Cardiovascular Imaging of Remote Myocardial Ischemia
Detecting a Molecular Trace of Evidence Left Behind

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Intravenous microbubbles currently are approved by the Food and Drug Administration solely for the purpose of enhancing left ventricular cavity opacification. However, intravenous microbubbles also have been used in several clinical studies to detect myocardial perfusion. Ultrasound imaging of myocardial contrast to detect perfusion abnormalities has significant advantages over other imaging techniques in that it has higher resolution, does not require ionizing radiation, and can be performed at the patient’s bedside. Clinical studies have demonstrated that myocardial contrast echocardiography is useful for detecting coronary artery disease both in the emergency room setting and during stress echocardiography. In these settings, the intravenous microbubbles function as pure intravascular tracers and are not adherent to the vessel wall or taken up by the myocytes.

Ligands can be attached to the surface of microbubbles, which results in their binding to specific epitopes upregulated on the endothelial surface. Such binding was used in the study by Villanueva et al in the present issue of *Circulation*. In a rat model, these investigators were able to successfully attach the selectin-targeting tetrasaccharide sialyl Lewis to phospholipid-shelled microbubbles and to detect myocardial regions that were previously ischemic with a sensitive ultrasound detection scheme. Detection of contrast enhancement from retained microbubbles within the remotely ischemic perfusion bed was evident at both 30 minutes and 1 hour after relief of the ischemic episode. These findings have significant implications in that detection of remote ischemic events may be possible in the emergent evaluation of patients with a history of chest pain and nondiagnostic ECGs.

**Targeted Microbubbles: Potential Clinical Applications**

Villanueva et al were one of the first groups to report that microbubbles adhere to dysfunctional endothelium. However, the quantity of nontargeted microbubbles that adhered to the endothelium in these early observations was small and not detectable with conventional ultrasound pulse sequence schemes. Targeting ligands or antibodies have been attached to the microbubble shell surface or incorporated into the shell to increase their adherence to upregulated receptors on the vascular endothelial surface. For example, microbubbles have been conjugated with disintegrins (eg, echistatin). This serves to target them to a-integrins expressed on neovascular endothelium, resulting in their retention within regions of increased arteriogenesis that occurs in response to prolonged hindlimb ischemia. The arteriogenic effects of proliferative agents like fibroblast growth factor-2 have been assessed by measuring ultrasound signal intensity from retained microbubbles targeted to a-integrins upregulated on neovascular endothelium within ischemic perfusion beds. Phosphatidylinerine has been incorporated into the shell of microbubbles to increase complement-mediated avidity to activated leukocytes. These microbubbles have been shown to be retained within reperfused myocardium, and the signal intensity derived from these microbubbles has correlated with the severity and extent of postischemic inflammation. The retained signal intensity from microbubbles targeted to upregulated leukocyte adhesion molecules on the endothelial surface also has been used in animal studies to detect rejection after cardiac transplantation.

Microbubbles targeted to both a,β integrins and activated leukocytes have been used with myocardial contrast echocardiography to assess the effects of agents that inhibit endothelial integrin and platelet glycoprotein IIb/IIIa receptors in reducing myocardial infarct size. Bioconjugate ligands targeted to the glycoprotein IIb/IIIa receptor also have been used to target microbubbles to platelet-rich thrombi, creating the potential for improved detection and treatment of thrombi with ultrasound.

**Improvement in Ultrasound Detection Schemes**

Detection of retained microbubbles has been further enhanced by the development of sensitive ultrasound pulse sequence schemes. Instead of conventional ultrasound pulse sequences, these newer sequences submit pulses that have alternating polarity and amplitude. These sequences increase the nonlinear response from microbubbles and, when combined with tissue cancellation techniques, produce exquisite sensitivity for the detection of retained microbubbles on both arterial and venular endothelium. These advances on imaging are vital to the detection of retained microbubbles for ischemic memory imaging.

**P-Selectin Imaging With Ultrasound and Microbubbles**

Although the findings of Villanueva et al demonstrate that microbubbles targeted to upregulated P-selectin can detect remote ischemic events, potential problems may prevent this...
from becoming a bedside application. The model chosen for the study was in animals with no underlying atherosclerotic disease. The specificity of P-selectin imaging for the detection of remote myocardial ischemia needs to be tested in animal models with atherosclerosis and other inflammatory states in which this epitope may be expressed more ubiquitously.12 Second, the duration of remote myocardial ischemia may be a factor in determining whether P-selectin upregulation can be detected. In the present study, there appeared to be a reduced level of detectable myocardial contrast enhancement when the duration of remote myocardial ischemia was 10 compared with 15 minutes of ischemia, although no statistical comparisons were made. Finally, the safety and feasibility of targeted microbubble formulations need to undergo a rigorous clinical evaluation. It is evident that the sensitivity of the detection methods, the specificity of P-selectin upregulation for ischemic events, and the duration of ischemia required for upregulation to occur will play a role in the eventual clinical use of this method.

In summary, there are at least 2 important new findings in the article by Villanueva et al.1 First, the microbubble retention first observed by this group and others >10 years ago has been exploited into a potentially useful method of detecting the P-selectin upregulation that occurs in response to relatively brief myocardial ischemic events. Second, imaging of these events is still possible for up to 1 hour after the ischemic episode occurs. Therefore, the findings of this study indicate that newer advances in ultrasound detection and advances in molecular design of microbubble shells can be used to detect molecular traces of evidence left behind after myocardial ischemia. The implications of this molecular imaging modality are very important and may assist in the large clinical problem of detecting remote ischemic events in patients suspected of having acute coronary syndromes.

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References
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