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CHLOROQUINE- AND PRIMAQUINE-INDUCED ALTERATIONS OF GLUCOSE METABOLISM IN THE UNINFECTED RED CELL

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Abstract—The effects of chloroquine and primaquine on glucose metabolism in uninfected red cells were studied. The flux of glucose through the hexose monophosphate shunt was decreased by chloroquine and increased by primaquine; the flux through glycolysis was not altered significantly by either drug. Since the hexose monophosphate shunt was the metabolic pathway affected by chloroquine and primaquine, measurements were made of the intracellular concentration of NADPH, a major product of the hexose monophosphate shunt in red cells. After incubation with either drug in the absence of glucose, the concentration of NADPH was lower than in control red cells; in the presence of glucose, higher concentrations of NADPH were maintained. Primaquine was more potent than chloroquine in lowering the NADPH concentration. Neither chloroquine nor primaquine inhibited the capacity of the red cell to increase flux through the hexose monophosphate shunt in response to gradual infusion of hydrogen peroxide. No qualitative differences in the effects of chloroquine or primaquine on glucose metabolism were observed when experiments were carried out with red cells containing methemoglobin or carbonmonoxyhemoglobin. This observation leads to the reassessment of the role of oxyhemoglobin in the mechanism of action of primaquine. Since incubation with chloroquine significantly decreased glucose flux through the hexose monophosphate shunt but resulted in only slightly lower NADPH levels, and the turnover of NADPH in control red cells in 1 hr was about ten times the total NADPH content, it follows that chloroquine both decreases utilization of NADPH and inhibits flux through the hexose monophosphate shunt. The effects of primaquine, a significant increase in the flux through the hexose monophosphate shunt with significantly lower NADPH concentrations, can be explained by the capacity of primaquine to undergo oxidation–reduction reactions which result in increased NADPH utilization and, therefore, increased flux through the hexose monophosphate shunt. The observed alterations in metabolism of uninfected red cells may be relevant to understanding the mechanisms of prophylactic and therapeutic effects of antimalarial agents.

Antimalarial drugs, such as chloroquine and primaquine, can prevent malaria or at least limit the severity of infection when administered prophylactically to persons entering malarious areas [1, 2]. In contrast to the almost completely effective prophylactic effect, the progress of treatment of fulminant malarial infection with antimalarial agents is more difficult and unpredictable. Although the mechanism of prophylaxis is not understood, it is possible that the antimalarial agent acts to alter the biochemistry of potential host cells, making them an unfavorable environment to the malarial parasite. Metabolic alterations in both liver cells and red cells may be involved in the mechanism of prophylaxis. Various alterations of human red cell metabolism are known to protect against malaria. Friedman [3] has recently reported that red cells from carriers of various genetic traits are refractory to parasitic infection because of oxidant sensitivity. Similarly, Eckman and Eaton [4] have suggested that, since glucose-6-phosphate dehydrogenase deficient red cells are inefficient in supplying NADPH, and since the parasite does not appear to have a significant hexose monophosphate shunt, glucose-6-phosphate dehydrogenase deficient red cells would be incapable of maintaining the levels of reduced glutathione necessary for the parasite. To understand how antimalarial agents might prophylactically alter the metabolic environment of the red cell, making that environment unfavorable to the prospective parasite, we have studied the effects of antimalarial agents on the uninfected red cell. The results of this study may also be relevant to the mechanism of antimalarial action in combating pre-existing infection. We have begun this investigation by studying the effects of chloroquine, a 4-aminoquinoline, and of primaquine, an 8-aminoquinoline, on glucose metabolism in uninfected red cells.

MATERIALS AND METHODS

Red cell preparations. Adult human blood was drawn daily into 3.8% sodium citrate solution. After centrifugation, plasma and white cells were removed, and red cells were washed three times with phosphate-buffered saline (9 parts, 0.9% NaCl; 1 part, 0.1 M KH_2PO_4/K_2HPO_4, pH 7.4). A 25% (v/v) suspension of red cells was prepared in Krebs–Ringer

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phosphate buffer (100 parts, 0.154 M NaCl; 4 parts, 0.154 M KCl; 1.5 parts, 0.11 M $CaCl_2$; 1 part, 0.154 M $MgSO_4$; 21 parts, 0.1 M NaH_2PO_4/Na_2HPO_4, pH 7.4) with or without 5 mM D-glucose.

In experiments requiring gradual infusion of H_2O_2, wells containing 0.3 ml of 30% H_2O_2 were included in 25-ml stoppered Erlenmeyer incubation flasks containing 2 ml of red cell suspension. This procedure allowed for the gaseous diffusion of H_2O_2 from the center well to the red cells at a steady rate [5].

Red cells containing methemoglobin were prepared by mixing 1 vol. of red cells with 1 vol. of 1% $NaNO_2$ in phosphate-buffered saline, incubating at room temperature for 10 min, and washing the red cells five times with phosphate-buffered saline to remove the nitrite [6]. Red cells containing carbonmonoxyhemoglobin were prepared by exposing a suspension of red cells in Krebs–Ringer phosphate to an atmosphere of carbon monoxide until an absorbance maximum was observed at 569 nm. Red cells containing carbonmonoxyhemoglobin were then washed once with oxygenated phosphate-buffered saline. Red cells containing methemoglobin or carbonmonoxyhemoglobin were resuspended in Krebs–Ringer phosphate with 5 mM D-glucose.

Measurement of the flux of glucose metabolism. The flux of glucose through the hexose monophosphate shunt and glycolysis was measured by a modification of the method of Pescarmona *et al.** Two milliliters of a 25% red cell suspension in Krebs–Ringer phosphate with 5 mM D-glucose was added to a 25-ml Erlenmeyer flask. Primaquine diphosphate or chloroquine diphosphate (Sigma Chemical Co., St. Louis, MO) was added to the red cell suspension in a small volume of Krebs–Ringer phosphate with 5 mM D-glucose (brought to pH 7.4) to give a final concentration of 0.1, 0.5 or 1.0 mM. An appropriate amount of a solution of sodium phosphate in Krebs–Ringer phosphate with 5 mM D-glucose (brought to pH 7.4) was added to each flask to control for the phosphate contained in the aminoquinoline salts. The final phosphate concentration of all flasks after all additions was 18.5 mM. Blanks consisted of red cell suspensions that had been boiled for 10 min. D-[1-^{14}C]Glucose (0.3 ml) (Amersham Searle, Arlington Heights, IL) in Krebs–Ringer phosphate with 5 mM D-glucose (0.5 μCi/0.3 ml) was added to each flask. Flasks were stoppered with wells containing 0.2 ml of 2 M KOH and incubated in a shaking water bath at 37° for 1 hr. The reaction was stopped by the addition of 0.7 ml of 35% perchloric acid and incubated for 30 min to insure that released $^{14}CO_2$ was trapped in the KOH. Contents of the well (KOH with trapped $^{14}CO_2$) were transferred into 10 ml Oxosol (National Diagnostic, Somerville, NJ) and counted in a liquid scintillation counter. The flux through the hexose monophosphate shunt was calculated after subtracting blank values and expressed as μmoles CO_2 produced per hr per ml of red cells. After addition of, and incubation with, perchloric acid, the contents of the incubation flasks were transferred to centrifuge tubes, and the flasks were washed with 2 ml of distilled water, bringing the total volume in the centrifuge tube to 5 ml. After centrifugation at 8000 *g* for 10 min, 1 ml of the supernatant fraction was added to 1 ml of 0.5 M Tris, 0.78 M KOH and centrifuged to remove perchlorate as the potassium salt. Then 0.1 ml of this supernatant fraction was added to a 25-ml Erlenmeyer flask with 0.9 ml of 3.3 mM ATP, 1 mM NADP, 6.5 mM $MgCl_2$, 1.4 I.U. glucose-6-phosphate dehydrogenase, 0.1 M Tris (pH 7.6), and 0.05 ml containing 2.8 I.U. hexokinase and 0.24 I.U. 6-phosphogluconate dehydrogenase. Flasks were stoppered with wells containing 0.2 ml of 2 M KOH and incubated in a shaking water bath at 37° for 30 min. The purpose of this procedure is to decarboxylate the remaining glucose, leaving intermediates of glycolysis as the only radioactive species in the flask. The reaction was terminated by the addition of 0.2 ml of 7% perchloric acid, and the flasks were further incubated for 30 min. The flask contents were centrifuged and 0.25 ml of the supernatant fraction was transferred to 10 ml Oxosol and counted in a liquid scintillation counter. The flux through glycolysis was calculated after subtracting blank values and correcting for dilution and is expressed as μmoles of glycolytic intermediates produced per hr per ml of red cells.

Measurement of intracellular NADPH and NADP. For measurement of NADPH and NADP concentrations, red cells were suspended in Krebs–Ringer phosphate without glucose. A 25% red cell suspension (3 ml) was added to a 25-ml Erlenmeyer flask and was incubated at 37° for 30 min in the presence and absence of 1 mM chloroquine or 1 mM primaquine and in the presence and absence of 5 mM D-glucose. When no drug was present, the phosphate concentration was controlled by adding an appropriate amount of sodium phosphate. Ethanolic extraction and fluorometric determination of NADPH and NADP were carried out by the method of Sander *et al.* [7]. The mean value obtained for NADPH concentration (in nmoles/ml red cells) was 2.85 ± 0.13 (S.E. with $N = 6$); the mean value obtained for NADP concentration was 7.21 ± 0.29 ($N = 6$). Increases or decreases in red cell concentrations of NADPH were reflected in opposite changes in NADP concentrations. Data were expressed as the fraction of the total nucleotide present in reduced form: NADPH/(NADP + NADPH).

RESULTS

Dosages of chloroquine and primaquine for in vitro *experiments with red cells.* Therapeutic plasma concentrations of chloroquine range between 3×10^{-8} M and 2×10^{-6} M [8] and of primaquine between 9×10^{-9} M and 2×10^{-8}M [9]. To obtain observable metabolic changes in the short time periods of *in vitro* experiments, higher concentrations are required. Dosage levels of chloroquine and primaquine are limited by the chemical and biological properties of these agents. Concentrations of primaquine over 1 mM cause lysis of red cells *in vitro* [10, 11]. We observed no lysis after 90 min at 37° with 1 mM primaquine, whereas 2 mM primaquine

* G. P. Pescarmona, A. Bosia, P. Arese and M. L. Sartori (University of Torino, Italy; University of Sassari, Italy), personal communication.

Table 1. Effects of chloroquine and primaquine on the hexose monophosphate shunt

Additions	Flux through the hexose monophosphate shunt [μmoles glucose metabolized·hr^{-1}·(ml red cells)$^{-1}$]		
	Expt. 1*	Expt. 2	Expt. 3
None	0.092	0.144	0.095
Chloroquine (0.1 mM)	0.074	0.130	0.086
Chloroquine (0.5 mM)	0.068	0.106	0.076
Chloroquine (1.0 mM)	0.041	0.062	0.069
	Expt. 4	Expt. 5	Expt. 6
None	0.074	0.092	0.066
Primaquine (0.1 mM)	0.085	0.095	0.090
Primaquine (0.5 mM)	0.105	0.107	0.108
Primaquine (1.0 mM)	0.184	0.115	0.141

* Each numbered experiment was carried out using red cells from a different individual.

caused significant lysis in the same period. In contrast, 15 mM chloroquine caused no lysis after 24 hr at 37°. Lysis on incubation with primaquine is thought to be related to the primaquine-mediated intracellular production of H_2O_2 [12]. Although primaquine can cause red cell lysis *in vivo* in individuals with glucose-6-phosphate dehydrogenase deficiency [13], the characteristics of *in vitro* lysis are identical for glucose-6-phosphate dehydrogenase deficient and normal red cells [10]. The differences between *in vivo* and *in vitro* lysis caused by primaquine remain unexplained.

Incubation of red cells with chloroquine interfered with measurements of fluorescence of NADPH extracted from red cells. This problem could be avoided by proper choice of excitation and emission wavelengths. Concentrations of chloroquine above 1 mM resulted in background fluorescence too great for accurate measurement at any wavelength. Incubation of red cells with primaquine up to 1 mM did not interfere with measurements of fluorescence.

For the several reasons outlined above, concentrations of chloroquine and primaquine of 0.1, 0.5 and 1.0 mM were chosen for *in vitro* experiments with red cells.

Effects of chloroquine and primaquine on glucose metabolism. The effects of chloroquine and primaquine on the flux of glucose through the hexose monophosphate shunt are shown in Table 1. The flux of glucose through the hexose monophosphate shunt and the magnitude of changes induced by chloroquine or primaquine varied between different individuals and between experiments on different days with red cells from the same individual; the qualitative effects of chloroquine and primaquine, however, were consistent. The flux of glucose through the hexose monophosphate shunt decreased with increasing chloroquine concentration but increased with increasing primaquine concentration. The effects of chloroquine and primaquine on the flux of glucose through glycolysis are shown in Table 2. Although mean values for the flux through glycolysis were higher in the presence of chloroquine or primaquine as compared to controls, there was considerable variation among individuals and a paired *t*-test showed these changes to be non-significant.

Effects of chloroquine and primaquine on intracellular NADPH. Since both chloroquine and primaquine have major effects on the flux through the hexose monophosphate shunt, we investigated the effects of chloroquine and primaquine on intracellular NADPH concentration. The results are shown in Table 3. After a 30-min incubation with 1 mM chloroquine, the red cell NADPH concentration was slightly lower than in controls. In the presence of glucose, red cells maintained higher NADPH levels than controls, both with and without chloroquine. In contrast, incubation with 1 mM primaquine decreased NADPH levels to nearly zero. Red cells

Table 2. Effects of chloroquine and primaquine on glycolysis

Additions	Flux through glycolysis [μmoles glucose metabolized·hr^{-1}·(ml red cells)$^{-1}$]	
None	1.32 ± 0.08 (N = 10)*	NS†
Chloroquine (1.0 mM)	1.56 ± 0.14 (N = 10)	
None	1.42 ± 0.16 (N = 9)	NS
Primaquine (1.0 mM)	1.56 ± 0.29 (N = 9)	

* Values are means ± S.E.; N = the number of individuals tested.

† The difference between control cells and those incubated with chloroquine or primaquine, as determined by a paired *t*-test, was not statistically significant ($P > 0.1$).

Table 3. Effects of chloroquine and primaquine on cellular concentrations of NADPH

Additions	NADPH/(NADP + NADPH)		
	Expt. 1*	Expt. 2	Expt. 3
None	0.32	0.25	0.25
Glucose (5.0 mM)	0.45	0.46	0.49
Chloroquine (1.0 mM)	0.22	0.23	0.20
Chloroquine (1.0 mM) + glucose (5.0 mM)	0.41	0.43	0.49
	Expt. 4	Expt. 5	Expt. 6
None	0.28	0.28	0.33
Glucose (5.0 mM)	0.42	0.49	0.44
Primaquine (1.0 mM)	0.05	0.06	0.00
Primaquine (1.0 mM) + glucose (5.0 mM)	0.29	0.28	0.30

* Each numbered experiment was carried out using red cells from a different individual.

incubated with glucose and primaquine maintained the NADPH concentration at about 60% compared to red cells incubated with glucose alone.

Capacity of the hexose monophosphate shunt to respond to oxidative stress in the presence of chloroquine and primaquine. Both chloroquine and primaquine have significant effects on the flux through the hexose monophosphate shunt with little or no effect on glycolysis. In the presence of an oxidative stress, such as that presented by primaquine, the hexose monophosphate shunt is capable of a much greater response than glycolysis [14]. The glucose normally metabolized through the hexose monophosphate shunt is only about 10 per cent of the total glucose metabolized, the rest being metabolized by glycolysis [15, 16]. When the red cell is challenged by oxidative stress, flux through the hexose monophosphate shunt may increase as much as 20-fold (as measured using D-[1-^{14}C]glucose), whereas glycolysis can only triple its rate [14]. The data on the effects of chloroquine and primaquine on the flux through the hexose monophosphate shunt and on levels of NADPH do not provide information about the effects of chloroquine and primaquine on the

Table 4. Effects of chloroquine and primaquine on the hexose monophosphate shunt in the presence of oxidative stress or altered hemoglobin

Red cell preparation	Additions	Flux through the hexose monophosphate shunt [μmoles glucose metabolized·hr^{-1}·(ml red cells)$^{-1}$]	
		Expt. 1*	Expt. 2
Oxyhemoglobin	None	0.122	0.067
Oxyhemoglobin	H_2O_2†	0.287	0.226
Oxyhemoglobin	Chloroquine (1 mM)	0.085	0.040
Oxyhemoglobin	Chloroquine (1 mM) + H_2O_2	0.345	0.188
Oxyhemoglobin	Primaquine (1 mM)	0.193	0.284
Oxyhemoglobin	Primaquine (1 mM) + H_2O_2	0.486	0.500
		Expt. 3	Expt. 4
Oxyhemoglobin	None	0.100	0.109
Oxyhemoglobin	Chloroquine (1 mM)	0.090	0.066
Methemoglobin	None	0.454	0.393
Methemoglobin	Chloroquine (1 mM)	0.448	0.385
Carbonmonoxyhemoglobin	None	0.079	0.105
Carbonmonoxyhemoglobin	Chloroquine (1 mM)	0.051	0.071
		Expt. 5	Expt. 6
Oxyhemoglobin	None	0.125	0.118
Oxyhemoglobin	Primaquine (1 mM)	0.278	0.380
Methemoglobin	None	0.412	0.625
Methemoglobin	Primaquine (1 mM)	0.704	0.888
Carbonmonoxyhemoglobin	None	0.131	0.098
Carbonmonoxyhemoglobin	Primaquine (1 mM)	0.217	0.240

* Each numbered experiment was carried out using red cells from a different individual.
† H_2O_2 was added by gradual infusion as described in Materials and Methods.

capacity of the hexose monophosphate shunt to respond to additional stress, as might be provided in the presence of the malarial parasite. This problem was studied by measuring changes in the flux through the hexose monophosphate shunt in the presence of chloroquine or primaquine with the additional stress of externally added H_2O_2 (Table 4). Red cells were incubated in stoppered flasks provided with wells containing an H_2O_2 solution. This procedure allowed for the gaseous diffusion of H_2O_2 from the center well to the red cells at a steady rate [5]. The results in Table 4 show that H_2O_2 caused an increase in flux through the hexose monophosphate shunt, which is superimposed on the inhibitory effect of chloroquine and superimposed on the activating effect of primaquine. The increase in flux caused by H_2O_2 in the presence of chloroquine or primaquine was equal to or greater than the increase in flux caused by H_2O_2 observed with control red cells. The results show that chloroquine and primaquine did not inhibit the capacity of the red cell to increase flux through the hexose monophosphate shunt in response to H_2O_2. While the maximum increase in flux caused by H_2O_2, shown in Table 4, was about 8-fold compared to control red cells, a 30-fold increase in glucose consumption has been reported in red cells infected with *Plasmodium berghei* and *Plasmodium knowlesi* [17, 18]. In such extreme conditions, the inhibitory effect of chloroquine on the flux or the utilization of NADPH and glutathione resulting from primaquine-mediated oxidative stress may be significant enough to limit the NADPH and glutathione available to the parasite.

Role of hemoglobin in the effects of chloroquine and primaquine on glucose flux through the hexose monophosphate shunt. Since hemoglobin is the main organic component of the red cell, a possible role for hemoglobin should be considered when studying the mechanism of action of an agent affecting the red cell. Cohen and Hochstein [12] present evidence that the intracellular production of H_2O_2 mediated by primaquine requires the presence of oxyhemoglobin. We tested the possible role of hemoglobin in the effects of chloroquine and primaquine on flux through the hexose monophosphate shunt by carrying out experiments with red cell preparations containing carbonmonoxyhemoglobin or methemoglobin. Cells containing methemoglobin were used to exaggerate the effect of the small amount of methemoglobin present, or produced, in conditions where intracellular H_2O_2 is present. Cells containing carbonmonoxyhemoglobin approximate conditions where the heme group is unavailable for reaction; by the end of a 90-min incubation period, however, about 20 per cent of the hemoglobin reverted to oxyhemoglobin so that some effect of oxyhemoglobin can be expected in these experiments. Table 4 shows the flux of glucose through the hexose monophosphate shunt in red cell preparations containing oxyhemoglobin, carbonmonoxyhemoglobin and methemoglobin. In red cells containing methemoglobin, the flux through the hexose monophosphate shunt was increased about 4-fold over the flux in red cells containing oxyhemoglobin. It has been suggested that the increase in flux in red cells containing methemoglobin results mainly from the multiple washings required to rid the red cells of nitrite used to convert oxyhemoglobin to methemoglobin [19]. In our laboratory, however, multiple washings of control red cell preparations did not increase flux through the hexose monophosphate shunt. The activity of the methemoglobin reductase system may account for part of the increase in flux in red cells containing methemoglobin. Chloroquine (1 mM) decreased flux through the hexose monophosphate shunt in red cell preparations containing both oxyhemoglobin and carbonmonoxyhemoglobin. No significant decrease in flux through the hexose monophosphate shunt was observed when red cells containing methemoglobin were incubated with 1 mM chloroquine. Primaquine (1 mM) caused significant increases in flux through the hexose monophosphate shunt in all three red cell preparations studied. The greatest increase was observed in red cells containing methemoglobin and the smallest in red cells containing carbonmonoxyhemoglobin. No consistent changes were observed in the flux through glycolysis when red cells containing methemoglobin or carbonmonoxyhemoglobin were incubated with chloroquine or primaquine (data not shown).

DISCUSSION

Primaquine, but not chloroquine, can cause red cell lysis both *in vivo* [13] and *in vitro* [10]. Cohen and Hochstein have shown that primaquine causes production of H_2O_2 in the red cell whereas chloroquine does not, and it has been presumed that the hemolytic capacity of primaquine is due, in part, to the production of H_2O_2 [12]. The effects of chloroquine and primaquine on the flux through the hexose monophosphate shunt can be understood on the basis of the capacity of primaquine to undergo oxidation–reduction reactions together with the finding by Desforges *et al.* [20] and Cotton and Sutorius [21] that both chloroquine and primaquine inhibit glucose-6-phosphate dehydrogenase.

Chloroquine decreased the flux of glucose through the hexose monophosphate shunt but only slightly lowered NADPH levels in the presence of glucose. Since the turnover rate of NADPH in control red cells was about ten times the NADPH concentration per hour, it follows that chloroquine also decreased utilization of NADPH, thereby maintaining NADPH levels in the face of reduced flux. Although chloroquine decreased the flux through the hexose monophosphate shunt, the capacity of the shunt to respond to the presence of H_2O_2 was unaffected. Oxyhemoglobin was not required for the effect of chloroquine on the hexose monophosphate shunt since similar changes were observed with red cells containing carbonmonoxyhemoglobin. The inhibiting effect of chloroquine was overcome or masked by the 4-fold increase in flux through the hexose monophosphate shunt in red cells containing methemoglobin.

Inhibition of glucose-6-phosphate dehydrogenase by chloroquine [21] might have been the cause of the inhibition of overall flux through the hexose monophosphate shunt. The inhibition of glucose-6-phosphate dehydrogenase by chloroquine can be reversed completely by excess NADP [21]. Since

chloroquine does not induce H_2O_2 formation in red cells, increased utilization of NADPH and formation of NADP are not observed. Inhibition of flux through the hexose monophosphate shunt by chloroquine might then be primarily due to inhibition of glucose-6-phosphate dehydrogenase activity.

Incubation of red cells with primaquine resulted in decreased levels of NADPH and increased flux through the hexose monophosphate shunt. Glucose only partially maintained NADPH levels in the presence of primaquine. Primaquine did not inhibit the capacity of the hexose monophosphate shunt to respond to the further oxidative stress of H_2O_2 infusion. Primaquine increased flux through the hexose monophosphate shunt in red cells containing oxyhemoglobin, methemoglobin, or carbonmonoxyhemoglobin.

Primaquine has been shown to generate H_2O_2 in red cells containing oxyhemoglobin but not in red cells containing methemoglobin [12]. H_2O_2 is removed from the red cell by glutathione and catalase; NADPH, a product of the hexose monophosphate shunt, supplies reducing equivalents to maintain the activity of glutathione peroxidase [14] and catalase [14, 22]. The production of H_2O_2 by primaquine in the red cell would, therefore, increase the flux through the hexose monophosphate shunt to maintain NADPH levels. However, since we observed an increase in flux through the hexose monophosphate shunt in red cells containing oxyhemoglobin, methemoglobin or carbonmonoxyhemoglobin, the increase in flux cannot be explained on the basis of the simple removal of H_2O_2 produced by a primaquine–oxyhemoglobin interaction. We have carried out experiments measuring H_2O_2 formation by reaction of primaquine with purified oxyhemoglobin or methemoglobin.* H_2O_2 formation was measured by inactivation of catalase in the presence of 3-amino-1,2,4-triazole [12]. H_2O_2 was formed in the presence of primaquine plus oxyhemoglobin, primaquine plus iron-EDTA, oxyhemoglobin plus iron-EDTA, or primaquine plus oxyhemoglobin plus iron-EDTA. No catalase inactivation occurred in the presence of primaquine plus methemoglobin or of iron-EDTA plus methemoglobin, while methemoglobin protected against catalase inactivation when added to a mixture of primaquine plus iron-EDTA. Methemoglobin may protect catalase activity by scavenging H_2O_2 or by preventing H_2O_2 formation by scavenging other oxygen or primaquine intermediates. The increase in flux through the hexose monophosphate shunt caused by primaquine in red cells containing methemoglobin would seem to be independent of H_2O_2-mediated catalase inactivation. Similar results have been observed in red cells incubated with 6-hydroxydopamine [23], a catecholamine that autoxidizes, yielding H_2O_2 as one product [24]. In those experiments, accumulation of the peroxidative intermediate of catalase, Compound II, was used as a measure of intracellular H_2O_2 formation. While 6-hydroxydopamine significantly increased the flux through the hexose monophosphate shunt in red cells containing oxyhemoglobin or methemoglobin, Compound II accumulated in red cells containing oxyhemoglobin but not methemoglobin. Since 6-hydroxydopamine autoxidizes and causes an increase in flux regardless of the type of hemoglobin, it was concluded that methemoglobin may have a protective effect on catalase activity [23].

Based on the data presented here, we must reassess the hypothesis that the increase in hexose monophosphate shunt activity is due solely to reducing equivalents necessary for the reduction of H_2O_2. It is possible that primaquine acts in the red cell by a mechanism similar to that of methylene blue and/or ascorbate. Ascorbate is specifically reduced in the red cell by glutathione, whereas methylene blue is specifically reduced by NADPH [25, 26]. It is the reduced forms of ascorbate and methylene blue that react to form hydrogen peroxide [27, 28]. It is possible that primaquine (or its metabolites) when introduced into the red cell is present in various redox states, that some of these species are capable of one-electron transfer reactions (one product of which might be H_2O_2), and that the increase in hexose monophosphate shunt activity is, in large part, due to the reduction of primaquine or its metabolites by NADPH and/or glutathione.

Primaquine inhibits glucose-6-phosphate dehydrogenase [21] and, independently of other factors, might inhibit overall flux through the hexose monophosphate shunt. The inhibition of glucose-6-phosphate dehydrogenase by primaquine can be reversed completely by excess NADP [21]. In red cells treated with primaquine, NADP formed by constant oxidation of NADPH could reverse the inhibition of glucose-6-phosphate dehydrogenase and any overall inhibition of flux through the hexose monophosphate shunt. In fact, glucose partially maintains NADPH in the presence of primaquine by increasing flux through the hexose monophosphate shunt.

Chloroquine and primaquine differentially alter the glucose metabolism of the uninfected red cell. The possible relationship of the observed alterations and the prophylactic effects of antimalarial agents is not known. Both chloroquine and primaquine inhibit glucose-6-phosphate dehydrogenase [21] and decrease the intracellular concentration of NADPH, and the latter effect is only partially countered by glucose in the presence of primaquine. The requirement of the malarial parasite for products of the hexose monophosphate shunt [4] would be expected to further lower the intracellular concentration of NADPH and glutathione. The resulting intracellular milieu might not sustain the malarial parasite.

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