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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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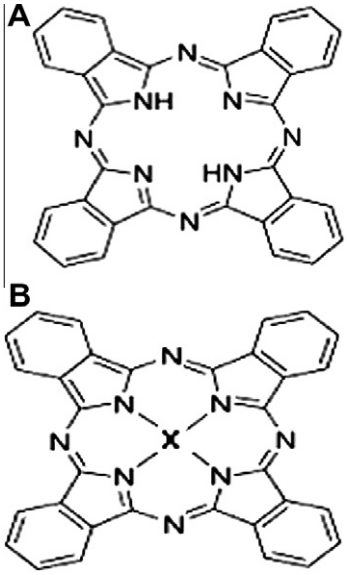

Fig. 1. The chemical structure of phthalocyanine (PC) (A). The chemical structures of MPCs [copper(II) phthalocyanine (CuPc), manganese(II) phthalocyanine (manganese-nPc), zinc phthalocyanine (zinc-Pc), iron(II) phthalocyanine (iron-PC)] were obtained by replacing X by one of the following metals: Cu^{2+},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