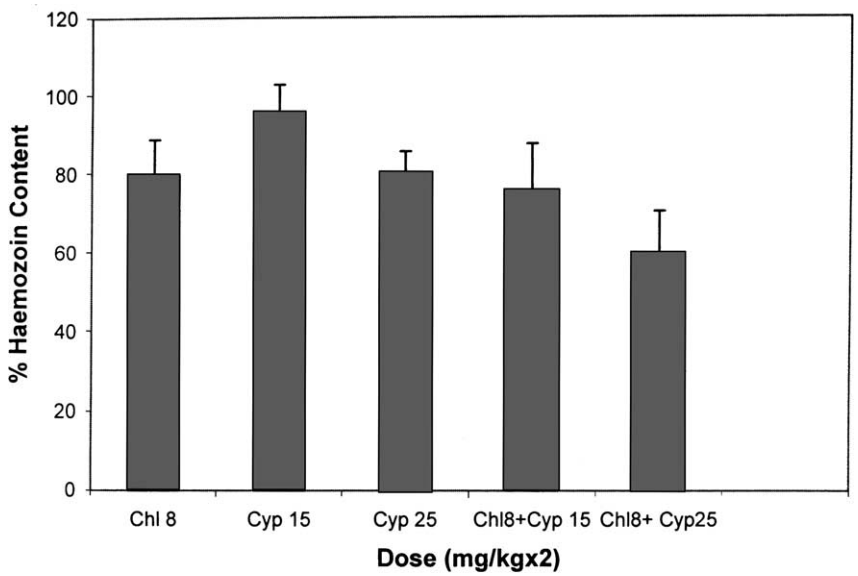

Fig. 4. Effect of chloroquine (chl), cyproheptadine (cyp), and the chl–cyp combination on the haemozoin content of *P. Yoelii nigeriensis*-infected erythrocytes, *in vivo*. Each bar represents the mean ± SD of at least four observations from six separate animals. The control value of haemozoin content was 0.25 ± 0.05 nmol haem/hr.

4. Discussion

The original observation demonstrating antimalarial activity of antihistaminic drugs was made by Pan *et al.* [13]. Subsequently, inhibitory effects of the antihistaminics on the growth of *P. falciparum in vitro* also were reported [5]. Later the reversal of CQ resistance by some antihistaminic compounds was confirmed [14]. The results reported in this paper provide additional *in vivo* information on the antimalarial action of cyproheptadine on a multidrug-resistant strain of *P. yoelii nigeriensis*. The action of cyproheptadine appears to be direct. The molecular mechanism of the schizontocidal action of cyproheptadine in the blood has not been reported in the literature. Verapamil and desipramine reverse chloroquine resistance by blocking the efflux of the drug, resulting in an increase in the steady-state concentration of chloroquine within the parasite food vacuole, the site of antimalarial action. These compounds, however, did not show noticeable antimalarial action *in vivo* (Agrawal *et al.*, unpublished observation).

During intraerythrocytic development and proliferation, malaria parasites depend largely on host cell haemoglobin for fulfilling nutritional requirements [15]. Digestion of haemoglobin by specific proteases results in the continuous generation of free haem as a toxic by-product [16]. Malaria parasites detoxify haem through a specific metabolic reaction of haem polymerization that results in the formation of haemozoin. The mechanism of formation of haemozoin by the parasite is still under debate [17,18]. Initially the process was shown to be an enzymatic reaction involving haem polymerase [19]. Some workers have shown that the preformed haemozoin or β-haematin (the synthetic polymer of haem) can also promote subsequent haem polymerization without the presence of any parasite material [20]. Later, the malarial histidine-rich proteins II and III [21] were demonstrated to nucleate haem binding and initiate haemozoin formation. However, it was confirmed recently that haem polymerization is not a spontaneous reaction but requires the presence of the parasite extract [22]. The haem polymerization function of malaria parasites has been demonstrated unequivocally as a potential target for schizontocidal action of several classes of antimalarials in the blood [15]. Our data suggest that the schizontocidal action of cyproheptadine in the blood also seems to involve a similar mode of action of inhibition of haem polymerization function. Incubation of intact *P. yoelii* (MDR)-infected erythrocytes in the presence of chloroquine alone did not show a marked effect on intracellular haemozoin content, even though chloroquine produced potent inhibition of haem polymerization in a cell-free system. Less accumulation of the drug in intact erythrocytes may be the cause of refractoriness of the parasite to chloroquine action, as reported earlier in the case of *in vitro P. falciparum* cultures [23,24]. Cyproheptadine also caused inhibition of haem polymerization activity in the cell-free parasite extract and a significant decrease in haemozoin content in intact erythrocytes. Interference of cyproheptadine with the haem detoxification function of the malaria parasite was also confirmed *in vivo*. The IC_{50} values for verapamil/desipramine have been found to be very high, showing that these two agents do not cause inhibition of haem polymerization. The inhibition of haem detoxification seems to be associated specifically with blood schizontocidal activity and is also one of the biochemical modes of action of cyproheptadine. Although the therapeutic range of cyproheptadine as an antihistaminic is 4–16 mg daily/human body weight, the results of the present study in which a higher dose was used will certainly provide a new lead that will be useful in the development of more potent antimalarials for the treatment of drug-resistant cases of the disease.

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