



Cavitation Biology and the Safety of Contrast-Aided Diagnostic Ultrasound

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ABSTRACT

Contrast agents for diagnostic ultrasound consist of stabilized microbubbles designed to enhance the echogenicity of blood filled regions and reveal tissue perfusion. The availability of these agents has enabled the study of cavitation biology in diagnostic ultrasound, a topic of non-ionising radiation biology which was previously a matter of conjecture. Bioeffects are engendered by intermittent imaging at high peak rarefactional pressure amplitudes (PRPA), which nucleates cavitation within tissue capillaries and allows refill of the capillaries with agent between high PRPA images. The magnitude and extent of the bioeffects depends not only on the agent dosage and PRPA, but also on ultrasound timing parameters and tissue of interest. Cavitation bioeffects, which have been demonstrated using commercial contrast agents and diagnostic ultrasound in animal models, include heart capillary rupture with cardiomyocyte killing and kidney glomerular capillary haemorrhage into the urinary space with consequent acute tubular necrosis. The medical significance of such randomly scattered bioeffects is uncertain, but cardiac arrhythmia and hematuria are clinically detectable manifestations. The potential for cavitation biological effects, particularly with common patient conditions which might aggravate otherwise minor health impacts, requires careful risk-benefit considerations for diagnostic ultrasound imaging with contrast agents.

INTRODUCTION AND BACKGROUND

Diagnostic ultrasound imaging utilizes pulsed ultrasound, which typically involves temporal average intensities too low for substantial heating and thermal bioeffects. However, questions arise as to whether nonthermal biological effects might occur, owing to the relatively high pulse amplitudes (high temporal peak intensity). Indeed, a potent nonthermal mechanism for bioeffects of ultrasound exists in the form of acoustic inertial cavitation, which depends predominantly on the peak rarefactional pressure amplitude (PRPA). Inertial cavitation involves the growth and inertial collapse of a bubble or gas body (stabilized microbubble) in response to PRPAs in excess of the particular inertial cavitation threshold. The threshold in any given medium is related to the population of cavitation nuclei and their size distribution. Theoretically, for blood having a population of nuclei with optimum sizes for frequencies in the 1-10 MHz range (including diagnostic ultrasound imaging), the PRPA threshold is given approximately by 0.4 MPa times the square-root of the frequency in MHz [1,2]. In terms of the Mechanical Index (MI), a clinical on-screen exposure index, this threshold is approximately $MI=0.4$ which is well below the USA guideline limit of $MI=1.9$ for diagnostic ultrasound.

The potential occurrence of cavitation in diagnostic ultrasound led to a search for cavitation *in vivo* [3,4]. Medical ultrasound can activate bodies of gas in lung and intestine, producing cavitation-like activity and deleterious bioeffects under some conditions [4]. However, inertial cavitation by pulsed diagnostic ultrasound appears to be rare, or non-existent for intact mammals, because the requisite optimum nuclei to not normally exist in tissue. Even for very high PRPA pulses used in lithotripsy, cavitation could not be detected in circulating blood [5]. Apparently, the cleansing and sterilization of blood and bodily tissues by normal life processes and immune surveillance create a dearth of cavitation nuclei *in vivo*. Thus, in general, the placement of the guideline upper limit well above the possible inertial cavitation threshold does not normally risk significant cavitation bioeffects.

This general rule is broken by diagnostic ultrasound contrast agents (UCA), which consist of suspended microscopic gas bodies. Injection of these agents into the body radically changes the population of cavitation nuclei from sparse to optimum. This circumstance has afforded ultrasound bioeffects researchers opportunities to study inertial cavitation biology for diagnostic ultrasound, a unique new topic of non-ionising radiation biology which was previously a matter of conjecture. This topic previously has been the subject of comprehensive reviews of earlier work [2, 4, 6]. The purpose of this invited presentation is to review briefly recent research on the potential biological effects associated with inertial cavitation inception during contrast aided diagnostic ultrasound, with emphasis on biological and dosimetric aspects.

CONTRAST AIDED DIAGNOSTIC ULTRASOUND

Ultrasound contrast agents (UCA) for diagnostic ultrasound imaging have been developed over the last two decades and have gradually improved in lung passage after intravenous injection and in their persistence in the circulation. Several extensive reviews describe UCA developments and applications [7, 8, 9]. Commercial UCAs now have been approved in different countries; for example, Definity® (perflutren lipid microsphere injectable suspension, Bristol-Myers Squibb Medical Imaging, N. Billerica MA USA) contains up to $1.2 \cdot 10^{10}$ microbubbles/ml of 1.1-3.3 μm diameter. These agents were designed to enhance conventional ultrasound imaging when the results were non-diagnostic. For example, UCA are approved in the USA for use in patients with sub-optimal echocardiograms to opacify the left ventricular chamber and improve the delineation of the left ventricular endocardial border. The safety of these applications appears to be good, particularly in terms of pharmacological side effects.

Greater promise for widespread clinical applications of UCA has been shown for contrast-specific modes of imaging. These imaging modes involve the instability of UCA in various ways. For images with high PRPAs, the gas bodies are destabilized and can be rapidly destroyed. For conventional real-time imaging, the agents typically are destroyed in large blood vessels. However, if high PRPA images are spaced intermittently, then the gas bodies can penetrate the capillary bed, which allows imaging of tissue perfusion at the capillary level (i. e. much below the resolution of conventional Doppler imaging of blood flow). UCA perfusion imaging is of diagnostic value in detecting infarcts, measuring ischemia and evaluating tumours. Perfusion imaging can be accomplished by taking intermittent high PRPA images with different refill times, or by using low PRPA imaging methods to see the contrast agent refill a region of tissue. The low PRPA methods nevertheless utilize high PRPA pulses to destroy and clear the contrast agent gas bodies from the region of interest, which allows fresh examination of a new region or repeated refill of the same region.

CAVITATION BIOLOGY OF CONTRAST AIDED DIAGNOSTIC ULTRASOUND

In conventional diagnostic ultrasound imaging, UCA may be destroyed in the blood within relatively large vessels. This may entail some hemolysis (lysis of red blood cells), but this bioeffect is unlikely to be medically important. In contrast-specific modes; however, the gas bodies enter tissue capillaries, which transforms the picture for contrast ultrasound induced bioeffects. High PRPA exposure of UCA with nucleation of cavitation in tissue leads to capillary injury, leakage or event rupture. Whether or not such tissue effects might be important depends on several biological factors, such as the relative perfusion of an organ, the possible death of parenchymal cells, which may be irreplaceable, or the amplification of the initial capillary injury within a specific organ. Typically, PRPAs are measured or estimated at the *in situ* point of bioeffect evaluation, which compensates for differences in attenuation in small animal exposure. Therefore, equivalent PRPA (or MI) values reported in research should correspond with clinical on-screen MI values, which estimate *in situ* values in patient tissue.

Myocardial contrast echocardiography

UCAs have shown promise for imaging of myocardial perfusion using gas body destruction in myocardial contrast echocardiography (MCE). Due to the potential for widespread usage of MCE in cardiology, most *in vivo* bioeffects research has centered on the heart. Microvascular effects of MCE have been studied in isolated rabbit hearts using 1.8 MHz ultrasound and images triggered at 1 Hz [10]. Scanning at MI=1.6 led to indications of capillary damage and erythrocyte extravasation. Optison® or Definity® were studied for *in vivo* rat hearts at 1.3 MHz with ECG triggering of images each 4 cardiac cycles [11]. Elevations of Troponin T in blood plasma, indicating myocardial damage, were detected after 30 min for MIs of 1.2 and 1.6.

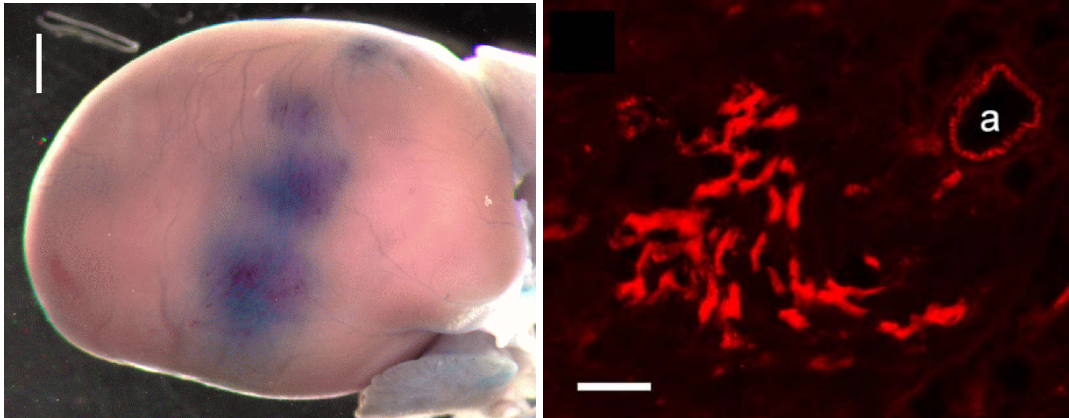


Figure 1. A rat heart (left) after MCE at 1.5 MHz with Evans blue leakage in the scan plane: scale bar 2 mm. Vital staining of cardiomyocytes (right) seen in fluorescent red in a frozen histological section with a small artery (a): scale bar 50 μm .

The rat heart provides a convenient model system for exploring the range of potential bioeffects of MCE and their dependence on various dosage and exposure parameters. Figure 1 shows a rat heart after 1.7 MHz intermittent imaging each fourth heartbeat at a PRPA of 2.0 MPa and a recommended bolus of 10 : l/kg of Definity® [12]. Microvascular injury was detected by leakage of Evans blue dye, and petechiae could be counted on the heart surface. Different UCAs, including Optison®, Definity® and Imagent®, produced similar bioeffects for similar exposure and equal numbers of microbubbles in the contrast agent dose [12]. Histological examination of heart tissue after MCE showed evidence of microvascular injury and cardiomyocyte death with inflammatory cell infiltration after 24 h [13]. As shown in Fig. 1, the irreversibly injured cardiomyocytes were revealed by Evans blue vital staining, which produces bright red fluorescence of the dead cells in frozen sections after 24 h [14]. Similar bioeffects have also been seen in a canine model of MCE at 1.5 MHz and Definity® infusion at 2 : l/kg/min for 10 min [15]. The occurrence of PVCs in association with many triggered images represented a clinically detectable indication of the strong interaction of diagnostic ultrasound with UCAs during MCE [14, 15].

Kidney perfusion imaging

The effects of contrast aided diagnostic ultrasound in rat kidneys have been examined using Optison® and several experimental agents [16]. Rats were scanned at 1.8, 4 and 6 MHz with diagnostic ultrasound probes placed on skin. Glomerular capillary haemorrhage was induced from the glomerular tuft into Bowman's capsule and proximal convoluted tubules. This effect was thought to be unlikely in clinical examinations.

However, recent research using 1.5 MHz ultrasound with scanning through a tissue mimicking attenuator and with the rat in a water bath indicated that glomerular capillary haemorrhage might be expected in humans for similar high PRPA imaging [17]. Capillary haemorrhage was induced in 37 % \pm 5 % of glomeruli counted in histological sections, and blood filled tubules were visible on the kidney surface, as shown in Figure 2. The PRPA was 1.8 MPa at the kidney for 10 : l/kg of Definity® infused *via* a tail vein during 1 min of intermittent imaging at 1 s intervals. The kidney is particularly susceptible to this injury owing to the relatively high blood perfusion of kidney and the relatively high blood pressure of the glomerular capillaries (the primary filter of the nephron). Glomerular capillary haemorrhage into the urinary space greatly enhances the consequences of the capillary injury by diagnostic ultrasound cavitation activity, because the blood fills and tends to block the tubules [18]. Thrombus-like fibrinous clots were seen in many haemorrhaged glomeruli and persisted for 24 hr, at which time indications of tubular epithelium injury with possible acute tubular necrosis were evident. Hematuria was a clinically detectable manifestation of glomerular capillary haemorrhage induced by high PRPA intermittent imaging with UCA destruction.

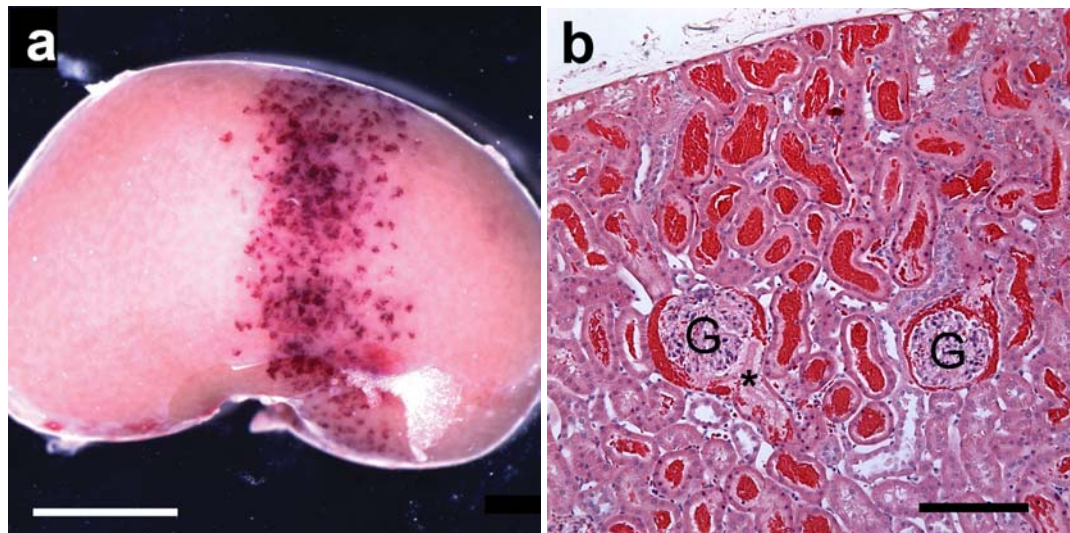


Figure 2. A rat kidney (a) scanned with 1.5 MHz diagnostic ultrasound and with UCA infused *via* tail vein. The blood-filled tubules within the scan plane are visible on the surface of the kidney: scale bar 5 mm. Histology of a kidney (b), scanned as in (a), showing blood-filled tubules near the surface and glomeruli (G) with capillary haemorrhage filling Bowman's capsule. A thrombus-like fibrinous clot had formed in one Bowman's capsule (*), which extended into the neck of the proximal convoluted tubule: scale bar 100 : m.

Other UCA associated bioeffects

Other tissues examined with contrast aided diagnostic ultrasound involving cavitation nucleation in the microcirculation might also be expected to suffer microvascular injury. Platelet aggregation in liver sinusoids and endothelial cell damage in samples taken 5 hr after scanning has been reported for diagnostic ultrasound of rat liver at 8 and 12 MHz with Levovist® [19].

Contrast aided diagnostic ultrasound can be used for detection and examination of tumours in tissue. The fearsome but conceivable risk of enhanced metastatic spread of cancer cells was investigated for mouse tumour models under conditions similar to intermittent perfusion imaging with UCA [20]. There was no significant increase in lung metastasis from tumours scanned on the hind leg with UCA in the circulation, relative to sham exposure.

The reliable production of bioeffects by diagnostic ultrasound with UCA has opened opportunities for therapeutic applications. The microvascular leakage effect noted above has been studied as a drug delivery method from the blood pool to the interstitium [21]. Possible nanoparticle delivery to the heart using diagnostic imaging with a non-commercial UCA has been reported [22]; however, functional impairment of the heart was encountered as the parameters were elevated for therapeutic efficacy. Sonoporation during ultrasonic cavitation can be used to transfer DNA into cells, which opens a potential application to gene therapy for diagnostic ultrasound [23, 24]. Reports of therapeutic applications of contrast-aided diagnostic ultrasound indicate the substantial nature of the possible bioeffects and raises uncertainty about the clear separation of diagnostic and therapeutic applications of medical ultrasound.

DOSIMETRIC FACTORS IN UCA CAVITATION BIOLOGY

The potential for bioeffects from contrast-aided diagnostic ultrasound depends on a number of user-controlled parameters. The most important is the PRPA of the imaging pulses. Exposure-response trends are shown in Fig. 3 for intermitted scanning with Definity® injection for rat heart, leading to cardiomyocyte death, and rat kidney, leading to glomerular capillary haemorrhage. The cardiomyocyte death threshold was determined from the plotted data with 50 : l/kg infused for 5 min and images triggered each 4 heartbeats (about 1 s) [14], together with data from 100 : l/kg infusion [25]. Apparent thresholds were 0.4 MPa for petechiae and 1.0 MPa for PVCs for 1.7 MHz MCE [12]. The glomerular haemorrhage was determined for 1 s interval imaging with 10 : l/kg infusion over 1 min, with the threshold confirmed with 100 : l/kg infusion for 10 min of scanning. For both plots in Fig. 3, the scale extends to 2.3 MPa, which corresponds to the Mechanical Index upper limit (USA) of 1.9.

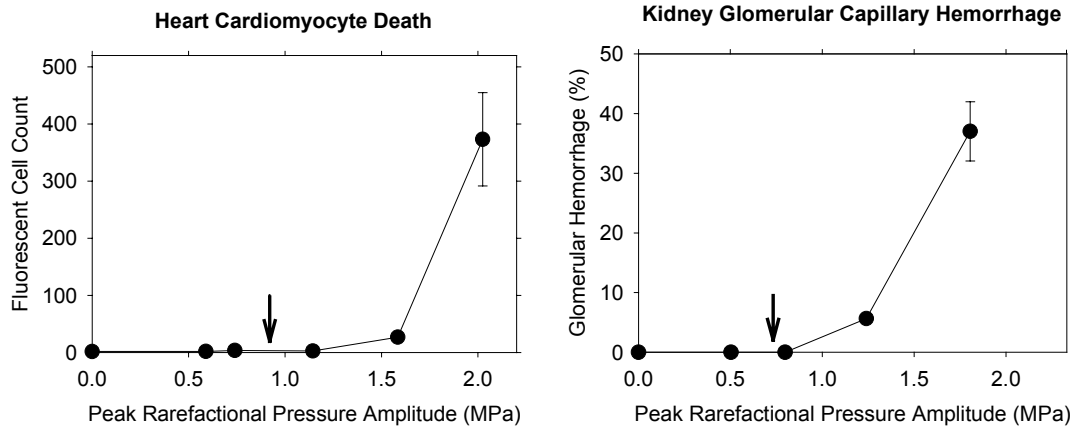


Figure 3. Exposure-response data for vital staining of cardiomyocytes with Evans blue for 1.5 MHz MCE (left) and for glomerular capillary haemorrhage for 1.5 MHz intermittent imaging (right). Both bioeffects had a strong dependence on PRPA above apparent thresholds, which are indicated by the arrows.

The microvascular effects increased in proportion to agent dosage at low doses, but tended to level-off for high doses [12, 17]. When expressed in terms of the number of gas bodies contained in the volume doses, there was no apparent difference between Optison® and Imagent® or Definity® used for MCE.

Timing parameters are also important, including, at least, ultrasonic frequency, pulse duration, pulse repetition frequency and intermittent interval. The effects decrease with increasing ultrasonic frequency, but information is limited as to whether or not the Mechanical Index (with $1/\sqrt{\text{frequency}}$ dependence) accurately describes this trend. The intermittent imaging interval is important to allow for refill of the tissue with UCA: real time imaging may yield no microvascular bioeffects (nor image contrast effect). A 1 s interval between frames yields substantial refill and relatively large bioeffects in highly perfused tissue [12, 17], although longer intervals may be needed for low perfusion tissues with slower refill times. The bioeffects occur incrementally with each intermittent high PRPA image and accumulate throughout the examination.

DISCUSSION AND CONCLUSIONS

For diagnostic ultrasound with UCA, microvascular bioeffects can be induced by cavitation nucleation of the contrast agent gas bodies. *In vivo* testing has involved doses and equivalent MI values within the clinically relevant range. In skeletal and heart muscle, microvascular leakage and petechiae have been observed for intermittently triggered imaging modes. Premature ventricular contractions were induced for rat and canine models of MCE with approved UCAs. Cardiomyocyte death with development of histologically defined micro-lesions has been demonstrated for triggered diagnostic imaging with gas body destruction within the myocardium. This effect is biologically significant because these indispensable cells are not replaced. Research in liver and kidney has also revealed microvascular bioeffects. The kidney may be the most sensitive tissue to UCA related bioeffects, because the rupture of a glomerular capillary yields haemorrhage into the urinary space, from which blood is normally excluded. This effect is biologically significant because the initial capillary injury is amplified to impact the entire nephron (functional unit) with possible acute tubular necrosis. The bioeffects of diagnostic ultrasound with UCA depend not only on the agent dose and imaging PRPA, but also on other user-controlled parameters such as agent delivery method and image trigger interval.

Since doses and tissue equivalent MIs in animal research were often within the clinical range, microvascular bioeffects could be induced during clinical diagnostic ultrasound examination using UCAs. Clinically observable manifestations include PVCs and hematuria. Although the bioeffects are certainly biologically significant, the medical significance of the experimentally observed bioeffects is presently uncertain. These microscale bioeffects might have medically significant consequence under some conditions, particularly in patients who are aged, diabetic (or have other disease states) or under drug treatment. The cavitation biology of contrast-aided diagnostic ultrasound is a complex and challenging new topic of non-ionising radiation biology.

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