Affinity-Targeted Contrast Agents

Samuel A. WICKLINE[1], Gregory M. LANZA[2]
[1]Washington University School of Medicine, Cardiology Division, 216 South Kingshighway Boulevard, Saint Louis, Missouri United States;
[2]Washington University, ;

Introduction

The emerging field of "molecular imaging" refers to in vivo diagnosis of complex pathological processes by specific detection of unique molecular signatures of disease states either at the cellular, subcellular, or gene level.[1] Unlike a blood pool agent, a site directed contrast agent specifically enhances the signal from pathological tissue that would otherwise be difficult to distinguish from surrounding normal tissue. This concept, which might be considered an example of in vivo immunohistochemistry, is being extended to noninvasive imaging of important molecular or cellular moieties present in nano- and picomolar concentrations. An ideal targeted contrast agents would exhibit: 1) long circulating half-life (ideally greater than 30-60 minutes); 2) long residence time at targeted site; 3) sensitive and selective binding to epitopes of interest; 4) prominent contrast-to-noise enhancement; 5) acceptable toxicity profile; 6) ease of production and clinical use; 7) applicability with standard commercially available imaging modalities; and 8) promise for adjunctive therapeutic delivery.

Targeting ligands may be chemically attached directly to the contrast agents by a variety of methods depending upon the nature of the particle surface. Monoclonal antibody and fragments have been conjugated to liposomes, emulsions and other microbubble particles to provide active, site-specific targeting. Phage display techniques are now used to produce recombinant human monoclonal antibody fragments against a large range of different antigens without involving antibody-producing animals. Peptides, like antibodies, may have high specificity and epitope affinity.[2-3] Asialoglycoproteins have been used for liver-specific applications due to their high affinity for ASG receptors located uniquely on hepatocytes.[4,5]. Aptamers are high affinity, high specificity RNA or DNA-based ligands generated from random sequences of 20 to 30 nucleotides, selectively screened by absorption to molecular antigens or cells, and enriched to purify specific high affinity binding ligands.

Targeted nanoparticle emulsion agents

We have developed a ligand-targeted, lipid-encapsulated, nongaseous perfluorocarbon emulsion that can be used for both ultrasound and magnetic resonance applications.[6,7] The nanoparticles have long circulatory half-lives (1-1.5 hour elimination rates) due to their small size and inherent in vivo stability. Preliminary data suggest that this novel agent elicits specific and sensitive contrast enhancement within an hour of intravenous injection and persists at the site for many hours after binding.

Nanoparticles targeted to thrombi with an anti-fibrin antibody fragment exhibit marked acoustic enhancement both in vitro and in vivo, at frequencies typically used for clinical transcutaneous scanning (3.5 to 7.5 MHz) as well as at higher frequencies used for intravascular ultrasonic imaging (12 to 30 MHz).[6] The mechanism of contrast enhancement depends on augmentation of the acoustic impedance mismatch at a surface only after specific binding of nanoparticles Up to 30 dB of ultrasound contrast enhancement can be achieved with binding of a single layer (putative 250 nanometers) of nanoparticles.[8] Other proteins imaged in vivo with ultrasound include "tissue factor," a 43 kD, transmembrane glycoprotein that is induced in medial smooth muscle cells after angioplasty and contributes to restenosis.[9,10] The small particle size allows penetration of the contrast nanoparticles through microfractures created by balloon stretch injury into to the media where they bind and reside.

Nanoparticles prepared for MRI may contain tens of thousands of lipid-conjugated gadolinium-DTPA complex in the surfactant layer [7], resulting in dramatic T1- and T2-weighted contrast effects. Experimental arterial and venous thrombi are imaged readily by MRI with fibrin targeting ligands.[11-13] Only a single layer of particles is sufficient to cause marked contrast enhancement after binding, despite the partial volume dilution for typical clinical MRI voxel dimensions. We have sought to optimize the concentration of gadolinium on the particle surface, and we have extended the use to fluorine spectroscopy and imaging for simultaneous characterization of unique selected molecular epitopes.[11-13] Background blood signal T1w enhancement is negligible due to the low circulating concentrations of the nanoparticle contrast agent.

We and others also have demonstrated noninvasive MRI detection in vivo of $\alpha_v\beta_3$ integrin expression on neovascular endothelial cells in experimental growth factor-induced angiogenesis.[14,15] For the nanoparticle preparations, the contrast effect requires about an hour to develop after an intravenous injection, and is both sensitive and specific for angiongenesis as demonstrated by in vivo competition experiments with free antibody against $\alpha_v\beta_3$. Finally, we have recently incorporated classic anti-proliferative agents such as paclitaxel and doxorubicin into the nanoparticle formulation and have demonstrated their inhibitory effects on cell proliferation in culture (unpublished).

References

1. Weissleder R. Molecular imaging: Exploring the next frontier. Radiology 1999;212:609-614.
2. Wright WJ, McCreery T, Krupinski E, Lund P, Smyth S, Baker M, Hulett R, Unger E. Evaluation of new thrombus-specific ultrasound contrast agent. Acad Radiol. 1998;5(Suppl 1):S240-S242.
3. Reimer P, Weissleder R, Shen T, Knoefel W, Brady T. Pancreatic receptors: initial feasibility studies with a targeted contrast agent for MR imaging. Radiology. 1994;193:527-531.
4. Reimer P, Bader A, Weissleder R. Preclinical assessment of hepatocyte-targeted MR contrast agents in stable human liver cell cultures. J Magn Reson Imaging. 1998;8:687-689.
5. Leveille-Webster C, Rogers J, Arias I. Use of an asialoglycoprotein receptor-targeted magnetic resonance contrast agent to study changes in receptor biology during liver regeneration and endotoxemia in rats. Hepatology. 1996; 23:1631-1641.
6. Lanza G, Wallace K, Scott M, Cacheris W, Abendschein D, Christy D, Sharkey A, Miller J, Gaffney P, Wickline S. A novel site-targeted ultrasonic contrast agent with broad biomedical application. Circulation. 1996;94:3334-3340.
7. Lanza G, Lorenz C, Fischer S, Scott M, Cacheris W, Kaufman R, Gaffney P, Wickline S. Enhanced detection of thrombi with a novel fibrin-targeted magnetic resonance imaging agent. Acad Radiol. 1998;5(suppl 1):s173-s176.
8. Lanza G, Trousil R, Wallace K, Rose J, Hall C, Scott M, Miller J, Eisenberg P, Gaffney P, Wickline S. In vitro characterization of a novel, tissue-targeted ultrasonic contrast system with acoustic microscopy. J Acoust Soc Am. 1998;104:3665-3672.
9. Lanza GM, Abendschein DR, Hall CH, Scott MJ, Scherrer DE, Houseman A, Miller JG, Wickline SA. In vivo molecular imaging of stretch-induced tissue factor in carotid arteries with ligand-targeted nanoparticles. J Am Soc Echo. 2000;13:608-614.
10. Lanza GM, Abendschein DR, Hall CH, Marsh JN, Scott MJ, Scherrer DE, Wickline SA. Molecular imaging of stretch-induced tissue factor expression in carotid arteries with intravascular ultrasound. Invest Radiol. 2000;35:227-234.
11. Flacke S, Fischer S, Hall C, Scott M, Marsh J, Gaffney P, Lorenz C, Wickline S, Lanza G. Targeted magnetic resonance contrast agent for detection of thrombus. J Cardiovasc Magn Reson. 1999;1:353.
12. Flacke S, Fischer S, Hall C, Scott M, Marsh J, Gaffney P, Lorenz C, Wickline S, Lanza G. Molecular imaging of thrombus with a new targeted magnetic resonance contrast agent. In: 8th Scientific Meeting of International Society for Magnetic Resonance in Medicine. Denver, Colorado; 2000.
13. Yu X, Song S-K, Chen J, Scott M, Fuhrhop R, Hall C, Gaffney P, Ackerman J, Wickline S, Lanza G. High resolution MRI characterization of a novel fibrin-targeted paramagnetic nanoparticle. Magn Reson Med. 2000; 44:867-872.
14. Anderson SA, Rader RK, Westlin WF, Null C, Jackson D, Lanza GM, Wickline SA, Kotyk JJ. Magnetic resonance contrast enhancement of neovasculature with avb3 targeted nanoparticles. Magn Reson Med. 2000;44:433-439.
15. Sipkins DA, Cheresh DA, Kazemi MR, Nevin LM, Bednarski MD, Li KC. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. Nature Med. 1998;4:623-626.