Objective Outcome Measures Following Sonodynamic Photodynamic Therapy – A Case Series

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Abstract

Sonodynamic Photodynamic Therapy (SPDT) is a novel cancer treatment approach utilising a photosensitive agent with reported ultrasound-activated properties (Sonnelux-1), which is administered prior to light and low-intensity ultrasound exposure. SPDT has previously demonstrated significant tumour cell inhibition in animal studies and several case reviews have reported clinical benefits in metastatic cancer patients. This current case series presents the objective outcome measures of six patients with a variety of cancer diagnoses following SPDT, providing further evidence of beneficial treatment outcomes. The importance of immunoediting and the tumour microenvironment as well as the potential role of Sonodynamic Photodynamic Therapy to modify anti-tumour immunity are also outlined.

Introduction

Photodynamic Therapy

Photodynamic Therapy (PDT) utilises specific wavelengths of light to activate a pre-loaded photosensitiser. PDT has been studied extensively and is used for a variety of pre-cancerous and malignant pathologies [1,2,3,4,5]. Photosensitisers are typically based on chlorophyll or porphyrin ring structures [6] which are inherently light sensitive. Absorption of light by the sensitizer is capable of inducing a transfer of absorbed energy to molecular oxygen, with subsequent singlet oxygen and free radical production, producing activated tumour cell necrosis [6]. Photosensitisers have demonstrated the clinically useful capacity of preferential uptake and retention in malignant cells leading to accumulation selectively at tumour sites and are non-toxic unless activated [6,7]. This combination allows for targeted cytotoxicity with minimal effect to healthy surrounding tissue and the ability to repeat treatment without total dose limitations.

Sonodynamic Therapy

Sonodynamic Therapy (SDT) refers to the use of low-intensity ultrasound as an activation stimulus for a pre-loaded sensitiser. [8,9,10]

Light-activation in Photodynamic Therapy is limited by the absorption and scatter of light in surrounding tissues. This can be partially compensated by using agents sensitive to longer wavelengths of light [11], but currently limits Photodynamic Therapy for use in superficial malignancy or to sites capable of endoscopic light-access.

The potential to use ultrasound as an additional activation stimulus with significantly greater tissue penetrance combines the advantages of Photodynamic Therapy with the ability to activate a pre-loaded sensitizer within deep-sited and metastatic tumours. Ultrasound propagation into deep tissues has been well established, with half-value layers sufficient to achieve ultrasound exposure to deep-sited organs [12,13,14] and a potential mechanism for ultrasound propagation in bone [15]. Indeed, ultrasound at low-intensity is used widely in medical diagnostics and physiotherapy for its safety profile and for the deep soft-tissue effect that can be achieved [16].

Previous pre-clinical studies of existing photosensitive agents have demonstrated a synergistic effect with ultrasound exposure as little as 0.51 W/cm2 at 1.0 MHz for 10 minutes [17]. Jin et al demonstrated that the combination of light and ultrasound exposure (PDT and SDT) significantly improved inhibition of tumour growth (92-98% - additive effect) as compared to either single treatment (27-77%). Also, the

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median survival period from irradiation to death of PDT+SDT treated mice (>120 days) was significantly greater than that in single treatment groups (77-95 days) and histological changes revealed that combination therapy could induce tumour necrosis much deeper than PDT alone [17].

Proposed mechanisms of the synergistic effect of ultrasound with sonosensitisers in Sonodynamic Therapy include generation of sensitiser-derived free radicals which initiate chain peroxidation of membrane lipids, the physical destabilization of the cell membrane by the sonosensitizer thereby rendering the cell more susceptible to shear forces and ultrasound enhanced drug transport across the cell membrane (sonoporation) [8,18].

Specific ultrasound-sensitivity varies widely between the photosensitiser compounds [19]. The development of new agents modified to increase ultrasound sensitivity has enabled further study and application of the sonodynamic approach.

Sonnelux-1 is a modified metallo-chlorin agent with an average molecular weight of 942 and light absorption peaks at wavelengths 402 nm and 646nm [20].

Previous animal studies of Sonnelux-1 SDT have demonstrated marked sensitivity to low-intensity ultrasound, leading to significant dose-dependent reduction in tumour volume when ultrasound was applied at 1 MHz varying from 0.3W/cm2 to 1.2W/cm2 for 3 minutes after systemic sonnelux-1 administration [20]. The greatest anti-tumour effect was seen at exposure to 1.2W/cm2 compared to 0.3W/cm2 with marked necrosis on histology. Minimal or no tumour effect was seen when ultrasound or the sensitiser was used alone.

Several case reports have been published using the same ultrasound intensity, with light and ultrasound activation 24-48 hours after systemic Sonnelux-1 administration.

A series of cases in human patients with metastatic tumours has been previously published by an Oncology Department in China which documented evidence including PET imaging showing reduced tumour mass post treatment. [21,22] and our previous publication reviewed 115 consecutive patients with many examples of patients surpassing predicted median survival times [23]. We now present five cases with objective outcomes in more detail.

SPDT Treatment Protocol

The Sonnelux-1 sensitiser is administered by the patient sublingually. After 48 hours the patient is then exposed to a light bed containing 48 panels of LED’s emitting a combination of visible and infra-red light at the frequencies 660nm and 940nm (+/- 30nm). Light bed exposure time varies with a shorter initial exposure duration which is titrated upward according to the physical status and diagnosis of the patient.

Ultrasound is then applied using a hand-held manipule at 1W/cm2 and a frequency of 1MHz at sites of known malignant disease, with time titrated on a case by case basis. Light and ultrasound activation is repeated on three consecutive days. This process is then repeated with further Sonnelux-1 administration and three consecutive days of light and ultrasound administration to complete one SPDT treatment cycle. Patients with significant tumour mass are treated with dexamethasone 2mg twice a day which commences on the first day of treatment and continues for a total of four weeks.

Ozone Autohaemotherapy and Tumour Hypoxia

Tumour hypoxia is a well-recognised factor in cancer treatment resistance to chemotherapy and radiotherapy as well as PDT, which requires singlet oxygen production [24]. Therefore any method of improving local hypoxia within the tumour environment should increase the efficacy of Sonodynamic and Photodynamic Therapy.

Ozone auto-haemotherapy is the use of medicinal grade oxygen to generate ozone (O3) that at a set volume dose is externally exposed to the blood of the patient via an anti-coagulated sterile IV infusion kit. A previous study
measured the effect of ozone auto-haemotherapy on tumour hypoxia in patients with accessible metastases or advanced tumour. Tumour oxygenation status was measured directly via polarographic needle probes [25]. Areas of low PO2 within the tumour significantly improved following treatment. The number of PO2 values ≤10 mmHg at baseline decreased significantly after ozone therapy, P = 0.002. Ozone auto-haemotherapy is administered shortly before each light bed exposure, aiming to increase PO2 at the tumour site.

Cases

The following cases received Sonodynamic Photodynamic Therapy following informed consent and full explanation of the treatment.

Case 1

This 82 year old lady presented in June 2006. She had a previous history of right-sided breast cancer in 2002 treated by lumpectomy, with no chemotherapy or radiotherapy. The tumour was oestrogen and progesterone receptor negative. She smoked heavily until the age of 50.

Since December 2005 she had been complaining of a persistent cough and recurrent chest infections. A Chest X Ray was performed in January 2006 which revealed a 1.8 cm soft-tissue density in the left upper lobe.

She was subsequently referred urgently for further investigation and was given the diagnosis of a breast secondary or further lung primary. TB testing was performed for completeness, but was negative. It was felt that biopsy was not possible due to the location of the tumour and associated bleeding risk.

Subsequently, she underwent a follow up Chest X Ray which revealed the mass had doubled in size between January and May 2006. She underwent a PET scan which did not show any other focal changes. At this time a fractionated course of radiotherapy was advised and at the same time the patient attended for SPDT review. The patient broke her arm in a fall and felt unable to attend for a four week daily radiotherapy regimen. The patient therefore declined radiotherapy. SPDT was also discussed and the patient made a decision to commence SPDT in July 2006. She tolerated treatment very well.

She has undergone regular Chest X Rays since SPDT which show that the previously enlarging mass is stable:

“The lesion situated within the left upper zone has not altered in size since the previous chest radiograph dated 30/11/2007. The lungs are otherwise clear.” 28/5/2008.

Her chronic cough resolved after SPDT and she stopped having regular chest infections. No other active treatments were commenced and the patient remains fit and well. Follow up 6 monthly Chest X Rays remain stable to the time of writing, with no evidence of progressive disease. A clear change in the progression of her mass was visualised on imaging following SPDT as the sole-intervention.

Case 2

This 56 year old female was diagnosed with squamous cell carcinoma of the anus in April 2006. She underwent chemotherapy and radiotherapy with an excellent response. No evidence of recurrent local or distant metastatic disease was seen on two follow up scans.

In August 2007 a CT revealed a 16 mm lesion in segment 7 of her liver. This increased to 3 cm by October 2007. Radiotherapy was not offered due to her previous treatment and she was offered a partial hepatectomy. She refused neo-adjuvant chemotherapy. At this time in October 2007 she attended for SPDT review and was strongly encouraged to consider surgical management.

She decided to undertake SPDT as a neo-adjuvant treatment prior to right hemi-hepatectomy. She tolerated the treatment very well. After SPDT, ultrasound appearances showed cavitation in the liver lesion.
Histology confirmed “extensive tumour cell necrosis” and showed 3 tumours in the resected section, each tumour had extensive central necrosis. Some tumour cells were detectable, but there was marked necrotic change. She remains well and disease free.

**Case 3**

This 22 year old male was diagnosed with a Ewing’s Sarcoma originating from his left pubis in June 2000. He underwent chemotherapy and radiotherapy with a good initial response. He had extensive recurrent local disease which was diagnosed as a Malignant Peripheral Nerve Sheath Tumour on histology and was also found to have pulmonary metastases in November 2000. He underwent further chemotherapy and radiotherapy as well as a right lobectomy. He presented in April 2009 with a 15 x 11.7 cm mass in right lower hemithorax. He underwent further chemotherapy and SPDT. Following treatment he developed a moderate increase in right sided chest pain over 4-6 weeks. Follow up CT imaging significant reduction in tumour mass to 10.6 x 8.6 cm with evidence of central cavitation. He underwent a second course of SPDT along with low dose cyclophosphamide 50mg once daily on alternate weeks. Following SPDT he experienced a further increase in right sided chest pain which gradually reduced over 6-8 weeks. Follow up imaging, 4 weeks after SPDT, showed an increase in tumour size during the inflammatory phase post SPDT and subsequent imaging revealed a reduction in tumour mass after 3 months. He is continuing to undergo active follow up.

**Case 4**

This 58 year old female presented in May 2007. She complained of a persistent cough over the previous 10 months with an ongoing 40 pack-year smoking history. After recurrent courses of antibiotics she had a Chest X Ray which revealed a 6 cm left upper lobe mass. She was diagnosed following a scan and a biopsy as having a non-small cell lung cancer in the left lung. The CT scan showed an adrenal metastasis on the right side. On examination breath sounds were absent in the left lung.

At presentation her clinical status was poor. She had been given a prognosis of a few weeks and referred to palliative care nurses. No active intervention had been advised following Oncology review. The patient completed SPDT in July 2007. The cough symptomatically improved and breath sounds were detectable again in the left lung approximately 2 months after SPDT. She requested a further scan but due to there being no conventional active treatment this request was refused.

The persistent cough recurred after five months. On examination air entry was again reduced on the left lung and she was experiencing marked right loin pain from the adrenal metastasis. A further course of SPDT was completed in December 2007. The cough again resolved and clinically air entry returned to her left lung. During the second SPDT cycle she experienced pain in the left chest and tiredness, though this was controlled symptomatically and resolved over 4-6 weeks. The pain she had experienced in the right loin fully settled post SPDT.

She has not had a follow up scan until recently. She developed visual symptoms and had a CT scan that showed a solitary brain metastasis. The lung primary was stable from previous imaging over 2 years ago and the previous pleural effusion had almost fully resolved. She reports to us that it also demonstrated a significant reduction in the adrenal metastasis and is due to send us a copy of this report. She recently completed whole brain radiotherapy.

This shows sustained symptomatic improvement following SPDT, stable lung disease and improved adrenal pathology on follow up imaging 2 years after diagnosis.

**Case 5**

A number of cases have demonstrated an initial visible inflammatory reaction in tumour tissue providing visible evidence of tumour-related changes which settle after a period of weeks.
This 46 year old female presented in August 2008. She had a right-sided breast cancer in November 2004 which was treated with mastectomy. The tumour was oestrogen receptor positive. She refused radiotherapy, chemotherapy and Tamoxifen. She had local recurrence along the scar line 9 months after mastectomy and then developed secondary lymphadenopathy in her neck. She agreed to commence Tamoxifen in 2006.

On first review she had multiple enlarged lymph nodes in the right and left supravacular fossa and widespread tumour across the right side of the chest, extending very deeply, together with a fungating lesion in the centre of the chest. SPDT was performed in September 2008. The patient developed a marked inflammatory response to the SPDT with erythema and tenderness over the affected chest wall which appeared one week after treatment and gradually resolved over a period of 8-12 weeks. The patient completed a further one week cycle of SPDT in January 2009. Again a marked inflammatory response occurred. This inflammatory response took 5 months to settle down completely and she is left with fibrous tissue all the way around the right side of her chest, with no active tumour seen over the area which received ultrasound. There are some small areas showing signs of active recurrence, but they are above the area where the ultrasound was applied. At this stage a further one week cycle of SPDT is planned.

Case 6

This 77 year old male suffered nocturia and was found to have an enlarged nodular prostate on rectal exam. He was diagnosed on biopsy with prostate cancer, gleason 3+4, and underwent a laparoscopic non-nerve sparing radical prostatectomy in October 2004. He presented for SPDT review in November 2008 with a rising PSA (10.9 in May 2008 and 19.4 in November 2008). He refused hormone therapy and did not want a CT scan. He initially opted for treatment with high dose IV Vitamin C. Following IV Vitamin C treatment the PSA level continued to increase to 26.1.

He underwent SPDT treatment in June 2009, which he tolerated well. Follow up PSA in November 2009 had normalised to 1.9 with no other active intervention.

The Role of Immunoediting?

It has long been hypothesized that host immune function is a primary defence against the progression of malignancy. Long-term follow up studies confirm that those taking immunosuppressant medication and people with poor innate immune function have higher rates of a wide variety of cancers [26, 27]. This forms part of an increasing body of evidence, including the occurrence of spontaneous remissions, epidemiological studies and the prognostic role of tumour-infiltrating lymphocytes, supporting a surveillance role for the immune system in the elimination of pre-clinical cancers, and in powerfully modifying the immunogenicity and behaviour of clinically evident cancers [28,29,30] This interactive process between immune response and selection pressure of heterogeneous cells at the tumour microenvironment is termed “Immunoediting” with three proposed outcomes of tumour elimination, equilibrium and escape [30].

The presence of tumour infiltrating lymphocytes and specifically CD8+ cytotoxic T cells within tumour mass and lymph node material has been strongly associated with favourable prognosis in a wide variety of cancers [31,32,33,34]. With this in mind, the development of treatments capable of modifying the immune-tumour microenvironment to increase CD8+ effector T cell populations may offer survival advantages.

Tumour cell death induced by conventional treatments releases a host of tumour associated antigens, with the potential to prime an antitumour immune response. However, both radiotherapy and chemotherapy have been assumed to antagonise any priming of the immune system, through the inhibition of
lymphocyte division and the induction of lymphocyte death. Furthermore, tumour cell apoptosis induced by both treatments has not been considered to be immunogenic [30,35,36].

It is proposed that anti-tumour immunity will only develop if tumour cells possess unique antigens and activate co-stimulation from antigen presenting cells via pattern recognition receptors. According to the ‘danger’ theory, the potential of tumour cells to offer an alarm signal is of key importance to eliciting an anti-tumour immune response [37]. ‘Healthily’ growing tumours are more likely to induce immune tolerance and therefore immune escape.

The balance of positive and negative signals received by dendritic cells within the tumour microenvironment is likely to alter during the natural history of tumour progression. Local inhibitory mechanisms may explain the common occurrence of tumour progression in an immunocompetent environment [38,39], but areas of hypoxia and necrosis in some tumours may activate the immune system.

Can PDT and SDT modify the anti-tumour immune response?

Numerous pre-clinical studies have shown that local PDT treatment of tumours enhances anti-tumour immunity [40] and may act as the necessary “danger” signal. It is suggested that unlike immunologically silent genotoxic damage produced by radiotherapy and chemotherapy, photo-oxidative cytotoxic lesions generated by PDT are extra-nuclear and result in a rapid cell death that alerts the host’s innate immune system [41].

Local PDT treatment can result in wide-spread effects including systemic neutrophilia [42], induction of acute-phase proteins [42,43], increased circulating levels of complement proteins [44] and systemic release of pro-inflammatory cytokines [43,45], all of which indicate the presence of a systemic inflammatory response.

Subsequent studies showed that local PDT treatment of murine tumours results in the induction of anti-tumour immunity with control of local and distant disease mediated by increased CD8+ T cell population numbers within tumour microenvironment [46,47]. Increased CD8+ cell populations have also been noted in clinical studies post PDT, both at the treated (local) sites [48] and at non-treated (distant) sites [49]. This demonstrates in human cases a potential mechanism for beneficial modification of the immune microenvironment following PDT-induced photo-oxidative necrosis.

It has been observed in people with cancer, that numbers of regulatory T-cells are significantly increased in blood, bone marrow and at tumour sites [50]. It is becoming clear that cancer growth, development and resistance to treatment may be aided by this element of our own immune systems [51,52]. In this situation regulatory-T cells act in a tolerogenic fashion potentially aiding tumour escape by preventing effective CD8+ T cell mediated anti-tumour immunity.

The prognostic role of CD8+ and CD4+ regulatory T-cells have been assessed in biopsy studies of human ovarian cancer [53], with clear prognostic benefit associated with a high number of CD8+ tumour infiltrating lymphocytes (TIL). This study also demonstrated that CD4+ TILs down-regulated the beneficial effects of CD8+ TIL. This unfavorable effect of CD4+ T cells on prognosis was found to be due to CD25+ forkhead box P3 (FOXP3) regulatory suppressor T cells, as indicated by survival of patients with high versus low CD8+ /Treg ratios (median=58 versus 23 months; hazard ratio=0.31; C.I.=0.17 0.58; P=0.0002) [53].

CD4/25+ regulatory T-cell populations can be monitored using techniques such as Flow Cytometry. Certain approaches such as low dose cyclophosphamide have been proposed as a means of modifying regulatory T-cell populations in a dose specific manner [54].

Approaches that can reduce the dominant effect of regulatory T-cells and bring their population
numbers back to the normal reference range may increase immune recognition of the tumour and make immunotherapy treatments more effective [55].

Thus, a combination of PDT and SDT along with modification of regulatory T-cell populations may optimise tumour immugenicity and subsequent anti-tumour immunity as a standalone approach or alongside other immunotherapy strategies [56].

Summary

This review of SPDT cases and anti-tumour immunity demonstrates a number of mechanisms to support the clinical outcomes which include stabilisation of progressive disease on imaging, necrosis on histological follow up and normalisation of tumour markers.

SPDT is a non-invasive and well tolerated treatment that may be capable of controlling tumour progression by directly inducing inflammatory necrosis in a variety of deep and superficial tumours, including those that have proven refractory to chemotherapy.

Thus, Photodynamic and Sonodynamic Therapy along with modification of regulatory T-cell populations may represent excellent tools in the generation of the relevant “danger” signal required to up-regulate tumour-associated antigen expression and consequently an effective dendritic cell reponse. In turn this may lead to increased tumour-infiltrating lymphocytes with the capacity of generating tumour regression or stabilising disease progression.

References


3. Morton, CA; Whitehurst, C; McColl, JH; Moore, JV; MacKie, RM. Photodynamic Therapy for Large or Multiple Patches of Bowen Disease and Basal Cell Carcinoma. Arch. Dermatol. 2001;137:319–324.


19. QuanHong Liu · Xiaobing Wang · Pan Wang · LiNa Xiao · Qiao Hao Cancer Chemother Pharmacol. 2007; 60:671–680.


32. Sandeep S et al. Prediction of Survival in Follicular Lymphoma Based on Molecular Features of Tumor-Infiltrating Immune Cells.


47. E Kabingu1, L Vaughan, B Owczarzak, KD Ramsey and SO Gollnick. CD8+ T cell-mediated control of distant tumours following local photodynamic therapy is independent of CD4+ T cells and dependent on natural killer cells British Journal of Cancer. 2007. 96(12), 1839 – 1848.


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