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Effects of Amodiaquine, Chloroquine, and Mefloquine on Human Polymorphonuclear Neutrophil Function In Vitro

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This study concerns the in vitro interaction with human polymorphonuclear neutrophils (PMNs) of amodiaquine, chloroquine, and mefloquine, three antimalarial drugs currently in use for the treatment and prophylaxis of malaria. It was found that mefloquine (100 and 50 μg/ml) significantly altered PMN viability while the other two drugs did not. Neutrophil chemotaxis was impaired by chloroquine (100 μg/ml) and mefloquine (>10 μg/ml) but not by amodiaquine. Phagocytosis was decreased by about 50% in the presence of chloroquine (100 μg/ml) or mefloquine (10 μg/ml). The three antimalarial drugs altered neutrophil oxidative metabolism as assessed by luminol-amplified chemiluminescence. The strongest effect was observed with mefloquine, which abolished almost completely the neutrophil burst at concentrations of >10 μg/ml whatever the stimulus used. This effect was not reversed by washing. Chloroquine and amodiaquine also impaired this PMN response by approximately 80 and 50%, respectively, but only at the highest concentration used (100 μg/ml). In the case of amodiaquine, the neutrophil response was restored by washing, except for stimulation with opsonized particles. After washing, the depressive effect of chloroquine was reversed completely in the case of phorbol myristate acetate stimulation and partly in the case of opsonized particle stimulation, but the formylmethionyl-leucyl-phenylalanine-induced response was not restored. These data show that although they are structurally related, amodiaquine and chloroquine exhibit qualitatively and quantitatively different depressive effects on PMN function and probably interfere at different points of cell activation, although the precise mechanisms are as yet unresolved.

Polymorphonuclear neutrophils (PMNs) play a crucial role in a variety of infections caused by bacteria, fungi, and parasites. Indeed, the involvement of PMNs in host defense against *Plasmodium falciparum* is becoming well documented both in vitro (4, 5, 21, 40) and in vivo (1, 39, 48). However, alterations of the host defense system (12, 28, 44) and in particular the depression of PMN function (33) have been reported in acute human malaria. As a consequence, a decreased ability to deal with a secondary invading pathogen has been observed in malaria-suppressed mice (22, 42) and in malaria-infected humans (12, 20). Taken as a whole, these data emphasize the need for PMN functions to remain intact for the successful resolution of malaria and to protect against intercurrent bacterial infections. However, available evidence indicates that many of the antimalarial drugs currently used in the prevention or treatment of human malaria significantly alter the immune response of the host in vitro and in vivo (2, 9, 10, 15, 16, 25, 34, 45). Furthermore, some of these drugs, in particular amodiaquine, have been reported to induce neutropenia, which in some cases has proved to be fatal (3, 26, 37). Since the in vitro interaction of amodiaquine, a 4-aminoquinoline, with human PMNs has not yet been investigated, the aim of this work was to analyze the effect of this molecule on human PMN function in comparison with those of chloroquine, another 4-aminoquinoline, and mefloquine, a new 4-quinoline methanol, which have been already studied in this context (10, 15, 19).

MATERIALS AND METHODS

Isolation of human PMNs. Human PMNs were isolated from heparinized venous blood of healthy volunteers by sedimentation on 2% dextran followed by hypotonic lysis of residual erythrocytes for oxidative metabolism studies and by sedimentation on dextran-Radioselectan (35) for chemotaxis assay.

Antimalarial drugs. The drugs used and their sources were as follows: mefloquine, Laboratoire Produits Roche, Neuilly, France; amodiaquine, Roussel Uclaf, Paris, France; and chloroquine diphosphate, Sigma Chemicals, St. Louis, Mo. The drugs were made up to a stock solution of 1 g/liter in sterile distilled water and further diluted in buffer (47; Krebs Henseleit; pH 7.4) to give final concentrations of 100 to 0.1 μg/ml.

Neutrophil functions. (i) Viability. PMN viability was assessed by the trypan blue exclusion method after incubation of the cells (10^6/ml) in the presence of buffer or antimalarial drugs for 30 min at 37°C.

(ii) Chemotaxis. Chemotaxis was assayed by the under-agarose method of Nelson et al. (32). Formylmethionyl-leucyl-phenylalanine (FMLP; 10^{-7} M) and normal human serum were used as chemoattractants. Amodiaquine, chloroquine, and mefloquine, diluted as indicated above, were used as follows: (i) after incubation with PMNs for 30 min at 37°C and (ii) directly incorporated in agarose as previously described (23, 35). Control PMNs were run in parallel; random migration and chemotaxis were compared for antimalarial-treated and untreated PMNs.

(iii) Phagocytosis. Ingestion of heat-killed and radiolabeled *Klebsiella pneumoniae* cells was tested as previously described (47). The technique measures PMN-associated bacteria, i.e., ingested plus membrane-adherent bacteria.

(iv) Oxidative metabolism. Oxidative metabolism was measured by luminol-enhanced chemiluminescence (LACL) as previously described (24). LACL was recorded in a Packard Picolite luminometer. A 100-μl sample of the PMN preparation (10^6/ml) was incubated in the apparatus at 37°C in the

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TABLE 1. PMN viability after incubation in the presence of antimalarial drugs

Antimalarial drug and concn (μg/ml)	% of dead cells (mean ± SEM)
Control (buffer)	7.8 ± 1.33 (11)[a]
Chloroquine	
100	8.8 ± 3.36 (5)
10	11.2 ± 2.35 (4)
Amodiaquine	
100	10.7 ± 1.57 (5)
10	7.0 ± 4.59 (3)
Mefloquine	
100	84.5 ± 11.85[b] (4)
50	41.6 ± 11.28[b] (4)
25	13.8 ± 6.31 (3)
10	10.8 ± 5.48 (3)

[a] The number of experiments is shown in parentheses.
[b] $P < 0.05$ (Student's t test for unpaired data).

dark with stirring in the presence of buffer or of the antimalarial drugs (100 to 0.1 μg/ml) for 30 or 5 min. Then 50 μl of luminol (4×10^{-4} M) and 50 μl of stimulating agent (opsonized zymosan [0.67 g/liter], phorbol myristate acetate [PMA; 1 μg/ml], FMLP [5×10^{-7} M], or opsonized *Pseudomonas aeruginosa* [10^8 CFU/ml]) were added, and the light emitted was recorded for 30 to 40 min until a peak value and a decreasing slope were obtained.

LACL cell-free system. The LACL cell-free system was adapted from the work of Riley and Robertson (38) designed to study oxidative killing mechanisms in vitro. The reaction mixtures were obtained by mixing 90 μl of a crude granule extract of human PMNs prepared by the method of Watanabe et al. (51) in citrate buffer (pH 5.5) with 10 μl of buffer or 10 μl of the antimalarial drug (1 g/liter), plus 50 μl of luminol and 10 μl of NaI (0.2 mM); the samples were placed in the luminometer at 37°C and stirred. Light emission was measured after the addition of 40 μl of H_2O_2 (17.5 μM). This cell-free system measures the activity of myeloperoxidase (MPO) contained in the crude granule extract of PMNs.

RESULTS

(i) PMN viability. After incubation for 30 min in the presence of chloroquine or amodiaquine (up to 100 μg/ml), the viability of PMNs was not impaired compared with that of control PMNs incubated with buffer. On the contrary, mefloquine altered significantly the viability of the cells at concentrations of 100 and 50 μg/ml (Table 1), with approximately 84.5 and 41.6% dead cells, respectively, compared with 7.8% for control PMNs ($P < 0.05$; Student's t test for unpaired data). Consequently, in the PMN function assays, mefloquine was used at concentrations of <50 μg/ml.

(ii) Chemotaxis. Up to 100 μg/ml, amodiaquine altered neither PMN random migration nor chemotaxis regardless of the chemoattractant and technique used (PMNs incubated with the drug or migration under amodiaquine-supplemented agarose) (Table 2). Chloroquine, however, which did not alter PMN migration following incubation with the drug, impaired migration under chloroquine-supplemented agarose. This effect was observed only with the highest concentration (100 μg/ml) and was not specific: random migration was reduced by about 50% and chemotaxis was reduced by 30% (FMLP-oriented chemotaxis) and 40% (activated serum-oriented chemotaxis). Mefloquine displayed the most depressive effect on chemotaxis, since not only the concentration which altered PMN viability (50 μg/ml) but also concentrations as low as 10 μg/ml impaired the migration of PMNs. As with chloroquine, migration under mefloquine-supplemented agarose was more impaired than in the preincubation experiment. In three experiments, PMNs were incubated in the presence of mefloquine (50 μg/ml) for 30 min and then washed before migration was assessed under untreated agarose. A complete reversion of inhibition was observed with percentages (means ± standard error of the mean) of migration of 83 ± 16.9, 92 ± 8.5, and 79 ± 4.6, respectively, for random migration, FMLP-induced chemotaxis, and activated serum-induced chemotaxis.

(iii) Phagocytosis. The phagocytosis of heat-killed radiolabeled *K. pneumoniae* cells was assessed in six different experiments. Concentrations of amodiaquine up to 100 μg/ml did not alter this PMN function, while chloroquine (100 μg/ml only) and mefloquine (10 μg/ml) impaired the phago-

TABLE 2. Effects of antimalarial drugs on PMN migration

Drug and concn (μg/ml)	% of control migration (mean ± SEM)					
	Random		Chemotaxis			
			FMLP		Activated serum	
	A[a]	B[b]	A	B	A	B
Amodiaquine						
100	96 ± 4.2 (6)[c]	106 ± 6.3 (8)	87 ± 9.1 (3)	89 ± 3.9 (4)	107 ± 3.8 (3)	97 ± 3.3 (4)
10	100 ± 0.0 (6)	110 ± 10.0 (5)	91 ± 5.9 (3)	100 ± 0.0 (3)	107 ± 9.6 (3)	87 (2)
Chloroquine						
100	92 ± 4.1 (6)	47[d] ± 4.1 (9)	104 ± 7.4 (3)	68[d] ± 7.8 (5)	100 ± 5.4 (3)	59[d] ± 8.5 (4)
10	100 ± 0.0 (6)	90 ± 10.0 (5)	104 ± 7.2 (3)	80 ± 10.4 (3)	106 ± 3.3 (3)	85 ± 7.5 (3)
Mefloquine						
50	47[d] ± 15.3 (8)	0[d] (6)	41[d] ± 12.8 (5)	0[d] (3)	56 ± 28.3 (3)	0[d] (3)
25	93 ± 7.1 (7)	32[d] ± 5.6 (8)	73 ± 8.4 (4)	53[d] ± 7.5 (4)	88 ± 3.8 (3)	50[d] ± 9.4 (4)
10	96 ± 3.8 (13)	45[d] ± 5.8 (10)	101 ± 3.6 (3)	72[d] ± 5.4 (5)	101 ± 2.8 (6)	65[d] ± 9.1 (5)
1	92 ± 8.3 (6)	91 ± 8.2 (6)	101 ± 4.7 (3)	96 ± 3.8 (3)	104 ± 3.8 (3)	77 ± 9.0 (3)

[a] A, PMNs were incubated for 30 min with the drug before assay.
[b] B, Antimalarial drug was directly mixed with the agarose.
[c] The number of experiments is shown in parentheses.
[d] $P < 0.01$ (Student's t test for paired data).

TABLE 3. Effects of antimalarial drugs on LACL of PMN (30 min of incubation in the presence of the drugs or buffer)

Drug and concn (μg/ml)	% of control PMN response (mean ± SEM)			
	Stimulating agent		FMLP	PMA
	Opsonized zymosan	Opsonized *P. aeruginosa*		
Amodiaquine				
100	52 ± 10.5[a] (7)[b]	46 ± 10.4[a] (6)	44 ± 11.8[a] (11)	41 ± 13.3[a] (7)
10	71 ± 14.3 (10)	199 ± 72.8 (6)	52 ± 5.8[a] (9)	93 ± 22.4 (8)
1	90 ± 13.2 (5)	103 ± 9.5 (5)	101 ± 6.1 (3)	61 ± 14.9 (6)
0.1	69 ± 19.1 (3)	112 ± 32.5 (3)	90 ± 15.8 (3)	95 ± 17.5 (4)
Chloroquine				
100	3 ± 1.3[a] (8)	13 ± 4.4[a] (8)	15 ± 6.2[a] (11)	17 ± 4.0[a] (7)
10	69 ± 10.3 (9)	109 ± 27.4 (9)	82 ± 16.8 (9)	82 ± 11.6 (8)
1	77 ± 14.7 (5)	108 ± 6.0 (4)	91 ± 1.6 (3)	84 ± 18.4 (6)
0.1	85 ± 20.2 (3)	144 ± 33.0 (3)	112 ± 16.4 (3)	77 ± 8.2 (4)
Mefloquine				
50, 25	<0.1[a] (5)	<0.1[a] (5)	<0.1[a] (5)	<0.1[a] (5)
10	0.8 ± 0.3[a] (7)	2.0 ± 1.6[a] (4)	5 ± 1.3[a] (10)	5.0 ± 1.4[a] (9)
1	80 ± 3.4[a] (5)	111 ± 31.9 (3)	117 ± 11.6 (3)	78 ± 7.2[a] (8)
0.1	71 ± 14.8 (3)	111 ± 30.0 (3)	110 ± 0.0 (3)	103 ± 3.5 (3)

[a] $P < 0.05$ (Student's t test for paired data).
[b] The number of experiments is shown in parentheses.

cytic ability of PMNs. The mean percentages of control phagocytosis (± standard error of the mean) were 87 (±7.0) for amodiaquine (100 μg/ml), 56 (±9.3) for chloroquine (100 μg/ml), and 47 (±8.1) for mefloquine (10 μg/ml).

(iv) LACL. The results of the LACL assay after 30 min of incubation of PMNs in the presence of the drugs are given in Table 3. Amodiaquine and chloroquine impaired the PMN response only at concentrations of 100 μg/ml. The depressive effect was more marked for chloroquine (87 to 97% inhibition) than for amodiaquine (48 to 59% inhibition). Amodiaquine at a concentration of 10 μg/ml also depressed the PMN response by 50% but only in the case of FMLP stimulation. Mefloquine, as was the case for the other PMN functions assayed, severely depressed the PMN response: the PMN LACL was completely abolished at concentrations equal or superior to 25 μg/ml, and the inhibition was approximately 95 to 99% after incubation with 10 μg of mefloquine per ml. At a concentration of 1 μg/ml, mefloquine slightly but significantly reduced the response of PMNs to opsonized zymosan and PMA. The influence of incubation time was tested for amodiaquine and chloroquine (100 and 10 μg/ml) and mefloquine (0.1 to 50 μg/ml) (Table 4). With a short (5-min) incubation time, amodiaquine (100 μg/ml) reduced the response of PMA-stimulated PMNs to the same extent as after 30 min of incubation. Chloroquine (100 μg/ml) also altered the PMN response but to a lesser extent than with a 30-min incubation period. The effect of mefloquine (≥25 μg/ml) did not vary with the time of incubation, with the exception of opsonized zymosan stimulation. A 5-min incubation in the presence of 10 μg of mefloquine per ml had less

TABLE 4. Effect of antimalarial drugs on LACL of PMN (5 min of incubation in the presence of the drugs or buffer)

Drug and concn (μg/ml)	% of control PMN response (mean ± SEM)			
	Stimulating agent		FMLP	PMA
	Opsonized zymosan	Opsonized *P. aeruginosa*		
Amodiaquine				
100	100 ± 12.7 (3)[a]	106 ± 11.5 (4)	92 ± 28.9 (3)	47 ± 6.6[b] (3)
10	78 ± 22.4 (3)	103 ± 32.2 (6)	105 ± 15.4 (3)	96 ± 5.8 (3)
Chloroquine				
100	47 ± 2.7[b] (3)	14 ± 7.3[b] (4)	28 ± 4.0[b] (3)	73 ± 22.8
10	119 ± 26.1 (3)	95 ± 17.7 (6)	124 ± 20.7 (3)	105 ± 3.4 (3)
Mefloquine				
50	<0.1[b] (4)	<0.1[b] (4)	<0.1[b] (4)	<0.1[b] (4)
25	14 ± 1.5[b] (3)	ND[c]	<1[b] (3)	<1[b] (3)
10	24 ± 6.6[b] (3)	8 ± 4.5[b] (4)	3 ± 1.5[b] (3)	18 ± 4.5[b] (3)
1	79 ± 19.1 (3)	60 ± 17.6 (3)	90 ± 3.6 (3)	109 ± 5.4 (3)
0.1	88 ± 11.9 (3)	104 ± 2.1 (3)	111 ± 8.1 (2)	72 ± 1.0 (2)

[a] The number of experiments is shown in parentheses.
[b] $P < 0.05$ (Student's t test for paired data).
[c] ND, Not determined.

TABLE 5. Effect of washing on LACL inhibition induced by antimalarial drugs

Drug and concn (μg/ml)	% of control PMN response (mean ± SEM)							
	Stimulus				FMLP		PMA	
	Opsonized zymosan		Opsonized *P. aeruginosa*					
	A[a]	B[b]	A	B	A	B	A	B
Amodiaquine (100)	61 ± 11.1 (3)[c]	49 ± 21.9 (3)	57 ± 7.2 (3)	52 ± 10.2 (3)	20 ± 4.4 (6)	72[d] ± 16.4 (6)	19 ± 4.2 (3)	217[d] ± 52.5 (3)
Chloroquine (100)	2 ± 0.8 (4)	26[d] ± 11.9 (4)	11 ± 7.3 (3)	30[d] ± 6.7 (3)	25 ± 8.8 (7)	39 ± 14.1 (7)	14 ± 6.2 (3)	151[d] ± 59.2 (3)
Mefloquine (10)	0.13 ± 0.06 (4)	0.5 ± 0.3 (4)	ND[e]	ND	3 ± 1.0 (6)	6 ± 1.2 (6)	6 ± 2.9 (4)	10 ± 5.5 (4)

[a] A, PMNs were incubated for 30 min with the drug before the LACL assay.
[b] B, PMNs were incubated for 30 min with the drug and washed before the LACL assay.
[c] The number of experiments is shown in parentheses.
[d] $P < 0.05$ (Student's t test for unpaired data).
[e] ND, Not determined.

effect than 30 min of incubation but still strongly inhibited the PMN response (76 to 97% inhibition).

In another set of experiments, PMNs were incubated with the drugs or buffer for 30 min (amodiaquine and chloroquine, 100 μg/ml; mefloquine, 10 μg/ml) and then extensively washed prior to the LACL assay (Table 5). The inhibition of the PMN response by amodiaquine was reversed in the case of FMLP or PMA stimulation but not in the case of opsonized zymosan or *P. aeruginosa* stimulation. With regard to chloroquine, only the PMA-stimulated response of PMNs was restored, while zymosan-stimulated LACL was significantly increased after washing but still very low compared with that of control PMNs; the FMLP-stimulated response was not increased after washing. In the case of mefloquine, no reversion of inhibition was obtained by washing, regardless of the stimulus used.

LACL cell-free system. The altered LACL response of PMNs in the presence of antimalarial drugs could be the result of either an interaction between the drug and the cells or interference with other components of the light-generating system. To determine whether the observed alterations were due to cellular interaction, the effects of each drug on the chemiluminescence generated in a cell-free system consisting of human MPO (contained in a crude granule extract), H_2O_2, and NaI were studied. Three different crude granule extract preparations were used (protein concentrations ranging from 0.2 to 2.6 mg/ml). The peak value for the control MPO, H_2O_2, and NaI mixture was obtained in the first 60 s and varied from 10^7 to 7.7×10^7 counts per 30 s per mg of protein, depending on the crude granule extract preparation. This was followed by a progressively decreasing slope. None of the drugs affected the kinetics or the mean peak value (data not shown).

DISCUSSION

Although a number of antimalarial drugs were previously examined for their effects on immune response (2, 9, 16, 45, 46), their interaction with the host phagocytic system has only been marginally studied (10, 15, 19). Unquestionably, such information is of major importance since not only do phagocytes appear to be key cells in the resolution of malarial infection but they also protect malaria-suppressed patients from a secondary bacterial or parasitic infection.

The data reported here confirm and extend the work of others concerning the inhibition of phagocyte function by chloroquine and mefloquine. Furthermore, the interaction of amodiaquine with human PMNs in vitro was analyzed. We chose to analyze three PMN functions which are crucial in the defense against pathogens, i.e., chemotaxis, phagocytosis, and respiratory burst. This last function was measured by the LACL assay, which is a global technique and reflects the complex cascade of events from the activation of the PMN membrane to the release of active oxygen species. Several researchers (7, 8) have shown that LACL depends mainly on MPO activity either in phagosomes or released in the assay medium; the LACL assay should thus measure, partly at least, degranulation of PMNs either induced by phagocytosis (particulate stimulus) or any soluble stimuli which do not lead to phagocytosis. The cell-free system MPO-H_2O_2-NaI serves as a control not only of radical scavenging by the drugs but also of their direct interference with MPO.

Amodiaquine at concentrations up to 100 μg/ml did not impair PMN migration either after preincubation of PMNs in the presence of the drug or under amodiaquine-supplemented agarose. The phagocytosis of radiolabeled *K. pneumoniae* cells was also unaltered by amodiaquine up to 100 μg/ml. On the contrary, the PMN oxidative burst was depressed by about 50% when the cells were preincubated for 30 min with amodiaquine (100 μg/ml), whatever the stimulus. This effect was time dependent except in the case of PMA stimulation: a similar inhibition was observed after 5 and 30 min of incubation (PMN response, 47 versus 41%, respectively; Tables 3 and 4). This depressive effect of amodiaquine was reversed by washing, except in the case of stimulation by opsonized particles. The generation of oxidative species by the cell-free system (MPO-H_2O_2-iodide) was not depressed by amodiaquine. Amodiaquine interfered with PMN oxidative metabolism in a specific manner. The effect on the PMA-induced response was immediate but was reversed by washing. The effect on the FMLP-induced response was delayed but was reversed by washing. However, the effect of amodiaquine on the response induced by opsonized particles was also delayed but not reversed by washing. It is known that PMA interaction with PMNs is mediated by direct activation of protein kinase C (43), while FMLP or opsonized particles require fixation onto a specific receptor and transmembrane signaling for the induction of the PMN response (30, 31, 41). The data reported here suggest that amodiaquine could interfere at least at two different sites, one of which might be the protein kinase C system (with possible reversion of action) and the other of which might be membrane receptors with irreversible modification. The precise mechanisms of these phenomena are

under study. The observation that auto-oxidative forms derived from amodiaquine selectively react with thiol groups of proteins (29) is a possible explanation. In comparison to the mild depression observed with amodiaquine, mefloquine led to a severe impairment of PMN integrity and function. First, PMN viability was significantly decreased after incubation of the cells in the presence of mefloquine at concentrations of 50 and 100 μg/ml. Second, PMN migration was also decreased by concentrations of mefloquine as low as 10 μg/ml, but this effect could be reversed by washing. Phagocytosis was decreased by about 50% in the presence of mefloquine at a concentration of 10 μg/ml. This was also shown by Kharazmi and Eriksen (19), who found a dose-dependent effect from 50 to 0.5 μg/ml without alteration of total bactericidal activity. Finally, the most impressive effect was on PMN oxidative metabolism: at 10 μg/ml, mefloquine abolished almost completely the PMN response, whatever the stimulus added. An incubation time of 5 min also had a strong effect, and removal of the drug by washing did not reestablish the PMN oxidative response. Mefloquine did not interfere with the cell-free LACL system. A similar depressive effect of mefloquine on PMN function was also observed by Ferrante et al. (10). They reported that mefloquine may have a greater effect on the degranulation process than NADPH oxidase activity, since the iodination reaction was more impaired by mefloquine than was hexose monophosphate shunt activity. We also observed that compared with LACL, cyanide-insensitive oxygen uptake was less affected by mefloquine (data not shown). Thus, our results are likely to confirm the previous data (10), in particular that PMN degranulation may be particularly sensitive to mefloquine. The fact that mefloquine binds tightly to phospholipids (6), in particular membrane phospholipids, could explain the impairment of PMN function through a modification of membrane fluidity and receptor function with an alteration of cell integrity at very high concentrations.

In terms of the depressive effect on PMNs, chloroquine gave results intermediate between those of amodiaquine and mefloquine. This drug did not alter PMN viability and impaired PMN migration only at the highest concentration tested (100 μg/ml) and only in the presence of a uniform concentration (chloroquine-supplemented agarose). When PMNs were first incubated with chloroquine (100 μg/ml) and then were assayed for migration under control agarose, the drug could diffuse into the agarose, and the chloroquine concentration within and around the PMNs decreased, resulting in normal migration of PMNs. These data indicate a reversive effect of chloroquine on PMN migration. The inhibition of phagocytosis was also observed only with the highest concentration (100 μg/ml). The alteration of the PMN oxidative burst by chloroquine was very strong (83 to 97% inhibition), but only at 100 μg/ml, and was time dependent (Table 4). The removal of the drug by washing restored this PMN function only in the case of PMA stimulation (Table 5), and chloroquine did not modify the LACL generated in a cell-free system. The mechanisms of the interaction of chloroquine with phagocyte function have not yet been fully explained. One hypothesis for such interference has been based upon the effects of the drug on phospholipases (11, 27); another hypothesis is the alkalinization of the phagolysosomes as reported by Styrt and Klempner (B. Styrt and M. S. Klempner, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstract no. 60, 1983), since other lysosomotropic amines have been shown to reduce superoxide anion generation by PMNs (49). Finally, a depression of protein phosphorylation by high concentrations of chloroquine has been reported in yeast cells (17) and could explain the effect of this drug on a final common pathway for stimulus transmission of information in PMNs. All three mechanisms could be involved in PMN function blockade and explain the differences observed with regard to reversion by washing according to the stimulus used.

Since immunosuppression is the hallmark of many protozoal diseases, including malaria, chemotherapy with drugs which further compromise such an immunological defect is undesirable. However, it should be noted that amodiaquine, chloroquine, and mefloquine, while affecting the PMN response, do so at far higher concentrations than those attained in plasma during treatment. For mefloquine, there is considerable interindividual and racial variability in pharmacokinetic parameters (18): the highest peak concentrations in plasma are observed in Thai subjects (range, 638 to 2,494 ng/ml) after a single oral dose (250 mg), while in healthy adult Caucasians it varies from 240 to 431 ng/ml. For chloroquine, after an oral intake of 600 mg the mean peak concentration in plasma is 374 ± 56 ng/ml (49). Negroes and Caucasians do not differ markedly with respect to chloroquine kinetics (13). After oral administration (200 to 600 mg), amodiaquine achieves only low concentrations in plasma (51 to 136 ng/ml) (53), while after intravenous injection in human volunteers (3 mg/kg [body weight] over 10 min), there is considerable variation in plasma concentration profiles (peak ranging from 65 to 1,921 ng/ml) (52). However, the results presented here may be relevant in cases of drug overdose. Furthermore, pharmacokinetics studies of chloroquine outline the exceptionally large apparent volume of distribution of this drug due to its high concentrations in organs and tissues (50) and in particular leukocytes (36), exceeding the concentration observed in plasma. Ferrante et al. (10) have also suggested that, because of the high affinity of mefloquine for plasma membrane lipids, it is likely that leukocytes accumulate the drug. It is also possible that longer-term exposure of leukocytes to lower concentrations of the drug, such as occurs in treatment of malaria, may reduce PMN function. Extreme care should be taken in extrapolating these laboratory data to the clinical situation, since chemotherapy remains the principal means of treating malaria. Relevance to clinical situations needs further study, in particular concerning the functions of neutrophils from patients undergoing antimalarial therapy. This work is under way in our laboratory. Nevertheless, changes in immune status caused by antimalarial drugs must be borne in mind when parasitic diseases are treated (14). Furthermore, these drugs may prove to be useful tools in investigating the mechanisms of PMN function.

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