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● *Original Contribution*

EFFECT OF MICROBUBBLE-ENHANCED ULTRASOUND ON RADIOFREQUENCY ABLATION OF RABBIT LIVER

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Abstract—Microbubble-enhanced ultrasound (MEUS) can non-invasively disrupt and block liver blood perfusion. It may potentially overcome the heat sink effect during a thermal ablation and consequently enhance radiofrequency ablation (RFA) of the liver. We propose a new strategy combining RFA with MEUS. For ultrasound treatment, an 831-kHz air-backed focused transducer directed 400-cycle bursts at 4.3 MPa to the liver at a 9-Hz rate. The treatment was nucleated by a lipids microbubble forming MEUS. Eighteen surgically exposed rabbit livers were treated using MEUS combined with RFA; the other 32 livers were treated using MEUS (n = 14) or RFA (n = 18) alone and served as the controls. Contrast ultrasound imaging confirmed that MEUS treatment significantly reduced liver blood perfusion by cutting contrast peak intensities in half (44.7%–54.1%) without severe liver function damage. The ablated liver volume treated using MEUS combined with RFA was 2.8 times greater than that treated using RFA alone. In conclusion, RFA of the liver can be safely and greatly enhanced by combination with MEUS pre-treatment. (E-mail: liuzhengs@hotmail.com)

Key Words: **Microbubble, Ultrasound, Liver, Thermal ablation, Radiofrequency ablation.**

INTRODUCTION

Radiofrequency ablation (RFA) is widely used in clinical settings to treat small hepatocellular carcinomas (HCCs) because it is less invasive, highly reproducible and effective (Bleicher et al. 2003; Cha et al. 2009; Chen et al. 2016; Weis et al. 2013). It causes irreversible thermal necrosis of the tissue through the delivery of electromagnetic energy. RFA has become the major alternative method in clinical settings for treating small HCCs less than 3 cm in diameter. Several long-term studies have indicated that RFA is on a par with surgical resection for very early and early stage HCCs, even with underlying hepatic insufficiency (Forner et al. 2012; Kim et al. 2013; Mazzaferro et al. 2004). However, the limitation of RFA is obvious. Previous studies have indicated that the complete ablation response of small liver tumors less than 3 cm could be as high as 90%–100%, but the complete response decreased to 45%–70% for tumors 3–5 cm in diameter and 23%–45% for tumors >5 cm in size (Peng et al. 2008; Shiina et al. 2012; Yan et al. 2008). Therefore, RFA is recommended only for tumors smaller than 3 cm in diameter (Forner et al. 2010).

It is generally accepted that the heat-sink effect is one of the major factors influencing ablation size and shape. Blood flow through tumors or major vessels near the tumors promotes heat loss and prevents heat deposition (Lu et al. 2002; Poch et al. 2016; Sheiman et al. 2012). As a result, it is difficult for larger HCCs to be completely ablated after RFA treatment. Moreover, larger tumors often develop minute, local, intra-hepatic satellite lesions (Kanai et al. 1987) nearby. To achieve tumor-free margins (*e.g.,* using surgical resection), a 2-cm circumferential ablation adjacent to the tumor is needed in RFA treatment (Chen et al. 2004). Acquiring a sufficient ablation volume for HCC treatment has become a major issue in use of the RFA technique.

One of the strategies used to achieve a larger RFA ablation area is blocking the blood flow of tissues before RFA. Trans-arterial embolization or chemo-embolization (TAE/TACE), which can reduce blood perfusion and increase heat retention, has been performed in combination

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with RFA, resulting in an improved complete ablation response and long-term survival rate (Duan et al. 2015; Lee et al. 2012).

Microbubble-enhanced ultrasound (MEUS) has been confirmed as able to disrupt tumor microvasculature by several studies (Burke et al. 2011; Liu et al. 2012; Wood and Sehgal 2015). Inertial cavitation induced by high-amplitude, low-intensity ultrasound and microbubbles can severely damage small vessels and vasculature, resulting in the cessation of circulation in relevant tissues (Hu et al. 2012; Hwang et al. 2006; Wood et al. 2008). In our previous study, we applied MEUS to disrupt tumor microvasculature and arrested tumor perfusion up to 24 h (Liu et al. 2012). The combination of MEUS and percutaneous ethanol ablation (PEA) can increase the necrosis rate of rat tumor significantly from 81.0% to 97.5% (Gao et al. 2016). In normal rabbit liver, MEUS can also block the circulation for 15–60 min and enlarge the PEA ablation volume up to 10-fold (Liu et al. 2013). MEUS facilitates PEA by disrupting tissue circulation and delaying ethanol washout. A high ethanol concentration within the target liver, therefore, enhances the chemical ablation of absolute ethanol.

This study investigated the possibility, safety and effectiveness of MEUS-induced perfusion blockage to enhance RFA of the liver *in vivo*.

METHODS

Animals

The study protocol was approved by the institutional review board of the hospital. A total of 50 healthy New Zealand rabbits aged 3–6 mo and weighing 1.8–2.5 kg were enrolled. A 21-gauge needle was inserted into the proximal auricular vein of one ear for intravenous injection, and the animals were anesthetized using 2% pentobarbital at 0.3 mL/kg before surgery. The animals were then placed in a supine position, with the upper abdominal hair removed. After a middle surgical incision of the abdominal wall, the middle and right lobes of the liver were exposed and fixed *ex vivo in situ* with gauze pre-soaked with normal saline.

Pulsed therapeutic ultrasound device

The therapeutic ultrasound (TUS) transducer was driven by a wave generator and a specifically designed power amplifier (CZ960, Mianyang Sonic Electronic Ltd, Mianyang, China). The transducer comprised an air-backed, spherical disk (25 mm in diameter) with a 160-mm radius of curvature. To provide acoustic coupling, the front chamber (10 mm long) of the disk was filled with degassed water. The geometric focus of the transducer was 150 mm from the tip. The transducer was operated at a frequency of 831 kHz, with a 400-cycle pulse length and a pulse repetition frequency of 9 Hz. A needle hydrophone (TNU001 A, NTR, Seattle, WA, USA), adjusted by a precision 3-D motion stage, was set up to measure the acoustic output of the transducer in a range of 10 mm outside the tip. The results indicated that the acoustic pressure (peak negative pressure) output was 4.3 MPa. The transducer was working in an intermittent mode of 4 s on and 4 s off. The actual working duty cycle was approximately 0.22%, and the corresponding actual acoustic intensity (I_{SPTA}) was approximately 0.4 W/cm^2 (Zhao et al. 2012).

Microbubbles

Lipid-coated microbubbles (Zhifuxian) were used both to enhance diagnostic ultrasound imaging and to act as cavitation nuclei during the ultrasound therapeutic procedure. Zhifuxian was prepared by lyophilization of a two-lipid suspension and was agitated with perfluoropropane gas using a high-speed mechanical amalgamator. The size distribution and concentration of MBs were measured using a RC-3000 Resistance Particle Counter (OMEC Technology, Guangdong, China). The MBs had a mean particle diameter of 2 µm and a bubble concentration of 2–9 × 10^9/mL (Gao et al. 2017). For MEUS treatment, Zhifuxian was constantly administered for 5 min for a total dose of 0.05 mL/kg (diluted in 5 mL saline).

Contrast-enhanced ultrasonography

Contrast-enhanced ultrasonography (CEUS) of liver was performed using a commercial diagnostic ultrasound system (VINNO 70, Vinno Technology Co. Ltd, Suzhou, China) using a X12-4 L linear array transducer. The system was equipped with a low-mechanical-index (MI = 0.04) contrast imaging modality. For CEUS, an intravenous bolus injection of Zhifuxian at 0.05 mL/kg was used. Depth, gain and other settings were the same throughout the CEUS studies.

Radiofrequency ablation

Radiofrequency ablation (RFA) was performed using the RITA 1500 system (RITA Medical Systems, Mountain View, CA, USA) and a monopolar electrode with 100-mm, 18-gauge cannulas and 5-mm active tips. Two circuit pads were placed on both shaved hind legs of the rabbit. The electrode was inserted into the connection of the middle and right lobes from the lateral side under ultrasound guidance. RFA was performed with an output power of 10 W for 90 s.

Treatment protocols

The 50 healthy New Zealand rabbits were divided randomly into three groups to receive either MEUS alone (n = 14), RFA alone (n = 18) or MEUS + RFA (n = 18) combination treatment.