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Comparing the efficacy of photodynamic and sonodynamic therapy in non-melanoma and melanoma skin cancer

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ABSTRACT

Sonodynamic therapy (SDT) involves the activation of a non-toxic sensitiser drug using low-intensity ultrasound to produce cytotoxic reactive oxygen species (ROS). Given the low tissue attenuation of ultrasound, SDT provides a significant benefit over the more established photodynamic therapy (PDT) as it enables activation of sensitisers at a greater depth within human tissue. In this manuscript, we compare the efficacy of aminolevulinic acid (ALA) mediated PDT and SDT in a squamous cell carcinoma (A431) cell line as well as the ability of these treatments to reduce the size of A431 ectopic tumours in mice. Similarly, the relative cytotoxic ability of Rose Bengal mediated PDT and SDT was investigated in a B16-melanoma cell line and also in a B16 ectopic tumour model. The results reveal no statistically significant difference in efficacy between ALA mediated PDT or SDT in the non-melanoma model while Rose Bengal mediated SDT was significantly more efficacious than PDT in the melanoma model. This difference in efficacy was, at least in part, attributed to the dark pigmentation of the melanoma cells that effectively filtered the excitation light preventing it from activating the sensitiser while the use of ultrasound circumvented this problem. These results suggest SDT may provide a better outcome than PDT when treating highly pigmented cancerous skin lesions.

1. Introduction

Photodynamic therapy (PDT) is currently approved as a first line treatment for non-melanoma skin cancer and has been trialled in the treatment of other cancers such as head and neck, oesophageal, bladder and prostate.[1,2] PDT requires the presence of three distinct components before a cytotoxic effect can be observed; (i) a sensitising drug, (ii) light of an appropriate wavelength and (iii) molecular oxygen. The combination of all three components result in the generation of singlet oxygen and other reactive oxygen species (ROS), that when generated in sufficient quantities, result in cell death.[3] The attraction of PDT over other cancer therapies is that generation of the cytotoxic species can be controlled by careful positioning of the illumination source. This targeting is further enhanced when treating skin cancer as the sensitiser prodrug aminolevulinic acid (ALA) is converted to the active sensitiser protoporphyrin IX (PpIX) more effectively by cancer cells when compared to non-cancer cells. PpIX is naturally generated during the cellular heme cycle and is meticulously controlled to prevent its natural accumulation.[4] This negative feedback system is thought to be modified in cancer tissues due to enzymatic defects that lead to an increase in protoporphyrinogen IX oxidase and/or reduced activity of ferrochelatase.[5] Another advantage of PDT is the excellent cosmetic outcome observed post treatment when compared to surgery or topical chemotherapy treatment (i.e., 5-fluorouracil).[6,7] However, the limited penetration of light through mammalian tissue has restricted the use of PDT to the treatment of superficial lesions and reduces its effectiveness in treating more deeply-seated or highly pigmented lesions.[8] Indeed, while PDT is routinely used to treat superficial basal cell carcinoma (BCC), it is not indicated for the treatment of malignant melanoma.[9] The dark pigmentation associated with melanotic lesions acts as a filter for the light used to activate the majority of conventional sensitizers that absorb in the visible range of the electromagnetic spectrum.[10] To compound matters further, melanin has natural anti-oxidant properties thereby acting as a scavenger for ROS generated during PDT.[11,12] The development of near-infrared (NIR) absorbing sensitizers that possess absorption maxima in a region where melanin

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does not absorb have produced impressive results in treating melanoma, suggesting the anti-oxidant effect of melanin may be overcome when using PDT.[13–16]

Sonodynamic therapy (SDT) has recently emerged as an alternative to PDT and uses low intensity ultrasound instead of light to activate the sensitiser. This interaction of the sensitiser with an acoustic field generates ROS that result in cytotoxic effects similar to those observed in PDT.[17,18] The major benefit of using SDT instead of PDT is that ultrasound is clinically approved as a safe and effective imaging modality and unlike light can achieve penetration depths in soft tissue in the region of tens of centimetres.[19] Therefore, SDT offers the potential of treating more deeply seated solid tumours than currently possible using PDT.

In this manuscript, we compare the effectiveness of PDT and SDT at treating non-melanoma and melanoma skin cancer in preclinical models. Specifically, we determine the effect of ALA mediated PDT/SDT in treating A431 squamous cell carcinoma and Rose Bengal mediated PDT/SDT in treating B16-F10-Luc2 melanoma cells both in vitro and in vivo. The resulting efficacy of these treatments is then discussed.

2. Experimental

2.1. Materials and reagents

ALA and Rose Bengal were purchased from Aldrich at the highest grade possible. Metvix cream was purchased from Galderma. All other chemicals were purchased from commercial sources at the highest possible purity and used as received. Human epidermoid carcinoma A431 cell line was obtained from ATCC and the B16-F10-Luc2 cell line was purchased from PerkinElmer. SCID and athymic nude mice were obtained from Harlan Laboratories. In these studies all animals were treated humanely and in accordance with licensed procedures under the UK Animals (Scientific Procedures) Act 1986.

2.2. ALA mediated PDT and SDT treatment of A431 cells in vitro

A431 cells, maintained in DMEM medium supplemented with 10% (v/v) foetal bovine serum at 37 °C in a humidified 5% (v/v) CO_2 atmosphere were seeded into wells of a 96-well tissue culture plate at a concentration of 5×10^3 cells per well and incubated overnight. The medium was then removed and replaced with 100 μL of ALA solution in PBS at concentrations of 1 or 2 μM. The cells were then incubated for a further 4 h at 37 °C in a humidified 5% (v/v) CO_2 atmosphere, the ALA solution removed, the wells washed with PBS and treated with either red light using a Phototherapeutics Paterson BL1000A lamp (630 nm ± 15 nm, total dose = 5 J/cm^{-2}) or ultrasound (Sonidel SP 100 sonoporator, frequency = 1 MHz, a power density = 3.0 W cm^{-2}, 50% duty cycle (pulse repetition frequency = 100 Hz, total dose = 90 J/cm^{-2}) from the underside of the plate. The wells were washed with PBS and 100 μL aliquots of DMEM medium supplemented with 10% (v/v) foetal bovine serum were dispensed into each well. The plates were then incubated in a humidified 5% CO_2 atmosphere at 37 °C for 21 h and cell viability then determined using an MTT assay.[20] A similar procedure was repeated using ALA at the same concentrations without stimulus. Results were compared against control experiments where cells were treated with only the vehicle (i.e., PBS).

2.3. DPBF assay of PpIX and Rose Bengal

An EtOH/H_2O (50:50 v/v) (10 mL) solution was prepared containing PpIX or Rose Bengal (0.5 μM) and 1,3-diphenylisobenzofuran (DPBF) (20 μM). The solutions were then irradiated for 30 min with white light (Fenix LD01 LED, 50 mW output) or ultrasound (Sonidel SP 100 sonoporator, frequency = 1 MHz, a power density = 3.0 W cm^{-2}, 50% duty cycle, pulse repetition frequency = 100 Hz). Aliquots were taken at specific time points and the absorbance at 410 nm recorded using a Cary 50 UV–Vis spectrometer. Control experiments in the absence of drug (i.e., DPBF + stimulus) were also performed for comparative purposes.

2.4. PDT and SDT treatment of human xenograft A431 tumours in SCID mice using topical Metvix cream

A431 cells were maintained in DMEM medium supplemented with 10% foetal bovine serum. Cells were cultured at 37 °C in a humidified 5% (v/v) CO_2 atmosphere. The cells (1×10^6) were re-suspended in 100 μL of Matrigel and implanted into the rear dorsum of female SCID mice. Tumour formation occurred approximately 2 weeks after implantation and tumour measurements were taken daily using calipers. Once the tumours had reached an average volume of 205 mm^3, calculated from the geometric mean diameter using the equation, tumour volume = $4\pi R^3/3$, animals were randomly distributed into 3 groups ($n = 3$) which included (i) no stimulus group (control), (ii) a PDT treated group and (iii) a SDT treated group. Following induction of anaesthesia (intraperitoneal injection of Hypnorm/Hypnovel), a 1 mm thick layer of Metvix cream was topically applied to the tumour and 5–10 mm of the surrounding skin.[21] The residual cream was then removed 4 h later, and the tumours treated with (i) no stimulus (ii) red-light (total dose: 40 J/cm^{-2}) or ultrasound (at a frequency of 1 MHz using 3.5 W/cm^2 and a duty cycle of 30% – total dose: 220 J/cm^{-2}). After treatment, animals were allowed to recover from anaesthesia and tumour volume and body weight were recorded on a daily basis for 7 days.

2.5. PDT and SDT treatment of RIF-1, HeLa and B16 melanoma cells using Rose Bengal

B16-F10-Luc2 melanoma and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) Foetal Bovine Serum (FBS) while RIF-1 cells were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640) supplemented with 10% (v/v) FBS. All cell lines were incubated at 37 °C in a humidified 5% (v/v) CO_2 atmosphere. Cells were seeded at a concentration of 5×10^3 cells per well in 96-well tissue culture plate for 24 h. The medium was then removed from each well and replaced with 100 μL of Rose Bengal at a concentration of 0.5 μM. The cells were incubated for 3 h at 37 °C in a humidified 5% (v/v) CO_2 atmosphere. Individual wells were washed with PBS to remove excess Rose Bengal before treatment with either white light (parameters as in Section 2.3: 30 s, total dose: 11.4 J/cm^{-2} or 60 s, total dose 22.8 J/cm^{-2}) or ultrasound (parameters as in Section 2.2, 30 s, total dose: 45 J/cm^{-2} or 60 s, total dose: 90 J/cm^{-2}) for 30 or 60 s. Wells were washed again with PBS and 100 μL aliquots of the respective medium supplemented with 10% (v/v) foetal bovine serum were dispensed into each well. Plates were then incubated in a humidified 5% CO_2 atmosphere at 37 °C for 21 h. The cell viability was then determined using a MTT assay. A similar procedure was repeated using RB at the same concentration in the absence of light or ultrasound and for stimulus alone. Results were compared against control experiments where cells were treated with only the vehicle (i.e., PBS).

2.6. PDT and SDT treatment of ectopic B16 melanoma tumours in nude mice using Rose Bengal

B16-F10-Luc2 cells (3×10^5) maintained as described in Section 2.4, were re-suspended in 100 μL of PBS and implanted into