Journal of Photochemistry and Photobiology B: Biology 151 (2015) 142–152

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Efficiency of photodynamic therapy on WM35 melanoma with synthetic porphyrins: Role of chemical structure, intracellular targeting and antioxidant defense

Ioana Baldea [a], Diana Elena Olteanu [a,*], Pompei Bolfa [b,c], Rodica Mariana Ion [d], Nicoleta Decea [a], Mihai Cenariu [e], Manuela Banciu [f], Alina Viorica Sesarman [f], Adriana Gabriela Filip [a]

[a] University of Medicine and Pharmacy, Department of Physiology, Clinicilor 1, Cluj-Napoca, Romania
[b] University of Agricultural Sciences and Veterinary Medicine, Department of Pathology, Calea Manastur 3-5, 400372 Cluj-Napoca, Romania
[c] Ross University School of Veterinary Medicine, Department of Biomedical Sciences, PO Box 334, Basseterre, Saint Kitts and Nevis
[d] National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM, Nanomedicine Research Group, 202 Splaiul Independentei, Sector 6, Bucharest, Romania
[e] University of Agricultural Sciences and Veterinary Medicine, Department of Biochemistry, Calea Manastur 3-5, 400372 Cluj-Napoca, Romania
[f] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, Clinicilor Street 5-7, 400006 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form 23 July 2015
Accepted 28 July 2015
Available online 29 July 2015

Keywords:
Photodynamic therapy
Melanoma
Apoptosis
Melanogenesis
Oxidative stress
Meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin
Meso-5-(4-hydroxyphenyl)-10,15,20-tris (4-methoxyphenyl) porphyrin

ABSTRACT

Photodynamic therapy (PDT) could be an adjuvant therapy in melanoma, an aggressive cancer that arises from melanocytes. Several reports showed encouraging results of the efficacy of PDT in melanoma on experimental models and in clinical trials. Therefore, we studied the efficacy of two derivatives of tetraphenylporphyrin (TPP): meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin (THOPP) and mes o-5-(4-hydroxyphenyl)-10,15,20-tris (4-methoxyphenyl) porphyrin (THOMPP) as photosensitizers for PDT, compared to FDA approved delta aminolevulinic acid (ALA) against a lightly pigmented, melanoma cell line, WM35, *in vitro*. Both porphyrins were more efficient as photosensitizers, compared to ALA, without dark toxicity. The efficiency depended on the intracellular localization and the molecule structure. THOPP, the most efficient porphyrin localized mainly in mitochondria, while THOMPP accumulated in lysosomes; both showed melanosomal localization. The symmetric THOPP molecule was able to generate increased oxidative stress damage and apoptosis. THOPP also induced a low effect on the defense mechanisms like antioxidant enzyme SOD (superoxide dismutase), NF-kB (nuclear transcription factor kB) activation and MITF (microphthalmia transcription factor). The lower efficiency of the asymmetric molecule, THOMPP was probably due to a diminished photoactivation, which led to a lower ROS induced damage, combined with higher activation of the defense mechanisms.

1. Introduction

Malignant melanoma is a cancer derived from melanocytes, pigmentary cells located mainly in the epidermis. Although melanoma represents around 4% of all skin cancers, it has an aggressive behavior, leading to more than 79% of all deaths caused by skin cancers [1,2].

There are encouraging data concerning the efficacy of photodynamic therapy (PDT) in melanoma [3], both in experimental settings and clinical reports [4].

PDT is a minimally invasive two-stage procedure that requires administration of a photosensitizing agent (PS) followed by illumination of the tumor with visible light, of an appropriate wavelength, usually laser generated [5,6]. The PS molecule absorbs a light photon and becomes an excited triplet state [7,8]. The excited

Abbreviations: ALA, δ-amino levulinic acid; HPLC, high-performance liquid chromatography; ^{1}H NMR, proton nuclear magnetic resonance; DMSO, dimethyl sulfoxide; DSB, double strand brakes; EI-MS, Electron Impact Mass Spectra; FTIR spectra, Fourier transform infrared spectroscopy; MDA, malondialdehyde; MITF, microphthalmia-associated transcription factor; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetra-zolium, inner salt; NF-kB, nuclear transcription factor kB; PDT, photodynamic therapy; PI, propidium iodide; PMS, phenazine metosulphate; PS, photosensitizing agent; ROS, reactive oxygen species; SOD, superoxide dismutase; THOMPP, meso-5-(4-hydroxyphenyl)-10,15,20- tris (4-methoxy phenyl) porphyrin; THOPP, meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin; TNFα, tumor necrosis factor α.

* Corresponding author at: Department of Physiology, 1-3 Clinicilor Street, Cluj-Napoca, Romania.

E-mail addresses: baldeai@yahoo.com (I. Baldea), ariana_di@yahoo.com (D.E. Olteanu), pompei.bolfa@usamvcluj.ro (P. Bolfa), rodicaion2000@yahoo.co.uk (R.M. Ion), nicoleta_decea@yahoo.com (N. Decea), mihai.cenariu@usamvcluj.ro (M. Cenariu), manuela.banciu@ubbcluj.ro (M. Banciu), alina.sesarman@hasdeu.ubbcluj.ro (A.V. Sesarman), adrianafilip33@yahoo.com (A.G. Filip).

http://dx.doi.org/10.1016/j.jphotobiol.2015.07.019
1011-1344/

PS molecule can then transfer energy to molecular oxygen to produce singlet oxygen (type II reaction) or suffer electron transfer (type I reaction) to form superoxide radical anion and/or hydroxyl radicals [9].

These reactive oxygen species (ROS) can induce oxidative damage to cellular proteins, lipids, and nucleic acids [9], thus leading to direct tumor and vascular cell death, inside the treated tumor, which further leads to inflammation, hypoxia [4,9] and activation of an immune response [4].

Our study is concerned on testing the efficacy and biological effects of two derivatives of tetraphenylporphyrin (TPP): meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin (THOPP) and meso-5-(4-hydroxyphenyl)-10,15,20-tris (4-methoxyphenyl) porphyrin (THOMPP), compared to ALA approved for oncological therapy, against a radial growth phase melanoma cell line (WM35), *in vitro*. These porphyrins were specifically designed for use as photosensitisers in PDT, in order to overcome the melanoma resistance mechanisms to therapy like defects in the apoptotic pathways, pigmentation, sequestration of PS inside melanosomes and increased oxidative stress defense [4,10].

In this study we tested several biological effects of PDT using THOPP and THOMPP as PS in WM35 melanoma cells: the intensity of photokilling, cell death induction mechanisms like: apoptosis/necrosis ratio, the role of the inflammatory molecule, TNFα and NF-kB pathway activation, the intracellular localization of the compounds, the oxidative damage and defense mechanisms.

To explore more thoroughly the effects of the porphyrin mediated PDT in melanoma, we assessed the cell death mechanism, with focus on cell death induction. Previous reports found that both, apoptosis and necrosis were induced by PDT; however, apoptosis was the dominant form of cell death using various PS and cell types [11,12].

Several reports showed overexpression of the inflammatory molecule, tumor necrosis factor α (TNFα) following PDT in different experimental settings [4]. The roles of TNFα are variable. TNFα can trigger differentiation and apoptosis through increased ROS production and activation of the caspase cascade [13,14]. However, TNFα stimulation has also been involved in NF-kB (nuclear transcription factor kB) activation leading to survival, under oxidative stress [14,15]. In this study, we measured the TNFα secretion, caspase 3 activation and NF-kB expression, following PDT, in order to assess the influence of these pathways on the apoptotic/survival mechanism.

Oxidative damage induced by PDT was investigated by measurement of malondialdehyde (MDA), a marker of lipid peroxidation. Peroxidation of lipids is particularly destructive because the formation of lipoperoxidation products leads to a facile propagation of free radicals and membrane disintegration.

We also assessed the enzymatic activity of superoxide dismutase (SOD, EC 1.15.1.1) and hydrogen peroxide accumulation. ROS formation activates the antioxidant defense mechanisms, from which superoxide dismutase (SOD) stands out. SOD is the main free radical scavenger since it dismutes the superoxide anion radical, generating hydrogen peroxide. Thus, PDT efficiency depends on the free radicals level, and indirectly on the induction of SOD [16].

2. Materials and methods

2.1. Analytical characterization of the photosensitizers

The tested compounds, THOPP and THOMPP are derivatives of tetraphenyl porphyrins, a class of substances with good photodynamic activity and chemical stability [17,18].

2.1.1. General instrumentation

UV–visible spectra were recorded on a Carl Zeiss Jena M400 Spectrophotometer with double beam and microprocessor using MgO as reference solid powder. *The FTIR spectra* were recorded using a Perkin Elmer Spectrum GX spectrometer, by using KBr pellets and DRIFT techniques. Scans in the range of 400–4000 cm^{-1} were accumulated for each spectrum at a spectral resolution of 4 cm^{-1}. *Proton nuclear magnetic resonance* (^{1}H NMR) spectra was recorded on a Varian Gemini and a Bruker 300 MHz spectrometer at 300 MHz. Chemical shifts are reported in units of δ (from tetramethylsilane), as internal references, in $CDCl_3$, The chemical shifts are given in ppm and coupling constants in Hz in the indicated solvent. *Electron Impact Mass Spectra (EI-MS)* were taken with a mass-spectrometer Varian MAT 312 (450 °C, Tor, 70 eV) type operating in Electron Spray Ionization (ESI-MS) mode, Finnigan-Mat TSQ 700 type. The *m/z* values refer to the highest peak of the isotopic pattern ratio according to the natural abundance of the isotopes. *Elementary analysis* is done using a "2400 CHN Elemental Analyzer" by Perkin Elmer Series II. After proper calibration, the result was expressed as a weight percentage of nitrogen, carbon, hydrogen and sulfur or oxygen. All the chemicals from Aldrich and Merck (GR grade) were used without further purification. After opening the bottles, the solvents were stored over 4 Å molecule sieves.

2.1.2. Meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin (THOPP) characterization (Fig. 1)

TMOPP synthesized by the Lindsey method [19] was treated with pyridine hydrochloride, washed with hydrochloric acid, dryed over anhydrous sodium sulphate and filtered. The obtained product was purified by column chromatography on silica gel (100–200 mesh) using chloroform followed by 25% methanol in chloroform as eluent. Yield 1.2 g (88%).

^{1}*H NMR* (400 MHz, DMSO-doublet 6δ): 9.94 (singlet, 4H, ArOH), 8.86 (singlet, 8H, βH), 8.0 (doublet, 8H, ArH 2,6, *J* = 8.4 Hz), 7.21 (doublet, 8H, ArH3,5, *J* = 8.4 Hz), −2.88 (singlet, 2H, NH). The other peaks, corresponding to phenyl and pyrrole protons or to OH are resonating in low field region of 7.36–8.11 ppm (orto-and meta-H-Ph), 8.98 ppm (β-H-pyrrole), respectively to 10.01 ppm (OH).

IR (KBr): 3391, 3032, 1345 cm^{-1}. (3415 cm^{-1} is assigned to O—H stretching vibration; 3317 and 967 cm^{-1}-stretching and bending vibrations of N—H and C—N, respectively, which are the characteristic absorptions of porphyrin free base.; 1500–1600 cm^{-1} – stretching vibration of C=C in the benzene aromatic ring).

Mass spectrum (FAB) Found $(M + H)^+$ 678.253 C44H30N4O4 requires M + 1 = 678.250. Molecular ion (M+) at *m/e* value of 678; *m/e* = 307 fragment, corresponding to the double charged ion of the meso-tetraphenylporphyrin fragment; loss of water molecules and the appearance of remarkable abundant (55%) carbonylium fragments (*m/e* = 94).

UV–Vis spectrum: λ_{max}(MeOH, nm) (ε, M^{-1} cm^{-1}) 418 (143 000), 520 (10 000), 550 (9 000), 597 (5 100), and 651 (18 600).

2.1.3. Meso-5-(4-hydroxyphenyl)-10,15,20-tris (4-methoxy phenyl) porphyrin (THOMPP) characterization (Fig. 1)

THOMPP was synthesized by refluxing pyrrole, propanoic acid containing p-methoxybenzaldehyde and p-hydroxybenzaldehyde. The synthesized product was isolated and purified by column chromatography on silica gel (60–120 mesh) using hexane and ethyl acetate (2:8) as eluent. The Rf value on silica gel coated plate was found to be 0.53 in 3:7. Yield 1.082 g (78%).

^{1}*H NMR* (300 MHz in $CDCl_3$, δ in ppm): 8.84 (multiplet, 8H, pyrrole β-H), 8.01−8.12 (multiplet, 8H, phenyl o-H), 7.52 (multiplet, 8H, phenyl m-H), 4.08 (singlet, 9H, —OCH3) −2.65 (broad singlet, 2H, imino H).