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Echocardiographic Ischemic Memory Imaging Through Complement-Mediated Vascular Adhesion of Phosphatidylserine-Containing Microbubbles

Brian Mott, MD,[a] William Packwood, BS,[a] Aris Xie, MS,[a] J. Todd Belcik, BS, RCS, RDCS,[a] Ronald P. Taylor, PhD,[b] Yan Zhao, MD,[a] Brian P. Davidson, MD,[a] Jonathan R. Lindner, MD[a]

ABSTRACT

OBJECTIVES This study hypothesized that microvascular retention of phosphatidylserine-containing microbubbles (MB-PS) would allow detection of recent but resolved myocardial ischemia with myocardial contrast echocardiographic (MCE) molecular imaging.

BACKGROUND Techniques for ischemic memory imaging which can detect and spatially assess resolved myocardial ischemia are being developed for rapid evaluation of patients with chest pain.

METHODS MCE molecular imaging with MB-PS was performed 1.5 h, 3.0 h, and 6.0 h after brief (10 min) myocardial ischemia in mice; data were compared to selectin-targeted microbubbles. MCE molecular imaging with Sonazoid (GE Healthcare, Amersham, United Kingdom), a commercially produced phosphatidylserine (PS) – containing agent, was performed in separate mice at 1.5 h and 3.0 h after ischemia-reperfusion; and in dogs undergoing 135 min of ischemia and 60 min of reflow as well as in closed-chest nonischemic control dogs. The mechanism for MB-PS attachment was assessed by intravital microscopy of post-ischemic muscle and by flow cytometry analysis of cell-MB interactions.

RESULTS In mice undergoing ischemia-reperfusion without infarction, signal enhancement in the risk area for MB-PS and p-selectin glycoprotein ligand-1–targeted microbubbles was similar at reflow times of 1.5 h (23.3 ± 7.3 IU vs. 30.7 ± 4.1 IU), 3.0 h (42.2 ± 6.2 IU vs. 33.9 ± 7.4 IU), and 6.0 h (24.1 ± 4.3 IU vs. 25.5 ± 4.7 IU). For both agents, signal in the risk area was significantly ($p < 0.05$) higher than remote region at all reflow times. Sonazoid also produced strong risk area enhancement at 1.5 h (34.7 ± 5.0 IU) and 3.0 h (52.5 ± 4.5 IU) which was approximately 3-fold greater than in the control region, and which correlated spatially with the microsphere-derived risk area. In dogs, Sonazoid signal in the risk area was >5-fold higher than in closed-chest control myocardium (42.2 ± 8.1 IU vs. 7.9 ± 3.3 IU; $p < 0.001$). Mechanistic studies indicated that MB-PS attached directly to venular endothelium and adherent leukocytes which was dependent on serum complement components C1q and C3.

CONCLUSIONS Ischemic memory imaging with MCE is possible using MB-PS which may obviate the need for ligand-directed targeting. (J Am Coll Cardiol Img 2016;9:937–46) © 2016 by the American College of Cardiology Foundation.

The diagnosis of acute coronary syndrome (ACS) in symptomatic patients relies on clinical history, laboratory evaluation, and electrocardiogram which, except in the case of ST-segment elevation myocardial infarction, are often nondiagnostic on initial evaluation (1,2). To address this limitation, molecular imaging techniques for detecting ischemia-related molecular profiles

From the [a]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, Oregon; and the [b]Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, Virginia. Sonazoid was provided for this study by GE Healthcare, Amersham, United Kingdom. Dr. Lindner is supported by grants R01-HL120046, R01-HL078610, and R01-HL111969 from the NIH; and a grant from the Doris Duke Foundation. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

LAD = left anterior descending coronary artery

MB = microbubbles

MB-PS = phosphatidylserine microbubbles

MB-PSGL-1 = p-selectin glycoprotein ligand-1–targeted microbubbles

MCE = myocardial contrast echocardiography

PS = phosphatidylserine

PSGL-1 = p-selectin glycoprotein ligand-1

TTC = triphenyltetrazolium chloride

have been developed and may be useful for diagnosing ACS when initial tests are negative or when ischemia has resolved and not resulted in much myocardial necrosis (3). They could potentially also identify high-risk individuals based on the spatial extent of the area at risk.

Ultrasound is a practical approach for ischemic memory imaging because it is portable and rapid. Molecular imaging of resolved ischemia without infarction has been performed with myocardial contrast echocardiography (MCE) in rodent and nonhuman primate models using microbubbles targeted to the selectin family of endothelial cell adhesion molecules that are rapidly expressed in response to ischemia (4–7). Clinical translation of this approach has been slowed by the arduous regulatory process of testing efficacy and safety of a human-ready microbubble agent bearing a biologically active ligand. In this study, we propose an alternative approach through modulation of microbubble lipid shell content. Complement-mediated adhesion of lipid-shelled microbubbles to activated leukocytes and endothelial cells occurs in areas of inflammation (8–10). This process is amplified by the presence of anionic lipids, especially phosphatidylserine (PS) in the shell (11). Phosphatidylserine microbubbles (MB-PS) have been used to noninvasively image inflammation in both chronic limb ischemia and severe acute myocardial infarction (12,13).

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In this study we hypothesized that MB-PS can identify and assess the spatial extent of recent but resolved myocardial ischemia without infarction. Additional aims of the study were to: 1) compare MB-PS to a selectin-targeted microbubble agent bearing recombinant human p-selectin glycoprotein ligand-1 (PSGL-1); 2) evaluate the potential for performing ischemic memory imaging with a commercially produced PS-containing microbubble already used in humans; and 3) to further characterize the mechanism for MB-PS adhesion in regions of ischemia.

METHODS

MICROBUBBLE PREPARATION. Lipid-shelled MB-PS were prepared by sonication of a decafluorobutane gas-saturated aqueous suspension of 2 mg/ml distearoylphosphatidylcholine, 1 mg/ml polyoxyethylene-40-stearate, and 0.3 mg/ml distearoyl phosphatidylserine (Avanti Polar Lipids, Alabaster, Alabama). For p-selectin glycoprotein ligand-1–targeted microbubbles (MB-PSGL-1), biotinylated microbubbles were prepared and a PSGL-1 dimeric fusion protein on a human immunoglobulin G1 (Y's Therapeutics, Tokyo, Japan) was conjugated to the surface using a streptavidin link as previously described (6). For intravital microscopy and flow cytometry, MB-PS was fluorescently labeled by the addition of dioctadecyl tetramethylindocarbocyanine (DiI) or dioctadecyloxacarbocyanine (DiO) perchlorate. Clinical grade commercially produced decafluorobutane microbubbles with a shell composed of egg PS (Sonazoid, GE Healthcare, Amersham, United Kingdom) were reconstituted according to manufacturer's instructions. Microbubble size distribution and concentration was measured by electrozone sensing (Multisizer III, Beckman Coulter, Brea, California). Zeta potential was determined by measurement of their electrophoretic mobility (ZetaPALS, Brookhaven Instruments, Holtsville, New York) at pH 7.4.

MURINE MODEL OF MYOCARDIAL ISCHEMIA. Studies were approved by the Animal Care and Use Committee at Oregon Health & Science University. Brief myocardial ischemia or sham procedure was performed in C57Bl/6 mice (Jackson Labs, Bar Harbor, Maine) 10 to 15 weeks of age. Mice were anesthetized with inhaled isoflurane and placed on positive-pressure ventilation. A left lateral thoracotomy was performed and a 8-0 Prolene suture was placed around the left anterior descending (LAD) coronary artery. For the ischemic group, the ligature was secured for 10 min to produce myocardial ischemia confirmed by ST-segment elevation on electrocardiogram. The ligature was released and left in place, the chest wall was closed, and mice were extubated. For sham-treated animals, the ligature was placed but not secured. To exclude the presence of infarction, at the end of each study high-frequency (30 MHz) echocardiography (Vevo 770, Visualsonics, Toronto, Canada) was performed to exclude wall motion abnormality and the heart slice corresponding to the short-axis imaging plane was stained with 2,3,5-triphenyltetrazolium chloride.

MOLECULAR IMAGING. MCE molecular imaging of the midventricular short-axis plane was performed with a linear-array probe (Sequoia, Siemens Medical Systems, Mountainview, California) using multipulse phase-inversion and amplitude-modulation imaging at 7 MHz, a mechanical index of 1.4, and a dynamic range of 55 dB. End-systolic images were acquired 8 min after intravenous injection of microbubbles (5×10^6). Signal from retained microbubbles alone was determined as previously described by digital