Hemodynamic and Hematologic Effects of Histotripsy of Free-Flowing Blood: Implications for Ultrasound-Mediated Thrombolysis

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ABSTRACT

Purpose: To investigate the extent and consequences of histotripsy-induced hemolysis in vivo.

Materials and Methods: Porcine femoral venous blood was treated with histotripsy in 11 animals with systemic heparinization and 11 without heparin. Serum and hemodynamic measurements were obtained at 0, 2, 5, 10, 15, and 30 minutes and 48–72 hours after the procedure. Fisher exact test was used to determine differences in mortality between heparinized and nonheparinized groups. A linear mixed effects model was used to test for differences in blood analytes and hemodynamic variables over time.

Results: Of 11 animals in the nonheparinized group, 5 died during or immediately after histotripsy (45% nonheparin mortality vs 0% heparin mortality, $P = .035$). Serum hematocrit, free hemoglobin, lactate dehydrogenase (LDH), and right ventricular systolic pressure changed significantly ($P < .001$) over the treatment time. Serum hematocrit decreased slightly (from 32.5% ± 3.6% to 29.4% ± 4.2%), whereas increases were seen in free hemoglobin (from 6.2 mg/dL ± 4.6 to 348 mg/dL ± 100), LDH (from 365 U/L ± 67.8 ± to 722 U/L ± 84.7), and right ventricular systolic pressure (from 23.2 mm Hg ± 7.2 to 39.7 mm Hg ± 12.3). After 48–72 hours, hematocrit remained slightly decreased ($P = .005$), whereas LDH and free hemoglobin remained slightly increased compared with baseline (both $P < .001$).

Conclusions: Intravascular histotripsy applied to free-flowing venous blood is safe with systemic heparinization, causing only transient hemodynamic and metabolic disturbances, supporting its use as a future noninvasive thrombolytic therapy modality.

ABBREVIATIONS

LDH = lactate dehydrogenase, RVSP = right ventricular systolic pressure

Histotripsy, a form of pulsed cavitational ultrasound (US), discretely and noninvasively mechanically fractionates tissue (1). Animal studies have established several potential clinical applications, including deep vein thrombosis treatment, creation of palliative intracardiac communications in infants with congenital heart disease, and in utero palliation of congenital malformations (2–4). Other forms of therapeutic US with thermal effects cause hemolysis with increasing therapeutic intensity (5). In contrast to these other modalities, histotripsy uses short, high-intensity pulses for mechanical fractionation of tissue through acoustic cavitation and without thermal necrosis (1). Nevertheless, hemolysis remains a concern because in vitro studies have suggested acoustic cavitation may cause hemolysis in the absence of thermal effects (6).

Although the safety profile of histotripsy has been investigated generally, the effect of histotripsy on the

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Z.X. is a member of the clinical advisory board for Histosonics, Inc, and is named as an inventor on patents filed for applications of histotripsy. H.G. is named as an inventor on a patent filed for applications of histotripsy for thrombolysis by the University of Michigan. None of the other authors have identified a conflict of interest.

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circulating blood volume with regard to hemolysis has not been previously described. Determining the extent of hemolysis after histotripsy is clinically important because hemolysis can lead to acute anemia. Additionally, hemolysis increases serum free hemoglobin, potentially leading to endothelial dysfunction and secondary platelet activation and aggregation, renal injury, intravascular thrombosis, and pulmonary hypertension (7).

The purpose of this study was to establish the safety profile of histotripsy with respect to the circulating blood volume, primarily by evaluating reduction in serum hematocrit after histotripsy as a marker for clinically significant hemolysis. Secondary objectives included evaluation of clinically relevant sequelae of hemolysis, such as hyperkalemia, acute kidney injury, and pulmonary hypertension.

MATERIALS AND METHODS

Animal Model

The protocols described herein were approved by the University Committee on Use and Care of Animals at our institution. A porcine model was developed that maximizes the potential adverse effects of histotripsy, including hemolysis, on the circulating blood volume in vivo by focusing therapy on free-flowing blood through a femoral vein. Prior studies in our laboratory evaluating histotripsy-mediated thrombolysis and the creation of intracardiac defects routinely employed systemic heparinization during therapy (2,3). Because the target in the present study was not intracardiac tissue or intravascular thrombus, half of the study animals were not given systemic heparinization, as the risk for thrombosis or embolization was thought to be low. However, because heparin has been shown to mitigate endothelial cell dysfunction, we also sought to ameliorate potential adverse effects of histotripsy by administering heparin to half the animals (8,9). Because the precise effects of heparinization during histotripsy were unknown, sample size was determined based on statistical power to detect a hypothesized higher mortality rate in the nonheparinized group. With 11 pigs per group, there was 82% power to detect a $> 30\%$ increase in mortality in the nonheparinized group.

Animal Preparation

Animal preparation and the histotripsy therapy apparatus used with this technique have been previously described in detail (2). Juvenile pigs (mixed breed and purebreed) weighing 30–40 kg were sedated with 5 mg/kg tiletamine + zolazepam (Telazol; Fort Dodge Animal Health, Fort Dodge, Iowa) and 2.2 mg/kg xylazine (Lloyd Laboratories, Shenandoah, Iowa), followed by endotracheal intubation and rotation to a supine position. Isoflurane 0.5%–3.5% (VET ONE; MWI Veterinary Supply, Meridian, Idaho) was administered through the endotracheal tube for anesthesia. Animals were spontaneously breathing, although mechanical ventilation was initiated for animals that became apneic during histotripsy. A chemical depilatory (Nair; Church & Dwight Co, Inc, Princeton, New Jersey) was applied to the legs for 10 minutes for hair removal to provide adequate US transmission.

Using sterile technique, a 6-F sheath (Cook, Inc, Bloomington, Indiana) was percutaneously inserted into the right internal jugular vein or right femoral vein. A 5-F or 6-F balloon wedge catheter (Arrow International, Reading, Pennsylvania) was advanced through the venous sheath and into the right ventricle. A 5-F micropuncture introducer (Cook, Inc) was percutaneously inserted into the right femoral artery. Pressures from the right ventricle catheter and right femoral artery introducer were transduced simultaneously.

A hole was cut in the middle of a transparent polyethylene sheet, and Ioban (3M, St Paul, Minnesota) was affixed over this hole. The Ioban was then affixed to the skin overlying the left femoral vein, and a section of Ioban over the histotripsy target was cut away. The polyethylene sheet was used to line a bottomless bowl, and the bowl was filled with warm, degassed water to minimize prefocal cavitation. The use of this setup enabled direct US transmission to the skin overlying the femoral vein target, while ensuring an impermeable water bath sufficiently large to treat the target appropriately, given the fixed, 9-cm focal length of the therapy transducer (**Fig 1**).

A 1-MHz 10-cm-diameter spherically focused transducer (Imasonic, Besancon, France) was attached to a computer-controlled three-axis motorized positioning stage (Parker-Hannifin, Rohnert Park, California). The transducer had a 4-cm concentric hole, through which an 8-MHz phased array US imaging probe was placed (S8, SONOS 7500; Philips Healthcare, Andover, Massachusetts). The 8-MHz probe was used to align the therapy focus within the femoral vein and provide real-time visualization of histotripsy therapy.

Targeting, Treatment, and Evaluation

The focus of the therapy transducer was located on the US image by applying histotripsy pulses to an empty water bath. The center of the bright, percolating cavitation cloud was marked as the focal position (**Fig 2**). Next, the focus was aligned within the left femoral vein. The 11 anticoagulated animals received 100 U/kg of unfractionated heparin (Sagent Pharmaceuticals, Inc, Schaumburg, Illinois). Additional heparin was given before starting histotripsy, if necessary, to maintain activated clotting time > 200 seconds. The histotripsy transducer was driven to emit five cycle US pulses at 500 Hz for 30 minutes. The lowest transducer output necessary to produce a consistent bubble cloud was used for each treatment (**Fig 3**). The efficacy of this experimental setup was previously demonstrated for thrombolysis of acute deep vein thrombosis in the femoral vein in a porcine