Cardiovascular Molecular Imaging

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Ultrasound molecular imaging in cardiovascular disease

Cardiovascular diseases are the leading cause of mortality in the western world. Most important are complications of atherosclerosis, but other disease entities such as myocarditis contribute to a considerable disease burden particularly in young individuals. Noninvasive imaging plays an increasing role in diagnosis, risk stratification and assessment of treatment responses. Advances in image technology over the last years have allowed for depiction of the heart and blood vessels with ever increasing detail. Novel imaging technologies termed molecular imaging use detection of site-targeted contrast agents to depict the molecular footprint of a disease-relevant phenotype at the cellular level. It is thought that such techniques will in the future contribute to earlier detection of disease, to better risk stratification and to better assessment of treatment responses. Molecular imaging with ultrasound contrast agents relies on the detection of microbubbles within diseased tissue. Microbubbles produce an acoustic signal owing to their resonant properties in an ultrasound field. Microbubble targeting is accomplished by either manipulating the microbubble shell for attachment of microbubbles to activated leukocytes, or by conjugation of specific ligands to the microbubble surface (Fig. 1).

Ultrasound molecular imaging of myocarditis

Dilated cardiomyopathy as a consequence of viral myocarditis is a frequent cause for heart failure in young adults. In young patients presenting to the emergency department with either chest pain or signs of heart failure, myocarditis is a differential diagnosis. However, the diagnosis of myocarditis is difficult, as clinical signs, the electrocardiogram and biomarkers (troponins) lack sensitivity/specificity. Thus, there is a need for a rapid, non-invasive imaging tool for the detection of inflammatory events occurring in myocarditis. Using microbubbles targeted to leukocytes, to CD4+ lymphocytes and to the endothelial cell adhesion molecule P-selectin we were able to diagnose myocardial inflammation in a murine model of myocarditis even in the absence of effects on myocardial function. The specific detection of the recruitment of CD4+ lymphocytes which are important in driving autoimmune processes that lead to cardiac damage in myocarditis was possible using non-invasive ultrasound molecular imaging. Also, the signals obtained from microbubbles targeted to CD4+ lymphocytes correlated to CD4+ lymphocytes present in tissue as assessed on immunhistology.

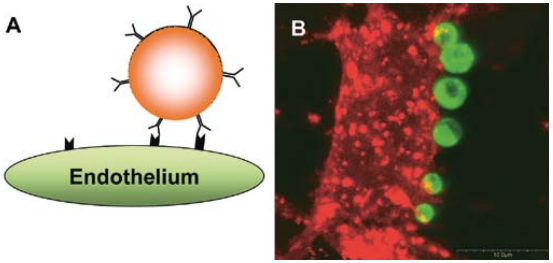

Fig. 1: Principle of site-targeting of microbubble contrast agents. **(A)** Antibodies or other ligands targeted to disease specific antigens are conjugated to the microbubble surface. **(B)** Attachment of microbubbles to VCAM-1 to an endothelial cell *in vitro*.

Ultrasound molecular imaging of atherosclerosis

Risk assessment for atherosclerosis relies on established clinical risk factors. This approach places a large proportion of individuals in an intermediate risk category. Therefore, tools to better assess the risk in these patients are needed. It is generally thought that noninvasive imaging of molecular events associated with atherosclerotic disease may serve this purpose. Previous studies have shown that contrast enhanced ultrasound (CEU) molecular imaging using microbubble contrast agents directed against vascular cell adhesion molecule 1 (VCAM-1), which is involved in inflammatory processes in atherosclerosis, is feasible in murine disease models. However, the ultrasound contrast agents used in these studies are not suitable for clinical translation, and there is a need for the development of microbubbles employing (a) clinically translatable strategies for conjugation of targeting moieties, and (b) targeting ligands that can readily be used in the clinical field. Nanobodies are small antibody fragments derived from heavy-chain-only antibodies. They are attractive for applications in molecular imaging, as they are highly specific, non-immunogenic and thus offer the potential for clinical translation. Likewise, Designed Ankyrin Repeat Proteins (DARPins) are potential candidates for clinical molecular imaging given their easy production and selection, high affinity and low immunogenicity. We are therefore currently developing and validating clinically translatable binders coupled to the microbubble surface using maleimide covalent bonding.

Selected Publications

Steinl M, Xu L, Khanicheh E, Ellertsdottir E, Ochoa-Espinosa A, Mitterhuber M, Glatz K, Kuster GM, Kaufmann BA. (2016) Non-invasive contrast enhanced ultrasound molecular imaging detects myocardial inflammatory response in autoimmune myocarditis. Circ Cardiovasc Imaging. Aug;9(8). pii: e004720

Steinl DC, Kaufmann BA. (2015) Ultrasound Imaging for Risk Assessment in Atherosclerosis. Int J Mol Sci. Apr 29;16(5):9749-9769

Khanicheh E, Qi Y, Xie A, Mitterhuber M, Xu L, Mochizuki M, Daali Y, Jaquet V, Krause KH, Ruggeri ZM, Kuster GM, Lindner JR, Kaufmann BA. (2013) Molecular Imaging Reveals Rapid Reduction of Endothelial Activation in Early Atherosclerosis With Apocynin Independent of Antioxidative Properties.. Arterioscler Thromb Vasc Biol. Aug 1

Khanicheh E, Mitterhuber M, Xu L, Haeuselmann SP, Kuster GM, Kaufmann BA. (2013) Noninvasive Ultrasound Molecular Imaging of the Effect of Statins on Endothelial Inflammatory Phenotype in Early Atherosclerosis. PLoS ONE 8(3): e58761. doi:10.1371/journal.pone.0058761

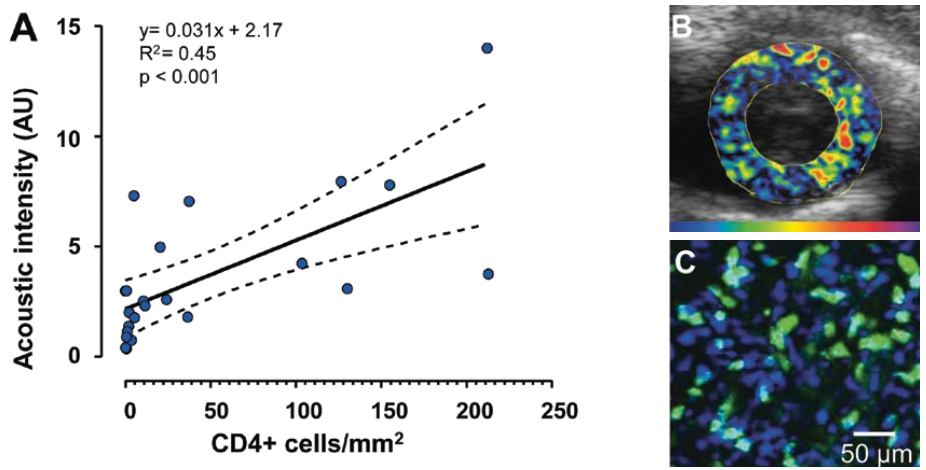

Fig. 2: Correlation of CEU molecular imaging data for CD4 targeted microbubbles with CD4+ T-lymphocyte counts in tissue **(A).** Example of background-subtracted signal for CD4 targeted microbubbles in an animal with myocarditis **(B).** Example of extensive myocardial CD4+ T-lymphocyte infiltration **(C).**