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The antioxidant properties of different phthalocyanines

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ABSTRACT

Oxidative stress is involved in the etiology of several chronic diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders. From this perspective, we have evaluated the possible antioxidant capacities of five different phthalocyanines (PCs), consisting of four metallophthalocyanines (MPCs) and one simple phthalocyanine (PC) in order to explore, for the first time, the potential antioxidant activities of these compounds. Our results show that all PCs tested in this study have significant antioxidant activity in lipid peroxidation assay, providing protection from sodium nitroprusside -induced oxidative damage to supernatant from the homogenized liver, brain, e rim of mice. Compared to the non-induced control, the PCs were generally more efficient in reducing malondialdehyde levels in all assays on lipid peroxidation induced by sodium nitroprusside; the order of approximate decrease in efficiency was as follows: manganese-PC (better efficiency) > copper-PC > iron-PC > zinc-PC > PC (worst efficiency). Furthermore, the copper-PC and manganese-PC compounds exerted a significant protective effect in deoxyribose degradation assays, when employing Fe^{2+}, $Fe^{2+} + H_2O_2$, and H_2O_2 solutions. In conclusion, all PCs tested here were shown to be promising compounds for future in vivo investigations, because of their potential antioxidant activities in vitro.

1. Introduction

Phthalocyanines (PCs) are macrocyclic complexes whose π systems (bonds in which the atomic orbitals overlap in parallel, forming an electron density cloud above and below the internuclear axis) (Graham Solomons and Fryhle, 2001; Pine et al., 1982) are delocalized over an arrangement of conjugated carbon and nitrogen atoms, providing for their unique chemical and physical properties (Fig. 1) (Leznoff and Lever, 2004; Mckeown, 1998). Due to the significance of the structural component of the π system in PCs, studies on the nature of the π system and attempts to modulate it have been intensively investigated (Day et al., 1975; Svetlana et al., 1996). Many of the properties of PCs are highly dependent on the extent of intermolecular π–π stacking interactions between the planar faces of the macrocycles. PCs as metal complexes, generated by replacement of the hydrogen atoms in the central cavity, are usually called metallophthalocyanines (MPCs), and these central metal ions play a critical role in regulating the properties of MPCs (Hanack et al., 2001). Thus, since the structural arrangement of MPCs is determined by the size and location of the metal ion center, in relation to the mean plane of the aromatic PC ligand, several conformations have been described (Barthel et al., 2002).

PCs and related macrocycles are of great interest due to the variety of interesting optoelectronic and coordination properties they display (Beltrán et al., 2004; Leznoff and Lever, 2004; Mckeown, 1998; Mitzel et al., 2004), and they serve as active components in several diverse fields (Cook and Mater, 1996; Emmelius et al., 1989). The applicability of these complexes has been investigated in different areas, especially in materials science (de la Torre et al., 1998; Farren et al., 2002; Loosli et al., 2005; Mizuguchi and Matsumoto, 1999; Nazeeruddin et al., 1998; Pandey and Herman, 1998; Sies, 1985) and in therapeutic medicine (Pandey and Herman, 1998); examples include photodynamic therapy (PDT) and catalytic therapy (CT). They are also emerging modalities for

Abbreviations: PCs, phthalocyanines; MPCs, metallophthalocyanines; simple PC, 29H, 31-phthalocyanine; CuPC, copper(II) phthalocyanine; MnPC, manganese(II) phthalocyanine; ZnPC, zinc phthalocyanine; FePC, iron(II) phthalocyanine; SNP, sodium nitroprusside; PDT, photodynamic therapy; CT, catalytic therapy; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; DMSO, dimethyl sulfoxide; MDA, malondialdehyde; S1, supernatant fraction; H_2DCF-DA, 2,7-dichlorodihydrofluorescein diacetate; DCF, 2,6-dicloroindophenol sodium salt hydrate; NO, nitric oxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TCA, trichloroacetic acid; TBA, thiobarbituric acid; RS, reactive species; BSA, bovine serum albumin.

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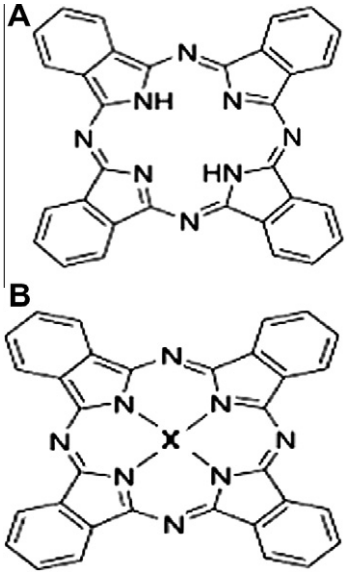

Fig. 1. The chemical structure of phthalocyanine (PC) (A). The chemical structures of MPCs [copper(II) phthalocyanine (CuPc), manganese(II) phthalocyanine (manganese-nPc), zinc phthalocyanine (zinc-Pc), iron(II) phthalocyanine (iron-PC)] were obtained by replacing X by one of the following metals: Cu^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+}, respectively (B).

the treatment of neoplastic and non neoplastic diseases such as cancer, skin disorders, and macular degeneration. Photodynamic therapy involves the administration of a photosensitizing drug (PCs) and its subsequent activation by light to produce reactive oxygen species and/or free radicals that selectively destroy target cells (Dougherty et al., 1998; Hasan et al., 2002).

Catalytic therapy (CT) is a cancer treatment modality that employs a transition metal complex as a catalyst and a second molecule as a substrate. Catalytic therapy is similar to photodynamic therapy (PDT), and is another approach to cancer treatment (Dougherty et al., 1998). This radiation-based approach for the treatment of solid malignancies involves the systemic or local administration of a photosensitizing agent (PCs), followed by irradiation with an appropriate wavelength of visible light. Photodynamic therapy has proved to be successful in the treatment of a broad range of diverse solid tumors; however, its use is limited to tissues and areas accessible to light or light-producing devices (Brown et al., 2004; Juzeniene et al., 2006; Triesscheijn et al., 2006). In contrast, CT is potentially a more versatile cancer treatment modality, which, although also based on the generation of reactive oxygen species (ROS), uses a combination of substrate molecules and a catalyst in place of light irradiation (Feofanov et al., 2000). Mechanisms underlying the antitumor action of CT are similar to X-ray therapy and PDT cancer treatments, in that CT's actions are dependent on the production of ROS, which subsequently induces oxidative degradation of critical cellular molecules and organelles (Fuchs et al., 2000; Heck et al., 2004, 2003; Plaetzer et al., 2005). However, until the present study, there have not been any studies depicting the possible antioxidant properties of the PCs.

It is important to consider that biomolecular reactions involving free radicals, and their relationship with oxidative stress, have been the subject of a multitude of scientific investigations, and this research consistently tops the list of current topics in health and medicine (Balentine, 1982; Ji, 1995). Oxidative stress is related to an imbalance between the production of reactive species and the strength of the antioxidant defenses, which can result in several impairments of cell function, culminating in cell death (Grune et al., 2001; Scott, 1997). It has been suggested that when exacerbated, oxidative stress, which is present during normal cell metabolism, is involved in the etiology of several chronic diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders (Grune et al., 2001; Scott, 1997). On the other hand, antioxidant intake has emerged as an alternative therapeutic approach for several pathological conditions related to oxidative damage in the biological systems responsible for normal cell functions (Scott, 1997; Simic and Karel, 1980).

Antioxidant defenses belong to two major groups: (1) those preventing the initiation of a peroxidative chain reaction, and (2) those slowing down the progression of a peroxidative chain reaction (Puntel et al., 2009; Simic and Karel, 1980). Research focused on the elucidation of the antioxidant and therapeutic properties of new chemical compounds have been continuously performed by our research group (de Avila et al., 2006; de Lima Portella et al., 2008; Puntel et al., 2009). Consistent with this line of research progress, we have conducted the present studies on the antioxidant potential of PCs, as well as the elucidation of the mechanisms of action of the PCs.

Thus, considering the relevance of oxidative stress in medicine in general, and the increasing interest in PCs compounds in particular, our research group is concerned with the elucidation of possible antioxidant potentials for five different PCs. To elucidate their potential use as antioxidant compounds, we have performed the present in vitro study which analyzed four MPCs and one PC.

2. Materials and methods

2.1. Drugs

Oxidant agents including hydrogen peroxide, and $FeSO_4$ were obtained from local suppliers. PCs [29H, 31-phthalocyanine (PC), copper(II) phthalocyanine (copper-PC), manganese(II) phthalocyanine (manganese-PC), zinc phthalocyanine (zinc-PC), iron(II) phthalocyanine (iron-PC)], sodium nitroprusside (SNP), the purity of each compound is respectively 98%, 97%, 90%, $\geqslant$90%, 90% and 99–102%, and other reagents were supplied by Sigma–Aldrich Chemical.

2.2. Animals

Untreated 40 adult male Swiss albino mice 50–60 days old, weighing 25–35 g, were used. These mice were obtained from our own breeding colony. The animals were maintained in an air conditioned room (20–25 °C) under a 12 h light/dark cycle, and with water and food provided ad libitum. All experimental procedures were conducted according to guidelines of the Committee of Care and Use of Experimental Animal Research of the Federal University of Santa Maria, Brazil.

2.3. Assays with tissue homogenates

2.3.1. Tissue preparation

Mice were sacrificed by cervical dislocation, and the liver, kidneys, and brain were quickly removed, placed on ice, and homogenized in 10 volumes of cold, Tris buffer (10 mM, pH 7.4). The homogenates were centrifuged at 4000×*g* at 4 °C for 10 min to yield a low-speed supernatant fraction (S1) for each tissue (liver, kidney and brain) that was used for SNP-induced lipid peroxidation and H_2DCF-DA assays.

2.3.2. SNP-induced lipid peroxidation assay

The antioxidant effect of the PCs was evaluated against production of SNP (5 μM)-induced thiobarbituric acid reactive substances (TBARS), using vehicle, dimethyl sulfoxide (DMSO), or PCs (1–100 μM). The S1 was pre-incubated for 1 h at 37 °C in a buffered medium with the PCs in the presence or absence of SNP. TBARS formation was determined spectrophotometrically at 532 nm,

using malondialdehyde (MDA) as a standard, according to Ohkawa et al. (1979). In this work we used the SNP as a mechanism of toxicity, in a concentration of 5 μM according to previously described (Puntel et al., 2009). In fact, sodium nitroprusside (SNP) is a good chemical inducer of lipid peroxidation in mice tissues (Rauhala et al., 1998), since it release in a short-lasting time NO. in tissue preparations.

2.3.3. Not-induced lipid peroxidation assay

The antioxidant effect of the PCs was evaluated against basal production of thiobarbituric acid reactive substances (TBARS), using vehicle, dimethyl sulfoxide (DMSO), or PCs (1–100 μM). The S1 was pre-incubated for 1 h at 37 °C in a buffered medium with the PCs. TBARS formation was determined spectrophotometrically at 532 nm, using malondialdehyde (MDA) as a standard, according to Ohkawa et al. (1979).

2.3.4. H_2DCF-DA assay

S1 was used for the 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) oxidation assay to evaluate levels of RS (reactive species). S1, in Tris buffer (10 mM, pH 7.4) was incubated with different PCs at concentrations of 1, 5, 10, 50, and 100 μM at 37 °C. After 1 h, aliquots were removed, H_2DCF-DA (7 μM) was added to the medium, and incubation was continued for 1 h in the dark. Fluorescence was determined using 488 nm for excitation and 520 nm for emission. A standard curve was created using increasing concentrations of 2,6-dicloroindophenol sodium salt hydrate (DCF) incubated in parallel (Pérez-Severiano et al., 2004). The results were analyzed as a percentage value in relation to the control group.

2.3.5. Protein determination

The protein content was determined according to Lowry et al. (1951), using bovine serum albumin (BSA) as a standard.

2.4. Assays without tissue homogenates

2.4.1. Nitric oxide (NO) scavenging assay

The scavenging of NO was assessed by incubating SNP (5 mM, in potassium buffer) with different PC concentrations at 25 °C. After 120 min, 0.5 mL of incubation solution was sampled and mixed with 0.5 mL of Griess reagent (Green et al., 1981), and the absorbance was measured at 550 nm. The amount of nitrite was calculated using different concentrations of sodium nitrite. A curve of sodium nitrite, constructed in the presence of the PCs in order to verify interaction with nitrite, depicted no interference of PCs with the color development after addition of Griess reagent. The values were compared to a control to determine the percentage of inhibition of nitrite reaction with Griess reagent, depicted by the PCs, as an index of the NO scavenging activity (Marcocci et al., 1994).

2.4.2. DPPH. radical scavenging activity assay

The measurement of a PC's scavenging activity against the radical (DPPH.) was performed in accordance with Choi et al. (2002). Briefly, 85 μM DPPH. was added to a medium containing different PCs concentrations. The medium was incubated for 30 min at room temperature, and the decrease in absorbance measured at 518 nm depicted the scavenging activity of the PCs against DPPH. (Puntel et al., 2009). The values are expressed as percentage of inhibition of DPPH. absorbance in relation to the control values without the PCs.

2.4.3. Deoxyribose degradation assay

The deoxyribose degradation assay was performed according to Puntel et al. (2005). Briefly, the reaction medium was prepared containing the following reagents at the final concentrations indicated: PCs (concentrations indicated in the figures), deoxyribose (3 mM) ethanol (5%), potassium phosphate buffer (0.05 mM, pH 7.4), $FeSO_4$ (50 μM), and H_2O_2 (500 μM). Solutions of $FeSO_4$ and H_2O_2 were made prior to use. Reaction mixtures were incubated at 37 °C for 30 min and stopped by the addition of 0.8 mL of trichloroacetic acid (TCA) 2.8%, followed by the addition of 0.4 mL of thiobarbituric acid (TBA) 0.6%. Next, the medium was incubated at 100 °C for 20 min and the absorbance was recorded at 532 nm (Gutteridge, 1981; Halliwell and Gutteridge, 1981). Standard curves of MDA were made for each experiment to determine the MDA generated by the deoxyribose degradation. The values are expressed as a percentage of control values (without PCs).

2.5. Statistical analysis

Statistical significance was assessed by one-way ANOVA, followed by the Student–Newman–Keuls test for post-hoc

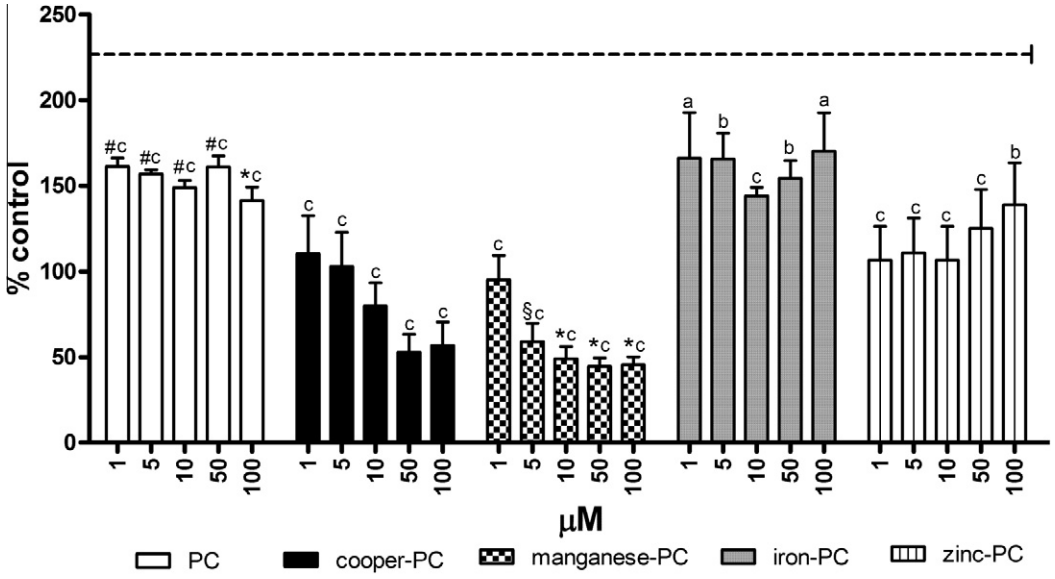

Fig. 2. Effect of PC (phthalocyanine), cooper-PC (copper(II) phthalocyanine), manganese-PC (manganese(II) phthalocyanine), iron-PC (iron(II) phthalocyanine), and zinc-PC (zinc phthalocyanine) on the SNP (sodium nitroprusside)-induced lipid peroxidation assay, in S1 from mouse liver. Values are expressed as % of control not induced, 36.6 nmol MDA/mg protein (C). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, compared to control induced (—) by SNP (5 μM). Data are presented as the mean ± S.E.M. ($n = 3$). $^{\#}p < 0.05$, $^{*}p < 0.01$, $^{\S}p < 0.001$, compared to control not induced by Student–Newman–Keuls test for post-hoc comparison.

comparison and two-way ANOVA. Results were considered statistically significant at values of $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

3.1. The chemical structures of PCs

The chemical structure of a PC is shown in Fig. 1A. The chemical structures of MPCs (copper-PC, manganese-PC, zinc-PC, and iron-PC) were obtained by replacing X with one of the following metals: Cu^{2+}, Mn^{2+}, Zn^{2+}, or Fe^{2+}, respectively (Fig. 1B).

3.2. SNP-induced lipid peroxidation assay

The PC significantly decreased the SNP-induced lipid peroxidation in liver, kidney, and brain tissues of mice at concentrations ranging from 1 to 100 μM (Figs. 2–4, respectively).

Similarly, cooper-PC (Figs. 2–4), and manganese-PC (Figs. 2–4) significantly decreased SNP-induced lipid peroxidation in liver, kidney, and brain at all tested concentrations (1–100 μM). Moreover, the manganese-PC was able to decrease the lipid peroxidation to levels lower than those of the controls, both in liver, and brain tissues (Figs. 2 and 4, respectively).

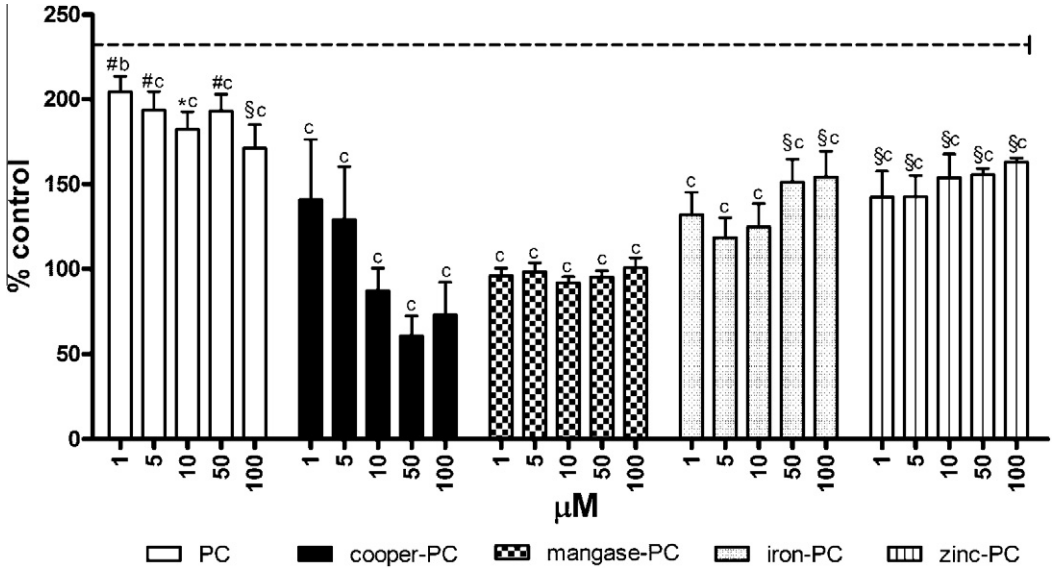

Fig. 3. Effect of PC (phthalocyanine), cooper-PC (copper (II) phthalocyanine), manganese-PC (manganese(II) phthalocyanine), iron-PC (iron(II) phthalocyanine), and zinc-PC (zinc phthalocyanine) on the SNP (sodium nitroprusside)-induced lipid peroxidation assay, in S1 from mouse kidney. Values are expressed as % of control not induced, 36.6 nmol MDA/mg protein. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, compared to control induced (—) by SNP (5 μM). Data are presented as the mean ± S.E.M. ($n = 3$). $^{\#}p < 0.05$, $^{*}p < 0.01$, $^{\S}p < 0.001$, compared to control not induced by Student–Newman–Keuls for post-hoc comparison.

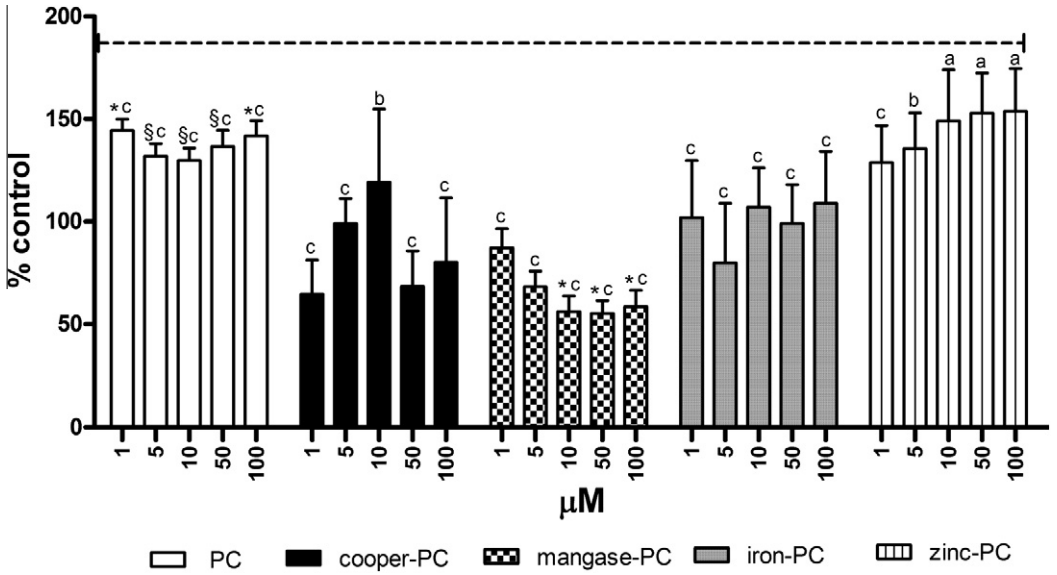

Fig. 4. Effect of PC (phthalocyanine), cooper-PC (copper(II) phthalocyanine), manganese-PC (manganese(II) phthalocyanine), iron-PC (iron(II) phthalocyanine), and zinc-PC (zinc phthalocyanine) on the SNP (sodium nitroprusside)-induced lipid peroxidation assay, in S1 from mouse brain. Values are expressed as % of control not induced, 36.6 nmol MDA/mg protein. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, compared to control induced (—) by SNP (5 μM). Data are presented as the mean ± S.E.M. ($n = 3$). $^{\#}p < 0.05$, $^{*}p < 0.01$, $^{\S}p < 0.001$, compared to control not induced by Student–Newman–Keuls test for post-hoc comparison.

The iron-PC at the same concentrations (1–100 μM) significantly decreased the SNP-induced lipid peroxidation in liver, kidney, and brain tissues of mice (Figs. 2–4, respectively) to the control levels. However, in kidney, the iron-PC at 50 and 100 μM, was not able to achieve the control levels.

The zinc-PC, at all tested concentrations, significantly decreased the SNP-induced lipid peroxidation in liver, kidney, and brain tissues of mice (Figs. 2–4 respectively) to the control levels. However, in kidney, the zinc-PC was less effective.

In the liver, manganese-PC and copper-PC induced lipid peroxidation levels that were significantly lower than that of PC at concentrations of 1, 5, 10, 50, and 100 μM (Fig. 2). Iron-PC and zinc-PC in the liver demonstrated no significant difference compared to PC at all concentrations used in this study (Fig. 2). In the liver, manganese-PC demonstrated reduction of SNP-induced lipid peroxidation levels that was lower than that of iron-PC at concentrations of 1, 5, 10, 50, and 100 μM (Fig. 2).

In addition, manganese-PC decreased the levels of lipid peroxidation in the liver at concentrations of 5, 10, 50, and 100 μM as

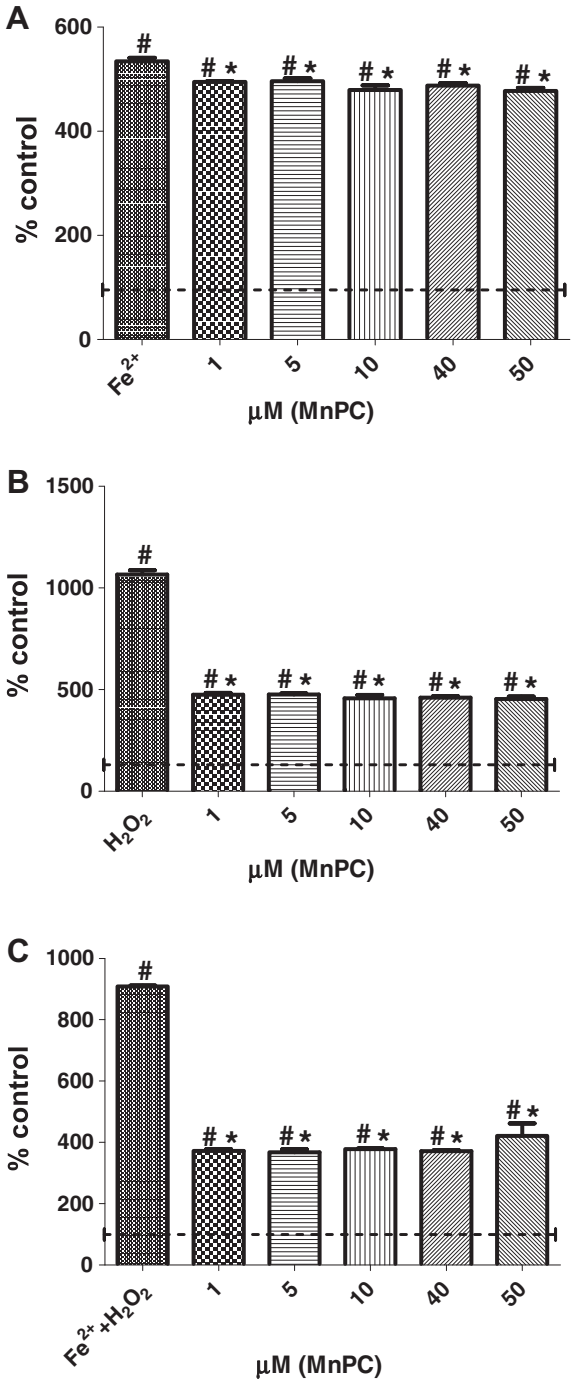

Fig. 5. Effect of manganese-Pc (manganese(II) phthalocyanine) on deoxyribose degradation assay. Values are expressed as % of control not induced, 0.94 μM/g deoxyribose (—). $^{*}p < 0.001$, compared to control induced by Fe^{2+} 50 μM (A), H_2O_2 500 μM (B), and Fe^{2+} 50 μM plus H_2O_2 500 μM (C). Data are presented as the mean ± S.E.M. ($n = 3$). $^{\#}p < 0.001$, compared to control not induced by Student–Newman–Keuls test for post-hoc comparison.

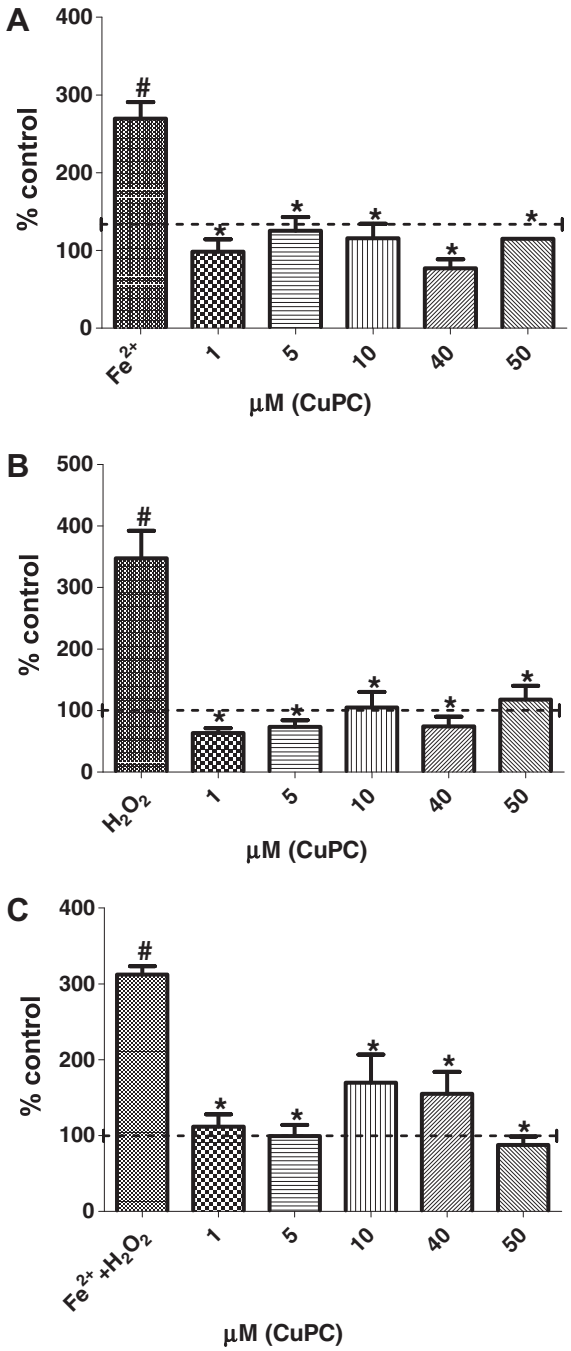

Fig. 6. Effect of cooper-Pc (copper(II) phthalocyanine) on deoxyribose degradation assay. Values are expressed as % of control not induced, 0.94 μM/g deoxyribose (—). $^{*}p < 0.001$, compared to control induced by Fe^{2+} 50 μM (A), H_2O_2 500 μM (B), and Fe^{2+} 50 μM plus H_2O_2 500 μM (C). Data are presented as the mean ± S.E.M. ($n = 3$). $^{\#}p < 0.001$, compared to control not induced by Student–Newman–Keuls test for post-hoc comparison.

compared with that of zinc-PC (Fig. 2). Copper-PC induced lower levels of lipid peroxidation in the liver at concentrations of 5, 10, 50, and 100 μM than iron-PC did (Fig. 2). In addition, copper-PC induced lower levels of lipid peroxidation in the liver at concentrations of 50 and 100 μM than zinc-PC did. There was no significant difference between copper-PC and manganese-PC in the liver at the concentrations used in this study (Fig. 2). At a concentration of 5 μM, iron-PC induced lipid peroxidation levels that were lower than that of zinc-PC (Fig. 2, $p < 0.05$).

In the kidney, PC increased levels of lipid peroxidation at concentrations of 1, 5, 10, 50, and 100 μM as compared to that of manganese-PC (Fig. 3). PC also increased levels of lipid peroxidation in the kidney at concentrations of 1 and 5 μM as compared to that of iron-PC, and demonstrated no difference compared to that of zinc-PC (Fig. 3, $p < 0.05$).

There was no significant difference between copper-PC and manganese-PC in the kidney at the concentrations used in this study (Fig. 3). In the kidney, copper-PC effected lower levels of lipid peroxidation than iron-PC did at concentrations of 50 and 100 μM (Fig. 3, $p < 0.05$).

In addition, copper-PC induced lower levels of lipid peroxidation in the kidney at concentrations of 10, 50, and 100 μM than zinc-PC did (Fig. 3). Manganese-PC induced no significant difference in the kidney in relation to that of iron-PC and zinc-PC (Fig. 3). There was no difference between iron-PC and zinc-PC (Fig. 3, $p < 0.05$).

In the brain, PC induced higher levels of lipid peroxidation compared to that of copper-PC and manganese-PC. There was no significant difference between PC compared to iron-PC and zinc-PC (Fig. 4, $p < 0.05$).

In the brain, manganese-PC effected lower levels of lipid peroxidation than zinc-PC did at concentrations of 5, 10, 50, and 100 μM, and at 10 μM compared to that of copper-PC (Fig. 4). Iron-PC resulted in no significant difference in the brain in relation to manganese-PC, zinc-PC, and copper-PC (Fig. 4). Compared to zinc-PC, copper-PC induced lower levels of lipid peroxidation in the brain at concentrations of 1, 50, and 100 μM (Fig. 4, $p < 0.05$).

3.3. Not-induced lipid peroxidation assay

The manganese-PC (Figs. 7 and 8) significantly decreased the basal lipid peroxidation in liver and brain at all tested concentrations (1–100 μM). Moreover, the manganese-PC was able to decrease the lipid peroxidation to levels lower than those of the controls, both in liver, and brain tissues (Figs. 7 and 8, respectively).

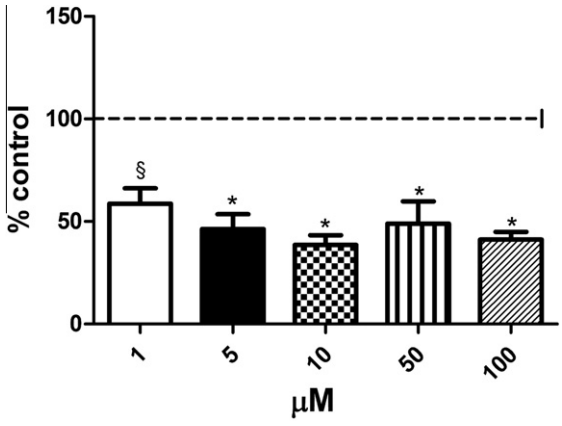

Fig. 7. Effect of manganese-PC (manganese(II) phthalocyanine) on the not-induced lipid peroxidation assay, in S1 from mouse liver. Values are expressed as % of control (—), 36.6 nmol MDA/mg protein. Data are presented as the mean ± S.E.M. ($n = 3$). $^{*}p < 0.01$, $^{§}p < 0.001$, compared to control by Student–Newman–Keuls test for post-hoc comparison.

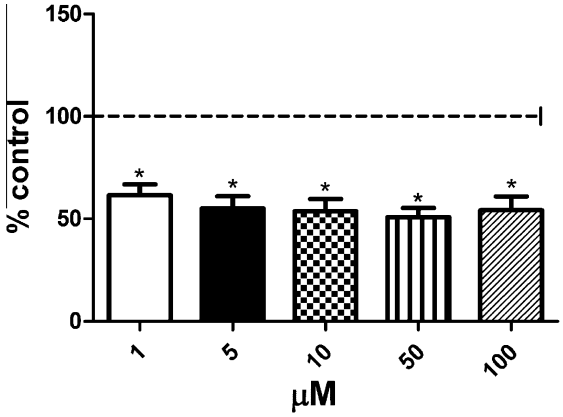

Fig. 8. Effect of manganese-PC (manganese(II) phthalocyanine) on the not-induced lipid peroxidation assay, in S1 from mouse brain. Values are expressed as % of control (—), 36.6 nmol MDA/mg protein. Data are presented as the mean ± S.E.M. ($n = 3$). $^{*}p < 0.01$, compared to control by Student–Newman–Keuls test for post-hoc comparison.

The PC, copper-PC, Zinc-PC, and iron-PC did not show any antioxidant effects in basal-lipid peroxidation (data not shown).

3.4. H_2DCF-DA, nitric oxide (NO) scavenging and DPPH. radical scavenging activity assays

The PC and MPCs did not show any antioxidant effects in tests involving H2DCF-DA, nitric oxide (NO) scavenging and DPPH. radical scavenging activities (data not shown).

3.5. Deoxyribose degradation assay

We evaluated the effect of manganese-PC and cooper-PC in the assay for degradation of deoxyribose, because these two compounds showed better results when tested in SNP-induced lipid peroxidation, compared to PC, zinc-PC, and iron-PC.

The manganese-PC (1–50 μM) significantly decreased the deoxyribose degradation induced by H_2O_2 (Fig. 5B), however it was less able to reduce the Fe-induced deoxyribose degradation (Fig. 5A). Additionally, the manganese-PC effect against $Fe^{2+} + H_2O_2$-induced deoxyribose degradation (Fig. 5C) was at the same magnitude as seen for Fe^{2+} alone, indicating that manganese-PC interferes with H_2O_2 without affecting Fe^{2+} chemistry.

In contrast, the copper-PC (1–50 μM) significantly decreased the deoxyribose degradation induced by Fe^{2+} or H_2O_2 alone, however, it showed no additional protective effect in the Fenton reaction ($Fe^{2+} + H_2O_2$) (Figs. 6A–C, respectively).

4. Discussion

In the current study, our research group investigated and clarified the antioxidant properties of four different MPCs and a PC, because of the relevance of these compounds in the contexts of oxidative stress, disease etiology, and for the progress of medicine (Balentine, 1982; Ji, 1995). The experiments performed in this study revealed a significant antioxidant capacity of PCs against lipid peroxidation induced by SNP in all tested tissues (Figs. 2–4). Results from the present study showed more significant antioxidant effects in trials using cooper-PC and manganese-PC (Figs. 2–4, respectively). Additionally, lipid peroxidation assays revealed that iron-PC and zinc-PC have less significant antioxidant effects in kidney samples (Fig. 3, respectively) compared with samples

of liver and brain (Figs. 2 and 4, respectively). Thus, we believe that some chemical change should have occurred in the extruded iron-PC and zinc-PC complexes, due to biological metabolism of the kidney enzymes, by mechanisms not yet known.

On the other hand, the PC and the MPCs did not prevent RS formation, as indicated by the H_2DCF-DA assay, at all concentrations tested (data not shown). This particular assay corresponds to a nonspecific assay, where generated RS oxidizes H2DCF-DA, resulting in the generation of a fluorescent sub-product (Pérez-Severiano et al., 2004), whose production was not prevented by the presence of a PC, or by the presence of the various MPCs employed in the study. Nevertheless, manganese-PC and cooper-PC showed antioxidant effects in the deoxyribose degradation assay (Figs. 5 and 6, respectively), thereby justifying the continuation of this study after the antioxidant results against lipid peroxidation induced by SNP were determined (Figs. 2–4).

Similar to the findings in the SNP-induced lipid peroxidation, manganese-PC and cooper-PC decreased oxidative stress induced by solutions of Fe^{2+}, H_2O_2, and Fe^{2+} + H_2O_2 in the deoxyribose degradation assay (Fig. 5A–C, and Fig. 6A–C, respectively). However, manganese-PC did not show antioxidant effects as expressive as those found in SNP-induced lipid peroxidation (Figs. 2–4), although it had a significant antioxidant effect in the deoxyribose degradation assay (Fig. 5). We believe that further studies are necessary to understand this matter.

In addition, the PC and the MPCs had no effect in the DPPH**.** and nitric oxide (NO) radical scavenging activity assays, (data not shown), which excludes the possibility that these PC compounds possess scavenging activity against the biologically relevant radicals, NO and DPPH**.**. Thus, we suggest that the PC and the MPCs may be acting by avoiding the generation of free radicals, or blocking the oxidative action of free radicals against the lipids present in the cells from biological samples, or against the deoxyribose moiety. Furthermore, these PCs compounds can act by directly degrading hydroperoxides such as H_2O_2, which is a common mechanism used by antioxidants (Scott, 1997; Simic and Karel, 1980).

We believe that the mechanism of action of the PC and the MPCs is deeply related to resonance that occurs in the π system located in these structures (Leznoff and Lever, 2004; Mckeown, 1998) (Fig. 1A and B). The π systems in these compounds correspond to bonds in which atomic orbitals overlap in parallel, comprising an electron density cloud above and below the internuclear axis, for example, as in the 2p orbital of nitrogen and d orbital of metal, called a pπ–dπ bond (Lee, 1999).

Thus, due to the fact that the cooper-PC and the manganese-PC showed better antioxidant effects against lipid peroxidation induced by SNP (Figs. 2–4) compared to the PC, the iron-PC and the zinc-PC (Figs. 2–4, respectively), we hypothesize here that the relative effects found are due to a total resonance around the ring, resulting in a twist of the coordination of the metal center, thus making a bridge between the opposite sides of the structure in the MPCs. Moreover, the difference in results of one metal complex compared to another, must be a consequence of a better resonance with Cu^{2+} and Mn^{2+} in comparison to Fe^{2+} and Zn^{2+} present in the other MPC structures (Barthel et al., 2002; Day et al., 1975; Hanack et al., 2001; Leznoff and Lever, 2004; Mckeown, 1998; Svetlana et al., 1996).

Manganese-PC displayed a significant antioxidant effect per se to reduce the basal levels of lipid peroxidation in the liver and brain, which confirms the results of the SNP-lipid peroxidation assay. We can deduce that manganese-PC not only reversed the SNP-induced lipid peroxidation but also act to prevent possible oxidative stress because it was able to decrease the basal levels of oxidative stress (Figs. 7 and 8, respectively).

Comparative analysis of manganese-PC and copper-PC in the liver demonstrated a statistically similar effect in preventing lipid peroxidation induced by SNP (Fig. 2). On the other hand, manganese-PC and copper-PC demonstrated better antioxidant activity than copper-PC, zinc-PC, and PC did in the liver, indicating that manganese-PC and copper-PC possess a better antioxidant mechanism for the prevention of SNP-induced lipid peroxidation (Fig. 2).

Copper-PC and zinc-PC in the liver presented very similar results and were superior to PC; together with the results of the other PC compounds, they support the existence of an antioxidant mechanism strongly reliant on the presence of metals in PC structure (Fig. 2).

Manganese-PC demonstrated an antioxidant activity similar to that of copper-PC, iron-PC, and zinc-PC in the liver (Fig. 3). On the other hand, copper-PC presented an antioxidant activity, prevention of lipid peroxidation, higher than that detected with the other PCs (Fig. 3). This indicates that the structure of copper PCs plays a key role in the reversal of renal cell lipid peroxidation (Fig. 3).

Copper-PC in the brain demonstrated a better antioxidant effect than PC and zinc-PC did in preventing SNP-induced lipid peroxidation (Fig. 4). In addition, manganese-PC in the brain yielded better results than zinc-PC did in the prevention of lipid peroxidation (Fig. 4). Other comparisons between PCs in the brain presented similar results, demonstrating that copper-PC and manganese-PC effected better antioxidant activities in brain structures than other PCs did, which is probably related to the presence of copper and manganese in the structure of the PCs (Fig. 4).

5. Conclusion

In conclusion, we believe that the PC and MPCs tested in this investigation deserve further attention as to their probable importance as antioxidants, especially due to the results obtained in assays of lipid peroxidation induced by SNP, lipid peroxidation not-induced and also due to the results of the deoxyribose degradation assay. In addition, our research group believes that cooper-PC and manganese-PC have promising antioxidant potentials, as evidenced by the positive effects observed in comparison to the other metal complexes tested in our assays.

6. Conflict of interest statement

The authors declare that there are no conflicts of interest.

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