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Novel Technology Enables Diagnostic Ultrasound Machine to Treat Hepatocellular Carcinoma in Mice

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Abstract

An off-the-shelf diagnostic transducer (ULTRASONIX C5-2) was modified with custom-built circuitry to enable the transducer to produce therapeutic ultrasound in order to ablate hepatocellular carcinomas grown in immunodeficient athymic nude mice (25-35 g; Charles River Laboratories, Wilmington, MA, USA). The therapeutic antivascular ultrasound (AVUS) produced by the off-the-shelf abdominal transducer was unfocused, continuous 2.8MHz ultrasound targeting contrast-enhancing perflutren lipid microbubbles within tumor vasculature. Previous research with a dedicated physiotherapy ultrasound machine (D150 Plus, Dynatronics Corp., Salt Lake City, UT, USA) targeting similar hepatocellular carcinomas showed disrupted tumor neovasculature and irreparable dilation of tumor capillaries with subsequent intercellular edema and hemorrhage.[1-3] In this study, the echointensity, peak enhancement (PE), perfusion index (PI), and area under curve (AUC) were measured using non-linear contrast B-mode images acquired before and after the sham or AVUS treatment. These measurements all showed the AVUS produced by the diagnostic transducer markedly decreased tumor blood flow (P < 0.001). In addition, tumor temperature measurement in the live mice showed that AVUS treatment markedly increased tumor temperature with a thermal dose (CEM43) delivered by ultrasound treatment of 124.02 min versus sham of 0 min. Finally, histochemical staining of the tumor samples taken after AVUS treatment revealed several hemorrhagic pools in tumors while the sham-treated tumors lack such hemorrhagic pools. This study demonstrates diagnostic transducers can be enabled to produce AVUS with the ability to target mural hepatocellular carcinomas.

Introduction

Hepatocellular carcinoma is the most common primary liver malignancy and is often treated with resection, systemic chemotherapy, and liver transplantation if the malignancy is caught early.[4] Many advanced tumors, however, require nonsurgical management with targeted local treatment methods such as transarterial embolization, thermal ablation, and radioembolization.[4] Antivascular ultrasound therapy defined as low-intensity ultrasound in combination with microbubble-containing contrast agent injected into tumor vasculature could become a localized, minimally-invasive, non-surgical, and inexpensive alternative to treating HCC as well as other cancers.[4] Antivascular ultrasound has been shown to be a potent tumor disrupting agent as opposed to sonication alone through mechanical and thermal destruction of capillaries in order to deprive tumors of nutriets.[1] Traditionally, ultrasound machines are separated into diagnostic machines or specialized ultrasound machines that can deliver enough power to induce these mechanical and thermal effects. This study shows that a traditional diagnostic transducer can be modified to produce these same effects (Figure 1 and 3) in one machine. This could have large clinical implications as an inexpensive upgrade to existing diagnostic transducers that unlocks a new therapeutic modality. In addition, having an all-in-one ultrasound probe allows for precise targeting of tumor tissue with real-time imaging feedback during the delivery of the cancer ablating therapy.

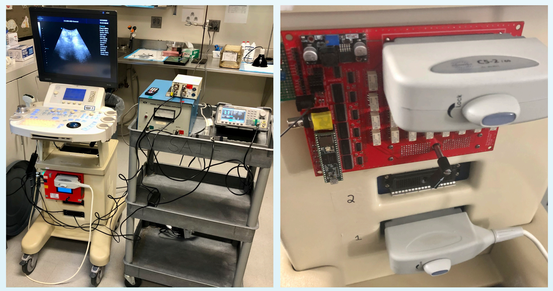


Figure 1. Left: The custom AVUS therapeutic system is turned off while UltrasonixRP is scanning in normal diagnostic B-mode. The AVUS system is only enabled when the operator has the transducer in desired position on the tumor. Right: A close-up image showing how the custom circuitry sits between the UltrasonixRP system and the transducer.

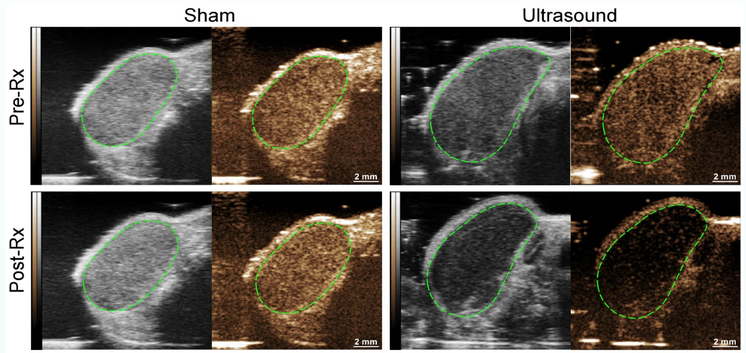


Figure 2. Left: Non-linear contrast B-mode images acquired before (Pre-Rx) and after (Post-Rx) the sham treatment showing no reduction in tumor echogenicity. Right: Non-linear contrast B-mode images acquired before (Pre-Rx) and after (Post-Rx) the AVUS treatment showing dramatic reduction in tumor echogenicity.

Methodology

HCC tumors were grown in (n = 5) adult male immunodeficient athymic nude mice (25-35 g; Charles River Laboratories, Wilmington, MA, USA). The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC # 804998). Briefly, mouse hepatoma cells Hepa1-6 (ATCC CRL-1830; American Type Culture Collection, Manassas, VA, USA) were injected subcutaneously in the right flank of the mice and grown until they were 10 mm in diameter. Prior to AVUS treatment, contrast enhanced B-mode images of each tumor were acquired (13–24 MHz; Vevo 2100 system - Fujifilm VisualSonics, Toronto, ON, Canada) following the intravenous tail-vein injection of 10 µL of the contrast-enhancing microbubbles. For the AVUS treatment, 50 µL of contrast-enhancing perflutren lipid microbubbles (Definity, Lantheus Medical Imaging, North Billerica, MA, USA) were injected and AVUS was cycled for 5 minutes on and 5 minutes off for a total of 30 minutes. After AVUS treatment, contrast enhanced B-mode images of each tumor were acquired again.

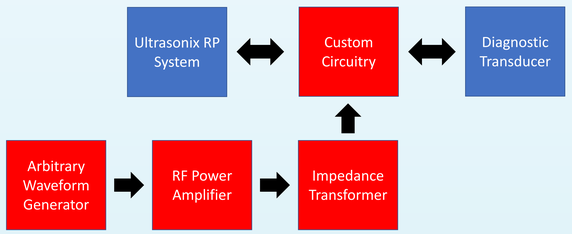


Figure 3. A block diagram of the AVUS system (Red), UltrasonixRP, and transducer. The therapeutic system's custom circuitry printed circuit board sits between the UltrasonixRP to either allow for normal diagnostic operation of the UltrasonixRP system or inject AVUS created by the arbitrary waveform generator, RF power amplifier, and impedance transformer.

Results

AVUS decreased tumor blood flow

Non-linear contrast (NLC) images acquired before (Pre-Rx) and following (Post-Rx) the sham or AVUS treatment demonstrated that the flow of the ultrasound-enhancing contrast agents was markedly decreased in the AVUS-treated groups. A qualitative examination of the NLC images revealed that, as opposed to the sham-treated groups where pre- and post-treatment groups have comparable NLC signals, the AVUS-treated groups have much reduced NLC signals post-treatment (Figure 2).

Quantitatively, the time-intensity curves, that represent the echointensity of the NLC images over time, revealed a marked increase in echointensity values of tumors after the injection of ultrasound-enhancing contrast agent before AVUS treatment. Notably, there is a significant decrease in the time-intensity curves after AVUS treatment (Figure 4-5). The time-intensity curves before and after sham-treatment remain did not change. Additionally, PE, PI, and AUC – expressed in arbitrary units (a.u.) – measured before (Pre-Rx) vs. after (Post-Rx) AVUS treatment showed that there is a substantial decrease in these parameters after AVUS treatment; PE: 23.8 (±1.5) vs. 1.8 (±0.4); PI: 24.3 (±1.1) vs. 1.11 (±0.1); and AUC: 2414.1 (±57.1) vs. 181.3 (±33.6). The difference in PE, PI, and AUC values between Pre-Rx vs. Post-Rx groups in the AVUS-treated tumors was statistically significant (P < 0.001). For the sham-treated controls, these parameters were comparable between Pre-Rx vs. Post-Rx; PE: 23.8 (±3.8) vs. 23.4 (±2.8); PI: 23.8 (±1.5) vs. 24.8 (±2.2); and AUC: 2339.2 (±350.3) vs. 2260.1 (±310.8).

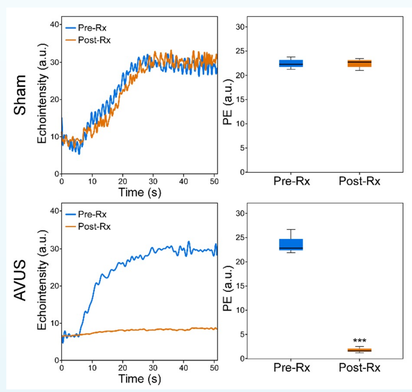
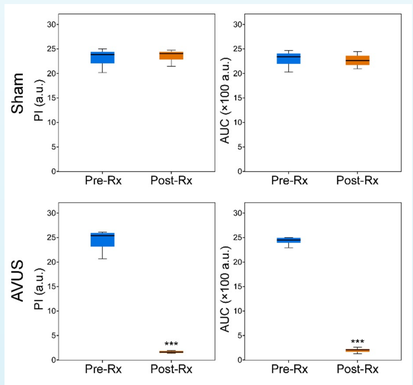


Figure 4. Echointensity and peak enhancement (PE) Pre-Rx and Post-Rx in sham versus AVUS shows large decrease in AVUS in all of the above measurements (P < 0.001).

Figure 5. Perfusion index (PI), and area under curve (AUC), Pre-Rx and Post-Rx in sham versus AVUS shows large decrease in AVUS in all of the above measurements (P < 0.001).



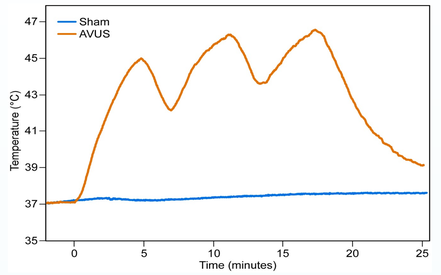


Figure 6. Sham (blue) showed no temperature increase measured inside the mural HCC while AVUS (orange) showed significant temperature increase

AVUS increased tumor temperature

Tumor temperature measurement in live mice showed that AVUS treatment markedly increased tumor temperature (Figure 6). The 0 min in the x-axis represents the onset of the treatment. Thermal dose (CEM43) delivered by ultrasound treatment was found to be 124.02 min.

AVUS increased tumor hemorrhage

Histochemical staining of the tumor samples taken after AVUS treatment revealed several hemorrhagic pools in tumors (Figure 7). The sham-treated tumors lack such hemorrhagic pools.

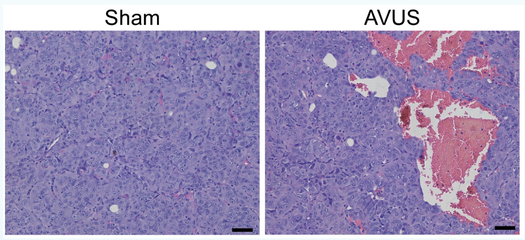


Figure 7. Left: Histopathology of mural HCC showing no hemorrhage. Right: Histopathology of mural HCC showing hemorrhage.

Conclusion

In this preclinical study, five mice with HCC were treated with AVUS from a diagnostic transducer. The dramatic reduction in tumor vasculature indicates this treatment is a potential viable alternative to several other HCC treatments. This study was a necessary step so that AVUS can eventually be translated into the clinical setting, which could provide a cost-effective upgrade to older ultrasound equipment that would enable a wide range of therapeutic applications.

References

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4. D'Souza JC, Sultan LR, Hunt SJ, et al. Microbubble-enhanced ultrasound for the antivascular treatment and monitoring of hepatocellular carcinoma. Nanotheranostics (Sydney, NSW). 2019;3(4):331-341.