

FERRIPROTOPORPHYRIN IX AND CELL LYSIS:
A PROTECTIVE ROLE FOR HYDROGEN PEROXIDE

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Summary

Two potentially lytic substances, ferriprotoporphyrin IX (FP) and hydrogen peroxide, may coexist and partially detoxify each other in sickle cells and in erythrocytes infected with malaria parasites. Since hydrogen peroxide can decompose FP, its effect on hemolysis induced by FP and by the complex of FP with chloroquine was investigated. Human erythrocytes suspended at a concentration of 0.5% in a 50 μ M solution of FP underwent approximately 42% hemolysis during the course of 2 hours. Twenty-five micromolar chloroquine potentiated hemolysis to 99%, and preincubation of 50 μ M FP with 25 μ M hydrogen peroxide for 5 minutes reduced hemolysis to 4%. Mixing either FP or hydrogen peroxide first with chloroquine abolished the effect of hydrogen peroxide. Detoxification of FP by hydrogen peroxide may be an important protective mechanism in certain hemolytic anemias, and inhibition of detoxification could account for the effectiveness of chloroquine in malaria.

Ferriprotoporphyrin IX (FP) is released when malaria parasites digest hemoglobin (1,2) and when hemoglobin is denatured in sickle cells (3) and other variant erythrocytes (4). It is lytic either by itself or when it exists as a complex with the antimalarial drug chloroquine (5-8). In fact, the toxicity of the FP-chloroquine complex may explain the antimalarial action of chloroquine (9). Excess generation of hydrogen peroxide also has been reported to occur in sickle cells (10) and in erythrocytes infected with malaria parasites (11). Because hydrogen peroxide penetrates cellular membranes (12) and can decompose FP (1,13), it was considered important to investigate the detoxification of FP by hydrogen peroxide and the effect of chloroquine on the process.

Methods

Blood was collected from healthy human volunteers who understood the risks of venipuncture and gave informed consent. Heparin was used as an anticoagulant. The blood was centrifuged, plasma and buffy coat were discarded, and the erythrocytes were washed 3 times with Tris medium [141 mM NaCl, 10 mM Tris-(hydroxymethyl)aminomethane, pH 7.4 at 37^o C]. Hemolysis was evaluated by preparing a 0.5% suspension of erythrocytes in Tris medium containing the desired concentrations of FP, hydrogen peroxide, and chloroquine, singly or in various combinations. These suspensions were incubated at 37^o C for 2 hours with shaking at a rate of 2.3 Hz. Percent hemolysis was calculated from the release of hemoglobin into the medium as has been described (5,8), except for a small correction for the absorbance of the appropriate incubation mixture without erythrocytes. A stock solution of FP was prepared by dissolving crystalline

hemin in 0.02 N NaOH as previously described (5,8); it was diluted with Tris medium to achieve the desired concentration just prior to use. Hemin, hydrogen peroxide, and chloroquine diphosphate were purchased from Sigma Chemical Company.

Results

The experiments summarized in Fig. 1 demonstrate both the potentiation of FP-induced hemolysis by chloroquine, as has been previously reported (8), and the detoxification of FP by small amounts of hydrogen peroxide. Addition of chloroquine after preincubation with hydrogen peroxide did not reverse this detoxification. However, addition of chloroquine prior to or at the same time as the addition of hydrogen peroxide prevented the detoxification of FP (Fig. 1). Although not shown in Fig. 1, the protective effect of chloroquine could be overcome with high concentrations of hydrogen peroxide (200 μ M or more).

Large amounts of hydrogen peroxide have been shown to decolorize hematin and malaria pigment (1,13). This effect is demonstrated for a low concentration of hydrogen peroxide by the decrease in the Soret band of the absorption spectra presented in Fig. 2A. Forming a complex of chloroquine with FP slowed the rate of reaction of hydrogen peroxide with FP (Fig. 2B). Other anti-malarial drugs including primaquine, quinine, pyrimethamine, and mefloquine did not slow the reaction of hydrogen peroxide with FP (results not shown). The way in which chloroquine protects against hydrogen peroxide was not explored in the present experiments, but there is evidence that chloroquine intercalates between μ -oxobridged dimers of FP (14,15) and, in so doing, it might deny access of the iron atom of FP to hydrogen peroxide.

Discussion

Since FP and hydrogen peroxide coexist in erythrocytes of people with certain hemolytic diseases (1-4,10,11), we propose that the detoxification of FP by hydrogen peroxide has physiological significance. For example, decomposition of FP by hydrogen peroxide in sickle cells would reduce the hemolytic effect of FP. Similarly, since hydrogen peroxide is decomposed by its interaction with FP (16), any toxicity of hydrogen peroxide also would be reduced. Toxicity would be expected to occur only with a relative excess of FP or hydrogen peroxide. The concentrations of hydrogen peroxide used in the experiments described in Fig. 1 were in the range of concentrations that can be produced by activated neutrophils (17). In these experiments, hydrogen peroxide alone did not cause significant hemolysis.

The opposing effects of hydrogen peroxide and chloroquine on FP toxicity are of special interest because of the hypothesis that chloroquine acts by delaying the sequestration of FP into malaria pigment and allowing the FP to exert its intrinsic cellular toxicity (9). This hypothesis is based on the following observations: (a) the FP in malaria parasites is stored as an insoluble nontoxic form of hematin in malaria pigment (1,2), (b) when malaria parasites degrade hemoglobin in the presence of chloroquine, they accumulate chloroquine with the affinity and specificity characteristic of chloroquine binding to FP (14,18), and (c) FP and the complex of chloroquine with FP are lytic for malaria parasites (6,7), but (d) chloroquine alone is not lytic (6,7). Since hydrogen peroxide exists in parasitized erythrocytes and can decompose FP, we now modify the hypothesis to indicate that malaria parasites can detoxify FP both by sequestering it and by decomposing it. In the latter case, chloroquine would cause the accumulation of a toxic chloroquine-FP complex by delaying the decomposition of FP by hydrogen peroxide. Finally, we recognize that malaria parasites could limit the accessibility of FP to chloroquine by increasing either sequestration or decomposition. Thus, increased

availability of hydrogen peroxide could cause chloroquine resistance in malaria.

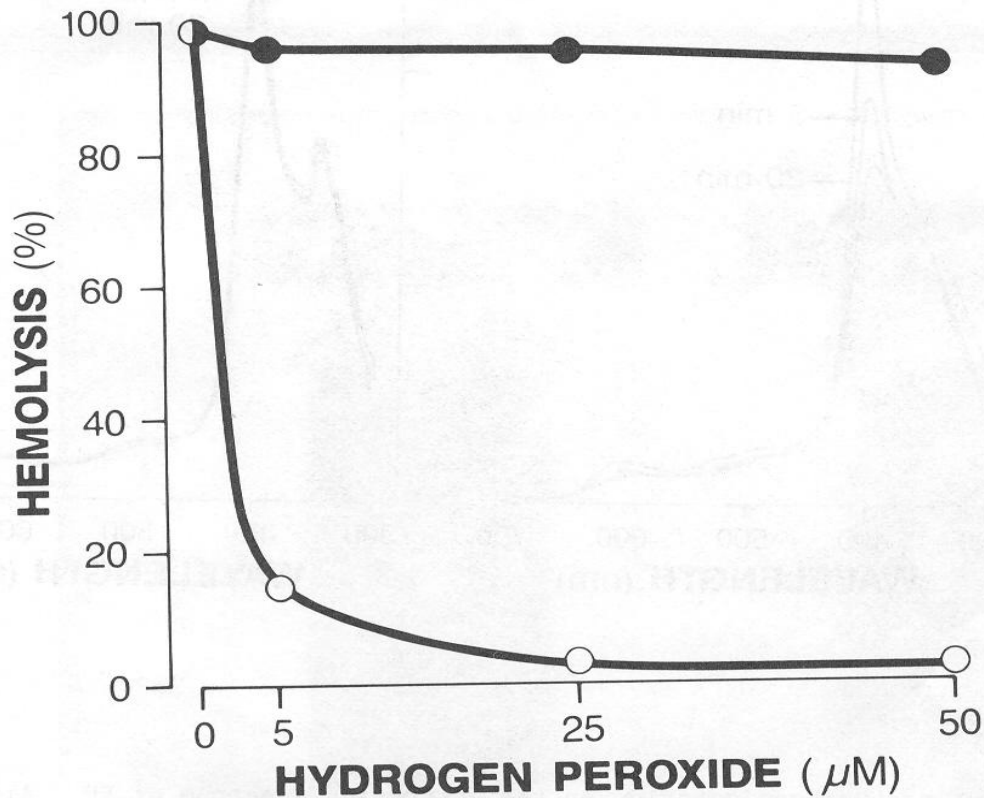


FIG. 1

Effects of hydrogen peroxide and chloroquine on FP-induced hemolysis. Representative results from one of 3 experiments are shown. In one series of incubation vessels (open circles), the indicated concentrations of hydrogen peroxide were each mixed with 50 µM FP and, after incubation at 37° C for 5 minutes, 25 µM chloroquine and erythrocytes were added for evaluation of hemolysis as described in the text. In another series of incubation vessels (closed circles), 25 µM chloroquine was added to the indicated concentrations of hydrogen peroxide prior to adding 50 µM FP for a 5 minute incubation at 37° C; finally, erythrocytes were added for evaluation of hemolysis. In other vessels (data not shown), chloroquine was first mixed with the FP before adding hydrogen peroxide, and the results were the same as those shown by closed circles. In the absence of hydrogen peroxide and chloroquine, erythrocytes suspended in 50 µM FP underwent approximately 42% hemolysis.

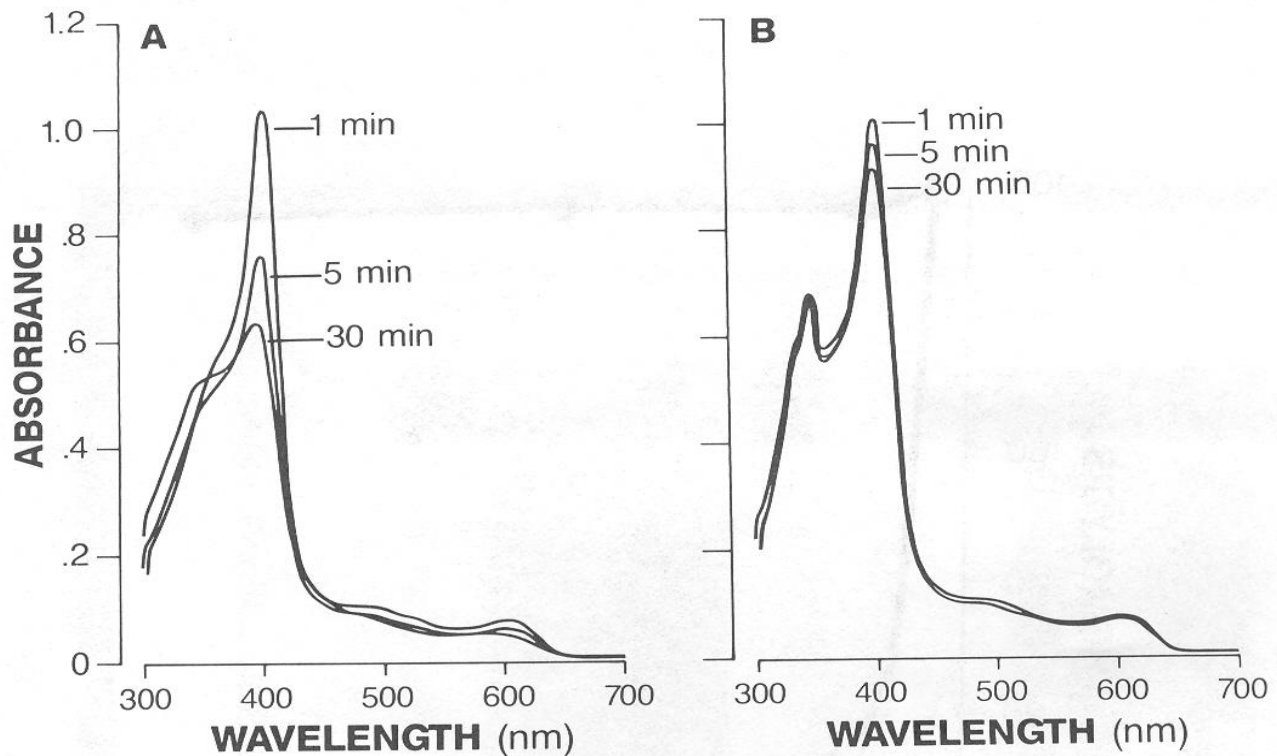


FIG. 2

Effect of hydrogen peroxide on the absorption spectrum of FP. Aliquots of hydrogen peroxide (A) or a mixture of hydrogen peroxide and chloroquine (B) were placed in plastic tubes. At time 0, FP was added and each mixture was incubated at 37° C for the indicated length of time before sufficient SDS was added to achieve a concentration of 2.5% and the absorption spectrum was recorded. The initial concentrations were 7.5 μ M hydrogen peroxide, 15 μ M chloroquine and 15 μ M FP. In control experiments, there was no effect of hydrogen peroxide on the absorption spectrum of chloroquine.

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