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Opposite Effects of Mn(III) and Fe(III) Forms of *meso*-Tetrakis(4-*N*-methyl pyridiniumyl) Porphyrins on Isolated Rat Liver Mitochondria

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The relevance of porphyrins as therapeutic drugs targeted to mitochondria has been widely recognized. In this work, we studied the action of *meso*-tetrakis porphyrins (TMPyP) on respiring rat liver mitochondria. Mn(III)TMPyP exerted a protective effect against lipid peroxidation induced by Fe(II) or the azo initiator 4,4′-azobis(4-cyanopentanoic acid) (ABCPA), which partition in the hydrophobic phospholipid moiety, and 2,2′-azobis(2-amidinepropane)dihydrochloride (ABAP), which partitions in the aqueous phase. In contrast, Fe(III)TMPyP itself induced an intense lipid peroxidation, accompanied by mitochondrial permeability transition. Both mesoporphyrins studied promoted a release of mitochondrial state-4 respiration, in the concentration range of 1.0–20 μM. Based on the relative effects of Mn(III)TMPyP against ABAP and ABCPA-induced lipid peroxidation, we believe that *meso*-tetrakis porphyrins must concentrate preferably at membrane–water interfaces.

KEY WORDS: Oxygen consumption; free radicals; oxidative stress; photodynamic therapy.

INTRODUCTION

The relevance of porphyrins and their related compounds as therapeutic drugs and targeting agents has been widely recognized (Richelli, 1995). However, biological effects of porphyrin derivatives vary according to their physicochemical properties. Highly hydrophobic porphyrins can penetrate in the lipid regions of membranes, while moderately hydrophobic ones are distributed into polar environments of the cell (Richelli *et al.*, 1993). Highly polar species exclusively partition into the aqueous compartments and their binding to cellular membranes is relatively poor (Richelli *et al.*, 1993). In spite of that, many water soluble porphyrins and their metal forms can bind to DNA (Gandini *et al.*, 1998; Sehlstedt *et al.*, 1994) and albumin, (Borissevitch *et al.*, 1997). The binding mechanisms seem to depend on the nature of the metal ion and on the size and location of substituent groups on the periphery of the porphyrin (Gandini *et al.*, 1998; Tominaga *et al.*, 1997). These porphyrin properties can be exploited as an approach to their use in photodynamic therapy (Tominaga *et al.*, 1997).

Biological membranes are important targets of photomodification by porphyrins (Shulok *et al.*, 1990), and mitochondrial membranes are the major sites of oxidative damage by cationic porphyrins such as hematoporphyrins (Richelli *et al.*, 1995). Therefore, damage to mitochondria is one of the earliest events in porphyrin photodynamic action (Hilf *et al.*, 1986), and may, subsequently, activate apoptosis, promoting tumor regression (Chatterjee *et al.*, 1997; Zaidi *et al.*, 1993).

In the present work, we studied the effects of water-soluble *meso*-tetrakis(4-*N*-methyl pyridiniumyl) porphyrin on mitochondrial physiology, either as a free

Key to abbreviations: ABAP, 2,2′-azobis(2-amidinopropane)dihydrochloride; ABCPA, 4,4′-azobis(4-cyanopentanoic acid); BHT, butylhydroxytoluene; CsA, cyclosporin A; DTT, dithiothreitol; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; TMPD, tetramethyl-*p*-phenalinediamine; TBARS, thiobarbituric acid reactive substances; TMPyP, *meso*-tetrakis(4-*N*-methyl pyridiniumyl) porphyrin.

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base (TMPyP) or in Fe(III)TMPyP and Mn(III)TMPyP forms. Experiments were designed to investigate whether the type of central metal atom and the substituent groups could alter the effects of water-soluble porphyrins on mitochondrial respiration, oxidative phosphorylation, membrane permeability, and lipid peroxidation in the dark. The location of these porphyrins in the mitochondrial membrane was approached by experiments analyzing the antioxidant effect of the manganese porphyrin against lipid peroxidation, induced either by water or lipid soluble azo initiators 2,2′-azobis(2-amidinopropane)dihydrochloride (ABAP) or 4,4′-azobis(4-cyanopentanoic acid) (ABCPA), respectively. ABCPA yields a negatively charged radical upon thermolysis at neutral pH. Thus, a neutral 4-cyano pentano-4-yl radical, or its corresponding peroxyl radical, should be thermodynamically more suitable to partition into the hydrophobic interior to initiate lipid peroxidation (Hanlon and Seybert, 1997). For this reason, ABCPA initiates peroxidation in phospholipid membranes in an efficient manner, while ABAP produces peroxidation through radical formation in the aqueous medium.

MATERIAL AND METHODS

Isolation of Rat Liver Mitochondria

Mitochondria were isolated by conventional differential centrifugation from the livers of adult Wistar rats overnight (Scheneider and Hogeboom, 1951). The homogenate was prepared in 250 mM sucrose, 1.0 mM EGTA, and 5.0 mM Hepes buffer, pH 7.2. The mitochondrial suspension was washed twice in the same medium containing 0.1 mM EGTA, and the final pellet was diluted in 250 mM sucrose to a protein concentration of 80–100 mg/mL. The protein concentration determined by the biuret method modified by the addition of cholate (Kaplan and Pedersen, 1983).

Standard Incubation Conditions

The experiments were performed in standard medium containing 250 mM sucrose, 10 mM Hepes pH 7.2 and oxidizing a cocktail of NAD-linked substrates at 5 mM (malate, pyruvate, α-ketoglutarate). For lipid peroxidation experiments, osmotic support was provided by 120 mM KCl, 2 mM phosphate and 10 mM Hepes, pH 7.2, to avoid sucrose interference with the method to estimate lipid peroxidation. Oxygen consumption experiments were performed in standard medium containing 250 mM sucrose, 10 mM Hepes pH 7.2, 2 mM phosphate, 1 mM $MgCl_2$, and 0.5 mM EGTA. Other additions are indicated in the figure legends.

Oxygen Uptake Measurements

Oxygen consumption was measured using a Clark-type electrode in a 1.3 mL glass chamber equipped with a magnetic stirrer.

Estimation of Lipid Peroxidation

Mitochondria (1 mg/mL) were added to 1 mL of medium, immediately followed by porphyrin additions under the conditions described in the figure legends. The samples were incubated for 20 min, with constant shaking, in an open chamber. After this incubation period, 0.1 mL of 50 μM BHT was added to prevent further lipid oxidation, and the samples were treated with 3 mL of 0.04 M H_2SO_4 and 2 mL of 0.8% thiobarbituric acid (TBA) in 0.1 M NaOH. The samples were boiled for 45 min at 100°C, and after cooling, 4.0 mL of n-butanol were added. The suspension was thoroughly mixed and centrifuged at 900 $\times$ g for 10 min. TBA-reactive substances (TBARS) were determined spectroscopically in the organic layer, at 535 nm. TBARS content was calculated from a standard curve of 1,1,3,3-tetraetoxypropane (Yagi, 1976).

Generation of Peroxyl Radicals

Peroxyl radicals were generated at a controlled rate by the thermal decomposition of the azo initiators 4,4′-azobis(4-cyanopentanoic acid) (ABCPA) and 2,2′-azobis(2-amidinepropane dihydrochloride) (ABAP) (Hanlon and Seybert, 1997). The azo initiator ABCPA was prepared as a concentrated stock solution in methanol, maintaining the final methanol concentration below 2% (v/v). The azo initiator ABAP was prepared in aqueous buffer solution, pH 7.0. Oxidation induced by both azo initiators (10 mM) was conducted for varying times, as indicated in the Table (Hanlon and Seybert, 1997).

Determination of Mitochondrial Swelling

Mitochondrial swelling was estimated from the decrease in the absorbance of the mitochondrial suspension at 520 nm measured in a DW-2000 spectrophotometer. This technique generates a light scattering variable β, which normalizes reciprocal absorbance for mitochondrial protein concentration, P (milligrams/mL), according to the formula:

$$\beta = P/\mathrm{Os}(A^{-1} - a)$$

where a is a machine constant and Os (equal to 0.5 mg/mL) is a constant introduced to make β dimensionless (Beaves and Vercesi, 1992).

Chemicals

All chemicals were purchased from Sigma Chemical Co. Porphyrins were obtained from Midcentury Chemicals.

RESULTS

Effect of Mesoporphyrins on Mitochondrial Respiration

The water-soluble cationic mesoporphyrins used in the present work exhibited different effects on rat liver mitochondria, depending on the presence and nature of the peripheral metal. In the concentration range from 1.0 to 20 μM a progressive release of state-4 (nonphosphorylating) respiration was observed (Table I). The strongest uncoupling effect was observed with the iron porphyrin, Fe(III)TMPyP. On the other hand, no effect on uncoupled respiration was observed when the mitochondrial respiration was supported by different respiratory substrates such as the NAD-linked substrates, succinate or TMPD/ascorbate (Table II). When the respiration was supported by TMPD/ascorbate, Fe(III)TMPyP promoted a significant increase in oxygen consumption possibly due to the iron-center reduction by ascorbate since a corresponding oxygen consumption from the reaction medium was observed in the absence of mitochondria (results not shown).

Table I. Effect of Porphyrins on Mitochondrial Respiration

Porphyrins (μM)	R.C.	State III[a]	State IV[a]
TMPyP			
—	5.1 ± 0.4	154 ± 4	30 ± 3
1.0	4.9 ± 0.3	154 ± 5	31 ± 5
2.5	4.5 ± 0.5	156 ± 6	35 ± 4
5.0	4.3 ± 0.5	165 ± 5	38 ± 3
7.5	4.4 ± 0.4	165 ± 6	38 ± 4
10.0	4.0 ± 0.5	166 ± 7	41 ± 3
20.0	3.5 ± 0.5	168 ± 7	48 ± 3
Fe(III)TMPyP			
—	5.1 ± 0.3	158 ± 5	31 ± 4
1.0	5.0 ± 0.4	163 ± 5	32 ± 3
2.5	4.9 ± 0.3	171 ± 5	35 ± 3
5.0	4.8 ± 0.4	178 ± 4	37 ± 3
7.5	4.0 ± 0.4	181 ± 4	45 ± 4
10.0	3.0 ± 0.6	181 ± 9	60 ± 6
20.0	1.9 ± 0.5	175 ± 8	90 ± 5
MN(III)TMPyP			
—	5.2 ± 0.3	158 ± 4	30 ± 3
1.0	5.2 ± 0.4	162 ± 4	31 ± 4
2.5	5.3 ± 0.5	173 ± 5	33 ± 5
5.0	5.0 ± 0.4	174 ± 5	34 ± 6
7.5	4.9 ± 0.3	176 ± 3	37 ± 4
10.0	4.6 ± 0.4	179 ± 4	39 ± 3
20.0	4.5 ± 0.5	180 ± 5	40 ± 3

Note. Rat liver mitochondria (0.5 mg/mL) were added to the standard medium containing 5 mM substrate cocktail (malate, pyruvate, α-ketoglutarate), in the presence of different porphyrins concentrations, as shown. State III respiration was initiated through the additon of ADP (200 nmoles/mg protein). The oxygen consumption was indicated as ng atom oxygen/(mg min). R.C. indicate the respiratory controls. Data values are average of $n = 3$ experiments ± SD.
[a]Values are in ng atom oxygen/mg min.

Table II. Lack Effect of Porphyrins on Oxygen Consumption by Uncoupled Mitochondria Respiring on NAD-Linked Substrates, Succinate, or TMPD/Ascorbate

Porphyrins (μM)	Substrate cocktail (5 mM)	Succinate (5 mM)	TMPD/ascrobate (0.1 mM/1 mM)
TMPyP			
—	100 ± 5	162 ± 4	177 ± 6
5.0	101 ± 4	163 ± 6	180 ± 6
10.0	102 ± 4	164 ± 5	182 ± 5
20.0	107 ± 3	164 ± 4	183 ± 4
Mn(III) TMPyP			
—	104 ± 6	165 ± 4	183 ± 5
5.0	105 ± 7	166 ± 5	186 ± 4
10.0	107 ± 5	167 ± 6	189 ± 3
20.0	106 ± 3	166 ± 4	190 ± 5
Fe(III)TMPyP			
—	108 ± 5	165 ± 5	175 ± 3
5.0	105 ± 6	169 ± 6	**
10.0	105 ± 4	168 ± 5	**
20.0	119 ± 3	169 ± 6	**

Note. Rat liver mitochondria (0.5 mg/mL) were incubated in a standard medium in the presence of 0.5 μM FCCP, respiratory substrates and porphyrins, as shown. Substrate cocktail includes 5 mM malate, 5 mM pyruvate, 5 mM α-ketoglutarate. The oxygen consumption was indicated as ng atom oxygen (mg min). Data values are average of $n = 3$ experiments ± SD.
**Fast oxygen consumption due to iron-center reduction by ascorbate.

Pro- and Antioxidant Actions of Metalloporphyrins on Isolated Rat Liver Mitochondria

We estimated the ability of the metalloporphyrins and the free base porphyrin (Fig. 1) to promote membrane lipid peroxidation by monitoring thiobarbituric acid reactive species (TBARS) formation. As shown in Fig. 2, Fe(III)TMPyP exerted a powerful pro-oxidant effect on mitochondrial suspensions, whereas Mn(III)TMPyP and free base TMPyP did not promote any effect.

In view of these results and taking into account literature data (Bloodsworth *et al.*, 2000; Castilho *et al.*, 1999) our next goal was to verify if Mn(III)TMPyP can prevent lipid oxidation (Day *et al.* 1999; Toyokuni, 1996) in mitochondrial suspensions submitted to iron salts (Nepomuceno *et al.*, 1997). Our results (Fig. 3) indicated

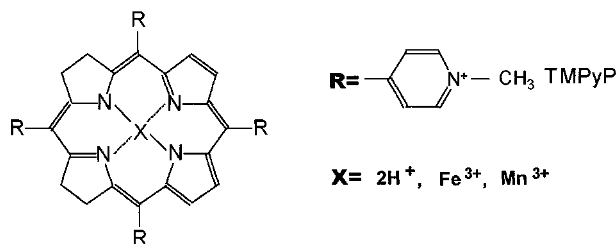

Fig. 1. Porphyrin structures—Chemical structure porphyrin as free base, or Mn(III) and Fe(III) forms.

that Mn(III)TMPyP, but not TMPyP, exerted a protective effect against lipid peroxidation induced by $FeSO_4$, with an IC_{50} close to 30 μM.

Mitochondrial Permeability Transition (MPT) in the Presence of Ca^{2+} and Fe(III)TMPyP

MPT is a nonselective permeabilization of the inner mitochondrial membrane typically promoted by the accumulation of excessive quantities of Ca^{2+} ions and stimulated by a variety of compounds or conditions (for a review see Gunter and Pfeiffer, 1990). The inner membrane permeabilization caused by MPT results in loss of matrix components, impairment of mitochondrial functionality and substantial swelling of the organelle, with consequent outer membrane rupture and cytochrome *c* release (Green and Reed, 1998; Kowaltowski and Vercesi, 1999; Zoratti and Szabò, 1995).

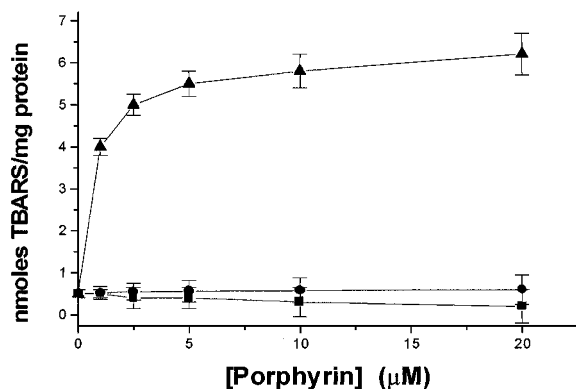

Fig. 2. Mitochondrial lipid peroxidation induced by Fe(III)TMPyP, but not Mn(III)TMPyP or TMPyP. Rat liver mitochondria (1 mg/mL) were incubated in standard medium supplemented with substrate cocktail (5 mM malate, pyruvate, α-ketoglutarate) in the presence of (Δ) Fe(III)TMPyP, (●) TMPyP, or (■) Mn(III)TMPyP, at the concentrations shown. TBARS content was determined as described in Materials and Methods. Data values are average of $n = 4$ experiments ± SD as shown by bars.

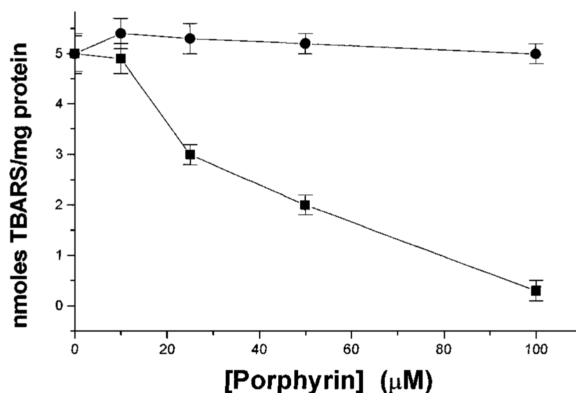

Fig. 3. Inhibition of $FeSO_4$ induced mitochondrial lipid peroxidation by Mn(III)TMPyP. Rat liver mitochondria (1 mg/mL) were incubated in the standard medium supplemented with substrate cocktail (5 mM malate, pyruvate, α-ketoglutarate) plus 0.5 mM $FeSO_4$ and (■) Mn(III)TMPyP, or (●) TMPyP, at the concentrations shown. TBARS content was determined as described in Material and Methods. Data values are the average of $n = 4$ experiments ± SD as shown by bars.

In isolated mitochondria, the amount of Ca^{2+} necessary to induce membrane permeability transition (Gunter and Pfeiffer, 1990) varies widely with experimental conditions and is largely reduced in the presence of "inducers" such as organic hydroperoxides, phosphate, and dithiol reagents (Gunter and Pfeiffer, 1990; Kowaltowski and Vercesi, 1999). The participation of reactive oxygen in this mechanism has also been demonstrated (Kowaltowski *et al.*, 1998, in press; Vercesi *et al.*, 1997). Since Fe(III)TMPyP showed a powerful oxidant activity on mitochondrial suspensions, its possible ability to act as a permeability transition "inducer" was analyzed in the swelling experiments depicted in Fig. 4. We observed that the iron (but not the manganese) porphyrin (5–10 μM) induced large amplitude mitochondrial swelling sensitive to the permeability transition inhibitors EGTA, cyclosporin A (CsA), dithiothretiol (DTT), and the radical scavenger butylhydroxytoluene (BHT).

Protective Effect of Mn(III)TMPyP Against Lipid Peroxidation Induced by Azo Initiators

The aim of the following experiments was to analyze whether the cationic manganese porphyrin was able to act as an antioxidant with different azo initiators which partition either in the aqueous phase (ABAP) or in the hydrophobic phospholipid moiety (ABPCA).

The results shown in Table III demonstrate that both azo initiators stimulated lipid peroxidation, and that Mn(III)TMPyP protected against this lipid oxidation. Protection was more pronounced against ABAP-induced lipid

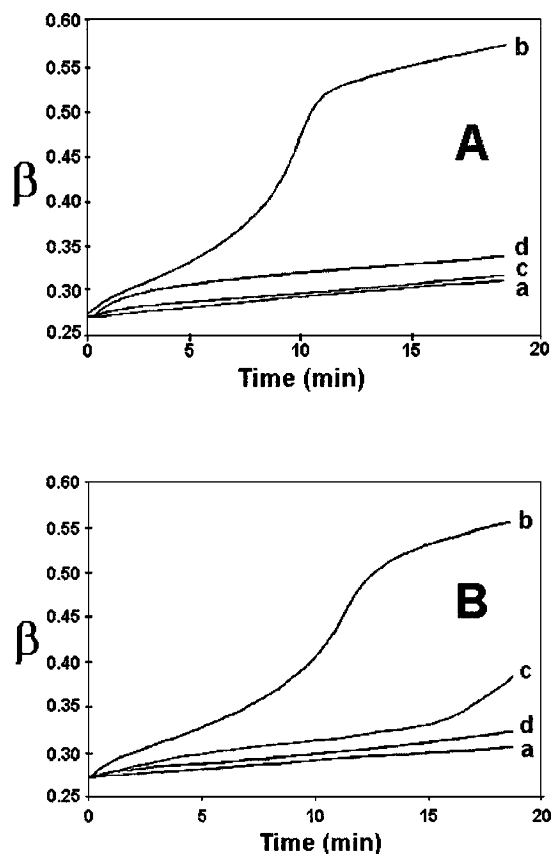

Fig. 4. Mitochondrial swelling induced by Fe(III)TMPyP in the presence of Ca^{2+}. Rat liver mitochondria (0.5 mg/mL) were added to standard medium with substrate cocktail (5 mM malate, pyruvate, α-ketoglutarate). After 2 min Ca^{2+} was added. Panel A: (a) 60 μM Ca^{2+}; (b) 10 μM Fe(III)TMPyP plus 60 μM Ca^{2+}; (c) 10 μM Fe(III) plus 60 μM Ca^{2+} and 0.5 mM EGTA; (d) 10 μM Fe(III) plus 60 μM Ca^{2+} and 2 μM CsA. Panel B: (a) 60 μM Ca^{2+}; (b) 10 μM Fe(III)TMPyP plus 60 μM Ca^{2+}; (c) 10 μM Fe(III) TMPyP plus 60 μM Ca^{2+} and 2 mM DTT; (d) 10 μM Fe(III) plus 60 μM Ca^{2+} and 200 μM BHT.

Table III. Mn(III)TMPyP Protects Against Lipid Peroxidation Induced by the Azo Initiators 2,2′-Azobis(2-amidinopropane)dihydrochloride (ABAP) or 4′,4-Azobis(4-cyanopentanoic acid) (ABCPA)

	Mn(III)TMPyP			
Time (min)	0 μM[a]	10 μM	35 μM	50 μM
0				
ABAP	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
ABCPA	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.2
30				
ABAP	1.1 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2
ABCPA	1.6 ± 0.1	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
60				
ABAP	1.7 ± 0.2	1.1 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
ABCPA	2.0 ± 0.2	1.8 ± 0.2	1.5 ± 0.3	1.3 ± 0.3
90				
ABAP	2.0 ± 0.3	1.6 ± 0.2	0.8 ± 0.3	0.8 ± 0.2
ABCPA	2.6 ± 0.3	2.3 ± 0.2	1.8 ± 0.2	1.6 ± 0.3
120				
ABAP	2.3 ± 0.3	2.8 ± 0.4	1.0 ± 0.2	1.0 ± 0.3
ABCPA	2.8 ± 0.3	2.1 ± 0.2	2.1 ± 0.3	2.0 ± 0.2

Note. Rat liver mitochondria (1 mg/mL) were incubated in the standard medium in the presence of 5 mM substrate cocktail (malate, pyruvate, α-ketoglutarate) containing 10 mM ABAP or ABCPA in the presence of Mn(III)TMPyP, at the concentrations indicated. Values are mean ± SD from four experiments.
[a]control; the numbers express nmol TBARS/mg protein/mL.

oxidation, suggesting that Mn(III)TMPyP is more effective in preventing lipid oxidation initiated in the water-soluble environment. It should be mentioned that in the absence of the azo initiators no significant production of TBARS occurred during the period of 2 h.

DISCUSSION

Previous studies have shown that a series of porphyrins promote mitochondrial dysfunction, rendering them useful drugs for photodynamic therapy (Atlante *et al.*, 1986; Salet *et al.*, 1991; Sanderberg and Romslo, 1980). However, the current drugs available for photochemotherapy present many deficiencies, including toxicity and low specificity (Chatterjee *et al.*, 1997). Therefore, it is very important to search for new compounds and cellular targets that could be exploited for rational development of improved photodynamic therapies. The recent characterization of mechanisms of cell death either by apoptosis or necrosis medicated by mitochondria (Kroemer *et al.*, 1995) pinpointed these organelles as important targets for cancer therpy. In this regard, the results presented here show that the water-soluble cationic mesoporphyrin, Fe(III)TMPyP, similarly to deuteroporphyrin (Koller and Romslo, 1978, 1980), released state-4 respiration in the concentration range of 1–20 μM. Mn(III)TMPyP had a much less pronounced effect. None of these porphyrins affected uncoupled respiration, but Fe(III)TMPyP promoted both permeability transition and lipid peroxidation. In contrast, MN(III)TMPyP exerted a protective effect against lipid peroxidation induced by $FeSO_4$, ABAP, or ABCPA. TMPyP in its free base form did not present any effect, either as a pro-oxidant or as an antioxidant.

The reason for the different behavior of these porphyrin complexes on mitochondria may be that, while both iron and manganese have three accessible valence states, manganese is a more effective metal center because iron porphyrins have a stronger preference for axial coordination (Day *et al.*, 1999). Indeed, iron, but not manganese,

porphyrins react with alkyl hydroperoxides below pH 10 leading to more oxidative products (Arasasingham and Bruice, 1991). On the other hand, the antioxidant effect of Mn(III)TMPyP could be attributed to its redox cycling between the +2 and +3 state (Bloodsworth *et al.*, 2000). Therefore, the metal-centered redox chemistry could explain the opposite effects of these porphyrins. Manganese porphyrins with positive redox potentials have high SOD-like activities and are potent inhibitors of lipid peroxidation (Day *et al.*, 1999). Although Fe(III)TMPyP also possesses SOD activity and a more positive redox potential than Mn(III)TMPyP (Day *et al.*, 1999), it is possible that iron porphyrins can initiate lipid peroxidation like iron-citrate (Castilho *et al.*, 1995) or iron-ascorbate chelates (Casalino *et al.*, 1996). In addition, the mitochondrial redox system may weaken iron binding to the porphyric nucleus, favoring Fenton catalysis and promoting lipid peroxidation through metal catalyzed multiplication reactions (Ryter and Tyrrel, 2000).

Mitochondrial damage is an important event in the photodynamic action of porphyrins (Atlante *et al*., 1986; Salet *et al*., 1991). Impaired mitochondrial functions may initiate a series of biochemical and metabolic events that lead to apoptosis or necrosis (Atlante *et al.*, 1986; Chatterjee *et al.*, 1997). We found that only Fe(III)TMPyP induced mitochondrial permeability transition, a process that may trigger apoptosis or necrosis (Zaidi *et al.*, 1993). In addition to the sensitivity to EGTA, ADP, Mg^{2+}, and CsA, well-known permeability transition inhibitors, BHT a free radical scavenger, also inhibited the transition, thus indicating that reactive oxygen species generated by Fe(III)TMPyP participate in this process. This is an indication that this effect will be strongly potentiated by photostimulation, since light increases the yield of excited species by these compounds (Girotti, 1990).

With regard to the location of these porphyrin effects on mitochondria, it is well-known that many biological effects of porphyrins and metalloporphyrins depend on their interaction with membranes (Richelli *et al.*, 1993; Sehlstedt *et al.*, 1994). The interactions also depend on substituent groups bound to the porphyric nucleus. Despite the suggestion that the soluble porphyrins are partitioned mainly in the aqueous compartments (Richelli *et al.*, 1993), the aggregation of tetraphenyl porphyrin derivatives (sulfoned or not) in micelles and their high affinity for low density lipoprotein (LDL), suggest that their may be taken up by cells (Gandini *et al.*, 1999; Kongshaug *et al.*, 1989), and possibly by mitochondria. In this regard, the possible interaction of Mn(III)TMPyP with the inner mitochondrial membrane was investigated through the analysis of its antioxidant action against lipid peroxidation induced by the azo initiators ABCPA and ABAP (Hanlon and Seybert, 1997). We observed that the antioxidant activity exerted by Mn(III)TMPyP against the aqueous soluble azoinitiator ABAP is significantly higher than that induced by the lipid soluble ABCPA. This is strong evidence for the location of this porphyrin at the membrane surface, where the accessibility to radicals generated in the aqueous phase would be high.

Other experiments are in course to evaluate the interactions of cationic and anionic water-soluble mesotetrakis porphyrins with rat liver mitochondria in the dark and upon photostimulation with the aim to find compounds suitable for photodynamic therapy, that is, with more intense and localized responses.

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