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PRE-CLINICAL RESEARCH

Detection of Antecedent Myocardial Ischemia With Multiselectin Molecular Imaging

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Objectives Our aim was to develop an echocardiographic molecular imaging approach for detecting recent myocardial ischemia by using recombinant P-selectin glycoprotein ligand (PSGL)-1 as a targeting ligand, which is a feasible approach for human use.

Background Ischemic memory imaging using human PSGL-1 as a targeting moiety may extend the time window for postischemic detection by targeting the early (P-selectin) and late (E-selectin) endothelial ischemic response.

Methods Lipid microbubbles bearing recombinant human PSGL-1 (MB_{YSPSL}) or P-selectin antibody (MB_{Ab}) were prepared. Targeted attachment was evaluated by using flow chamber and intravital microscopy. In vivo ultrasound molecular imaging was first performed in the hindlimb in wild-type and P-selectin–deficient ($P^{-/-}$) mice 45 to 360 min after brief ischemia-reperfusion injury. Myocardial contrast echocardiography molecular imaging was performed 1.5, 3, 6, and 18 h after brief left anterior descending coronary artery ischemia-reperfusion.

Results Microbubble attachment to P-selectin–immunoglobulin G fusion protein in flow chamber experiments (shear stress 0.5 to 8.0 dyne/cm^2) and to activated venular endothelium on intravital microscopy were similar for MB_{Ab} and MB_{YSPSL}. Intense enhancement was seen for MB_{Ab} and MB_{YSPSL} in postischemic muscle and was more stable over time for MB_{YSPSL}. On myocardial contrast echocardiography, both MB_{YSPSL} and MB_{Ab} produced similar signal enhancement at 90 min and 3 h after ischemia, which spatially correlated with the postischemic risk area. Signal significantly decreased but was still present at 6 to 18 h.

Conclusions Echocardiographic molecular imaging with a human multi-selectin–targeted contrast agent bearing recombinant human PSGL-1 can detect myocardial ischemia hours after resolution. This approach may potentially be used for rapid bedside evaluation of patients with recent chest pain. (J Am Coll Cardiol 2012;60:1690–7) © 2012 by the American College of Cardiology Foundation

There are well-recognized limitations in the algorithms currently used to diagnose acute coronary syndromes (ACS) in patients who present with symptoms but whose initial electrocardiogram does not show ST-segment elevation (1–3). Various noninvasive imaging techniques have been proposed for improving diagnostic accuracy in patients with possible ACS. Molecular imaging has been used to detect biochemical alterations that occur not only during ischemia but also persist after ischemia resolves. This approach, often referred to as ischemic memory imaging, may be particularly useful for detecting ischemia when the amount of necrosis is small or in patients who present after symptoms resolve or have pre-existing electrocardiogram or wall motion abnormalities. Ideally, molecular imaging should be able to detect and assess the spatial extent of ischemia hours after its resolution and provide information rapidly to the clinician.

Because of its portability and speed, myocardial contrast echocardiography (MCE) molecular imaging has been proposed as a point-of-care technique for rapidly detecting recent myocardial ischemia. MCE detection of myocardial ischemia after transient reduction in coronary flow has been achieved by targeting microbubble contrast agents to endothelial P-selectin (4,5). Selectins are a family of endothelial adhesion molecules that bind carbohydrate-bearing counterligands on leukocytes and are expressed in response to ischemia and other inflammatory stimuli (6,7). P-selectin is stored preformed in endothelial cells and expressed within minutes of ischemia or injury (8,9). However, the duration over which P-selectin imaging would be effective for detecting recent

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ischemia is uncertain because its surface expression tends to diminish over time after an ischemic insult. In the present study, we hypothesized that the time window for ischemic memory imaging could be extended by targeting not only P-selectin but also E-selectin, which exhibits delayed but more persistent expression for up to 24 h after endothelial activation (10–12). Accordingly, we developed a novel ultrasound contrast agent bearing a recombinant form of human P-selectin glycoprotein (PSGL)-1, an endogenous counterligand for both P- and E-selectin. The use of recombinant human PSGL-1 as a targeting moiety also represents an important step toward the development of a human-ready agent for myocardial ischemic memory imaging with echocardiography.

Abbreviations and Acronyms

ACS = acute coronary syndrome
IgG = immunoglobulin G
LAD = left anterior descending coronary artery
mAb = monoclonal antibody
MB_{Ab} = microbubbles bearing P-selectin antibody
MB_{YSPSL} = microbubbles bearing recombinant human P-selectin glycoprotein ligand-1
MCE = myocardial contrast echocardiography
MI = mechanical index
PEG = polyethylene glycol
PSGL = P-selectin glycoprotein ligand

Methods

Targeted microbubble preparation. Biotinylated lipid-shelled decafluorobutane microbubbles were prepared by sonication of an aqueous suspension of distearoylphosphatidylcholine, polyoxyethylene-40-stearate, and distearoylphosphatidylethanolamine-polyethylene glycol (PEG)(2000)-biotin in a 50:10:1 molar ratio. Conjugation of biotinylated ligand to the microbubble surface was performed by using a streptavidin bridge as previously described (13) to create the following agents: MB_{YSPSL}: bearing an immunoglobulin G (IgG) fusion protein with a dimeric recombinant form of the glycoprotein PSGL-1 (YSPSL, Y's Therapeutics Co., Ltd., Tokyo, Japan); MB_{Ab}: bearing rat anti-mouse P-selectin monoclonal antibody (mAb) (RB40.34, BD Pharmingen, San Jose, California); or MB_{Ctr}: bearing isotype control mAb (R3-34, BD Pharmingen). For flow chamber studies and intravital microscopy, MB_{Ab} and MB_{YSPSL} were fluorescently labeled by the addition of dioctadecyltetramethylindocarbocyanine or dioctadecyloxacarbocyanine perchlorate, respectively, to the microbubble shell. Perfusion imaging was performed with microbubbles lacking distearoyl-phosphatidylethanolamine-PEG(2000)-biotin. Microbubbles were analyzed for concentration and size distribution (Multisizer III, Beckman-Coulter, Brea, California). Intravascular half-life for each agent was determined by left ventricular cavity intensity on MCE after intravenous injection of 5×10^6 microbubbles.

Flow chamber attachment. Cell culture dishes were coated with an IgG fusion protein bearing murine P-selectin (BD Pharmingen Inc.) at a site density of approximately 100 μm^{-1}(14). The dishes were blocked with 3% bovine serum albumin and mounted on a parallel plate flow chamber (Glycotech Inc., Gaithersburg, Maryland) that was placed in an inverted position on a microscope (Axioskop2-FS, Carl Zeiss Inc., Thornwood, New York) for video recording. Suspensions of fluorescently labeled MB_{YSPSL} and MB_{Ab} (3×10^6 ml^{-1}) were drawn through the flow chamber at flow rates resulting in calculated shear stresses of 0.5, 1.0, 2.0, or 8.0 dynes/cm^2. The number of microbubbles attached to the plate was determined for 20 optical fields after 3 min of continuous flow. Experiments were performed in duplicate for each condition.

Animal preparation. Studies were approved by the Animal Care and Use Committee at Oregon Health & Science University. Male wild-type C57Bl/6 mice and P-selectin–deficient ($P^{-/-}$) mice (Jackson Labs) 10 to 15 weeks of age were studied. For intravital microscopy, mice were anesthetized with an intraperitoneal injection (12.5 $\mu l/g$) of a solution containing ketamine hydrochloride (10 mg/ml), xylazine (1 mg/ml), and atropine (0.02 mg/ml). For molecular imaging, mice were anesthetized with inhaled isoflurane (0.75% to 1.5% for maintenance). A jugular vein was cannulated for injection of microbubbles.

Intravital microscopy. A cremaster muscle in wild-type mice (n = 3) was prepared as previously described (15). Video recordings were made with a microscope and recorded with a charge-coupled device camera (C2400, Hamamatsu Photonics). Muscle preparations were studied 20 to 30 min after exteriorization, and P-selectin expression from surgical trauma was confirmed by the presence of leukocyte rolling in postcapillary venules (9). Fluorescently labeled MB_{YSPSL} and MB_{Ab} (1×10^7) were simultaneously injected intravenously. Microbubble attachment in 10 to 15 randomly selected optical fields was quantified 2 to 4 min after injection using fluorescent epi-illumination.

Contrast-enhanced ultrasound. Imaging was performed with a linear array transducer (15L8) interfaced with a Sequoia ultrasound system (Siemens Medical Systems, Mountain View, California). A multipulse algorithm using phase and amplitude modulation was used to detect the nonlinear fundamental component of the microbubble signal. Imaging was performed at a centerline frequency of 7 MHz.

Hindlimb molecular imaging protocol. Contrast-enhanced ultrasound molecular imaging of the proximal hindlimb adductor muscles was performed after unilateral ischemia-reperfusion injury produced by tourniquet occlusion for 10 min. Ischemia was confirmed by a >95% reduction in flow according to contrast-enhanced ultrasound perfusion imaging. Molecular imaging was performed in the postischemic limb at 45, 90, 180, and 360 min after ischemia in 20 wild-type and 9 $P^{-/-}$ mice and bilaterally in 3 control wild-type mice not undergoing ischemia-reperfusion. In 4 additional $P^{-/-}$ mice, imaging was performed at 360 min after blocking E-selectin by intravenous injection of rat anti-mouse E-selectin mAb (UZ4, Millipore Billerica,