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Targeted Venous Occlusion Using Pulsed High-Intensity Focused Ultrasound

Joo Ha Hwang [Member IEEE],

Division of Gastroenterology, Department of Medicine, Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle, WA 98195 USA

Yufeng Zhou [Member IEEE],

Division of Gastroenterology, Department of Medicine, University of Washington, Seattle, WA 98195 USA

Cinderella Warren,

Division of Gastroenterology, Department of Medicine, University of Washington, Seattle, WA 98195 USA

Andrew A. Brayman, and

Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle, WA 98105 USA

Lawrence A. Crum [Member IEEE]

Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle, WA 98105 USA

Joo Ha Hwang: jooaha@medicine.washington.edu; Yufeng Zhou: yufengz@medicine.washington.edu

Abstract

Targeted vascular occlusion is desirable for clinical therapies such as in the treatment of esophageal and gastric varices and varicose veins. The feasibility of ultrasound-mediated endothelial damage for vascular occlusion was studied. A segment of a rabbit auricular vein was treated *in vivo* with low duty-cycle, high peak rarefaction pressure (9 MPa) high-intensity focused ultrasound (HIFU) pulses in the presence of intravenously administered circulating microbubbles, followed by fibrinogen injection, which resulted in the formation of an acute occlusive intravascular thrombus. Further investigation and refinements of treatment protocols are necessary for producing durable vascular occlusion.

Index Terms

High-intensity focused ultrasound; vessel occlusion; fibrinogen; bubble cavitation; endothelial damage

I. Introduction

The application of high-intensity focused ultrasound (HIFU) for inducing vascular occlusion by thermal coagulation has previously been demonstrated [1–3]. HIFU-induced vascular occlusion is highly effective and is currently being evaluated by the US Army for emergent treatment of major bleeding in the battlefield. However, a significant downside to this form of

therapy is the collateral damage that can occur to surrounding tissue due to thermal injury. For example, thermal damage to nerves adjacent to many large vessels has been demonstrated to cause permanent nerve damage resulting in paralysis [4].

Several clinical situations exist where it is desirable to occlude a vessel for therapeutic purposes. One such application is in the treatment of gastric varices. Gastric varices are venous dilations that develop under the gastric mucosa as a result of portal hypertension. The most common cause of portal hypertension is end-stage liver disease due to cirrhosis of the liver. The incidence and prevalence of cirrhosis is expected to increase dramatically over the next 2 decades due to the prevalence of hepatitis C infections [5]. Once gastric varices form, they are at risk of rupturing, and the mortality from a gastric variceal bleed is reported to be 20% within the first 6 weeks following the bleed [6]. The most effective therapy for the prevention of gastric variceal hemorrhage is a transjugular intrahepatic portosystemic shunt (TIPS) procedure which is associated with several potential complications, and is therefore generally reserved for patients who have previously experienced a variceal bleed [7]. Thermal coagulation of gastric varices is ineffective and causes ulcerations of the gastric mucosa. Sclerotherapy appears to be the most promising endoscopic treatment for occluding gastric varices; however, complications from sclerotherapy, such as thromboemboli, can be life-threatening [8–11]. Therefore, a safe method that selectively occludes a targeted vessel segment is needed.

The vascular endothelial surface can be damaged by exposing a vessel to high-intensity pulsed ultrasound (US) in the presence of circulating ultrasound contrast agent (UCA) [12,13]. If sufficiently treated, a non-occlusive fibrin thrombus can be formed along the luminal surface of the vessel. This nidus of fibrin clot is anchored to the damaged endothelial surface. The hypothesis of this study is that selective occlusion of a vein is possible by initially damaging the endothelial surface of a targeted segment of vein by applying low duty factor HIFU pulses in the presence of circulating UCA followed by a local injection of fibrinogen. Injection of fibrinogen into a vessel with a damaged endothelial surface will result in conversion of fibrinogen to fibrin that will then polymerize with the existing fibrin clot. Propagation of this clot with a sufficient amount of fibrinogen will lead to an occlusive thrombus in the targeted vein lumen.

II. Methods and Materials

A. Ultrasound Exposure

The transducer used in this study was assembled in our laboratory with center frequency of 1.17 MHz [12,13] and Optison® (Amersham Health, Princeton, NJ) was used for the UCA. Ultrasound exposure conditions were +27/−9 MPa peak pressures, 5000-cycle pulse, 1 Hz pulse repetition frequency (PRF), 0.43% duty factor, and 23 W/cm² spatial peak-temporal average intensity (I_{SPTA}). The water tank was filled with degassed water and maintained at ~37 °C using a circulating water heater (VWR International, West Chester, PA). An acoustic absorber was placed along the distal tank wall to minimize reflections from the tank wall and thus reduce standing wave formation (see Fig. 1).

B. Experimental Protocol

New Zealand white rabbits weighing 4–5 kg each were used for these experiments, which were carried out according to NIH guidelines under a protocol approved by the Institutional Animal Care and Use Committee at the University of Washington. Following initial sedation with a subcutaneous injection of an acetylpromazine (1.0 mg/kg)/ketamine (22 mg/kg) cocktail, the auricular surfaces were shaved and depilated to facilitate ultrasound coupling. A 21 gauge catheter was inserted into the proximal auricular vein of one ear for intravenous (IV) access.

The animals were anesthetized with an IV ketamine (35–40 mg/kg)/xylazine (5 mg/kg) cocktail and placed in a lateral decubitus position in preparation for treatment. After achieving anesthesia, the rabbit was placed on a platform connected with a 3D motion stage with the ears mounted to a custom-built holder such that the targeted region of the auricular vein could be reliably positioned at the focus of the HIFU transducer. Targeted vessels were exposed at two sites 4 mm apart for 60 seconds each.

Each animal had 3 vessel segments targeted for treatment including two control treatments: 1) Fibrinogen injection only (no ultrasound, no UCA), and 2) US exposure following IV injection of UCA (0.5 ml of Optison®) without fibrinogen injection (Tisseel VH fibrin sealant, Baxter Healthcare Corp., Westlake Village, CA). Fibrinogen is a serum protein that is the precursor to fibrin and the conversion of fibrinogen to fibrin requires thrombin and is the final step in the coagulation cascade [14]. For the treatment arm of the study animals were administered 0.5 ml of Optison® IV followed by US exposure, then injection of fibrinogen into the lumen of the targeted vessel (up to 0.2 cc).

C. Evaluation of vascular occlusions

Following treatment, the targeted vessels were evaluated for evidence of vascular occlusion using a vascular Doppler US probe (pdAccess, Escalon Vascular Access, New Berlin, WI) and recorded as presence or absence of venous flow. In addition, injection of Evan's blue dye upstream to the treated segment was performed as a method of visual angiography to evaluate occlusion (no flow of blue dye through the treated vessel segment) or no occlusion (blue dye visualized flowing through the treated segment).

At the conclusion of the experiment, one animal was euthanized with an IV injection of sodium pentobarbital (120 mg/kg) and the remaining six animals were recovered and monitored for 14 days following treatment then euthanized. The vessels segments were then dissected and placed in fixative (10% buffered formalin). The vessel segments were then prepared for light microscopy to assess for the presence of a vascular thrombus. A single pathologist, who was blinded to the treatment protocol for each vessel, graded each vessel as occlusive thrombus, non-occlusive thrombus, or no thrombus.

D. Statistical Analysis

The primary endpoints of the study were acute vessel occlusion, and vessel occlusion at day 14 following treatment. Fisher's exact test was performed to compare the outcome of the treatment arm (US + UCA + fibrinogen injection) to the 2 control arms (US + UCA only and fibrinogen injection only) for both time points (acute and 14 day survival).

III. Results

A. Acute Vascular Occlusion

The results of the treatment arm and control arms are given in Table 1. Acute vascular occlusion occurred in all 7 vessels treated with US + UCA followed by intravascular injection of fibrinogen, which was demonstrated by the absence of venous flow on Doppler evaluation. Injection of Evan's blue dye confirmed vessel occlusion with redistribution of flow (Fig. 2D). Vessels that were injected with fibrinogen only did not occlude with the injection of fibrinogen. Furthermore, no acute systemic reactions occurred as a result of fibrinogen injection. Vessels that were targeted with US after injection of UCA (US + UCA only) did not demonstrate any evidence of vessel occlusion. The p -values in comparing the difference in outcomes between the treatment arm and either of the control arms were $p = 0.0006$ each.

B. Vascular Occlusion at Day 14

Vascular occlusion at day 14 following treatment was determined by examining histology and representative histological slices are shown in Figure 3. In both control arms (US + UCA only and fibrinogen only) there was no evidence of vascular occlusion or any injury to perivascular injury on histology (Fig. 3A). In the treatment arm (US + UCA + fibrinogen injection) there were four vessels identified to have non-occlusive vascular thrombus present remaining in the vessel lumen (Figs. 3B and 3C); however, two of the treatment vessels showed no evidence of vascular occlusion. There was a statistically significant difference ($p = 0.03$) in the number of vessels with partial occlusion by residual intravascular thrombus in the treatment arm (US + UCA + fibrinogen injection) compared with either control arm (US + UCA only or fibrinogen only, see results in Table 2).

C. Adverse Effects

There was no evidence of adverse systemic effects (acute or after 14 days) from injection of fibrinogen. All six rabbits in the survival group survived the entire 14 days. There was no evidence of ulceration or tissue damage in the US treated vessel segments 14 days following treatment. There was also no evidence of infection at the injection sites.

IV. Discussion

This study demonstrates the ability to selectively occlude a targeted segment of a vein by initially targeting the vessel segment with pulsed ultrasound in the presence of circulating UCA followed by local injection of fibrinogen. The characterization of the vascular bioeffects demonstrated that the vascular endothelial surface can be sufficiently damaged from exposed to low duty factor HIFU pulses in the presence of circulating UCA, which results in non-occlusive intravascular thrombus formation without thermal injury to perivascular tissue [12, 13]. The mechanism causing this damage appears to be inertial cavitation (IC) since the degree of endothelial surface damage correlates with the IC activity and only occurs when UCA is present in the circulation, which has been reported by us previously [15]. Although the presence of microbubbles can increase heating in tissue through non-linear effects, blood flow serves as a “heat sink” and low duty factor of HIFU pulses lead to an estimated temperature rise of less than 1 °C. Furthermore, histology performed on the treated tissues show no evidence of thermal coagulative effects.

Damage to the endothelium then initiates the intrinsic and extrinsic clotting cascades and activates platelets [14], which adhere to the damaged endothelium and become activated. Activation of platelets leads to the local release of fibrinogen [16] and an initial deposition of fibrin in the region of the US-induced damage resulting in a non-occlusive fibrin clot. This initial non-occlusive intravascular fibrin clot then can serve as the base for further propagation of the fibrin clot. Thrombin then converts fibrinogen to fibrin, which then polymerizes to form a fibrin clot. In the process of fibrin polymerization erythrocytes are trapped within the fibrin matrix. However, the US-induced vascular injury appears to be insufficient to result in an occlusive thrombus in the rabbit auricular vein. Increasing the local concentration of fibrinogen by injection appears to provide sufficient substrate to yield an occlusive fibrin thrombus. Injection of fibrinogen alone does not lead to vessel occlusion and distant thromboemboli since fibrinogen is not inherently thrombogenic. It appears that initial US-induced injury to the endothelial surface provides a sufficient milieu of endothelial damage, platelet aggregation and activation, and local clotting factors such that a local high concentration injection of fibrinogen results in an occlusive fibrin clot.

Survival studies demonstrate that the vessel occlusion is not durable over 14 days, which is not an unexpected since the biologic response to an occlusive thrombus is to reestablish normal

blood flow. In the case of clots primarily consisting of fibrin, the local activation of the fibrinolytic system regulates the extent of intravascular thrombus formation and its degradation [17]. Methods of the successful long-term occlusion of vessels generally involve intense inflammation. The primary mechanism by which inflammation promotes clot stability is thought to be due to interleukin-1 release from inflammatory cells resulting in up regulation of plasminogen activator inhibitor synthesis by endothelial cells which results in down regulation of fibrinolytic activity [18]. Histology of US treated vessels demonstrates that an intense inflammatory response does not occur as a result of US treatment. Therefore, in order to obtain a durable vascular occlusion, an additional agent that elicits an intense local inflammatory response, such as absolute alcohol, may be necessary.

This novel method of vessel occlusion has significant potential benefits over current modalities of vascular occlusion. Injection sclerotherapy often results in distant thromboemboli or other potentially lethal complications [8–11]. Catheter embolization is a labor intensive, highly technical procedure, requiring catheterization of a major vessel and the use of fluoroscopy [19]. US targeted vessel occlusion has potential to be safer, simpler, and more effective than current methods of vascular occlusion. Additional studies investigating methods for obtaining a durable vascular occlusion are needed.

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Fig. 1.
Diagram of the experimental setup.

Fig. 2. Serial photographs demonstrating the treatment of a segment of the auricular vein (A) prior to treatment, (B) following treatment with US in the presence of circulating UCA, (C) following injection of fibrinogen, and (D) injection of Evan's blue dye demonstrating vessel occlusion with redistribution of vascular flow due to obstruction.

Fig. 3. Representative image H&E histology obtained from vessels 14 days following treatment A) injected with fibrinogen only, treated with US and UCA followed by fibrinogen injection demonstrating B) a non-occlusive intravascular thrombus (arrow) and C) an intravascular thrombus that is bridging between the near and far walls of the vessel (arrow) (400 μ m scale bar).

TABLE I

Observed frequencies of acute vascular occlusion by treatment group

	US+UCA ONLY	US + UCA + Fibrinogen	Fibrinogen
Occlusion	0	7	0
No Occlusion	7	0	7
<i>Totals</i>	7	7	7

TABLE II

Observed frequencies of partial vascular occlusion at day 14 by treatment group

	US+UCA ONLY	US + UCA + Fibrinogen	Fibrinogen ONLY
Partial Occlusion	0	4	0
No Occlusion	6	2	6
<i>Totals</i>	6	6	6