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● *Original Contribution*

EXAMINATION OF INERTIAL CAVITATION OF OPTISON IN PRODUCING SONOPORATION OF CHINESE HAMSTER OVARY CELLS

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Abstract—The objective of this project was to elucidate the relationship between ultrasound contrast agents (UCAs) and sonoporation. Sonoporation is an ultrasound-induced, transient cell membrane permeability change that allows for the uptake of normally impermeable macromolecules. Specifically, this study will determine the role that inertial cavitation plays in eliciting sonoporation. The inertial cavitation thresholds of the UCA, Optison, are compared directly with the results of sonoporation to determine the involvement of inertial cavitation in sonoporation. Chinese hamster ovary (CHO) cells were exposed as a monolayer in a solution of Optison, 500,000 Da fluorescein isothiocyanate-dextran (FITC-dextran), and phosphate-buffered saline (PBS) to 30 s of pulsed ultrasound at 3.15-MHz center frequency, 5-cycle pulse duration and 10-Hz pulse repetition frequency. The peak rarefactional pressure (P_r) was varied over a range from 120 kPa–3.5 MPa, and five independent replicates were performed at each pressure. As the P_r was increased, from 120 kPa–3.5 MPa, the fraction of sonoporated cells among the total viable population increased from 0.63–10.21%, with the maximum occurring at 2.4 MPa. The inertial cavitation threshold for Optison at these exposure conditions has previously been shown to be in the range 0.77–0.83 MPa, at which sonoporation activity was found to be 50% of its maximum level. Furthermore, significant sonoporation activity was observed at pressure levels below the threshold for inertial cavitation of Optison. Above 2.4 MPa, a significant drop in sonoporation activity occurred, corresponding to pressures where >95% of the Optison was collapsing. These results demonstrate that sonoporation is not directly a result of inertial cavitation of the UCA, rather that the effect is related to linear and/or nonlinear oscillation of the UCA occurring at pressure levels below the inertial cavitation threshold. (E-mail: mforbes@uiuc.edu) **© 2008 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Chinese hamster ovary cells, Sonoporation, Ultrasound contrast agent, Inertial cavitation, Thresholds.**

INTRODUCTION

A significant problem in cancer therapy is the compromised quality of life experienced by the patient because of the side effects of the therapeutic compounds. Delivery of molecular medicine to solid tumors is often inefficient and, as a result, the patient's healthy cells and tissues are subject to the toxic effects of the drugs. Thus, it is important to develop approaches that deliver drugs only to the appropriate cells within the patient in a specific, efficient and safe manner. One such method, termed *sonoporation*, involves the use of ultrasound (US) to enhance cell permeabilization. With this method, it is possible, using US and contrast microbubbles, to noninvasively deliver therapeutic compounds into specific target cells.

Sonoporation alters the permeability of cell membranes in a transient fashion (McNeil 1989), leaving the compounds trapped inside the cell once US exposure is complete. Small compounds (Brayman et al. 1999; Guzman et al. 2001; Keyhani et al. 2001), macromolecules (Bao et al. 1997; Greenleaf et al. 1998; Guzman et al. 2002; Miller et al. 1999; Wyber et al. 1997) and other therapeutic compounds (Harrison et al. 1996; Keyhani et al. 2001; van Wamel et al. 2004; Wu et al. 2006) have successfully been delivered into cells using US. Ultrasound can also deliver protein (Mukherjee et al. 2000; Weimann and Wu 2002) and DNA (Amabile et al. 2001; Lawrie et al. 2000; Miller et al. 2003; Miller and Song 2003) into tissues. Low- and high-frequency US treat-

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ment of cells in the presence of plasmid DNA has been shown to cause mammalian cell transfection *in vitro* (Bao et al. 1997; Frenkel et al. 2002; Kim et al. 1996; Tata et al. 1997) and *in vivo* (Endoh et al. 2002; Miller et al. 1999, 2003; Taniyama et al. 2002). Thus, sonoporation has great possibilities in both targeted gene and drug delivery.

Little is known about the mechanism of sonoporation, both physically and biologically. Tachibana et al. (1999) and Meheir-Humbert et al. (2005) have shown that large pores form in a cell membrane after US exposure. Schlicher et al. (2006) provided evidence that these membrane disruptions are similar to those formed by other physical stresses and are resealed by an active process of vesicle fusion with the cell membrane. Cellular and molecular damage to human red blood cells occurs as a result of US exposure (Kawai and Iino 2003), although the role this damage plays in pore formation is unknown. It has been shown that the enhanced membrane permeability in sonoporation is transient (Bao et al. 1997; Brayman et al. 1999; McNeil 1989; Taniyama et al. 2002) and the recovery rate does not vary significantly with US parameters or the maximum amplitude of the transmembrane current (Deng et al. 2004). In addition, hyperpolarization of the cell membrane occurs in the presence of US and ultrasound contrast agent (UCA), most likely because of the activation of channels sensitive to mechanical stresses and nonspecific ion channels (Tran et al. 2007). However, this hyperpolarization does not explain the presence of the pores in the membrane.

The presence of a UCA is necessary to induce a significant sonoporation event (Bao et al. 1997; Greenleaf et al. 1998; Kim et al. 1996). This UCA requirement has led to the identification of inertial cavitation (IC), which is the rapid collapse of a bubble, as the probable sonoporation mechanism, theorized by several studies (Bao et al. 1997; Greenleaf et al. 1998; Hwang et al. 2005; Koch et al. 2000; Lai et al. 2006). However, the data provided in the literature are only circumstantial, not direct evidence that collapse cavitation is the sonoporation mechanism. UCAs have a complex dynamic behavior in an ultrasonic field. The major behaviors are linear oscillation, nonlinear oscillation and IC. Determining whether oscillation or IC of UCAs is involved in producing sonoporation is essential for determining the physical phenomenon responsible for this biological effect (Fig. 1).

Most UCAs are gas-filled, encapsulated microbubbles designed to increase acoustic reflectivity. As acoustic waves are incident on the UCA, it grows and shrinks because of the time-varying pressure of the wave. The behavior of the UCA is dependent on US frequency (Ammi et al. 2006b; Chen et al. 2003; Chomas et al. 2001; Giesecke and Hynynen 2003) and peak rarefactional pressure. At low-level acoustic pressure amplitudes, linear oscillation of the UCA occurs. These oscillations lead to local steady flows that are termed microstreaming. When the UCA is close to a cell, this microstreaming can lead to shearing motions on the cell membrane. Theoretical and experimental studies have shown that microstreaming near a cell boundary can adversely affect a cell membrane. The critical stress (in terms of viscous stress) for hemolysis is well defined (Rooney 1970; Williams et al. 1970). In addition, microstreaming from a vibrating Mason horn demonstrated a threshold for enhanced membrane permeability (12 ± 4 Pa at 21.4 kHz) (Wu et al. 2002). Also, under single-bubble controlled conditions (10 kPa at 180 kHz), Marmottant and Hilgerfeldt (2003) demonstrated experimentally that linear microbubble oscillations were sufficient to rupture lipid membranes because of large-velocity gradients. Thus, microstreaming as a result of linear oscillation of UCAs could play a role in sonoporation.

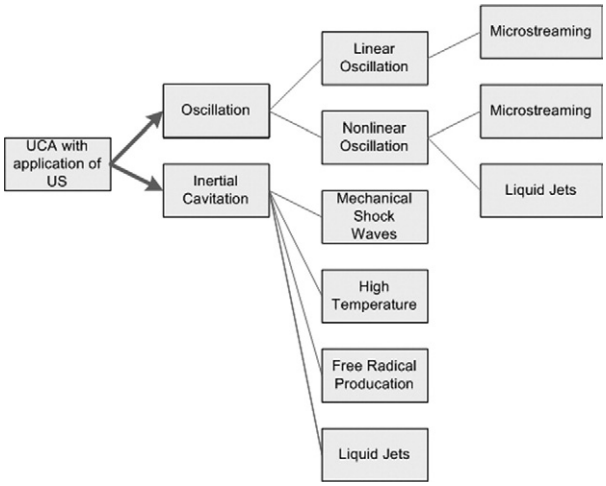

Fig. 1. The numerous UCA responses to US and the possible bioeffects of each response, thus emphasizing the critical junction of IC *vs.* oscillation in determining the mechanism for sonoporation.

At higher pressure amplitudes, UCAs exhibit nonlinear oscillation. During these conditions, the UCA expands slowly during rarefaction and is followed by a rapid contraction, but not collapse, during compression. Nonlinear oscillation produces microstreaming, as well as the potential for liquid jets (also known as *microjetting*). Liquid jets are formed as a result of the asymmetric behavior of the UCA in the presence of a pressure gradient near a surface, such as a cell. These jets can then impinge on the cell membrane at high speeds. Liquid jets provide increased transport of heat and gas by streaming and have the capacity to puncture the cell membrane, producing openings that could allow for the transport of extracellular material into the cell (Prentice et al. 2005).