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● *Original Contribution*

INFLUENCE OF SCAN DURATION ON PULMONARY CAPILLARY HEMORRHAGE INDUCED BY DIAGNOSTIC ULTRASOUND

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Abstract—Diagnostic ultrasound can induce pulmonary capillary hemorrhage (PCH) in rats and display this as "comet tail" artifacts (CTAs) after a time delay. To test the hypothesis that no PCH occurs for brief scans, anesthetized rats were scanned using a 6-MHz linear array for different durations. PCH was characterized by ultrasound CTAs, micro-computed tomography (μCT), and measurements of fixed lung tissue. The μCT images revealed regions of PCH, sometimes penetrating the entire depth of a lobe, which were reflected in the fixed tissue measurements. At −3 dB of power, PCH was substantial for 300-s scans, but not significant for 25-s scans. At 0 dB, PCH was not strongly dependent on scan durations of 300 to 10 s. Contrary to the hypothesis, CTAs were not evident during most 10-s scans ($p > 0.05$), but PCH was significant ($p = 0.02$), indicating that PCH could occur without evidence of the injury in the images. (E-mail: douglm@umich.edu) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Pulmonary ultrasound, Comet tail artifact, Pulmonary capillary hemorrhage, Pulmonary micro-computed tomography, Diagnostic ultrasound safety.**

INTRODUCTION

Diagnostic ultrasound can induce capillary hemorrhage in mammalian lung, depending on the physical and biological conditions. Pulmonary capillary hemorrhage (PCH) induced by ultrasound has been studied intermittently since its discovery by Child et al. (1990). Over this period, transthoracic ultrasound for pulmonary examination has grown to be an important tool for diagnosis in the point-of-care settings of emergency and intensive care (Lichtenstein 2014; Volpicelli 2013). Features in the diagnostic images, such as comet tail artifacts (CTAs), are indicative of diseases involving the alveolar–interstitial interphase, pulmonary edema and pulmonary effusion, among other problems. When PCH occurred, the causative diagnostic ultrasound image displays the development of PCH during scanning in rats as the initiation and growth of CTAs (Miller 2012), a remarkable exhibition of the power of the ultrasonic energy transmitted into the chest and of the sensitivity of ultrasonic imaging in detection of pulmonary injury. The PCH bio-effect is not yet fully understood relative to the complex mix of clinical diagnostic ultrasound modes and patient conditions examined. More information is urgently needed to develop the best possible safety advice for sonographers.

Early research indicated that ultrasonically induced PCH occurs in mice, rats and pigs (American Institute of Ultrasound in Medicine 2000; Church et al. 2008). PCH can be characterized by a threshold value of the ultrasonic pulse pressure amplitude in megapascals (or other relevant exposure metric) for a specific set of experimental conditions. PCH thresholds have been assessed for variations in pulsed ultrasound beam width, pulse length, frequency, total on-time and exposure duration (Church and O'Brien 2007). The age of the subject has also been evaluated as a risk factor, with some indication of increased sensitivity with age (Dalecki et al. 1997; O'Brien et al. 2003). Recently, we discovered that PCH was strongly influenced by anesthesia methods in our animal tests (Miller et al. 2015a), which adds complex biological parameters into the mix of physical parameters relevant to the PCH problem. Most early studies were performed using laboratory pulsed ultrasound systems configured for relevance to diagnostic ultrasound. The results of a comparison between PCH from diagnostic and laboratory

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systems indicated that the diagnostic scanning results were comparable to the earlier findings, which provides a large and reasonably consistent database (Miller et al. 2015b). The diagnostic ultrasound frequency does not strongly influence the threshold for PCH (Miller et al. 2015c), which questions the use of the on-screen mechanical index as a safety index for gauging the likelihood of PCH.

In addition to the dose–response trends for ultrasonic output parameters, exposure duration appears to be a key dosage parameter (Miller et al. 2015b). For example, a 2.0-MPa threshold for 2.8-MHz, 11.6-μs pulses, a 1-ms interval and 10-s exposure duration (O'Brien et al. 2003) was substantially higher than our 0.75-MPa threshold at 1.5 MHz for 10-μs pulses, 25-ms interval and 300-s exposure duration, even though the numbers of pulses delivered and total on-time were similar. This observation suggests an important role for exposure duration in the PCH threshold, an unexpected finding because the longer duration greatly reduced the average intensity of the energy delivered. However, for constant pulsing parameters, Raeman et al. (1996) found that the threshold for PCH in mice was about the same for 20- to 300-s exposures. Interestingly, for diagnostic ultrasound, the influence of exposure duration on outcome may be directly determinable during scanning by observation of the initiation and growth of CTAs in the ultrasound image. Previous work (Miller 2012) indicated variation in the time delay between the start of scanning and the initiation of CTAs in the image for 5-min scans, which suggests that brief scans might allay the risk of injury. There may be an exposure-scan duration threshold for PCH. Indeed, monitoring the ultrasound image itself for CTA initiation might be a plausible safety strategy. However, for different ultrasonic frequencies, the detection of CTAs was found to be somewhat difficult for lower frequencies with lower resolution (Miller et al. 2015c).

This study was undertaken to provide information on the dependence of PCH on different exposure-scan durations, rather than just the time of CTA initiation during 5-min scans. PCH areas on the lung surface were measured as before, but were considered inadequate to fully describe the impact of PCH. The severity of some pulmonary conditions may be indicated by the length of the comets (Gargani et al. 2009; Weitzel et al. 2015). Also, the number of CTAs (also called "B-lines") appears to be correlated with the extent of parenchymal changes in CT images (Martelius et al. 2016). However, the CTAs do not actually provide quantitative data on PCH depth. The nature and depth of the PCH regions inside the lung were evaluated in the present study. Histologic evaluations can provide information on depth but do not clarify the nature of the PCH. To clarify the precise extent of the PCH relative to its appearance in radiologic observation, micro-computed tomography (μCT) images were obtained. In addition, PCH depths and volumes were measured in fixed tissue samples and confirmed as realistic for assessment of *in situ* PCH by comparison with the μCT images.

METHODS

All *in vivo* animal procedures were conducted with the approval and guidance of the University Committee on Use and Care of Animals (UCUCA) of the University of Michigan. In this study, a total of 46 female rats (Sprague Dawley, Charles River, Wilmington, MA, USA) weighing an average of 257 ± 23 g were tested; one rat was lost from the study because of anesthetic death. Anesthesia was induced by intraperitoneal injection of ketamine (75 mg/kg) plus dexmedetomidine (0.5 mg/kg). The specific anesthesia technique can be an important factor in studies of ultrasound-induced PCH (Miller et al. 2015a). Most PCH studies in rodents have used ketamine and xylazine (a veterinary sedative) for anesthesia. Here, dexmedetomidine, a common human clinical sedative and anxiolytic (Mason et al. 2011), which has similar PCH results even in the normal human dose range (Miller et al. 2016), replaced xylazine in the anesthetic mixture. The right thorax of all rats was shaved and depilated for ultrasound transmission. The rat was mounted on a plastic board and partly immersed in a 38°C degassed water bath together with the ultrasound probe. This exposure method has reliable ultrasound coupling, and maintains the body temperature of the rats. Physiologic data were collected at the time of ultrasound scanning using a pulse oximeter (SurgiVet V3395 TPR, Smiths Medical, St Paul, MN USA), and visual observation of respiration. The average values were: 244 ± 12 bpm heart rate, 80± 4% S_pO_2 and 73 ± 15 breaths/min. Anesthesia was maintained until sacrifice for sample collection 1 h after scanning.

Ultrasound scanning

A GE Vivid 7 Dimension (GE Vingmed Ultrasound, Horten NO) ultrasound machine was used for imaging and exposure scanning. The 7 L probe was used in B-mode at the 6.0-MHz setting with 4-cm depth, 1.56-cm focus and 63.1 frames/s (fps). For one group of rats, the frames were triggered intermittently at 5 fps. The probe was aimed with a −20-dB power setting approximately horizontally at the right side of the rat to position the lung surface at an image depth of 1.25–1.75 cm at the level of the cranial or middle lobes, avoiding the ribs as much as possible.

For a test scan, the power setting was raised to the desired level of 0 dB or −3 dB and timed for the desired duration. To accurately time 10-s scans, a paddle was