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The *in-vitro* proliferation-suppression of MCF-7 and HeLa cell lines mediated by differently substituted ionic phthalocyanines in sonodynamic therapy supplemented-photodynamic therapy

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A B S T R A C T

This work focuses on the study of the effects of the ultrasonic frequency (MHz) and power (W.cm^{-2}) on the stability, reactive oxygen species yields and cytotoxicity activities of differently substituted ionic phthalocyanines (Pcs) in sonodynamic therapy (SDT). Four ultrasonic parameters were investigated: *Par I* (1 MHz: 1 W.cm^{-2}), *Par II* (1 MHz: 2 W.cm^{-2}), *Par III* (3 MHz: 1 W.cm^{-2}) and *Par IV* (3 MHz: 2 W.cm^{-2}). A higher degradation of the Pcs was observed with increasing power at the *Par II*. Two reactive oxygen species (ROS) were detected in the ultrasound treated Pcs: singlet oxygen and hydroxyl radicals. Due to minimal degradation of most Pcs, *Par I* was chosen for SDT, photodynamic therapy (PDT), and photo-sonodynamic therapy (PSDT) against Michigan Cancer Foundation-7 and Henrietta Lacks cancer cell lines. PSDT generally showed improved therapeutic efficacies of the Pcs compared to the SDT and PDT mono treatments.

1. Introduction

Photodynamic therapy (PDT) has shown promising results as an alternative anticancer modality to the conventional chemotherapy, surgery, and irradiation [1]. However, the penetrability of the light used in PDT is limited to up to 10 mm past the epidermis [2–4]. Thus, the efficient use of PDT is limited to the eradication of superficial tumours. Ultrasound has since been developed as a supplementary, and sometimes alternative to light for sensitizer activation, as it has improved tissue penetrability [4–6]. This technique is known as sonodynamic therapy (SDT). The ultrasound in SDT may also be focused within a narrow region, thus allowing for specific tumour eradication [3]. The ultrasound used in SDT has also demonstrated reduced to no harm to tissue in regions where the sensitizer has not accumulated [5]. SDT, therefore, affords a controllable and minimally invasive therapeutic technique which may be used to treat deep tissue seated tumours [4,5]. When supplementing PDT with SDT, the technique is known as photo-sonodynamic combinatorial therapy (PSDT) [7,8].

The mechanism of action in both PDT and SDT have been defined in the literature to involve the generation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and hydroxyl radicals (•OH) [1,4,8]. The ROS generated initiate cytotoxicity through oxidative stress [9,10]. For PDT, the generation of ROS using light has been well explained using the Jablonski diagram which summarizes the energy pathways from the light-absorbing sensitizers to the generation of ROS [11]. The mechanism of action in SDT is not yet clearly understood. However, a phenomenon known as acoustic cavitation has been reported [12]. The phenomenon suggests that during the ultrasonication of an aqueous medium, gas-filled bubbles form, grow and burst to emit light. This emitted light is known as sonoluminescence [13]. Nearby sensitizers may absorb the energy from the sonoluminescence, causing them to be excited.

For PSDT, sensitizers that can be activated by both light and ultrasound are favourable. In this work, phthalocyanines (Pcs) were studied as sensitizers for PSDT. Although Pcs have been applied to a much lesser extent in SDT compared to PDT, they have so far shown promising results as sensitizers for SDT through their impressive ROS yields under ultrasound exposure [9,14–16].

Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified eagle's medium; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; DPBS, Dulbecco phosphate-buffer saline; EPR, electron paramagnetic resonance; FBS, fetal bovine serum; HeLa, Henrietta Lacks; MCF-7, Michigan Cancer Foundation-7 (MCF-7); PBN, phenyl-*N-tert*-butylnitrone; PDT, photodynamic therapy; PSA, streptomycin-amphotericin-B-mixture; PSDT, photo-sonodynamic therapy; ROS, reactive oxygen species; SDT, sonodynamic therapy; TEMP, 2,2,6,6-Tetramethylpiperidine; TPP, triphenyl phosphine.

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