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Sonoporation with Acoustic Cluster Therapy (ACT®) induces transient tumour volume reduction in a subcutaneous xenograft model of pancreatic ductal adenocarcinoma

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers with survival averaging only 3 months if untreated following diagnosis. A major limitation in effectively treating PDAC using conventional and targeted chemotherapeutic agents, is inadequate drug delivery to the target location, predominantly due to a poorly vascularised, desmoplastic tumour microenvironment. Ultrasound in combination with ultrasound contrast agents, i.e., microbubbles, that flow through the vasculature and capillaries can be used to disrupt such mechanical barriers, potentially allowing for a greater therapeutic efficacy. This phenomenon is commonly referred to as sonoporation. In an attempt to improve the efficacy of sonoporation, novel microbubble formulations are being developed to address the limitation of commercially produced clinical diagnostic ultrasound contrast agents.

In our work here we evaluate the ability of a novel formulation; namely Acoustic Cluster Therapy (ACT®) to improve the therapeutic efficacy of the chemotherapeutic agent paclitaxel, longitudinally in a xenograft model of PDAC. Results indicated that ACT® bubbles alone demonstrated no observable toxic effects, whilst ACT® in combination with paclitaxel can transiently reduce tumour volumes significantly, three days posttreatment ($p = 0.0347$–0.0458). Quantitative 3D ultrasound validated the calliper measurements. Power Doppler ultrasound imaging indicated that ACT® in combination with paclitaxel was able to transiently sustain peak vasculature percentages as observed in the initial stages of tumour development. Nevertheless, there was no significant difference in tumour vasculature percentage at the end of treatment. The high vascular percentage correlated to the transient decrease and overall inhibition of the tumour volumes.

In conclusion, ACT® improves the therapeutic efficacy of paclitaxel in a PDAC xenograft model allowing for transient tumour volume reduction and sustained tumour vasculature percentage.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most deadly cancers with survival averaging only 3 months if untreated following diagnosis [1,2]. The current clinical regimes are chemotherapy and/or surgery, with or without radiation therapy. Whilst surgery remains the only potential for cure, it is rarely an option due to its late diagnosis and invasive nature [3]. Even the most effective chemotherapeutic regime (FOLFIRINOX) results in a median overall survival of 26.6 months in patients with locally advanced pancreatic cancer, *i.e.*, no metastasis [4]. If a tumour is downgraded following treatment allowing for the possibility of surgery, overall survival increases to 35.4 months [5] indicating the importance of tumour volume reduction.

A major limitation in effectively treating such solid tumours using therapeutic agents, such as chemotherapeutics, is the inadequate delivery to the target location whether due to tumour interstitial fluid pressure [6], lack of vascularisation or perfusion [7], or the presence of a dense stromal matrix [8,9]. The stromal microenvironment is a complex

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structure composed of an extracellular matrix (ECM), activated fibroblasts and myofibroblasts, inflammatory cells and blood and lymphatic vessels that distort the normal architecture of pancreatic tissue. The complex interplay between tumour cells and stroma promotes cancer cell motility, resistance to hypoxia and stromal neovascularization [8]. These physical barriers result in inaccessibility to the tumour for most chemotherapeutic agents. As a result, irrespective of how good the targeted approach, all chemotherapeutic treatments suffer the same fate *in vivo*, whilst increasing dosage to compensate only exasperates systemic side effects [10]. Thus, mechanical disruption of the tumour or normalisation of tumour vascularisation to permit enhanced delivery of therapeutics may provide greater clinical promise. Novel therapeutic options specifically targeted at treating solid tumours are currently under investigation, including nanoparticles [11,12], molecular targeting [13,14], and ultrasound- and microbubble-mediated therapy [15–17] *i.e.*, sonoporation.

Sonoporation is a novel methodology where gas microbubbles are injected into the vasculature and excited by ultrasound (US) to invoke biomechanical effects that increase the permeability of the vascular barrier and extravasation of drug in a specific location [18,19]. Microbubbles are stabilized gas bubbles (2–3 μm in diameter) that are injected intravascularly and are typically stable for up to 5 min *in vivo* with no known side-effects. Upon the application of ultrasound, these microbubbles volumetrically oscillate and interact with nearby cells forming small pores in the cell membrane via mechanical disruption and even been observed to enter the cells *in vitro* [20]. This interaction permits increased intracellular drug uptake [21] whilst also allowing therapeutic agents deeper penetration into the tissue than the vascular barrier alone [22]. As a result, sonoporation can improve the therapeutic efficacy of intravascularly injected therapeutic agents.

Previously, we have demonstrated that combining sonoporation with gemcitabine in an orthotopic xenograft model of gemcitabine resistant PDAC could inhibit tumour growth up to four-fold in comparison to gemcitabine monotherapy [16]. A subsequent Phase I clinical trial also indicated that sonoporation has the potential to transiently decrease tumour volume in patients with locally advanced or metastatic pancreatic ductal adenocarcinoma (PDAC) [15,17]. Whilst these studies show promise, the true potential of sonoporation is limited due to the use of commercially available microbubbles designed and optimised for ultrasound imaging, not therapy. As a result, substantial research is focusing on developing “next-generation” microbubbles optimised for ultrasound-target and -enhanced therapy. Specifically, the major limitation has been that currently sonoporation requires the co-administration of the therapeutic agent; resultantly similar systemic side effects are experienced. A secondary limitation is the size of microbubbles. It has been hypothesised that smaller bubbles are required to enter the sub-micron sized fenestrations presented in tumours [23]. However, it has also been suggested that larger bubbles may have a stronger sonoporation effect leading to deeper penetration of the drug and enhanced therapeutic efficacy [24].

To address the current limitations of commercial bubbles for therapy, a range of novel microbubbles are being developed [25–36]. Acoustic Cluster Therapy is a novel concept for ultrasound mediated, targeted drug delivery (ACT®, Phoenix Solutions AS, Oslo, Norway) [37].

ACT® is an ultrasound-activated formulation combining negatively charged, commercially available and clinically employed microbubbles (Sonazoid, GE Healthcare, Little Chalfont, United Kingdom) (Fig. 1-A1) with positively charged microdroplets (Fig. 1-A2). A mixture of these microbubbles and microdroplets results in small microbubble-microdroplets clusters held together by the electrostatic forces (Fig. 1-A3). The microdroplets consist of a fully or partially fluorinated or halogenated oil component (PF-X) that has a boiling temperature of <50 °C, low blood solubility. The ACT® cluster dispersion is intended for co-administration with a drug. A lipophilic therapeutic agent may optionally be embedded into the microdroplets. When these clusters are insonated with ultrasound (in the clinical diagnostic regime) the

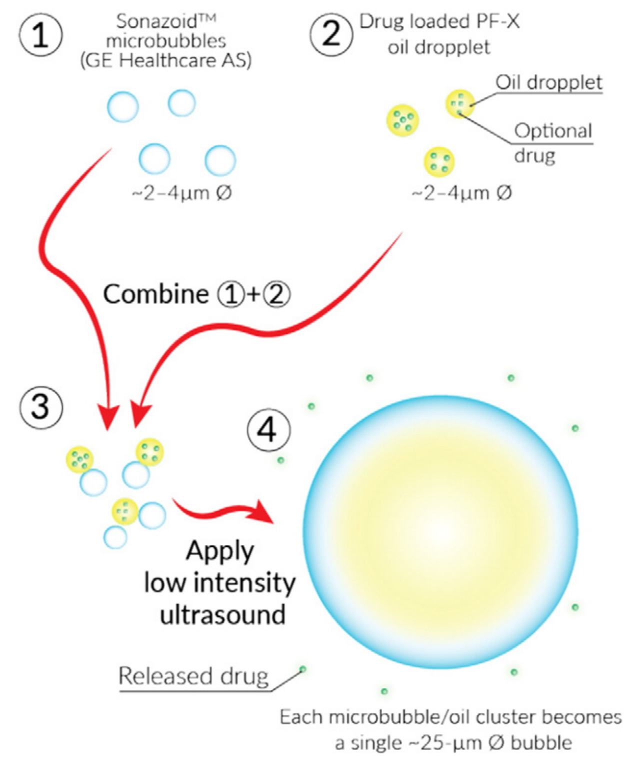

Fig. 1. Graphical representation of the ACT® principle. Sonazoid™ microbubbles are combined with a PF-X oil droplet that can be loaded with a drug. Upon low intensity ultrasound application, the oil droplet vaporises releasing the drug and forming a single large microbubble.

volumetrically oscillating microbubbles transfer energy to the microdroplet initiating vaporisation [37]. These 20–30 μm ACT® bubbles have been shown to form in capillary sized vessels in vivo [38,39] and can be used to induce sonoporation by exciting them at low frequencies (0.3–1.0 MHz). In addition, if a therapeutic agent is embedded in the oil, it will be released in the activation region resulting in ultrasound guided and targeted release.

In our work here we evaluate the ability of ACT® to improve the therapeutic efficacy of the chemotherapeutic agent paclitaxel (PTX) in a longitudinal xenograft model of PDAC. We evaluated the therapeutic benefit of ACT® on tumour volume and vasculature over 5 treatment cycles (5 weeks).

2. Materials and methods

Ultra-high-speed imaging was used to visualise the transition from ACT® clusters to single large microbubbles. The ability of ACT® to induce targeted, ultrasound-enhanced chemotherapy therapy was evaluated in a longitudinal study using a subcutaneous xenografted pancreatic cancer murine model. Tumour development was evaluated using 3D B-mode and Power Doppler ultrasound, calliper based tumour volume measurements, and full body bioluminescence imaging. Body weight measurements were performed to evaluate treatment toxicity.

2.1. Ultra-high-speed imaging

Ultra-high-speed imaging of ACT® was performed using a previously described setup. Specifically, the ACT® compound was injected into a 200-μm outer diameter cellulose capillary and imaged through a 60× water immersion objective (NA = 1.0, working distance = 2 mm) (Olympus, Tokyo, Japan). A Kirana ultra-high-speed imaging camera (Specialised Imaging Ltd., Hertfordshire, United Kingdom) was used as the video capture source. A pulsed laser with a 100 ns illumination time was used as the light source and defined the exposure time. Images