Outcome Measures Following Sonodynamic Photodynamic Therapy – A Case Series

Julian Norman Kenyon* and Richard James Fuller

The Dove Clinic for Integrated Medicine, The Old Brewery, High Street, Twyford, Winchester, Hampshire SO21 1RG UK

Abstract: Sonodynamic Photodynamic Therapy (SPDT) is a novel cancer treatment approach using a photosensitive agent (Sonnelux-1) with reported ultrasound-activated properties. The sensitiser is administered prior to a cycle of light and low-intensity ultrasound exposure. Ultrasound has the advantage of significantly greater tissue penetrance compared to light, which potentially allows non-invasive activation of the sensitiser within deep-sited tumours. Sonnelux-1 has previously demonstrated significant tumour cell inhibition following ultrasound administration in animal studies, and several case reviews have been published reporting clinical benefits in metastatic cancer patients. This current case series presents outcome measures of five patients with a variety of cancer diagnoses following SPDT, providing further evidence of beneficial treatment outcomes.

Keywords: Sonodynamic therapy, photodynamic therapy, immunoediting, cancer, ultrasound.

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 [1-5]. Photosensitisers are typically based on tetra pyrrole 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, leading to activated tumour cell necrosis [6]. Photosensitisers are non-toxic unless activated and have demonstrated the clinically useful capacity of preferential uptake and retention in malignant cells, leading to accumulation selectively at tumour sites [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 sensitisier [8-10].

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 activation stimulus with significantly greater tissue penetrance than light combines the advantages of Photodynamic Therapy with the ability to activate a pre-loaded sensitiser 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 [12-15]. Indeed, ultrasound is used widely at low-intensity 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 photo-sensitive 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 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 sensitisier-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]. The ultrasound-mediated effect on the sensitiser is not fully understood, but may be caused directly by the ultrasound producing a sonochemical reaction with the sensitiser. Alternately, it may be produced indirectly via “sonoluminescence”, which involves the rapid generation of light following tissue exposure to a sound wave of sufficient intensity to induce a gaseous cavity to collapse quickly [19].

Specific ultrasound-sensitivity varies widely between the photosensitiser compounds [20]. The development of new agents modified to increase ultrasound sensitivity has en-
able 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 [21]. Sonnelux-1 animal safety studies have been published showing an excellent safety profile [22].

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 [21]. The greatest anti-tumour effect was seen at exposure to 1.2W/cm2 compared to 0.3W/cm2 with marked necrosis on histology. No tumour effect was seen when ultrasound or the sensitiser were 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. [23, 24] and the authors’ previous publication reviewed 115 consecutive patients with examples of patients surpassing predicted median survival times [25].

**CAN PDT AND SDT MODIFY ANTI-TUMOUR IMMUNE RESPONSE?**

The presence of tumour infiltrating lymphocytes and specifically CD8+ cytotoxic T cells within tumour mass and regional lymph nodes has been strongly associated with favourable prognosis in a wide variety of cancers [26-30]. 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 [30-32].

Numerous pre-clinical studies have shown that local PDT treatment of tumours enhances systemic immune response [33] and may act as the necessary “danger” signal to improve the host’s anti-tumour immune response [34]. It is suggested that unlike more immunogenically silent genotoxic damage produced by radiotherapy and chemotherapy, photo-oxidative cytotoxic lesions generated by PDT are extranuclear and result in a rapid cell death that alerts the host’s innate immune system [35].

Local PDT treatment can result in wide-spread effects including systemic neutrophilia [36] induction of acute-phase proteins [36, 37], increased circulating levels of complement proteins [38] and systemic release of pro-inflammatory cytokines [37, 39], all of which indicate the presence of a systemic inflammatory response.

Subsequent studies have reported 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 the tumour microenvironment [40, 41].

Increased CD8+ cell populations have also been noted in clinical reports post PDT, both at the treated (local) sites [42] and at non-treated (distant) sites [43]. This demonstrates in human cases a potential mechanism for beneficial modification of the immune microenvironment following PDT-induced photo-oxidative necrosis which may help generate stable disease or remission.

The interplay of Sonodynamic Therapy with anti-tumour immunity is yet to be evaluated but given the proposed similarity of light and ultrasound activation it is logical to assume a similar capacity to generate an effective immune response [44].

**SPDT TREATMENT PROTOCOL**

SPDT was provided following informed written consent for both treatment and inclusion in anonymous case presentation. Treatment was performed in a clinic regulated by the Care Quality Commission. The Sonnelux-1 sensitiser is an unlicensed medicine imported under licence from the MHRA.

It 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 infrared 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 [45]. Therefore any method of improving local hypoxia within the tumour environment may 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 anticoagulated sterile IV infusion kit. A previous study measured the effect of ozone auto-haemotherapy on tumour hy-
poxia in patients with accessible metastases or advanced tumour. Tumour oxygenation status was measured directly \textit{via} polarographic needle probes [46]. Areas of low PO2 within the tumour significantly improved following treatment. The number of PO2 values \( \leq 10 \text{ mmHg at baseline} \) 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.

**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.

In December 2005 she complained 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. Despite discussing radiotherapy as the treatment option of choice, the patient declined radiotherapy. The patient made a decision to commence SPDT in July 2006 which she tolerated 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 conventional 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 60 year old lady presented in November 2009 with a history of vaginal spotting. Vaginal examination had been performed in October 2009 revealing a small endometrial polyp which was sent for histology, confirming grade 2 adenocarcinoma. Hysteroscopy and curettage biopsy was then performed which continued to show the presence of grade 2 carcinoma of the endometrium with an atrophic appearance. MRI scanning showed a thin endometrium and no further pathology.

She was advised to have a total hysterectomy and bilateral salpingo oophorectomy and in addition decided to undertake neo-adjuvant SPDT four weeks prior to the operation. During the SPDT treatment cycle she noticed an occasional sharp and prickling sensation in her low abdomen and pelvis. This fully settled after two weeks and was tolerated well without analgesia.

Total hysterectomy was performed at the end of December 2009 without complication. Pathology results reported no malignant change within the uterus though there was some atypical hyperplasia. This is in contrast to the confirmation of malignant changes on curettage biopsy pre-SPDT.

**CASE 4**

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 had 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 supraclavicular 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 completely and she is left with fibrous tissue over 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 5**

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 was advised regarding conventional management but opted to have 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.

**DISCUSSION**

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 appears worthy of further study. The treatment 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.

Sonodynamic Photodynamic Therapy may also represent a valuable future tool in the generation of targeted tumour cell necrosis to provide the relevant “danger” signal required to up-regulate an effective anti-tumour immune response alongside other immunotherapy approaches [33-35, 44, 47].

**REFERENCES**


[29] Sato E, Olson S, Ahn J, et al. Intraperitoneal CD8+ tumour-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio
are associated with favorable prognosis in ovarian cancer. PNAS 2005; 102(51): 18538-43.


