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● *Original Contribution*

IMPACT OF MICROBUBBLE-ENHANCED ULTRASOUND ON LIVER ETHANOL ABLATION

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Abstract—Ethanol ablation (EA) is a safe and effective method for treating small liver cancer. However, the ethanol is rapidly washed out by blood perfusion, preventing its accumulation within tumors. Microbubble-enhanced ultrasound (MEUS) is capable of disrupting tumor and liver circulation. We hypothesized that this disruption could be used to enhance EA of normal liver tissue. We treated surgically exposed rabbit liver with a combination of MEUS and EA. The controls were treated with only MEUS or 0.05 mL EA. MEUS treatment was administered with a high-pressure-amplitude, pulsed therapeutic ultrasound device and intra-venous injection of microbubbles. Therapeutic ultrasound was delivered at an acoustic pressure of 4.3 MPa and a duty cycle of 0.22%. Contrast-enhanced ultrasound was performed to estimate liver blood perfusion. Livers were harvested for necrotic volume measurements 48 h after treatment. Contrast-enhanced ultrasound demonstrated that liver perfusion was temporally arrested, with a significant peak intensity decline from −46.9 ± 3.8 to −64.0 ± 3.3 dB, after MEUS treatment. The mean volume ablated in MEUS + EA-treated livers (3.3 ± 2.3 cm^3) was more than 10 times larger than that in livers treated only with EA (0.3 ± 0.2 cm^3). The volume of liver ablated by MEUS treatment alone was minor, scattered and immeasurable. These results indicate that MEUS disruption of the liver circulation can greatly promote EA of liver. (E-mail: liuzhengs@hotmail.com) © **2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Liver, Ethanol ablation, Microbubble-enhanced ultrasound.**

INTRODUCTION

Percutaneous ethanol ablation (PEA) was first introduced by research teams in the 1980s (Livraghi et al. 1986; Sigura et al. 1983). They demonstrated that absolute ethanol was able to induce chemical ablation of small, nodular hepatocellular carcinoma (HCC) when administered as ultrasound-guided injections. The efficacy of PEA was later confirmed by numerous reports, and it was widely accepted for clinical use. Several long-term studies have shown that PEA is a safe and effective method for treating small liver cancer (≤3 cm), with a 5-y survival rate ranging from 47% to 60.3% and negligible complications (Ebara et al. 2005; Lencioni et al. 1997; Livraghi et al. 1995). PEA can completely ablate 80% of small HCCs smaller than 3 cm in diameter, but only 50% of tumors 3–5 cm in diameter (Ebara et al. 2005; Lencioni et al. 1997; Livraghi et al. 1995; Sala et al. 2004). Histopathologic studies found that PEA results in complete coagulation necrosis in approximately 70% of tumors smaller than 3 cm in diameter (Shiina et al. 1991).

The limitations of PEA are also obvious. The anti-tumor effect of PEA is based on chemical ablation by ethanol. The ethanol is rapidly washed out by the tumor or liver circulation (Koda et al. 2000), making its accumulation at a high concentration at the injection site difficult. Thus, patients with hypervascular small HCCs tend to have a worse long-term prognosis and a higher local recurrence rate than patients with non-hypervascular HCCs who are treated with PEA (Toyoda et al. 1997). Generally, for liver tumors, PEA has been restricted to tumors smaller than 3 cm in diameter. HCCs usually develop minute, local intra-hepatic metastases with an incidence that increases with tumor size (Kanai et al. 1987). When treated by PEA, patients with tumors larger

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than 3 cm in diameter have significantly increased local recurrence rates and decreased 3- to 5-y survival rates compared with smaller tumors (Khan et al. 2000; Livraghi et al. 1995; Sala et al. 2004). This makes PEA inferior to radiofrequency ablation in treating HCCs (Cho et al. 2009; Orlando et al. 2009). The PEA technique also is limited by the multiple treatment sessions necessary, the uncertainty of the ablation zone and a high local progression rate of 17%–38% within 3 y (McWilliams et al. 2010). Thus, a technique that can enlarge or extend the ablation volume of PEA is desirable.

Acoustic cavitation is one of the major physical effects of ultrasound (US). Cavitation is the formation and oscillation of cavities, such as gas or vapor bubbles, present in the medium. Inertial cavitation, which is the violent collapse of bubbles, can cause mechanical damage depending on the magnitude of the expansion and collapse during bubble oscillation (Miller 2007). Possible mechanical effects include high pressure, shock waves, microjets and light emissions (Prentice et al. 2005). A number of studies indicate that microbubble ultrasound contrast agent (UCA) can nucleate inertial cavitation; that is, the microbubbles serve as nuclei inducing cavitation (Hwang et al. 2005).

Microbubble-enhanced ultrasound (MEUS) using high-pressure-amplitude US has been shown to induce intra-vascular inertial cavitation at low acoustic intensities; it can also cause severe mechanical damage to the endothelium of capillaries or small vessels (Hwang et al. 2005, 2006). Additionally, we previously found that macroscopic liver blood perfusion can be temporally arrested for 15–30 min with microbubble-enhanced, pulsed therapeutic ultrasound (Gao et al. 2012), which could be used to delay the rapid ethanol washout by the liver circulation during PEA.

We hypothesized that the combination of ethanol ablation (EA) and MEUS would result in ablation of a larger volume of liver tissue, possibly because the circulation blockage induced by MEUS prevents the rapid washout of the ethanol injected. In this study, we demonstrate that MEUS disruption of liver circulation can significantly expand the volume of liver ablated by ethanol.

METHODS

Animals

We performed the study on 24 healthy New Zealand rabbits weighing 2.0–2.5 kg. Nine animals were used to study the joint ablative ability of MEUS and EA. Another nine animals were assigned to treatment with MEUS but not EA. The remaining six animals were subjected to EA alone. The latter two groups served as controls (Table 1). All procedures were performed with approval of the Institutional Animal Care and Use Committee (IACUC) of the hospital.

Table 1. Animal group assignment

Group	Volume of ethanol injected (mL)	Duration of MEUS treatment (min)
MEUS + EA (n = 9)	0.05	5 min
EA (n = 6)	0.05	0 min
MEUS (n = 9)	0	5 min

EA = ethanol ablation; MEUS = microbubble-enhanced ultrasound.

Microbubbles

An ultrasound contrast agent comprising lipid-coated microbubbles, called Zhifuxian (Gao et al. 2012), was used for both nucleation of US cavitation and contrast-enhanced ultrasonography (CEUS). Zhifuxian was prepared by lyophilizing two lipids in suspension, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and then agitating the suspension with perfluoropropane gas using a high-speed mechanical amalgamator. The microbubbles had a mean particle diameter of 2 μm; 98% of the particles measured less than 8 μm and the bubble concentration was 9×10^{10}/mL. For the CEUS study, a bolus of 0.02 mL/kg microbubble suspension was injected. For the nucleation for MEUS treatment, Zhifuxian was constantly administered at the rate of approximately 0.6 mL/min to a total dose of 0.1 mL/kg (diluted in 3 mL saline).

Therapeutic ultrasound device

The therapeutic ultrasound (TUS) transducer comprised an air-backed, spherically concave disk (25 mm in diameter, Kunshan Risheng Electronics, Kunshan, China) with a 160-mm radius of curvature (Liu et al. 2012a). A wave generator and a specially designed power amplifier (250–350 V peak-to-peak, Mianyang Sonic Electronics, Mianyang, China) drove the transducer. The transducer was built up with an aluminum shell, and the front aperture (28 mm in diameter) of the shell was covered with a polyimide membrane. To provide acoustic coupling, the 10-mm-long front chamber of the disk was filled with degassed water. The geometrical focus of the transducer was exactly 150 mm from the tip, where the diameter of the US beam was 28 mm. A needle hydrophone (TNU001A, NTR, Seattle, WA, USA) adjusted by a precision 3-D motion stage was set up to measure the acoustic output of the transducer at a range of 1 cm outside the tip. The transducer was operated at 831 kKHz with a 400-cycle pulse length and a pulse repetition frequency of 9 Hz. The acoustic pressure (peak negative pressure) output was 4.3 MPa. The