Ultrasound in Med. & Biol., Vol. 33, No. 7, pp. 1132–1139, 2007

0301-5629/07/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2007.01.005

● *Original Contribution*

# ENHANCED TARGETING OF ULTRASOUND CONTRAST AGENTS USING ACOUSTIC RADIATION FORCE

JOSHUA J. RYCHAK,* ALEXANDER L. KLIBANOV,† KLAUS F. LEY,*‡ and JOHN A. HOSSACK‡
*University of Virginia Cardiovascular Research Center, Charlottesville, VA, USA; †University of Virginia Department of Internal Medicine, Charlottesville, VA, USA; and ‡University of Virginia Department of Biomedical Engineering, Charlottesville, VA, USA

(*Received* 19 *June* 2006; *revised* 18 *December* 2006; *in final form* 2 *January* 2007)

**Abstract—Contrast-enhanced ultrasound has shown significant promise as a molecular imaging modality. However, one potential drawback is the difficulty that ultrasound contrast agents (UCA) may have in achieving adhesion to target molecules on the vascular endothelium. Microbubble UCA exhibit a lateral migration toward the vessel axis in laminar flow, preventing UCA contact with the endothelium. In the current study, we have investigated low-amplitude acoustic radiation as a mechanism to move circulating UCA toward targeted endothelium. Intravital microscopy was used to assess the retention of microbubble UCA targeted to P-selectin in the mouse cremaster microcirculation and femoral vessels. Acoustic treatment enhanced UCA retention to P-selectin four-fold in cremaster venules and in the femoral vein and 20-fold in the femoral artery. These results suggest acoustic treatment as a mechanism for enabling ultrasound-based molecular imaging in blood vessels with hemodynamic and anatomical conditions otherwise adversarial for UCA retention. (E-mail: jh7fj@virginia.edu)** **© 2007 Published by Elsevier Inc. on behalf of the World Federation for Ultrasound in Medicine & Biology.**

*Key Words:* **Acoustic radiation force, Microbubble, Ultrasound imaging, Contrast enhanced ultrasound.**

## INTRODUCTION

Targeted ultrasound contrast agents (UCA) have demonstrated utility for ultrasound-based molecular imaging. In a typical setting, the outer surface of a contrast agent is coated with a ligand that binds one or more target molecules of interest. Detection of agents accumulated at the target site using contrast-specific diagnostic ultrasound enables imaging of the targeted molecular event. This method has been used to image a number of physiological processes in animal models, including postischemic injury (Lindner et al. 2001), thrombus (Lanza et al. 1997; Hamilton et al. 2002; Schumann et al. 2002), tumor angiogenesis (Ellegala et al. 2003; Weller et al. 2005) and vascular remodeling in skeletal muscle (Leong-Poi et al. 2005).

Gas-encapsulated microbubbles are frequently used as targeted ultrasound contrast agents. The microbubble contrast agent was initially conceived as freely circulating blood tracer. The microvascular rheology of several nontargeted microbubble preparations has been shown to be very similar to that of erythrocytes (Ismail et al. 1996; Jayaweera et al. 1994; Lindner et al. 2002) and microscopic observations have confirmed that microbubbles tend to distribute toward the axis of the vessel (Keller et al. 1989). This characteristic enables the use of these agents as blood flow tracers for measuring vascular perfusion. However, for the purpose of molecular imaging, which requires that a circulating UCA come into contact with the targeted molecule at the vascular endothelium, lateral migration of an UCA toward the vessel axis is detrimental. In the microcirculation, hemodynamic and anatomical factors, such as postcapillary margination (Schmid-Schonbein et al. 1980), may facilitate UCA contact with the endothelium; however, such forces are largely absent in large blood vessels, especially in the arterial circulation. Thus, the frequent absence of UCA contact with the target surface may be a significant impediment to the widespread use of these agents for molecular imaging. This is especially pertinent for pathophysiology found in large vessels, such as thrombosis or atherosclerosis.

Address correspondence to: John A. Hossack, PhD, Department of Biomedical Engineering, MR5, 415 Lane Road, University of Virginia, PO Box 800759, Charlottesville, VA 22908-0759, USA. E-mail: jh7fj@virginia.edu

The application of low-intensity acoustic energy has been hypothesized as a mechanism to move freely flowing UCA toward the endothelium (Fowlkes et al. 1993; Dayton et al. 1997). The acoustic radiation force (also known as the Bjerkness force) consists of two components: these are a primary force directed away from the acoustic source and a secondary force, which is typically attractive between UCA. Dayton and colleagues verified that the primary radiation force was able to displace nontargeted UCA away from the vessel center in a flow chamber (Dayton et al. 1997, 1999a) and in the mouse microcirculation (Dayton et al. 1999b). The use of acoustic radiation force to enhance UCA adhesion to a target surface was subsequently examined *in vitro* (Rychak et al. 2005; Zhao et al. 2004). It was found that the retention of UCA targeted to the inflammatory protein P-selectin was increased significantly (up to 80-fold) by the application of acoustic radiation force and that this retention was specific. Significant clustering of UCA was observed after the application of acoustic radiation (Rychak et al. 2005), with up to 80% of the adherent UCA occurring in multiparticle aggregates. Acoustic radiation force has also been suggested as a mechanism to enhance the delivery of therapeutic substances (Lum et al. 2006; Shortencarier et al. 2004). In the current study, we examine the ability of applied acoustic radiation force to enhance the adhesion of UCA targeted to the inflammatory endothelial protein P-selectin *in vivo*. Contrast agent retention was examined in a model of inflammation in the mouse cremaster microcirculation and in the femoral artery and vein.

## MATERIALS AND METHODS

*Ultrasound contrast agents*

The microbubble UCA used in this study were composed of decafluorobutane encapsulated by a lipid monolayer. The preparation of these agents has been described in depth elsewhere (Klibanov et al. 1999). The fluorescent probe DiI (Molecular Probes, Eugene, OR, USA) was incorporated into the lipid shell to enable microscopic detection of the agents.

The anti-P-selectin monoclonal antibody Rb40.34 (Bosse and Vestweber, 1994), prepared from hybridoma supernatant at the University of Virginia Lymphocyte Culture Center (Charlottesville, VA, USA), and an isotype-matched control (clone R3-34; BD Biosciences, San Jose, CA, USA) were used as targeting ligands. Targeting ligands were attached to the microbubble by means of biotin-streptavidin chemistry, as in Lindner et al. (2001). The size distribution and concentration of microbubble dispersions was characterized by electrozone sensing using a Coulter Multisizer IIe (Beckman-Coulter, Miami, FL, USA).

*Animal preparation*

Ten male C57/Bl-6 mice (Hilltop Labs, Scottsdale, PA, USA) and four male P-selectin knockout mice (derived from the colony described by Bullard et al. 1995) were used to examine UCA retention in the cremaster muscle microcirculation. Six mice in which the gene for green fluorescent protein had been knocked-in to the Lys-M locus (Faust et al. 2000) were used to examine UCA retention in the femoral vessels. All mice were housed at the University of Virginia animal facility and experiments were performed according to institutional guidelines.

Anesthesia was induced by an IP injection of 125 mg/kg body weight ketamine (Parke-Davis, Morris Plains, NJ, USA), 12.5 mg/kg xylazine (Phoenix Scientific, St. Joseph, MO, USA) and 0.025 mg/kg atropine sulfate (Elkins-Sinn, Cherry Hill, NJ, USA). Body temperature was maintained with a heat pad. Contrast agents were administered through a cannula made of PE-20 tubing (Becton Dickenson, Sparks, MD, USA) inserted into the right jugular vein and secured with sutures.

*Intravital microscopy*

P-selectin expression in the cremaster microcirculation was induced by the surgical trauma of exteriorizing the muscle (Ley et al. 1995). After preparation, the cremaster muscle was pinned to the stage of a custom-fabricated bath mounted to a compound videomicroscope (Carl Zeiss, Thornwood, NY, USA). The bath was filled to a depth of 50 mm with superfusion solution (131.9 mM NaCl, 18 mM $NaHCO_3$, 4.7 mM KCL, 2.0 mM $CaCl_2$*$2H_2O$, 1.2 mM $MgCl_2$ equilibrated with 5% $CO_2$) that was continuously circulated through a heater to maintain a constant temperature of 38°C. The ultrasound transducer was bolted to the stage at the focal distance (20.3 mm) from the cremaster using a ring stand and transmission of ultrasound energy occurred through the bath (Fig. 1). A bolus of $10^7$ targeted or control UCA in

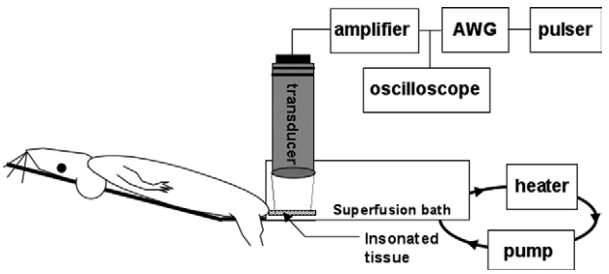


Fig. 1. Schematic of experimental apparatus. Acoustic energy was applied to the insonated tissue (femoral vessels or cremaster muscle) with a single element ultrasound transducer. The insonated tissue was immersed in a heated superfusion bath and the transducer was acoustically coupled to the tissue via the superfusion fluid.