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● *Original Contribution*

VASCULAR EFFECTS INDUCED BY COMBINED 1-MHz ULTRASOUND AND MICROBUBBLE CONTRAST AGENT TREATMENTS *IN VIVO*

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Abstract—Previous *in vivo* studies have demonstrated that microvessel hemorrhages and alterations of endothelial permeability can be produced in tissues containing microbubble-based ultrasound contrast agents when those tissues are exposed to MHz-frequency pulsed ultrasound of sufficient pressure amplitudes. The general hypothesis guiding this research was that acoustic (*viz.*, inertial) cavitation, rather than thermal insult, is the dominant mechanism by which such effects arise. We report the results of testing five specific hypotheses in an *in vivo* rabbit auricular blood vessel model: (1) acoustic cavitation nucleated by microbubble contrast agent can damage the endothelia of veins at relatively low spatial-peak temporal-average intensities, (2) such damage will be proportional to the peak negative pressure amplitude of the insonifying pulses, (3) damage will be confined largely to the intimal surface, with sparing of perivascular tissues, (4) greater damage will occur to the endothelial cells on the side of the vessel distal to the source transducer than on the proximal side and (5) ultrasound/contrast agent-induced endothelial damage can be inherently thrombogenic, or can aid sclerotherapeutic thrombogenesis through the application of otherwise subtherapeutic doses of thrombogenic drugs. Auricular vessels were exposed to 1-MHz focused ultrasound of variable peak pressure amplitude using low duty factor, fixed pulse parameters, with or without infusion of a shelled microbubble contrast agent. Extravasation of Evans blue dye and erythrocytes was assessed at the macroscopic level. Endothelial damage was assessed via scanning electron microscopy (SEM) image analysis. The hypotheses were supported by the data. We discuss potential therapeutic applications of vessel occlusion, *e.g.*, occlusion of at-risk gastric varices. (E-mail: jooha@u.washington.edu) © 2005 World Federation for Ultrasound in Medicine & Biology.

***Key Words*: Acoustic cavitation, Bioeffects, Blood vessels, Endothelial damage, Endothelial permeability, Extravasation, Hemorrhage, Inertial cavitation, Microbubbles, Optison®, Rabbit, Sclerotherapy, Thrombin, Ultrasound contrast agent.**

INTRODUCTION

Gas-filled microbubbles used as intravascular contrast agents (UCA) have enhanced the diagnostic capabilities of ultrasound (US) imaging, *e.g.*, the ability to visualize smaller caliber blood vessels, improved identification of tumors and visualization of the cardiac wall (Feinstein et al. 1990; Keller et al. 1987, 1989; Kitzman et al. 2000). However, UCAs greatly increase the potential for cavitation-related bioeffects *in vitro*, such as cell lysis (Brayman et al. 1996, 1997, 1999; Brayman and Miller 1999; Chen et al. 2003a, 2003b, 2003c; Dalecki et al. 1997a; Everbach et al. 1998; Feril, Jr. et al. 2003; Kudo et al. 2002; Miller and Quddus 2001; Miller and Thomas 1996; Miller et al. 2001), platelet activation (Poliachik et al. 2001), thrombolysis (Everbach and Francis 2000; Tachibana 1992; Tachibana and Tachibana 1995) and enhanced gene and drug delivery (Guzman et al. 2001a, 2001b; Huber et al. 2003; Seemann et al. 2002; Taniyma et al. 2002; Zderic et al. 2002). A small but growing body of studies indicates that UCAs can nucleate inertial cavitation (IC) *in vivo* and that mechanical bioeffects in the form of petechial hemorrhage due to capillary rupture (Dalecki et al. 1997b, 2000; Maruvada and Hynynen

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2004; Miller and Gies 1998b; Miller and Quddus 2000a; Price et al. 1998; Skyba et al. 1998a) and endothelial cell damage (Kobayashi et al. 2002; Shigeta et al. 2004) can be produced by US exposure when UCAs are present in the circulation. A large body of evidence from *in vitro* studies indicates that inertial acoustic cavitation is associated with the production of US-induced mechanical bioeffects. Nucleation of insonified samples with UCA microbubbles diminishes the pressure threshold for, and increases the magnitude of, such bioeffects (AIUM 2000; Miller et al. 1996). Blood and tissues which lack endogenous gas bodies are resistant to the induction of inertial cavitation (IC) activity unless exposed to extremely high US intensities and negative pressures (Deng et al. 1996). By providing gas body nuclei, UCAs significantly reduce the acoustic pressure threshold for the inception of IC and appear to increase intravascular cavitation activity.

In vitro studies have demonstrated that IC is associated with mechanical bioeffects and that the threshold for IC activity is significantly reduced in the presence of UCAs. Tachibana et al. (1999) used scanning electron microscopy (SEM) to demonstrate pore formation in membranes of cells after exposure to 255 kHz continuous-wave US exposure, presumably due to IC. Additional *in vitro* studies have shown that pulsed US in the presence of UCA results in the erosion of artificial endothelia (Brayman et al. 1999), disrupts cell membranes (Guzman et al. 2001b; Miller et al. 1999; Miller and Quddus 2000b, 2001; Ward et al. 1999, 2000) and induces apoptosis (Ashush et al. 2000; Feril, Jr. et al. 2003). Kudo et al. (2002) further demonstrated, using high-speed video microscopy, that IC of a single bubble can damage the membrane of an endothelial cell *in vitro*. Several studies have demonstrated that hemolysis can be enhanced by exposing whole blood to pulsed US in the presence of UCAs and that hemolysis is associated with IC activity detected using passive cavitation detection methods (Brayman et al. 1996, 1997; Brayman and Miller 1999; Chen et al. 2003b; Everbach et al. 1997; Miller et al. 1998; Miller and Gies 1998a; Miller and Thomas 1996; Miller et al. 2001). Additional evidence that a "dose response" exists between the magnitude of IC and a quantifiable mechanical bioeffect was provided by Chen et al. (2003a), who demonstrated that the magnitude of the IC dose (cumulative root-mean-squared of the broadband noise amplitude in the frequency domain) correlates directly with hemolysis in whole human blood.

US-mediated vascular bioeffects may have clinical relevance from diagnostic and therapeutic perspectives. From a diagnostic perspective, it is desirable that imaging studies result in minimal or clinically insignificant bioeffects. From a therapeutic perspective, it is desirable to achieve the intended therapeutic bioeffect using US without causing, or at least minimizing, collateral tissue damage. Several potential therapeutic applications of high-intensity US are currently being investigated, including: hemostasis of bleeding vessels (Hwang et al. 2003; Martin et al. 1999; Vaezy et al. 1998); thrombolysis (Everbach and Francis 2000; Kodama et al. 1999; Rosenschein et al. 1992, 2000; Tachibana and Tachibana 1995); arterial occlusion for the treatment of tumors and bleeding (Hynynen et al. 1996b; Wu et al. 2002); venous occlusion for the treatment of esophageal and gastric varices, hemorrhoids and varicose veins (Hwang et al. 2003); and drug and gene delivery (Lawrie et al. 2000; Miller et al. 2002; Ng and Liu 2002; Porter and Xie 2001; Price et al. 1998; Tachibana and Tachibana 2001; Taniyma et al. 2002; Unger et al. 2001, 2002). The bioeffects that occur *in vivo* as a result of US/UCA interactions will theoretically be a result of IC initiated within the intravascular space resulting in bioeffects produced intravascularly (*e.g.*, hemolysis and activation of platelets), at the endothelial surface (*e.g.*, endothelial cell damage) and possibly deeper structures of the vessel wall (*e.g.*, sonoporation of vascular smooth muscle cells).

Indirect evidence of the potential for vascular bioeffects resulting from insonation in the presence of UCA microbubbles was provided by *in vitro* studies of cell monolayer disruption discussed earlier. Direct evidence of microvessel rupture *in vivo* in response to acoustic treatment of tissues containing UCAs has been reported (Dalecki et al. 1995a, 1995b, 1997b; Miller and Quddus 2000a; Price et al. 1998; Skyba et al. 1998). The mechanism involved in microvessel damage has been assumed generally to be associated with the violent inertial collapse of microbubbles. Counter to this assumption, Zhong et al. (2001) have produced data which suggest that the mechanism of vessel rupture involves radial forces exerted on the vessel wall by microbubble expansion produced by the rarefaction phase of the pressure pulse, rather than being due to violent bubble collapse. These data were derived from high-speed imaging studies of bubbles exposed to shock-wave lithotripter (SWL) pulses while contained in simulated blood vessels. However, because the duration of the rarefaction pressure phase of SWL impulses is very long relative to conventional ultrasound, the bubbles remained in an expanded state over time scales of tens to hundreds of microseconds, rather than micro- or submicrosecond time scales. Whether or not forced radial expansion of microvessels over the much shorter time scales associated with MHz-frequency ultrasound is sufficient to produce microvessel rupture by this mechanism remains an open question.

The available literature concerning US/UCA bioeffects in medium- to large-caliber blood vessels is sparse. An *in vivo* study examining US-mediated gene transfer into rabbit carotid arteries has been reported (Huber et al. 2003). In this study, the investigators isolated the carotid