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A ferriprotoporphyrin IX-chloroquine complex promotes membrane phospholipid peroxidation

A possible mechanism for antimalarial action

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The effect of a ferriprotoporphyrin IX -chloroquine complex on phospholipid membranes was investigated on the basis of iron-induced lipid peroxidation using microsomal phospholipid liposomes. The ferriprotoporphyrin IX -chloroquine complex remarkably promoted peroxidative cleavage of unsaturated phospholipids in liposomes, compared with ferriprotoporphyrin IX alone. Neither the combination of protoporphyrin IX with chloroquine nor the combination of Fe^{3+}-ADP with chloroquine promoted lipid peroxidation. DL-α-Tocopherol completely inhibited lipid peroxidation induced by the ferriprotoporphyrin IX -chloroquine complex.

Chloroquine; Ferriprotoporphyrin IX; Lipid peroxidation

1. INTRODUCTION

Chloroquine (7-chloro-4-(4-dimethylamino-1-methylbutylamino)quinoline) has been widely used as one of many antimalarial drugs. However, the mechanism of action of chloroquine in killing malaria parasites has not been understood. Chou et al. [1] and Fitch [2] have reported that FP IX, a main component of the malaria pigment, produces a co-ordination complex with chloroquine. On the other hand, Sugioka and Nakano [3] have demonstrated that the ferric ion-ADP-adriamycin co-ordination complex induces powerful membrane lipid peroxidation of liposomes prepared from microsomal phospholipids. Moreover, Tappel [4] has reported that FP IX itself could induce lipid peroxidation. These findings led us to speculate that peroxidative cleavage of vacuolar membranes in malaria parasites by the FP IX-chloroquine complex was the reason for the antimalarial action of chloroquine.

The present work was undertaken to prove a possible phospholipid peroxidation by the FP IX-chloroquine complex, using liposomes prepared from rat liver microsomal phospholipids as a lipid source.

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Abbreviations: FP IX, ferriprotoporphyrin IX; P IX, protoporphyrin IX

2. MATERIALS AND METHODS

2.1. *Reagents and enzymes*

Chloroquine, hemin (FP IX chloride) and P IX were purchased from Sigma. 1 mM FP IX stock solution in 0.02 N NaOH was prepared as described by Ginsburg and Demel [5]. The FP IX-chloroquine complex, in a molar ratio of 2:1, was

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prepared by mixing chloroquine in water with a FP IX stock solution. The Fe^{3+}-ADP complex was prepared by the method of Sugioka and Nakano [3]. DL-α-Tocopherol was kindly supplied by Eisai Co. Bovine red cell superoxide dismutase and bovine liver catalase were obtained from Sigma Chemicals. The catalase was dialyzed twice against 3 l of 0.01 M Na-phosphate buffer at pH 7.4, prior to use.

2.2. *Membrane phospholipids and incubation experiment*

Phospholipids were extracted from rat liver microsomes according to Folch et al. [6] and were freed from contaminants (free fatty acids, cholesterol and its ester) by silicic acid column chromatography [7]. Phospholipid liposomes were freshly prepared with or without α-tocopherol in 0.1 M Tris-HCl buffer at pH 7.4 as described previously [8]. The incubation mixture contained liposomes (0.8 μmol of lipid phosphorus/ml) and 0.1 M Tris-HCl buffer at pH 7.4 in a total volume of 1.9 ml (for measurement of malondialdehyde) and 6.0 ml (for lipid analysis). In some cases, the mixture contained 8.8×10^{-5} M α-tocopherol. Unless otherwise noticed, the reaction was initiated by the addition of the FP IX (25 μM)-chloroquine (12.5 μM) complex. All the incubation experiments were carried out at 37°C with continuous agitation.

2.3. *Assays*

Lipid peroxides were expressed as a concentration of malondialdehyde (thiobarbiturate-reactive substance) in accordance with the method of Ohkawa et al. [9]. Fatty acid composition was determined by a modification of the method described by May and McCay [10] using a Shimadzu gas chromatography.

3. RESULTS

Absorption spectra of the FP IX-chloroquine complex, FP IX and chloroquine are shown in fig.1. The spectrum of the complex was essentially the same as that reported by Chou et al. [1].

As shown in fig.2A,B, phospholipid peroxidation induced by the FP IX-chloroquine complex was much stronger than that displayed by FP IX alone, while little or no lipid peroxidation occurred

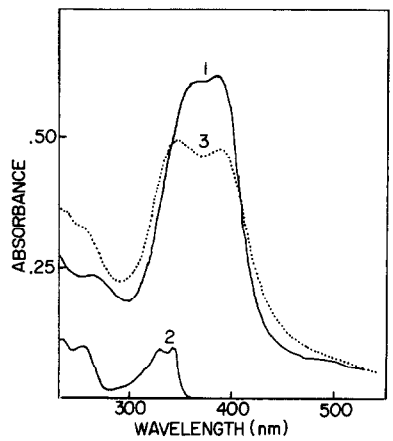

Fig.1. Absorption spectra of FP IX, chloroquine and the FP IX-chloroquine complex in 100 mM Tris-HCl buffer at pH 7.4. 1, 1×10^{-5} M FP IX; 2, 0.5×10^{-5} M chloroquine; 3, 1×10^{-5} M FP IX + 0.5×10^{-5} M chloroquine.

in the presence of chloroquine alone, the combination of P IX with chloroquine or the combination of Fe^{3+}-ADP with chloroquine. Each of the following substances, catalase at 20 μg/ml, 5×10^{-7} M superoxide dismutase and 50 mM sodium benzoate (an 'OH scavenger), did not inhibit the lipid peroxidation induced by FP IX or the FP IX-

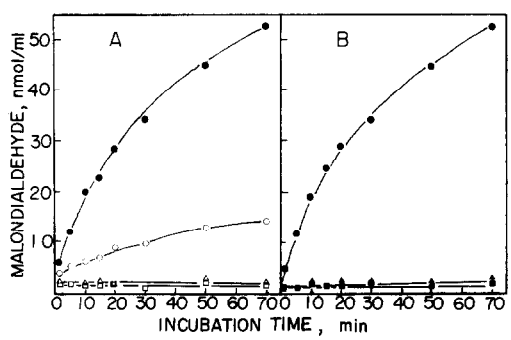

Fig.2. Time course of lipid peroxidation induced by the compound added. The reaction mixture, experimental conditions and assay were described in section 2. (A) The compounds added were: none (□), 12.5 μM chloroquine (△), 25 μM FP IX (○) and the FP IX (25 μM)-chloroquine (12.5 μM) complex (●). (B) The compounds added were: the FP IX (25 μM)-chloroquine (12.5 μM) complex (●), the Fe^{3+} (25 μM)-ADP (425 μM) complex with 12.5 μM chloroquine (▲) and the combination of 25 μM P IX with 12.5 μM chloroquine (■).

Table 1

Fatty acid composition in phospholipids incubated with various components for 60 min

Incubation system[a]	Fatty acid composition (mol/100 mol palmitic acid)					
	16:0	18:0	18:1	18:2	20:4	22:6
S1 Basal	100	112	41	88	128	24
S2 Basal + 12.5 μM chloroquine	100	106	43	86	130	25
S3 Basal + 25 μM FP IX	100	112	42	85	101	17
S4 Basal + 25 μM FP IX–12.5 μM chloroquine	100	113	40	78	73	11
S5 Basal + 25 μM P IX	100	112	42	85	120	22
S6 Basal + 25 μM P IX + 12.5 μM chloroquine	100	111	44	89	123	26
S7 S4 + 8.8×10^{-5} M α-tocopherol	100	109	42	89	127	22

[a] The reaction mixture and incubation conditions were the same as those described in section 2

chloroquine complex (not shown), indicating no participation of active oxygen species, such as O_2^-, H_2O_2 and $^{\cdot}OH$, on the lipid peroxidation. The replacement of 0.1 M Tris-HCl buffer with 0.01 M sodium phosphate buffer halved the lipid peroxidation induced by the FP IX-chloroquine complex (not shown).

To determine the direct fatty acid decomposition in liposomes during phospholipid peroxidation, fatty acid decomposition in liposomes after the 60-min incubation with or without various components was analyzed and compared with each other (table 1). The FP IX-chloroquine complex caused an obvious decomposition of unsaturated fatty acids in liposomes, especially arachidonic (20:4) and docosahexaenic (22:6) acids, while FP IX alone did not. No fatty acid decomposition occurred in the presence of chloroquine, P IX or the combination of P IX with chloroquine. Tocopherol completely protected FP IX-chloroquine-mediated fatty acid decomposition.

4. DISCUSSION

The importance of the iron moiety in the FP IX-chloroquine complex for promoting lipid peroxidation is supported by the following findings. (i) Neither the combination of P IX (iron-eliminated FP IX) with chloroquine nor that of Fe^{3+}-ADP with chloroquine promotes lipid peroxidation. (ii) FP IX itself causes slight, but detectable lipid peroxidation. At present, we do not know the valency of iron in the complex, either Fe^{III} or Fe^{II}, which initiates lipid peroxidation. Even though there are no reducing agents added exogenously in the system, the iron in the complex could be reduced by some manner during incubation and then act as an initiator of lipid peroxidation in the presence of O_2. It has been reported that the adriamycin-Fe^{3+}-ADP complex converts to an active form, the adriamycin-ADP-Fe^{2+} complex, by intramolecular self-reduction, which evokes lipid peroxidation [3]. The strong inhibitory effect of α-tocopherol on the FP IX-chloroquine complex-mediated lipid peroxidation suggests the participation of free radicals in the peroxidative cleavage of liposomes.

The peroxidative cleavage of phospholipid membranes induced by the FP IX-chloroquine complex might well explain the antimalarial action of chloroquine. That is, chloroquine, which is selectively localized within the parasite food vacuoles in endoerythrocytic stages [11], forms the complex with FP IX, a main metabolite from hemoglobin in vacuoles [12], and causes the peroxidative cleavage of phospholipid membranes of vacuoles. This process will lead to abnormal changes within the parasites, observed previously [11,13,14].

The detailed mechanism of lipid peroxidation induced by the FP IX-chloroquine complex will be described elsewhere.

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