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Short communication

Contrast enhanced ultrasound imaging can predict vascular-targeted photodynamic therapy induced tumor necrosis in small animals

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ABSTRACT

Aims: To evaluate the accuracy of contrast-enhanced ultrasound (CEUS) for monitoring tumor necrosis following WST-11 vascular targeted photodynamic therapy (VTP) using imaging-pathology correlation.
Methods: Renal adenocarcinoma cells were injected into the hindlimb of 13 BalB/c mice resulting in tumors ranging from 9.8 to 194.3 mm^3. US guidance was used to place a laser fiber into the tumor, and VTP was performed. CEUS was performed prior to animal sacrifice, 24 h post-VTP. Whole tumors were extracted for histopathologic analysis using H & E and TUNEL staining. Pathology samples corresponding to the CEUS imaging plane were prepared in order to compare the size and extents of tumor necrosis.
Results: Tumor necrosis following VTP appeared as a central region of non-enhancement on CEUS, while viable tumor appeared as patchy regions of enhancement in the tumor periphery. The region of tumor necrosis measured in mean 66% and 64.8% of total tumor area on CEUS and pathology respectively ($p = 0.2$). The size and location of the necrosis on CEUS images and pathology samples were found correlative with no inter-observer difference (weighted kappa of 0.771 and 0.823, respectively).
Conclusion: CEUS allows accurate monitoring of VTP induced tumor necrosis in a small animal model.

1. Background

In preclinical mouse studies, laser fiber placement and WST-11 Vascular Targeted Photodynamic Therapy (VTP) treatment monitoring are usually performed under direct visual guidance [1–3]. The lack of suitable imaging techniques that can be used to plan and perform VTP undermines the ability to study the biological effects of this technique.

We hypothesized that contrast-enhanced ultrasound (CEUS) may be used to guide laser fiber placement, monitor VTP mediated changes to tumor vasculature, and predict outcomes [3,4]. The high compressibility and resonance properties of gas-filled microbubbles makes them useful intravascular ultrasound contrast agents [5].

2. Aims

The purpose of this study was to examine the accuracy of CEUS in predicting tumor necrosis after VTP in a murine tumor model through radiologic-pathologic comparison.

3. Methods

3.1. Animal model and treatment

Thirteen BalB/c mice (Charles River Laboratory) underwent CEUS-guided VTP on subcutaneous flank tumors. A total of 5×10^6 Renca cells were injected into the left hind limb of 7–8 week old male mice. The mice were intravenously infused with WST-11 (Steba Biotech. France) at 9 mg/kg for 5 min followed by laser illumination for 10 min. A 10 mm cylindrical fiber (MedLight S.A., Switzerland) was placed into the tumor through a small incision on the skin, and was used to deliver 753 nm laser light at 150 mW/cm^2 generated with a diode laser (V-Gen. Israel). All animals were sacrificed 24 h after VTP.

3.2. Imaging

Lyophilized microbubbles (Vevo MicroMarker Contrast agents) were prepared by using techniques recommended by the manufacturer

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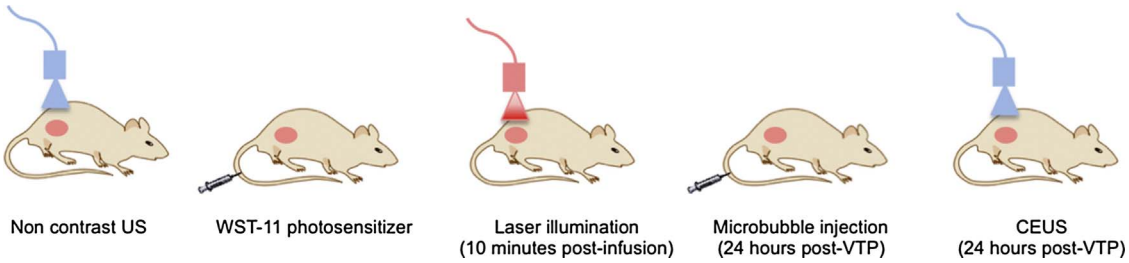

Fig. 1. Schematic detailing timeline and experimental workflow for VTP and CEUS.

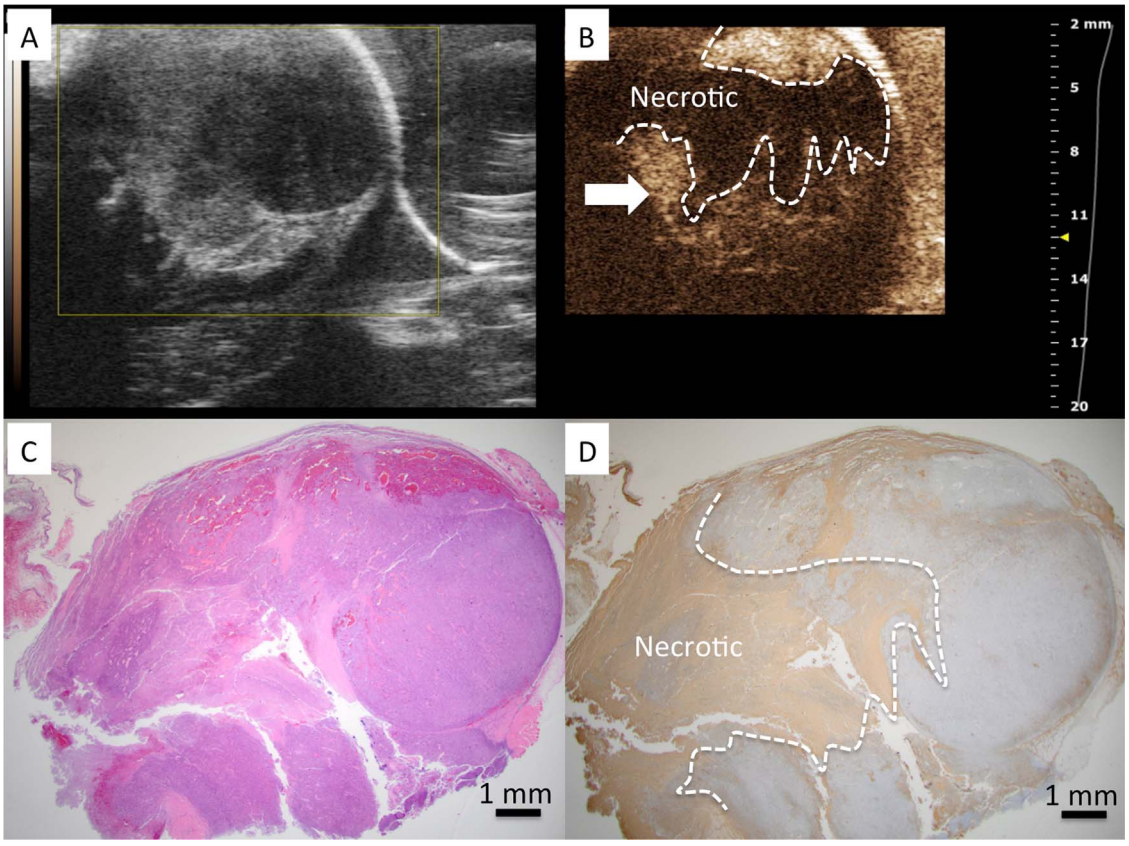

Fig. 2. A) US imaging shows tumor to have heterogeneous echo properties. B) CEUS identified a central necrotic area within the tumor after VTP (dashed line) C) H & E stained slide of the tumor corresponding to US imaging plane. D) TUNEL demonstrates a region of positive (brown) staining clearly demarcating a region of necrosis similar in size and shape to region of non-enhancing region observed on CEUS. Regions of enhancement on CEUS correspond to regions without positive staining for TUNEL and was interpreted to be viable tissue on H & E.

(Fujifilm Visualsonics, Toronto, Canada). A Vevo® 2100 high-resolution imaging system were used for placement of the laser fiber into the center of the tumor and for post-treatment CEUS imaging 24 h following VTP. For CEUS, a 50 µL bolus of 1.0×10^8 microbubbles was delivered into the tail vein of the animal using a 27 G needle. The tumor was scanned with two-dimensional US and images from the largest cross section of the tumor in the transverse plane was recorded. Fig. 1 shows the relative sequence of treatment and imaging in the experimental protocol.

3.3. Imaging and histological correlation of treatment response

Whole tumors were removed 24 h after VTP and fixed in 10% neutral buffered formalin paraffin embedded and then stained with hematoxylin-eosin (H & E) and TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling; DNA fragmentation and necrosis marker) for histopathological analysis. Non-enhancing regions on contrast-enhanced US images and regions staining positive for tumor necrosis on histopathology images were matched by reviewing the images on a reading software (Osirix, Switzerland) and compared. Size and extent (peripheral, central or patchy) of necrosis in the entire image was evaluated. A visual correlation between the area of the unenhanced zone on the CEUS images and the nonviable zone on gross images after ablation was analyzed by two independent observers. $P < 0.05$ was considered a statistically significant difference.

4. Results

4.1. Findings on CEUS of tumors post-VTP

Tumor volumes in the study ranged from 9.8 to 194.3 mm^3 (median: 24.6 mm^3). CEUS images demonstrated non-enhancing zones 24 h after VTP (n = 13). Such findings were not perceptible in the pre-treatment non CEUS images. CEUS showed non-enhancing zones in the center of the tumor for all animals (Figs. 2 B and 3B). In 8 cases, residual enhancement was observed in peripheral aspects of the tumor.