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● *Original Contribution*

ULTRASOUND IMAGING IN AN ANIMAL MODEL OF VASCULAR INFLAMMATION FOLLOWING BALLOON INJURY

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Abstract—Cardiovascular disease is a major cause of morbidity and mortality in the world and better prevention and treatment strategies are needed. Studies from this laboratory have shown that perfluorocarbon exposed sonicated dextrose albumin (PESDA) microbubbles bind to inflamed vasculature through interactions with scavenger receptors (SR). This current study details the use of PESDA as a tool for accessing and quantifying the extent of vascular inflammation. Angioplastied rat aortas were evaluated with low mechanical index microbubble imaging techniques contrast pulse sequencing (CPS); Siemens Acuson Sequoia 15L8, 7–15 MHz ultrasound probe with a mechanical index of 0.09 to detect microbubble binding. Real-time polymerase chain reaction (RT-PCR) analysis of angioplastied aortas demonstrated a significantly ($p < 0.01$) increased expression of both SRs and Interleukin 6 (IL-6). Vessel wall enhancement was quantified using densitometry of CPS ultrasound images and correlated with the upregulated expression of scavenger receptors, Toll-like receptors and IL-6. This study demonstrates that PESDA, in conjunction with CPS ultrasound, is an effective imaging technique to better detect early vascular inflammation and potential cardiovascular disease. (E-mail: danderso@unmc.edu) Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.

Key Words: **Perfluorocarbon exposed sonicated dextrose albumin, Scavenger receptors, Vascular inflammation, Atherosclerosis.**

INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in the world (Roger et al. 2011). An understanding of risk factors including: age, hypertension, dyslipidemia, diabetes and tobacco have improved patient outcomes (Wilson et al. 1998). Despite this, there is a significant need for a better understanding of both the progression and/or effective treatment of cardiovascular disease. In the last decade, one of the major predictors discovered for the development of atherosclerosis and cardiovascular disease is inflammation (Libby et al. 2011; Willerson and Ridker 2004). However, its role in the progression toward atherosclerotic lesions is not well understood. Importantly, these precursor lesions (or fatty streaks) have been shown to be present as early as childhood and adolescence, later leading to the development of mature atherosclerotic plaques (Corti and Badimon 2002; Falk et al. 1995). Histopathology of plaques have included active monocytes, macrophages, T cells and mast cells (Falk 2006). Inflammation has also shown to be a factor leading to both plaque stability and plaque instability (Hansson 2005; Libby 2001; Virmani et al. 2000). Plaque instability ultimately can lead to acute coronary syndrome and acute myocardial infarction (MI) (Libby 2008).

Studies have been reported, illustrating the potential for using microbubbles to image the vessel after injury (Anderson et al. 2007; Tsutsui et al. 2004). However,

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the mechanisms of bubble binding are currently unknown. Recently, we have shown perfluorocarbon exposed sonicated dextrose albumin (PESDA) microbubbles interact with and bind to sites of upregulated vascular inflammation (Anderson et al. 2010). The retainment of these PESDA microbubbles along vessel walls has been shown to be an effective method for noninvasive imaging combining the echogenic properties of microbubbles with a technique that uses low mechanical index (Tsutsui et al. 2004). Studies in our laboratory have shown that the binding of PESDA involves interactions between oxidized microbubble proteins and scavenger receptors of the innate immune system including: scavenger receptor A (SRA), scavenger receptor B1 (SRB-1), oxidized LDL receptor (LOX-1), CD36 and Toll-like receptors 2 and 4 (TLR2 and TLR4). The binding of these receptors could be useful in targeting their upregulation and thereby detecting potential disease. Thus, we have hypothesized that PESDA binding could be used to quantify the extent of vascular inflammation. This article describes the use of PESDA microbubbles as a technique for the noninvasive imaging and quantification of vascular inflammation.

METHODS

Animals

Male Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, USA) and maintained on a Purina rat chow diet. All animals were allowed free access to food and water up to 1 h prior to sacrifice. All procedures were approved by the Animal Subcommittee of the University of Nebraska Medical Center and are in accordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals.

Synthesis and injection of PESDA microbubbles

PESDA microbubbles were prepared according to the method previously described (Anderson et al. 2010). Briefly, a mixture of three parts 5% dextrose and one part 5% human serum albumin (total, 16 mL) was added to 8 mL decafluorobutane gas, hand-agitated and then sonicated with an electromechanical sonicator (Heat Systems Inc., Farmingdale, NY, USA) for 70 $\pm$ 5 s. The resulting microbubble size, confirmed by hemocytometry, was 4.6 $\pm$ 1.4 μm and mean concentration measured by a Coulter counter was 1.4×10^8 bubbles/m. As previously described, this formulation results in a microbubble concentration that shows the best stability binding and resolution (Porter et al. 1995).

Vascular injury models

Sprague-Dawley (SD) male rats were anesthetized with isoflurane. The left femoral artery was exposed via cut down and a 2.0×20 mm coronary balloon was inserted into the distal 2 cm of the infrarenal aorta over a 0.35 mm wire. Briefly, as previously described (Anderson et al. 2010), aortic diameter and flow velocities were documented using ultrasound. Balloon position verified and inflated to 6 ATM for 30 s while imaging with ultrasound (130%–150% original vessel diameter). Inflation of the balloon was repeated three times, balloon removed, femoral bleeding controlled by pressure, cut down incision closed and the animals were allowed to recover for 48 h. Control animals (*i.e.*, non-angioplastied animals) were not manipulated prior to imaging and imaging was performed using the same protocol as the angioplastied animals.

Following the 48-h recovery period, animals were anesthetized, the right femoral vein was exposed via cut down and a 22 gauge angio-catheter inserted and secured. The abdomen was opened while an ultrasound transducer was positioned 2 cm anterior to the infrarenal aorta using a normal saline bath. Microbubbles (synthesized as outlined above) were diluted 1:10 in normal saline and injected as a bolus of 0.1–0.2 mL (10^7 microbubbles per injection as determined by hemocytometer and/or Coulter counter) followed by a 0.5 mL saline flush into the femoral vein, imaged using contrast pulse sequencing (CPS) ultrasound and an additional intravenous injection of PESDA was given to allow for retention of microbubbles. Rats were euthanized 5 min later to ensure that non-retained microbubbles had cleared from the blood pool. The infrarenal aorta and remaining thoracic aorta were harvested for analysis by real-time polymerase chain reaction (RT-PCR), light microscopy and immunohistochemistry as previously published (Anderson et al. 2010).

Ultrasound imaging and vascular enhancement

Contrast intensity along the vessel wall was measured utilizing a low mechanical index (MI) pulse sequence scheme referred to as contrast pulse sequence (CPS) using a Siemens Medical Solutions 15L8 probe (Siemens, Munich, Germany). A 1:1 or 1:4 end systolic triggered imaging protocol at an MI of 0.09–0.10 was used to monitor the vascular wall. After catheter insertion, animals were anesthetized and prepared as noted above. The ultrasound probe was positioned to allow imaging of the infrarenal aorta. The final probe position was determined by using a combination of harmonic, color Doppler and pulse wave (PW) ultrasound. The probe was secured in a position, which minimized the overlap of the inferior vena cava (IVC) with the infra-renal artery. Harmonic, color Doppler and PW ultrasound images of this position were obtained to document the probe position and note any regions of overlap, allowing for the appropriate review and assessment of the images. Once in position, CPS images were taken at baseline prior to IV injection. CPS images were acquired