ANTIOXIDATIVE EFFECT OF SEVERAL PORPHYRINS ON LIPID PEROXIDATION IN RAT LIVER HOMOGENATES

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The effect of several porphyrins on Fe^{2+}-ascorbic acid-stimulated lipid peroxidation was examined in rat liver homogenates. Not only protoporphyrin IX (PP) but also mesoporphyrin IX and hematoporphyrin inhibited the lipid peroxidation. Some porphyrins, in which 6- and 7-carboxyethyl groups were esterified with a methyl group, such as protoporphyrin IX dimethyl ester and mesoporphyrin IX dimethyl ester, had no antioxidative effect. Hemin and zinc protoporphyrin IX, which are metal-chelated porphyrins, inhibited the lipid peroxidation while cobalt protoporphyrin IX and tin protoporphyrin IX showed no antioxidative effect. Thus, some of the porphyrins used in the present study showed an antioxidative effect as did PP, but the others did not show such an effect.

KEYWORDS porphyrin; metalloporphyrin; lipid peroxidation; rat liver homogenate

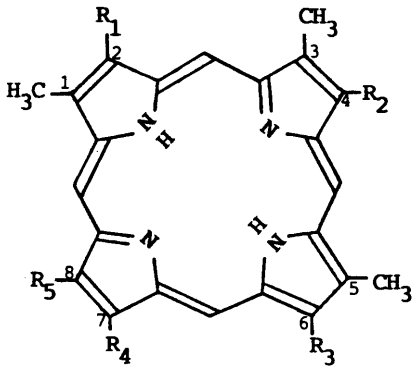
In our previous study, we have found that protoporphyrin IX (PP) inhibits the lipid peroxidation stimulated by Fe^{2+} and/or ascorbic acid (AsA) or a reduced nicotinamide adenine dinucleotide phosphate-generating system in rat liver homogenates, mitochondria and microsomes in vitro.[1] On the other hand, Wills reported that hemoproteins such as hemoglobin and cytochrome c catalyzed the oxidation of linoleic acid.[2] Kawashima, et al. reported that hemin accelerated the oxygen uptake of linoleic acid with increase in its concentration.[3] Das, et al. have reported that hematoporphyrin derivative, when given intraperitoneally to rats, enhances their hepatic microsomal lipid peroxidation under UV light irradiation.[4]

So, it was of interest to investigate whether porphyrins other than PP have an effect similar to PP on lipid peroxidation. In the present study, we used several porphyrins including their dimethyl esters and metal-chelated derivatives of PP to examine their antioxidative properties.

EXPERIMENTAL

Male Wistar rats, weighing about 200 g, were fed on a commercial chow and tap water, and were fasted for about 18 h before sacrifice, but were allowed free access to water. Liver homogenates were prepared in 150 mM KCl-10 mM Tris-HCl buffer (pH 7.4).

Protoporphyrin IX (disodium salt) and protoporphyrin IX dimethyl ester (PP-Me) were kindly donated by Tokyo Tanabe Co., Ltd., Tokyo. Mesoporphyrin IX dihydrochloride (MP), mesoporphyrin IX dimethyl ester (MP-Me), hematoporphyrin (HP), hemin (Type I, bovine crystalline) (Fe-PP) and cobalt protoporphyrin IX chloride (Co-PP) were obtained from Sigma Chemical Co. Ltd., St. Louis, MO., U.S.A. Etioporphyrin I dihydrobromide (EP), deuteroporphyrin IX dimethyl ester (DP-Me), diacetyl deuteroporphyrin IX dimethyl ester (DADP-Me) and zinc protoporphyrin IX (Zn-PP) were purchased from Aldrich Chemical Co. Inc., Milwaukee, WI., U.S.A. Tin protoporphyrin IX dichloride (Sn-PP) was obtained from Porphyrin Products, Logan, UT., U.S.A. The chemical structure of the porphyrins is shown in Fig. 1. These porphyrins were dissolved in pyridine just before use. The degree of lipid peroxidation was assayed by the 2-thiobarbituric acid (TBA) method as described previously.[1] The formation of lipid peroxides was expressed in terms of TBA value (absorbance at 532 nm/ml of incubation mixture).



Porphyrins	R_1	R_2	R_3	R_4	R_5
PP	$-CH=CH_2$	$-CH=CH_2$	$-CH_2CH_2COOH$	$-CH_2CH_2COOH$	$-CH_3$
PP-Me	$-CH=CH_2$	$-CH=CH_2$	$-CH_2CH_2COOCH_3$	$-CH_2CH_2COOCH_3$	$-CH_3$
MP	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_2COOH$	$-CH_2CH_2COOH$	$-CH_3$
MP-Me	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_2COOCH_3$	$-CH_2CH_2COOCH_3$	$-CH_3$
HP	$-CH(OH)CH_3$	$-CH(OH)CH_3$	$-CH_2CH_2COOH$	$-CH_2CH_2COOH$	$-CH_3$
EP	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_3$	$-CH_2CH_3$
DP-Me	$-H$	$-H$	$-CH_2CH_2COOCH_3$	$-CH_2CH_2COOCH_3$	$-CH_3$
DADP-Me	$-COCH_3$	$-COCH_3$	$-CH_2CH_2COOCH_3$	$-CH_2CH_2COOCH_3$	$-CH_3$

Fig. 1. Chemical Structure of the Porphyrins

RESULTS AND DISCUSSION

Antioxidative effect of the porphyrins which have different side chains was examined with rat liver homogenates in the presence of Fe^{2+} and AsA. The results are shown in Fig. 2. PP showed a marked decrease of TBA value as reported previously.[1] MP and HP also suppressed lipid peroxidation. Porphyrin dimethyl esters such as PP-Me, MP-Me and DADP-Me did not decrease the value, suggesting that esterification of both 6- and 7-carboxyethyl groups of the porphyrins with methyl group abolishes the antioxidative effect of the parent compounds. DP-Me, at a high concentration of 50 μM, significantly decreased the value. EP showed no inhibitory effect.

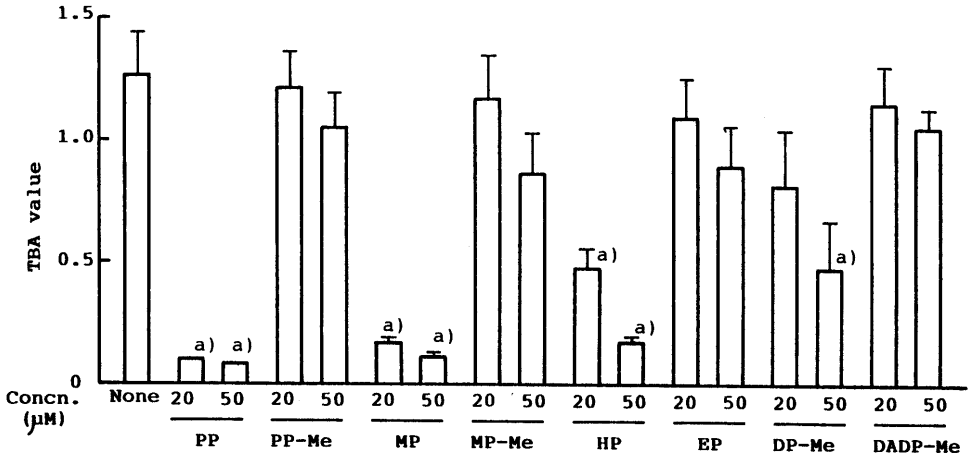


Fig. 2. Effect of Porphyrins on Lipid Peroxidation Stimulated by Fe^{2+} and AsA in Rat Liver Homogenates

Incubation mixture, consisting of 1 ml of 10% homogenate, 2.5 μM $FeSO_4$, 0.5 mM AsA, 90 mM KCl, 0.001% Tween 80, 5% (v/v) pyridine and 50 mM Tris-HCl buffer (pH 7.4) in a total volume of 2.0 ml, was incubated at 37°C for 30 min. Each bar represents the mean with S.E. (vertical bars) of 4 separate experiments. The control value without Fe^{2+} and AsA was 0.352. a) $p<0.05$.

From these results, the 6- and 7-carboxyethyl groups of the porphyrins seem to be essential for exerting their antioxidative effect but the 2- and 4- side chains do not. A weak antioxidative effect shown by DP-Me suggests that its 6- and 7-methyl carboxyethyl groups are subject to hydrolysis during incubation and that the hydrolysate may exert the effect. The weaker antioxidative effect of HP than PP or MP may be due to HP being less accessible to the membrane lipid domain, since HP has more hydrophilic side chains than PP or MP at positions 2 and 4.

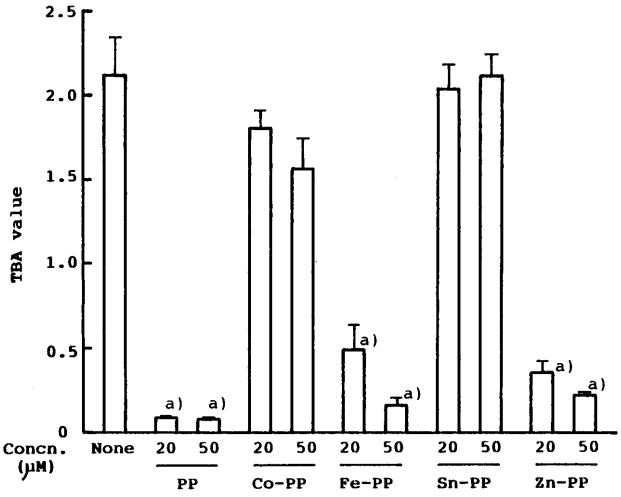


Fig. 3. Effect of Metalloprotoporphyrins on Lipid Peroxidation Stimulated by Fe^{2+} and AsA in Rat Liver Homogenates

Incubation mixture, consisting of 1 ml of 10% homogenate, 2.5 μM $FeSO_4$, 0.5 mM AsA, 90 mM KCl, 5% (v/v) pyridine and 50 mM Tris-HCl buffer (pH 7.4) in a total volume of 2.0 ml, was incubated at 37°C for 30 min. Each bar represents the mean with S.E. (vertical bars) of 3-4 separate experiments.
a) $p<0.05$.

We also examined whether some metalloprotoporphyrins inhibited lipid peroxidation. As shown in Fig. 3, Fe-PP and Zn-PP profoundly decreased the TBA value. Co-PP and Sn-PP showed no decrease in the value. In another experiment, we found that Fe^{2+} and Fe^{3+} increased the TBA value and Zn^{2+} had no effect on it (data not shown). These results indicate that the antioxidative effects of Fe-PP and Zn-PP were not brought about by the metal ions, even if they would be released from the parent porphyrins during incubation. The coordination type of Sn-PP and Co-PP is coplanar, but that of Fe-PP and Zn-PP is not.[5] These types may be involved in the antioxidative reaction.

Here, we have demonstrated that some porphyrins such as MP, HP, Fe-PP and Zn-PP as well as PP had an antioxidative effect, and in the case of metal-free porphyrins, the effect seems to be associated with 6- and 7- side chains, while metalloprotoporphyrins bearing the same side chains had different effects depending on the chelated metal species.

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