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● *Original Contribution*

ULTRASOUND-ENHANCED rt-PA THROMBOLYSIS IN AN *EX VIVO* PORCINE CAROTID ARTERY MODEL

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Abstract—Ultrasound is known to enhance recombinant tissue plasminogen activator (rt-PA) thrombolysis. In this study, occlusive porcine whole blood clots were placed in flowing plasma within living porcine carotid arteries. Ultrasonically induced stable cavitation was investigated as an adjuvant to rt-PA thrombolysis. Aged, retracted clots were exposed to plasma alone, plasma containing rt-PA (7.1 ± 3.8 μg/mL) or plasma with rt-PA and Definity® ultrasound contrast agent (0.79 ± 0.47 μL/mL) with and without 120-kHz continuous wave ultrasound at a peak-to-peak pressure amplitude of 0.44 MPa. An insonation scheme was formulated to promote and maximize stable cavitation activity by incorporating ultrasound quiescent periods that allowed for the inflow of Definity®-rich plasma. Cavitation was measured with a passive acoustic detector throughout thrombolytic treatment. Thrombolytic efficacy was measured by comparing clot mass before and after treatment. Average mass loss for clots exposed to rt-PA and Definity® without ultrasound ($n = 7$) was 34%, and with ultrasound ($n = 6$) was 83%, which constituted a significant difference ($p < 0.0001$). Without Definity® there was no thrombolytic enhancement by ultrasound exposure alone at this pressure amplitude ($n = 5$, $p < 0.0001$). In the low-oxygen environment of the ischemic artery, significant loss of endothelium occurred but no correlation was observed between arterial tissue damage and treatment type. Acoustic stable cavitation nucleated by an infusion of Definity® enhances rt-PA thrombolysis without apparent treatment-related damage in this *ex vivo* porcine carotid artery model. (E-mail: Christy.Holland@uc.edu) © **2011 Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Sonothrombolysis, Thrombolysis, rt-PA, Stable cavitation, Vascular bioeffects, Passive cavitation detection, *Ex vivo* vascular model.**

INTRODUCTION

Potent thrombolytic agents such as recombinant tissue plasminogen activator (rt-PA) have enabled effective clot lysis in occluded arteries in the brain and other sites, particularly for salvage of cardiac tissue in acute myocardial infarction (Mueller et al. 1985). For ischemic stroke, however, a higher rate of intracranial hemorrhagic complications is associated with higher rt-PA doses in clinical thrombolytic treatment (Cannon 2000). Thus, improved thrombolysis or greater safety could allow physicians to administer rt-PA to a greater portion of patients with ischemic stroke, including some who are now excluded because of concern for hemorrhagic complications. Researchers have examined the ability of ultrasound to enhance the efficacy of thrombolytic agents (Sobbe et al. 1974; Trubestein et al. 1976; Siegel et al. 2000). Investigations by Francis and his coworkers suggest that ultrasound accelerates enzymatic fibrinolysis by increasing transport of reactants through a cavitation-related mechanism (Francis et al. 1992; Blinc et al. 1993; Francis et al. 1995). Several investigators have used low-frequency, low-intensity ultrasound to accelerate rt-PA thrombolysis *in vitro* (Akiyama et al. 1998; Behrens et al. 1999; Saguchi et al. 2008). In addition, mechanistic studies *in vitro* have revealed that stable cavitation is correlated with enhanced rt-PA thrombolysis (Datta et al. 2006, 2008); yet strategies to optimize the occurrence of

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such bubble activity and avoid potential harmful bioeffects have yet to be identified.

Alexandrov et al. (2004) used 2-MHz transcranial Doppler ultrasound to monitor the middle cerebral artery in acute ischemic stroke patients. These authors observed an increase in the rate of sustained complete recanalization within 2 h after the administration of rt-PA in patients who were also monitored with ultrasound. However, another study carried out in patients using 300-kHz pulsed transcranial ultrasound in combination with rt-PA demonstrated an increased rate of cerebral hemorrhage, prompting the premature cessation of the study (Daffertshofer et al. 2005). Later simulations showed that this trial treatment may have resulted in rarefactional pressures in excess of 1 MPa (Baron et al. 2009) because of the formation of standing waves inside the skull, and exploration of treatments using less vigorous insonation are warranted.

The goal of this work was to determine whether ultrasonic stable cavitation could be sustained using a continuous infusion of a commercial contrast agent, Definity (Lantheus Medical Imaging, N. Billerica, MA, USA) in flowing plasma and to maximize this bubble activity to promote rt-PA thrombolytic enhancement. The study was carried out in living, excised porcine carotid arteries to simulate the cavitational milieu inside the body and allow monitoring of cavitation activity using a hydrophone. In addition, the biological effects of the combined ultrasound and thrombolytic treatments on the *ex vivo* arteries were observed.

MATERIALS AND METHODS

Porcine whole blood clots were formed in glass pipettes, placed within excised porcine carotid arteries and mounted in a flow system within a water tank to allow exposure to 120-kHz ultrasound. Perfusion with oxygen-rich pooled porcine plasma maintained viability of arterial tissue segments and enabled injection of rt-PA and an echo contrast agent, Definity. This apparatus was designed to allow detection of acoustic cavitation in a vascular environment that closely resembled the body, while permitting control of flow variables (Hitchcock et al. 2010).

Source transducer characteristics and calibration

A circular 120-kHz single-element transducer (Sonic Concepts Inc., Woodinville, WA, USA) with a 6.14-cm diameter was calibrated in water using a 0.5-mm hydrophone (Reson, TC 4038, Goleta, CA, USA) mounted on a computer-controlled three-axis positioner (Velmex, NF-90 series, Bloomfield, NY, USA). The transducer had a –3-dB beam width of 2.5 cm and a Rayleigh distance of 10.4 cm. The 120-kHz transducer was driven with a function generator (Agilent, 33120A, Palo Alto, CA, USA), power amplifier (75A250, AR Amplifier Inc., Souderton, PA, USA) and custom-built impedance matching network (Sonic Concepts Inc.).

The ex vivo flow system

The carotid arteries of 38 mature domestic pigs (6 to 10 months old) were excised within 20 min of exsanguination at an abattoir. The arteries were rinsed with cool (15°C) phosphate-buffered saline (PBS; Fisher Scientific, Hampton, NH, USA) to remove free blood and then stored in individual plastic bags in ice-cold PBS until use. All arteries were used within 72 h of excision (Thorne and Paul 2003). Whole blood was collected from abattoir pigs during exsanguination and immediately transferred to 1.9-mL Pasteur pipettes with tips sealed using surgical wax. The blood-filled pipettes were covered and incubated in a 37°C water bath for 3 h, and stored at 4°C for at least three days before use to allow clot retraction (Shaw et al. 2001; Holland et al. 2002; Datta et al. 2006). Fully retracted clots are more difficult to lyse with rt-PA and are typical of thrombi that have matured over prolonged periods in the heart (Marcu et al. 2007). These types of thrombi usually occur in the left atrium during atrial fibrillation and can lead to ischemic stroke. The relative resistance of clots to rt-PA thrombolysis *in vivo* is related to their origin and composition and varies widely (Undas et al. 2006a, 2006b; Mutch et al. 2010; Boulaftali et al. 2011). The choice of fully retracted clots for use in our study, though structurally different than some clots *in vivo*, is aimed at a worst-case scenario.

Each artery was weighed, and a clot was cut to 3.2 cm in length, weighed, gently injected into a 4.4-cm length of carotid artery and mounted in a special-purpose-built stainless steel and Delrin frame using 1/8 inch polypropylene barb-style tubing fittings as cannulae (EW-45500-34, Cole-Parmer, Vernon Hills, IL, USA). An acoustically transparent latex wall was placed around the frame to make an enclosed, leak-proof chamber, and the space between latex and artery was filled with degassed (1.73 ± 0.5 mg O_2/L) artificial cerebrospinal fluid (aCSF) to simulate the environment of the middle cerebral artery. The aCSF was composed of 128 NaCl, 3.0 KCl, 1.0 $MgSO_4$, 23.5 $NaHCO_3$, 0.5 NaH_2PO_4 and 30 glucose, all in mMol/L (Kehr et al. 2001). The cannulae were connected to a flow system through which 37 °C, citrated, oxygenated porcine plasma (Lampire Biological Products, Pipersville, PA, USA) circulated with a reciprocating pump as described by Hitchcock et al. (2010). The temperature of the plasma was maintained at 37 ± 0.5°C, the pH was 7.37 ± 0.03 and the dissolved oxygen content of the plasma was sustained at 23.3 ± 1.5 mg/L using a mixture of 95% oxygen and 5% carbon dioxide *via*