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● *Original Contribution*

INTRAVASCULAR INERTIAL CAVITATION ACTIVITY DETECTION AND QUANTIFICATION *IN VIVO* WITH OPTISON

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Abstract—Inertial cavitation (IC) is an important mechanism by which ultrasound (US)-induced bioeffects can be produced. It has been reported that US-induced *in vitro* mechanical bioeffects with the presence of ultrasound contrast agents (UCAs) are highly correlated with quantified IC "dose" (ICD: cumulated root-mean-squared broadband noise amplitude in the frequency domain). The ICD has also been used to quantify IC activity in *ex vivo* perfused rabbit ear vessels. The *in vivo* experiments reported here using a rabbit ear vessel model were designed to: (1) detect and quantify IC activity *in vivo* within the constrained environment of rabbit auricular veins with the presence of Optison and (2) measure the temporal evolution of microbubble IC activity and the ICD generated during insonation treatment, as a function of acoustic parameters. Preselected regions-of-interest (ROI) in the rabbit ear vein were exposed to pulsed focused US (1.17 MHz, 1 Hz PRF). Experimental acoustic variables included peak rarefaction pressure amplitude ([PRPA]: 1.1, 3.0, 6.5 or 9.0 MPa) and pulse length (20, 100, 500 or 1000 cycles). ICD was quantified based on passive cavitation detection (PCD) measurements. The results show that: (1) after Optison injection, the time to onset of measurable microbubble IC activity was relatively consistent, approximately 20 s; (2) after reaching its peak value, the IC activity decayed exponentially and the half-life decay coefficient ($t_{1/2}$) increased with increasing PRPA and pulse length; and (3) the normalized ICD generated by pulsed US exposure increased significantly with increasing PRPA and pulse length. (E-mail: juantu@u.washington.edu)

Key Words: ***In vivo*** **inertial cavitation, Passive cavitation detection, Ultrasound contrast agents, Microbubbles, Optison, Inertial cavitation dose.**

INTRODUCTION

Many studies have reported on ultrasound (US)-induced bioeffects both *in vitro* and *in vivo*, such as lung hemorrhage, microvascular hemorrhage, vascular endothelial cell damage, venous thromboses, platelet aggregation and adhesion, hemolysis and cell sonolysis (Coleman et al. 1987; Crum 1988; Vakil and Everbach 1993; Bailey et al. 1999; Brayman et al. 1999; Hwang et al. 2005; Miller and Quddus 2000; Sokolov et al. 2001; Maruvada and Hynynen 2004). An important mechanism of US-induced nonthermal bioeffects is inertial cavitation (IC), in which the insonated micrometer-sized bubbles expand from the negative pressure phase of the US field and then collapse in a violent implosion. The presence of ultrasound contrast agent (UCA) increases the potential for US exposure to generate inertial cavitation activity and related bioeffects by introducing gas-based cavitation nuclei, which lower the threshold for inertial cavitation (Apfel and Holland 1991; Miller and Thomas 1995; Deng et al. 1996).

Factors that influence the IC activity and related bioeffects have been reported previously. For instance, the threshold peak rarefaction pressure amplitude (PRPA) for significant hemolysis in whole blood was observed to be on the order of 0.5 MPa, with UCA at low MHz US frequency (Brayman et al. 1996) compared with ~4.5 MPa without UCA (Deng et al. 1996). Other studies have shown that, in the presence of UCA, US-induced hemolysis increased with increasing pressure levels, total exposure time, UCA concentration (Brayman et al. 1996; Miller and Thomas 1996; Brayman and Miller 1997; Brayman et al. 1999) and decreasing acoustic frequency (Brayman and Miller 1997). Other studies

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have provided additional evidence that bioeffects observed in *in vitro* systems are highly correlated either to the magnitude of IC activity or to acoustic parameters that are known to increase IC activity. Everbach et al. (1997) correlated hemolysis with IC activity in the presence of 3.5 v/v % Albunex. Miller et al. (2001) reported that more IC activity and hemolysis were produced with increasing PRPA, decreasing acoustic frequency and a UCA filled with a gas less soluble than air (*viz.*, Optison). Chen et al. (2003a, 2003b) correlated the quantified IC "dose" (ICD) with hemolysis produced *in vitro* with or without Optison. They concluded that hemolysis was significantly correlated with ICD, PRPA threshold for hemolysis and detectable ICD were ~ 0.5 MPa with UCA and hemolysis and ICD values were dependent on the initial Optison concentration, PRPA, pulse repetition frequency (PRF) and pulse length.

Although considerable experience has been accumulated in detecting IC activity and correlating the IC activity with US-induced bioeffects *in vitro*, the task of direct *in vivo* IC detection, especially quantitative measurements of IC activity, remains a challenging problem because of the complexity of the *in vivo* environment. Researchers have hypothesized that it is difficult to detect IC *in vivo* because there are not enough cavitation nuclei or the actual cavitation events with small scale and short duration are obscured in the noise of acoustic scattering from inhomogeneous tissues or structures in the body (Crum et al. 1992). To overcome these obstacles, a rabbit ear vessel model was selected here and Optison (GE Healthcare) microbubbles were introduced as cavitation nuclei. We have accomplished successful preliminary *ex vivo* studies (Tu et al. 2006) in the rabbit ear vessel system to verify that the IC activity does occur within the constrained environment of actual blood vessels and that the "amount" of IC activity under different treatment conditions could be quantified in terms of an ICD based on passive cavitation detection (PCD) measurement, with a good signal-to-noise ratio. The results of these *ex vivo* studies demonstrated the feasibility of detecting and quantifying the US-induced IC activity *in vivo* using the rabbit ear vessel model.

The general hypotheses for the present work were: (1) US-induced IC activity can be directly detected and quantified *in vivo* based on PCD measurements taken from rabbit ear blood vessels exposed to focused, pulsed US in the presence of UCA; and (2) the temporal evolution constants (*e.g.*, onset/decay time) and the "amount" of the US-induced IC activity are dependent on the PRPA and pulse length of the US source. Experiments investigating the IC activity onset/decay time and the ICD were conducted with variable treatment conditions (*e.g.*, PRPA and pulse length).

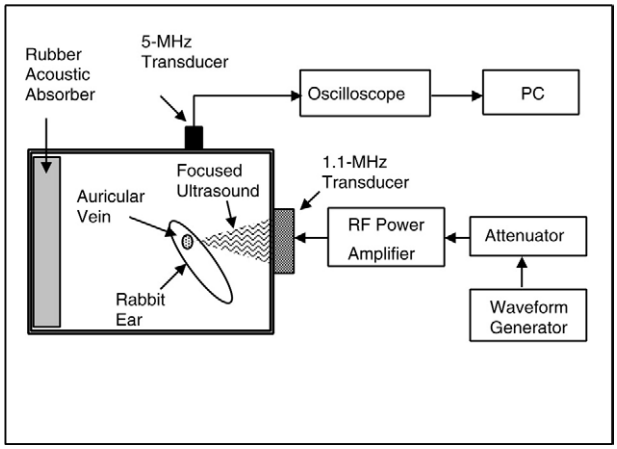

Fig. 1. Block diagram of the experimental apparatus.

MATERIALS AND METHODS

Animal preparation

Eighteen New Zealand white rabbits weighing 4.5 to 5.5 kg were used for these acute experiments, which were carried out according to National Institutes of Health guidelines under a protocol approved by the Institutional Animal Care and Use Committee at the University of Washington. After 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 24-gauge catheter was inserted into the proximal auricular vein of one ear for IV access. The animals were then anesthetized with an IV ketamine (35 to 40 mg/kg)/xylazine (5 mg/kg) cocktail and placed in a lateral recumbent position in preparation for treatment. Rabbits remained anesthetized throughout the entire experimental protocol.

Microbubble contrast agents

The Food and Drug Administration (FDA)-approved, commercially available ultrasound contrast agent (UCA), Optison, was used for these studies. According to the manufacturer's instructions, the vial was vented with a sterile 18-gauge needle before withdrawing the contents. Animals were injected with a bolus of 0.5 mL of Optison using a 1-mL syringe immediately before every treatment (corresponding to an intravascular void fraction of approximately 1.4×10^{-5}) followed by a flush of 2 mL of normal saline. The current maximum FDA-allowed dosage of Optison is 8.7 mL for human diagnostic purposes, which corresponds to a void fraction of approximately 10^{-5}. Therefore, the dose of Optison administered is comparable with the maximum dose allowed by the FDA for diagnostic purposes in humans. As Optison was withdrawn from the vial, an equivalent volume of perfluoropropane gas was injected simulta-