ELSEVIER

Ultrasound in Med. & Biol., Vol. 23, No. 6, pp. 961–965, 1997
Copyright © 1997 World Federation for Ultrasound in Medicine & Biology
Printed in the USA. All rights reserved
0301-5629/97 $17.00 + .00

PII S0301-5629(97)00067-7

●*Technical Note*

ULTRASONIC BIOMICROSCOPY OF VIABLE, DEAD AND APOPTOTIC CELLS

GREGORY J. CZARNOTA,* MICHAEL C. KOLIOS, HOMAYOUN VAZIRI, SAM BENCHIMOL, F. PETER OTTENSMEYER, MICHAEL D. SHERAR and JOHN W. HUNT
Department of Medical Biophysics, University of Toronto, and Ontario Cancer Institute, Toronto, Ontario, Canada

(*Received* 11 *September* 1996; *in final form* 10 *December* 1996)

Abstract—Ultrasonic imaging is frequently used in medical diagnosis to differentiate normal and tumour tissues. Here we investigate if distinct types of cell death can be discriminated through the use of ultrasound biomicroscopy. By using a well-controlled system *in vitro,* we demonstrate that this imaging modality can be used to differentiate living cells, dead cells and cells that have died by programmed cell death or apoptosis. The results indicate a greater than twofold ultrasound backscatter signal from apoptotic cells in comparison to viable cells, whereas heat-killed cells exhibit an intermediate level of ultrasound backscatter. The results have potential implications in the study of disease-related biological processes involving apoptosis. © 1997 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound biomicroscopy, High-frequency ultrasonic imaging, Apoptosis, Programmed cell death.**

INTRODUCTION

Apoptosis plays a significant role in both normal and disease-related biological processes (Fraser and Evan 1996; Hockenbery 1995; Majno and Joris 1995). The ability to differentiate apoptotic cells from living or otherwise dead cells noninvasively *in vivo* would potentiate clinical diagnoses and the understanding of disease processes. Ultrasound biomicroscopy has been used to investigate the living and hypoxic regions of cell spheroids, indicating the ability of this imaging modality to differentiate different types of cellular morphologies (Bérubé et al. 1992; Sherar et al. 1987). In this report, we determine whether ultrasound can be used to differentiate apoptotic, otherwise dead and viable cells. A cell culture system was used in conjunction with high-frequency ultrasonic imaging. The former permits the well-controlled induction of apoptosis or cell death, whereas the latter permits imaging at resolutions higher than that offered by conventional ultrasonic systems (Sherar et al. 1987). The results indicate visible and statistically significant differences in the ultrasound backscatter signal between viable cells, apoptotic cells and dead cells.

Address correspondence to: Dr. Gregory J. Czarnota, c/o Dr. F. P. Ottensmeyer, Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9.

MATERIALS AND METHODS

Cell culture

Apoptotic, viable and dead cells were prepared for ultrasonic biomicroscopy using a cell culture system. For each ultrasound imaging experiment, four batches of approximately 10^9 human acute myeloid leukaemia cells (AML-5) were grown at 37°C in α-minimal-media from frozen stock samples using 2-L roller flasks and 1.5 L of media. Culture growth was always initiated using frozen stock cells. To induce apoptosis, two batches of cells were treated with cisplatin, a DNA intercalater that causes p53-dependent apoptosis in this cell line, at a concentration of 10 μg/mL for 20 h. After 20 h light microscopy, gel electrophoresis showing DNA laddering and trypan blue staining were used to confirm that approximately 95% of the cells underwent apoptosis. Cells were washed in phosphate buffered saline (PBS), counted to ensure equal numbers of cells and pelleted in flatbottom cryotubes at 800g on a desktop swinging bucket centrifuge, producing pellets of the same size (diameter 1 cm, height 1 cm). Equal numbers of untreated cells were