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● *Original Contribution*

NON-INVASIVE THROMBOLYSIS USING MICROTRIPSY IN A PORCINE DEEP VEIN THROMBOSIS MODEL

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Abstract—Histotripsy is a non-invasive therapeutic technique that uses ultrasound generated from outside the body to create controlled cavitation in targeted tissue, and fractionates it into acellular debris. We have developed a new histotripsy approach, termed *microtripsy*, to improve targeting accuracy and to avoid collateral tissue damage. This *in vivo* study evaluates the safety and efficacy of microtripsy for non-invasive thrombolysis in a porcine deep vein thrombosis model. Acute thrombi were formed in left femoral veins of pigs (~35 kg) by occluding the vessel using two balloon catheters and infusing with thrombin. Guided by real-time ultrasound imaging, microtripsy thrombolysis treatment was conducted in 14 pigs; 10 pigs were euthanized on the same day (acute) and 4 at 2 wk (subacute). To evaluate vessel damage, 30-min free-flow treatment in the right femoral vein (no thrombus) was also conducted in 8 acute pigs. Blood flow was successfully restored or significantly increased after treatment in 13 of the 14 pigs. The flow channels re-opened by microtripsy had a diameter up to 64% of the vessel diameter (~6 mm). The average treatment time was 16 min per centimeter-long thrombus. Only mild intravascular hemolysis was induced during microtripsy thrombolysis. No damage was observed on vessel walls after 2 wk of recovery, venous valves were preserved, and there was no sign of pulmonary embolism. The results of this study indicate that microtripsy has the potential to be a safe and effective treatment for deep vein thrombosis in a porcine model. (E-mail: xizh@umich.edu) **© 2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Histotripsy, Microtripsy, Thrombolysis, Sonothrombolysis, *In vivo*, Deep vein thrombosis.**

INTRODUCTION

Deep vein thrombosis (DVT) is the most common form of venous thrombosis and can lead to pulmonary embolism (PE). DVT and PE affect more than 300,000 people and result in the deaths of 60,000 to 100,000 people each year in the United States (Beckman et al. 2010). In addition to anticoagulation, some DVT patients, especially those with severe symptoms, may require thrombolytic treatments, including systemic administration of thrombolytic drugs (Adams et al. 1996; Bates and Ginsberg 2004), catheter-directed infusion of thrombolytic drugs and mechanical thrombectomy. Systemic administration of thrombolytic drugs has limited effectiveness and requires prolonged time for effect (several hours to days) (Friedman et al. 1996). Catheter-directed thrombolysis can deliver drugs locally at the thrombosis site, but it is invasive and carries risks of bleeding, vascular damage, clot detachment and infection (Sharafuddin et al. 2003). In more severe cases, such as phlegmasia cerulea dolens, surgical or percutaneous mechanical thrombectomy may be performed (Karthikesalingam et al. 2011).

Histotripsy is a non-invasive tissue ablation method that mechanically fractionates soft tissue using ultrasound (Xu et al. 2007, 2008, 2010). High-intensity, microsecond-long ultrasound pulses are focused to generate well-controlled acoustic cavitation to fractionate target tissue without thermal necrosis. The feasibility of using histotripsy as a non-invasive and image-guided thrombolysis method was first illustrated by Maxwell et al. (2009, 2011a). Histotripsy was used to fractionate blood clots into acellular debris using ultrasound alone both *in vitro* and in an *in vivo* porcine DVT model. The safety concerns (primarily hemolysis) of histotripsy in circulating blood were also addressed by Devanagondi et al. (2015). In previous studies, multicycle (usually

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≥5 cycle) ultrasound pulses were used to generate acoustic cavitation *via* a shock scattering mechanism (Maxwell et al. 2011b). With use of the shock scattering mechanism, a shock front scatters from individual sparse bubbles formed from weak nuclei, resulting in an inverted shock wave. This inverted shock wave combines with the incoming negative pressure phase to create very high negative pressures exceeding the cavitation threshold and generates a cavitation cloud. Because the shock scattering approach relies on the weak nuclei that often reside in the vessel wall, cavitation generated by this approach is usually less confined and forms near the vessel wall. This poor localization within the clot can result in vessel damage and hemolysis.

Microtripsy is a new histotripsy approach that has recently been evaluated for its use in thrombolysis (Zhang et al. 2015a, 2015c, 2016). Microtripsy uses the intrinsic threshold mechanism, by which acoustic cavitation is generated *via* single-cycle ultrasound pulses with the negative pressure phase directly exceeding the cavitation threshold intrinsic to the medium. The intrinsic threshold mechanism does not rely on pre-existing weak nuclei and is more reproducible and predictable than the shock scattering mechanism (Lin et al. 2014; Maxwell et al. 2013). Our *in vitro* microtripsy thrombolysis study indicated that cavitation can be precisely generated and confined to the vessel lumen without contacting the vessel wall, which allows for creation of a precise flow channel within the clot while minimizing the risk of vessel damage (Zhang et al. 2015c).

This *in vivo* study investigated the safety and efficacy of microtripsy-mediated thrombolysis in a porcine DVT model. Thrombi were created in the femoral veins of juvenile pigs and then treated using an integrated, portable microtripsy thrombolysis system. Ultrasound images were used to guide and monitor the treatment. Ultrasound cross-sectional scans of the femoral vein were acquired before and after each thrombolysis treatment for qualitative and quantitative assessments of treatment efficacy. To evaluate treatment safety, vessel damage was examined by histology. Blood samples were collected to evaluate the degree of hemolysis induced during microtripsy thrombolysis. In addition to acute pigs, which were euthanized right after therapy, four pigs were allowed to survive for 2 wk to assess the subacute extent of any vessel damage and/or hemolysis.

METHODS

Animal preparation and thrombus formation

The protocols involved in this study have been approved by the University Committee on Use and Care of Animals at our university. A porcine DVT model, previously described by Maxwell et al. (2011a), was used in this study. Juvenile pigs (mixed breed) weighing approximately 35 kg were selected as the subjects of the study. First, the animal was sedated with 6 mg/kg tiletamine + zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA, USA) and 2.2 mg/kg xylazine (Lloyd Laboratories, Shenandoah, IA, USA). The animal was then endotracheally intubated and rotated to a supine position onto a medical-grade, water-filled heating pad to maintain body temperature. Isoflurane 0.5%–3.5% (VetOne, Meridian, ID, USA) was administered through the endotracheal tube for anesthesia. The animal was attached to monitoring equipment for continuous monitoring of core body temperature, pulse, SpO_2 levels and respiratory rate throughout the procedure. A chemical depilatory (Nair, Church & Dwight, Princeton, NJ, USA) was applied on the legs and the lower quadrant for 10 min, followed by a surgical preparation consisting of three applications of betadine scrub followed by a sterile saline rinse and an application of iodine. The area was then draped with a sterile drape.

Two 5-Fr wedge occlusion balloon catheters (AI-07124, Arrow International, Reading, PA, USA) were introduced percutaneously into the left femoral vein from the distal side of the desired location for thrombus formation (Maxwell et al. 2011a). The two balloons were positioned and inflated to occlude a 1.5-cm segment at the desired location in the vein. Thrombin (0.5 mL, 1000 IU/mL) was then infused through the distal catheter into the occluded region in the vein to stimulate thrombus formation. Heparin (200 U/kg, intravenously [IV]) was administered systemically through an ear-vein catheter immediately after thrombin infusion to prevent blood coagulation outside the occluded region. The balloons remained inflated for 2 h to allow the thrombus to become fully formed (Ryan et al. 1999). The balloons were then deflated, and the catheters were removed from the vein. Blood pressure was continuously monitored from the carotid artery. Additional heparin (200 U/kg, IV) was administered every hour from the start of thrombus formation to the end of treatment to maintain an activated clotting time (ACT) of blood greater than 200 s. High-dose pentobarbital (140–160 mg/kg, IV) was given to those animals euthanized right after treatment. For the animals that were euthanized 2 wk after treatment, carprofen (2–4 mg/kg, subcutaneously) was given before treatment for pain relief, and protamine (0.5 mg/kg, IV) was given at the end of treatment to lower the ACT. The animals were continuously monitored until they recovered from anesthesia and were returned to the housing facility when they were fully mobile. Animals were observed twice daily for 48 h for pain levels, activity levels, feeding activity and any evidence of bleeding. Carprofen (2–4 mg/kg, orally) was given every 12–24 h as needed until the animal returned to baseline activity levels.