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● *Original Contribution*

EFFECTS OF NON-FOCUSED MICROBUBBLE-ENHANCED AND HIGH-INTENSITY FOCUSED ULTRASOUND ON HEMOSTASIS IN A RABBIT MODEL OF LIVER TRAUMA

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Abstract—Uncontrolled hemorrhage after trauma to the liver can lead to death. The present study compared the effects of non-focused microbubble-enhanced ultrasound and high-intensity focused ultrasound on hepatic hemostasis in the injured liver. Blood perfusion level, serum liver enzyme levels and the aspartate transaminase/alanine transaminase ratio differed between the two types of treatment (all p values < 0.05). Hepatic cells in the microbubble-enhanced ultrasound group exhibited edema and compressed the hepatic sinus and blood vessels in the portal area. Coagulation and necrosis, inflammatory cell infiltration, and fibrous tissue encapsulation were observed in the high-intensity focused ultrasound group at later stages. The groups also differed in degree of ultrastructural damage and recovery time. Thus, microbubble-enhanced ultrasound has less of an impact on blood reperfusion and surrounding normal tissue than high-intensity focused ultrasound and is a better choice for the treatment of liver trauma. (E-mail: taoli39@163.com) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Microbubble, Ultrasound therapy, Liver, Trauma, High-intensity focused ultrasound, Hemorrhage, Hemostasis, Comparative study.**

INTRODUCTION

The liver is the second most frequently injured solid intra-abdominal organ, after blunt force trauma to the abdomen, which can lead to uncontrolled hemorrhage and death (Slotta et al. 2014). Surgery has traditionally been used to treat liver trauma, but minimally invasive intervention techniques have gained popularity in the past several decades, including therapeutic transcatheter arterial embolism (Wang et al. 2011), ultrasound (US)- or computed tomography-guided percutaneous drainage (Lv et al. 2012), percutaneous radiofrequency coagulation (Felekouras et al. 2004; Maroulis et al. 2013) and microwave coagulation (Takasu et al. 2004; Tang et al. 2008). These alternative approaches can improve the outcome of patients with hemodynamically compromised liver injuries. Even when surgery is indicated, the principle of minimal intervention can influence patient outcome, and any therapeutic procedure should aim to control bleeding and minimize tissue resection or damage.

High-intensity focused US (HIFU) has therapeutic effects in the treatment of liver hemorrhage (Burgess et al. 2007; Vaezy et al. 1997). HIFU can induce vascular occlusion and stimulate hemostasis (Vaezy et al. 1999a; Zderic et al. 2006b), and is completely non-invasive. However, it can also cause necrosis of the treated core region as a result of coagulation in blood vessels and direct exposure to high temperature (Chung et al. 2012; Vaezy et al. 2004); temperatures >70°C often lead to dehydration and deformation of adjacent tissue structures through transfer of heat to non-treated surrounding tissue (Dubinsky et al. 2008).

It was recently reported that microbubble-enhanced US (MEUS) can temporarily disrupt regional blood flow

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in the liver for 1 to 48 h (Liu et al. 2012; Wu et al. 2011) and induce hemostasis at a low acoustic intensity in a rabbit hepatic trauma model (Feng et al. 2014; Zhao et al. 2012). Cavitation-related effects caused by microbubbles can lead to mechanical damage to the endothelium of capillaries or tissues (Gao et al. 2012; Wang et al. 2013). To date, few reports have investigated longer recovery periods after MEUS in the treatment of liver trauma. Here, we compared the effects of MEUS and HIFU during post-operative recovery and euthanasia for post-treatment times up to 30 d in a rabbit model of liver injury.

METHODS

Animals and groups

In total, 48 New Zealand white rabbits weighing 2.06 ± 0.09 kg were obtained from the Animal Center of Daping Hospital. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Third Military Medical University (Chongqing, China) (Permit No. SYXK-PLA-2007035). Rabbits were housed in a temperature-controlled room on a 12-h/12-h light/dark cycle and were fed standard rabbit chow and water for 12 h before surgery. The rabbits were randomly divided into two groups of 24. The non-focused MEUS group was treated with low-intensity, non-focused US combined with microbubble administration, and the HIFU group was treated with HIFU without microbubbles.

Therapeutic US device

A custom-made, non-focused US device was designed by the Institute of Ultrasound Imaging, Chongqing Medical University, Chongqing, China. The 1.6-cm-diameter transducer was composed of an aluminum cylindrical shell (Fig. 1a). US was generated by a piezoelectric wafer active sensor, which formed an approximate plane wave in the near field. The acoustic output power was adjustable to the following values: 0.11, 0.22, 0.33, 0.44, 0.55, 0.66, 0.77, 0.88, 0.99, 1.1, 1.34, 1.58, 1.82, 2.06, 2.30, 2.54, 2.78, 3.02, 3.26 and 3.50 W. Other US parameters were as follows: frequency = 1 MHz; acoustic pressure (peak negative pressure) = 0.19 MPa; pulse repetition frequency = 1000 Hz; pulse duration = 500 μs; duty ratio = 50%; and irradiation time = 5 min.

HIFU apparatus

The Seapostar HIFU therapeutic apparatus (CZF, Chongqing Haifu Technology, Chongqing, China) used to treat rabbits in the HIFU group consisted of a power generator, therapeutic transducer, user console and circulating degassed water system. The focused US energy

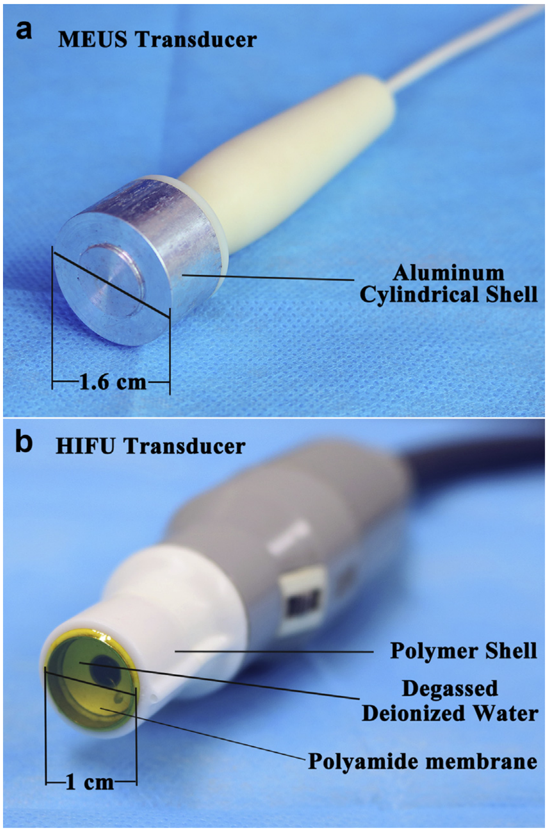

Fig. 1. Transducer used in the experiment. (a) The non-focused microbubble-enhanced ultrasound transducer (MEUS) was composed of an aluminum cylindrical shell with a diameter of 1.6 cm. (b) The high-intensity focused ultrasound (HIFU) transducer was composed of a concave transducer (diameter = 1 cm, focal length = 15 mm, distance from membrane to focal point = 6 mm) with a polymer shell; the tip was covered with a transparent polyamide membrane, and the front chamber was filled with degassed de-ionized water.

was emitted by a concave transducer (diameter = 1 cm, focal length = 15 mm) with an ellipsoidal focal region of 1 × 4 mm operating at frequency of 8–12 MHz. The acoustic power was 3–5 W. The transducer was built with a polymer shell, and the tip was covered with a transparent polyamide membrane. To provide acoustic coupling, the front chamber was filled with degassed de-ionized water (Fig. 1b). The US parameters used in the experiment were as follows: working frequency = 10.40 MHz; acoustic power = 3 W; pulse repetition frequency = 1000 Hz; pulse duration = 600 μs; and treatment time = 30 s.

Microbubbles

Lipid-coated microbubbles composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine and perfluoropropane gas (Feng et al. 2014) were provided by the Department