The effects of ultrasound on the cells of the vascular wall

Submitted by **Eleanor Martin**, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Physics, June 2010

I certify that all material in this thesis which is not my own work has been identified and that no material has been previously submitted and approved for the award of a degree by this or any other University.

(Signature) ……

Abstract

Investigations into the safety of diagnostic ultrasound and mechanisms of therapeutic ultrasound have provided evidence of a number of cellular responses to ultrasound. These studies have mainly concentrated on cells in culture, while work on intact tissue employed mainly kHz ultrasound fields, although diagnostic and many therapeutic procedures are performed using MHz ultrasound. Vascular tissue is known to respond to a variety of physical and chemical signals, and so arteries were used as a model system in this thesis to study the effects of MHz ultrasound *in vitro*.

Rings of equine carotid and lateral cecal mesenteric artery exhibited reversible, repeatable contraction on exposure to both pulsed and continuous wave 3.2 MHz ultrasound at acoustic powers up to 145 mW. Wall stress increased by up to 1.5% in carotid arteries and up to 2% in mesenteric arteries during exposure, and returned to basal levels after approximately 10 minutes. Contraction was endothelium independent, and was not affected by changes in the pulsing regime. The magnitude of contraction was dependent on the acoustic power, and the change in wall stress increased with increasing acoustic power in a linear fashion. The acoustic power dependence suggested the response was thermally mediated and this was confirmed by investigation of the response of arteries to non ultrasound generated heating, which also induced contraction. The effects of ultrasound and heating were also investigated in 1st order branches of the lateral cecal artery, as a model of a small artery. No response was observed in either case.

In order to determine the cellular basis of the response of carotid and mesenteric arteries, the involvement of potassium ion channels in the response was investigated using a potassium channel blocker. The response of arteries to ultrasound was increased by inhibition of inward-rectifier potassium channels, which would otherwise help to return the cell membrane potential to the normal level. The change in wall stress was increased by 42% on average, confirming the involvement of these channels in the response. Contraction of arteries is mediated by an increase in intracellular calcium. The ion channel activity during non ultrasound generated heating was examined further by observation of intracellular calcium concentration using a fluorescent calcium sensitive dye. Increases in intracellular calcium were observed in carotid and large mesenteric arteries, which confirmed the thermal influence on ion channel function in these vessels. No such effect was observed in the smaller vessels.

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Glossary of abbreviations

ΔT	Change in temperature
AIUM	American Institute of Ultrasound in Medicine
ATP	Adenosine triphosphate
$BaCl_2$	Barium chloride
CW	Continuous wave
DMSO	Dimethyl sulfoxide
EDHF	Endothelium derived hyperpolarising factor
FDA	Food and Drug Administration
I_{SPPA}	Spatial-peak pulse-average acoustic intensity
I_{SPTA}	Spatial-peak temporal-average acoustic intensity
LIPUS	Low intensity pulsed ultrasound
MI	Mechanical Index
NEMA	National Electrical Manufacturer's Association
NO	Nitric oxide
Np	Nepers
p_-	Peak negative acoustic pressure
p_+	Peak positive acoustic pressure
PVDF	Polyvinylidene fluoride
ROS	Reactive oxygen species
TI	Thermal Index
TPx	Polymethylpentene
TTO	Thermal Test Object
US	Ultrasound
VEGF	Vascular endothelial growth factor