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Interaction of photosensitizers with liposomes containing unsaturated lipid

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Abstract

Small unilamellar liposomes were made of dipalmitoyl-phosphatidylcholine and dioleoyl-phosphatidylcholine, and photosensitized by a symmetrically or an asymmetrically substituted glycosilated tetraphenyl-porphyrin derivative. As differential scanning calorimetry and electron paramagnetic resonance spectroscopy (EPR) revealed these porphyrin derivatives were localized in different depth within the lipid bilayer. Both porphyrin derivatives were able to induce photoreaction and consequent structural changes in the membrane. 5-, 12-, or 16-doxyl stearic acid labeled lipid bilayers were applied and the efficiency of photoinduced reaction was followed by the decay of their EPR signal amplitude. Light dose-dependent destruction of nitroxide radical proved to be dependent on the position of spin label. In this process the porphyrin localized in closer connection with the double bond of unsaturated fatty acid was more effective. EPR signal decay was also dependent on the unsaturated fatty acid content of the liposome and the oxygen saturation of the solvent.

Keywords: Photosensitizer; Porphyrin; Liposomes; Electron paramagnetic resonance; Spin labeling; Differential scanning calorimetry

1. Introduction

Photodynamic therapy (PDT) is the oxygen-mediated, tumoricidal combination of non-thermal power densities of visible light and non-toxic, light-absorbing chemicals. The modality is now well established as an anti-cancer therapy under investigation. First publications based on systematic experiment concerning the possible application as a therapeutic tool appeared in the early 1970s (Diamond et al., 1972; Dougherty, 1974). Since that time numerous in vitro and in vivo experiments were based on this technique (Agrez et al., 1983) and the method was already introduced into the clinical practice (Kessel, 1992; Schroder et al., 1988; Hill et al., 1990) PDT can be applied not only in tumor therapy, but also e.g., in the treatment of non-malignant skin disorders, such as psoriasis (Lui and Anderson, 1993; Nyamekye, 1996) or actinic keratosis (Leman and Morton, 2002) and in case of choroidal neovascularisation (Husain et al.,

Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocoline; DSC, differential scanning calorimetry; EPR, electron paramagnetic resonance; PDT, photodynamic therapy; PBS, phosphate-buffered saline; TGPP, 5,10,15,20-tetrakis(4-β-D-glucosylphenyl)-porphyrin; TPFP, 5,10,15-(4-β-D-galactosylphenyl),20-(2′,3′,4′,5′,6′-pentafluorophenyl)-porphyrin; TPP, tetraphenyl-porphyrin; 5-DSA, 5-doxyl stearic acid; 12-DSA, 12-doxyl stearic acid; 16-DSA, 16-doxyl stearic acid

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1996; Ladd et al., 2001) or age-related macular degeneration (Fong, 2000). It can also be used as antibacterial or antiviral agent (Wainwright, 1998; Gaspard et al., 1995).

The photodynamic effect is believed to be mediated largely, but almost certainly not exclusively, by the generation of singlet excited oxygen. This species has a short half-life and short diffusion path (Leman and Morton, 2002; Peeler and Valenzeno, 1979; Moan et al., 1979; Moan, 1990), so that primary damage is mainly expressed in the immediate vicinity of the photosensitizer molecule. Therefore, the efficiency of the photodynamic process depends strictly on the pattern of dye localization in cells and subcellular compartments (Teiten et al., 2003; Sparrow et al., 2003; Uzdensky et al., 2004).

The site of localization of photosensitizer within the cell can be various; however, membranes appear to be a primary site of photodynamic action (Ricchelli, 1995; Santus and Reyftmann, 1986; Kessel et al., 1995; Kunz and Stark, 1997; Trannoy et al., 2002). In this context the investigation of the binding of porphyrin derivatives to membrane constituents and their distribution between the membrane compartments is particularly important. A number of factors, including the physicochemical properties, hydrophilic/hydrophobic character and symmetry of the photosensitizer molecule, etc. (Kessel, 1986; Dougherty et al., 1998; Wiehe et al., 2001; Konan et al., 2003) are already known to be significant in the dye–membrane interactions. Many conclusions presented in this field are based on the data obtained on membrane model systems such as micelles or liposomes, which can mimic specific situations occurring in the cells (Dougherty, 1974; Saija et al., 1995; Yu et al., 2002).

Recently we have investigated the association of symmetrically and asymmetrically substituted tetraphenylporphyrin (TPP) derivatives to unilamellar liposomes made of saturated lipids (Csík et al., 1998; Voszka et al., 1999, 2005). As the results of fluorescence and EPR spectroscopic studies revealed the TPP derivatives were localized in different depth within the membrane.

In the present study we extend our investigation to the liposomes containing unsaturated fatty acids. The goal of this work is to establish a relationship between structure and localization of photosensitizer and the consequent oxidative damages in unsaturated membrane lipids.

Several strategies have been proposed to improve the tumor selectivity and efficiency of photosensitizers. An approach developed by several groups is to modulate the amphiphilicity of the photosensitizer (MacDonald and Dougherty, 2001). The combination of hydrophobic and hydrophilic substituents in the sensitizer structure results in an intramolecular polarity axis. This property can produce a better accumulation and a strict positioning of sensitizers at sensitive compartments of the cell (Moser et al., 1996). Structural modifications induced by glycoconjugation of the tetrapyrrole system appear as an effective means to create a balance between hydrophilicity and hydrophobicity. Recently, Zheng et al. (2001) established a quantitative structure–activity relationship for galactosylated chlorins, demonstrating that the presence and the position of the sugar substituent is crucial to photobiological activity. Following this approach, in the last decade, a series of neutral tri- and tetra-glycosylated porphyrins in which mono- or disaccharides are linked via the phenyl groups at the *meso* position of 5,10,15,20-tetraarylporphyrins were prepared and evaluated in vitro for their phototoxicity (Laville et al., 2003). It became clear that a little modification in symmetry or amphiphilicity can influence the photobiological activity of these molecules. For the better understanding of this phenomenon we investigated the strict positioning of two glycosylated porphyrin derivatives. One of them is a symmetrically substituted derivative having four carbohydrate moieties linked to the macrocycle. The other one was selected as asymmetrically substituted amphiphilic molecule with slightly different hydrophobic character due to the different ligand moieties.

Electron paramagnetic resonance (EPR) spectroscopy was used in the localization studies. Spin labeling, a frequently used method of the EPR spectroscopy can be a versatile tool to detect localization of the porphyrins or other photosensitizers along the hydrocarbon chain of the lipid components of biological or model membranes (Hadjur et al., 1997; Yang et al., 1999; Damoiseau et al., 2001). The lipids containing spin label in different positions of hydrocarbon chain reveal the site of interaction between membrane lipid components and other molecules, like porphyrins.

The structural changes of lipid bilayer induced by the incorporation and by the photoinduced reaction of porphyrin molecules were followed by EPR spectroscopy and by differential scanning calorimetry. The alterations in lipid phase transition parameters can give information about the strength of interaction between membrane lipids and other molecules interacting with them, the homogeneity or heterogeneity of the membrane structure and the energetic state of the lipid bilayer (Saija et al., 1995; Voszka et al., 1999; Biltonen and Lichtenberg, 1993; Kostecka-Gugala et al., 2003).