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● *Original Contribution*

REAL-TIME *IN VIVO* PHOTOACOUSTIC IMAGING IN THE ASSESSMENT OF MYOCARDIAL DYNAMICS IN MURINE MODEL OF MYOCARDIAL ISCHEMIA

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Abstract—Photoacoustic imaging (PAI) is an evolving real-time imaging modality that combines the higher contrast of optical imaging with the higher spatial resolution of ultrasound imaging. We utilized dual-wavelength PAI for the diagnosis and monitoring of myocardial ischemia by assessing variations in blood oxygen saturation estimated in a murine model. The use of high-frequency ultrasound in conjunction with PAI enabled imaging of anatomic and functional changes associated with ischemia. Myocardial ischemia was established in eight mice by ligating the left anterior descending artery (LAD). Longitudinal results reveal that PAI is sensitive to acute myocardial ischemia, with a rapid decline in blood oxygen saturation ($p < 0.001$) observed after LAD ligation (30 min: 33.05 ± 6.80%, 80 min: 36.59 ± 5.22%, 120 min: 36.70 ± 9.46%, 24 h: 40.55 ± 13.04%) compared with baseline (87.83 ± 5.73%). Variation in blood oxygen saturation was found to be linearly correlated with ejection fraction (%), fractional shortening (%) and stroke volume (μL), with Pearson's correlation coefficient values of 0.66, 0.67 and 0.77, respectively ($p < 0.001$). Our results indicate that PAI has the potential for real-time diagnosis and monitoring of acute myocardial ischemia. (E-mail: tvarghese@wisc.edu)

Key Words: **Photoacoustic imaging, High-frequency ultrasound, Myocardial ischemia, Murine model.**

INTRODUCTION

Coronary heart disease, including myocardial infarction (MI), is one of the leading causes of mortality, accounting for one in seven deaths in the United States, with more than 360,000 fatalities each year (Benjamin et al. 2017). MI results from necrosis of heart cells, typically caused by ischemia—a diminished supply of blood that causes hypoxia in cardiac muscle cells (Thygesen et al. 2007). Conventional diagnostic tools for MI include electrocardiography (ECG), laboratory tests such as creatine kinase MB (CK-MB) assay and non-invasive imaging such as Echocardiography (Murray and Alpert 1994). However, ECG is not always specific on the extent of ischemia, and CK-MB assays may take up to 12 h, ruling out the possibility of real-time diagnosis and treatment of acute MI (Puleo et al. 1994).

Non-invasive imaging methods include clinical echocardiography (Eaton et al. 1979; Pfeffer et al. 1979; Kanno et al. 2002), tissue Doppler and myocardial strain imaging (Bauer et al. 2011; Bhan et al. 2014; Konofagou et al. 2002; Ma et al. 2016; Varghese et al. 2003). These methods assess cardiac performance by tracking structural changes in heart muscle movement after a MI, but do not provide specific information regarding perfusion of cardiac muscle, which might be more definitive in indicating the extent of ischemia. Position emission tomography (PET) and contrast-enhanced magnetic resonance imaging (MRI) have also been used for 3-D mapping of perfusion (Kober et al. 2005; Schwaiger and Muzik 1991; Wiemer et al. 2009). However, these methods do not function in real time and are time intensive and expensive. Myocardial contrast echocardiography (MCE) has been used to evaluate myocardial perfusion and identify perfusion defects in ischemia models

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(Gao et al. 2011). MCE involves intravenous injection of contrast agents (microbubbles) to enhance the myocardial B-mode image (Gao et al. 2011). However, performing MCE in a small animal model with a rapidly beating heart is quite demanding in terms of both surgical procedures and image acquisition with a high-resolution scanner (French et al. 2006).

Photoacoustic imaging (PAI) is an evolving real-time biomedical imaging modality that combines optical imaging contrast with ultrasonic spatial resolution (Beard 2011; Hu and Wang 2010; Mallidi et al. 2011; Su et al. 2010; Wang and Hu 2012; Xu and Wang 2006). PAI illuminates tissue with short pulses of electromagnetic radiation typically ranging from 700 to 900 nm (Needles et al. 2013; Rich and Seshadri 2016). Light energy is selectively absorbed by endogenous chromophores present in tissue, resulting in rapid thermoelastic expansion (Beard 2011; Rich and Seshadri 2016; Su et al. 2010; Wang and Hu 2012; Xu and Wang 2006). This thermoelastic expansion generates broadband acoustic waves, which are detected using ultrasound transducers (Needles et al. 2013). Photoacoustic (PA) images provide anatomic information with ultrasound imaging coupled with tissue-specific information such as oxygen saturation *via* the optical absorption contrast.

In recent years, efforts have concentrated on *in vivo* quantitative imaging by capitalizing on the absorption spectra of endogenous contrast agents such as hemoglobin (Wang and Hu 2012; Xu and Wang 2006), leading to use of dual-wavelength PA to estimate percentage blood oxygen saturation (sO_2). Differing absorption spectra of oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) enable quantification of blood oxygenation with this approach (Beard 2011). The use of sO_2 to depict organ microvasculature (Beard 2011; Laufer et al. 2012; Mallidi et al. 2011; Wang and Hu 2012; Yao et al. 2011) and hypoxia (Arthuis et al. 2017; Gerling et al. 2014; Rich and Seshadri 2016) in real time has been described. Compared with MCE, dual-wavelength PA enables the study of myocardial perfusion changes without requiring any exogenous contrast agent. Real-time PAI and its sensitivity to blood oxygenation levels, coupled with the recent development of PA-integrated micro-ultrasound systems (Needles et al. 2013), make it suitable for diagnosis and monitoring of myocardial ischemia *in vivo*. Initial reports of PAI for murine cardiovascular dynamics have been published (Zemp et al. 2008). They utilized a 30-MHz linear array to image the beating heart of athymic nude mice at ~50 frames per second. Li et al. (2011) tried to establish a correlation between the extent of myocardial ischemia and the variation in PA signal intensity in rats submerged in water under tracheal intubation, on a section of the left ventricular wall. They used a wavelength of 532 nm and a single-element transducer with a center frequency of 3.5 MHz for reception (Li et al. 2011). They reported an exponential decay in the PA signal intensity with time after left anterior descending artery (LAD) occlusion.

Here, we describe the use of a commercially available PA imaging system for the diagnosis and monitoring of myocardial ischemia in murine models. Dual-wavelength PAI was utilized to generate parametric maps of blood oxygen saturation that were overlaid on high-resolution high-frequency ultrasound images of the myocardium. PAI was found to be sensitive to changes in myocardial oxygenation associated with acute myocardial ischemia.

METHODS

Animal models

Ten 10- to 12-wk-old male BALB/CJ mice obtained from Jackson Laboratories (Bar Harbor, ME, USA) were studied using PAI and high-frequency ultrasound (HFUS) imaging. Myocardial ischemia was established in each murine model using the procedure described below. All *in vivo* procedures were performed under an approved protocol by the Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin—Madison.

Murine model of myocardial ischemia

After induction of isoflurane anesthesia (3%), the mouse was intubated with an 18-gauge catheter, placed on a ventilator at 120−130 breaths/min with a stroke volume of 150 μL and maintained on 2% isoflurane. A left lateral incision through the fourth intercostal space was made to expose the heart. After visualization of the left coronary artery, a 7-O clear Prolene suture was placed through the myocardium in the anterolateral wall and secured (Kumar et al. 2005; Singla et al. 2006). Coronary artery entrapment was confirmed by observing blanching of the distal circulation (ventricular apex) and ECG changes indicative of myocardial ischemia. The lungs were overinflated, and the ribs and muscle layers were closed with absorbable sutures. The skin was closed by additional suturing using 6-O clear nylon or silk sutures. The mouse was then allowed to recover from anesthesia and extubated.

Photoacoustic and high-frequency ultrasound imaging

Longitudinal variations in perfusion and cardiac function of the heart after ischemia were evaluated using PAI and HFUS. Imaging sessions were performed before LAD ligation (baseline) and 30 min, 80 min, 120 min and 24 h after LAD ligation. The objective of imaging after LAD ligation (from 30 min to 24 h) was to study the ability of PAI in the early detection of ischemia in the ventricular wall. All imaging was performed using a