

Technology Insight: targeting of biological molecules for evaluation of high-risk atherosclerotic plaques with magnetic resonance imaging

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SUMMARY

Identification of high-risk atherosclerotic lesions prone to rupture and thrombosis may greatly decrease the morbidity and mortality associated with atherosclerosis. The development of magnetic resonance imaging contrast agents that specifically target components of the atherosclerotic plaque might enable non-invasive detection of high-risk lesions. This review discusses a variety of molecules present in atherosclerotic plaque that could serve as targets for specific contrast agents. Ultimately, such agents may allow the identification of high-risk atherosclerotic lesions in patients and enable treatment of these patients before lesion progression and complications.

KEYWORDS atherogenesis, biological markers, contrast agents, high-risk atherosclerotic plaque, magnetic resonance imaging

REVIEW CRITERIA

All articles were found by performing literature searches on the MEDLINE and PubMed databases. The search keywords, used in different combinations, were "atherosclerosis", "magnetic resonance", "molecules", "targets" and "imaging". All referenced articles were in English, not limited by the year of publication, and the full-text articles were retrieved from either electronic libraries or our institution's medical library.

INTRODUCTION

Since the first clinical manifestation of atherosclerosis is unheralded sudden death or myocardial infarction in over half of affected individuals,¹ the detection of high-risk plaques before the occurrence of cardiovascular events such as acute coronary syndromes or stroke may greatly decrease morbidity and mortality. A great deal of research has been conducted on HIGH-RISK ATHEROSCLEROTIC PLAQUES to determine which local and systemic factors are present in the lesions that progress to cardiovascular events. Besides elucidating the pathological processes involved in the development of atherosclerotic plaque and the characteristics leading to atherothrombosis, numerous biological markers of atherosclerosis have been identified. These biological markers may serve as targets for noninvasive detection of atherosclerotic disease and also serve as therapeutic targets. The purpose of this review is to propose biological markers of atherothrombotic lesions that may serve as targets for noninvasive detection using MRI.

ATHEROSCLEROTIC PLAQUE PROGRESSION

Endothelial dysfunction from local injury or from the retention of atherogenic lipoproteins is thought to initiate the formation of atherosclerosis. Regions of disrupted flow and decreased shear stress, often occurring in branch or bifurcation points of the arterial tree, have decreased production of nitric oxide and develop endothelial dysfunction and atherosclerosis.² The decrease in shear stress leads to increased expression of adhesion molecules and increased uptake of lipoproteins.³ Oxidized lipoproteins contain monocyte chemoattractant factors such as lysophosphatidylcholine and also are thought to trigger the release of macrophage-chemoattractant protein-1 by endothelial cells and smooth-muscle cells.⁴ These events result in the recruitment of monocytes that enter the subendothelium with the help of adhesion molecules such as vascular-cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin and P-selectin.

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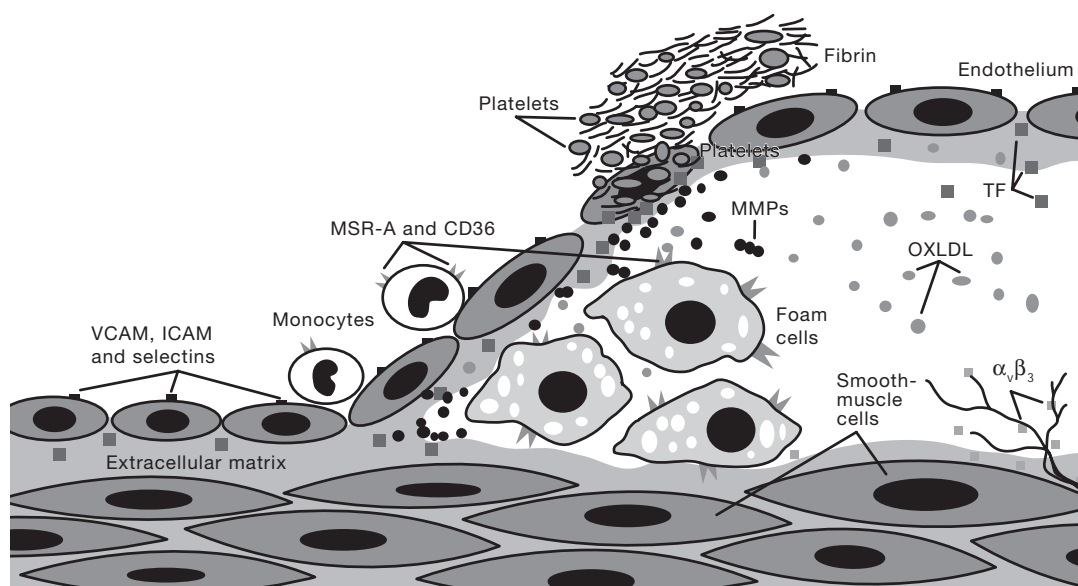


Figure 1 Potential biological targets in a high-risk atherosclerotic lesion that has ruptured at the shoulder. Monocytes adhere to the integrin proteins of dysfunctional endothelium then enter the subendothelial layer. They differentiate into macrophages and uptake oxidized LDL within the plaque via their scavenger receptors. This leads to their conversion into foam cells, which, along with smooth-muscle cells, begin to produce elevated levels of MMPs that degrade the extracellular matrix and lead to the destabilization of the plaque. Eventually the plaque ruptures, exposing tissue factor, oxidized LDL, and other prothrombotic molecules to the circulation. The coagulation cascade is activated, causing deposition of fibrin and platelets, and blockage of the artery. Neovascularization from the vasa vasorum is also seen, with vessels expressing $\alpha_v\beta_3$. ICAM, intercellular adhesion molecule; MMP, matrix metalloproteinases; MSR-A, anti-human macrophage scavenger receptor; OxLDL, oxidized low-density lipoprotein; VCAM, vascular-cell adhesion molecule.

Once inside the subendothelial space, monocytes differentiate into macrophages and internalize oxidized lipoproteins that bind to macrophage scavenger receptors.⁵ This process is thought to lead to the conversion of monocyte-derived macrophages into foam cells.⁶ Although early lesions (i.e. fatty streaks) consist mainly of foam cells, eventually the subendothelial accumulation of modified lipoproteins and foam cells leads to the formation of an **ATHEROMATOUS** core, which is hypocellular, avascular and devoid of supporting collagen. Foam cells become necrotic, releasing their highly thrombogenic, enzymatically modified lipids and **TISSUE FACTOR** into the extracellular space.

The growing atherosclerotic plaque is stabilized by the migration of medial smooth-muscle cells into the intima, where they deposit extracellular matrix, resulting in the formation and thickening of a fibrous cap. The shoulder region of plaques, or the junction between the plaque and the adjacent vessel wall, is believed to be the region most prone to rupture,⁷ it has a thin fibrous cap and is heavily infiltrated by foam cells⁸ that produce elevated levels of **MATRIX METALLOPROTEINASES** (MMPs), which are thought to erode the fibrous cap, leading to plaque rupture or erosion.⁹

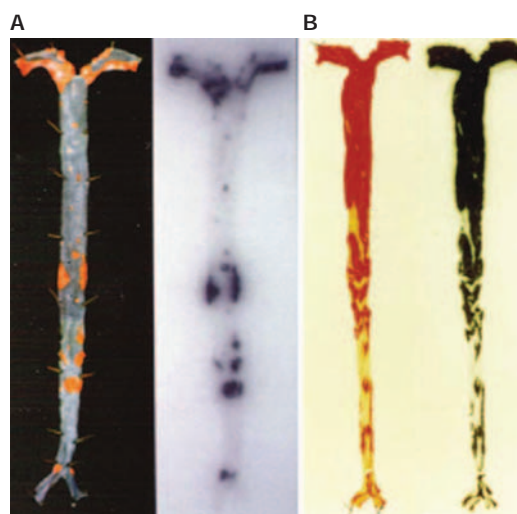


Figure 2 En-face preparations of Sudan-stained aortas. **(A)** Sample from an apolipoprotein E knockout mouse; red color signifies the presence of lipid within the atherosclerotic plaque. **(B)** Sample from a Watanabe heritable hyperlipidemic rabbit injected with 125-iodine-labeled MDA2; red color in the corresponding autoradiograph signifies the presence of oxidized low-density lipoprotein. Adapted with permission from Tsimikas S (2002) Noninvasive imaging of oxidized low-density lipoprotein in atherosclerotic plaques with tagged oxidation-specific antibodies. *Am J Cardio* **90** (suppl): 22L–27L.

GLOSSARY

HIGH-RISK ATHEROSCLEROTIC PLAQUE

Has a thin fibrous cap, large lipid core, numerous inflammatory cells, and a high thrombosis, rethrombosis and rapid stenosis potential

ATHEROMATOUS

Relating to lipid deposits in the artery intima that produce a yellow swelling on the endothelial surface characteristic of atherosclerosis

TISSUE FACTOR

A cell-surface glycoprotein coagulation factor that initiates coagulation via the extrinsic pathway of coagulation

MATRIX

METALLOPROTEINASES Endopeptidases that control the extracellular matrix composition by degradation of collagen and other extracellular proteins

GLOSSARY

PARAMAGNETIC

Material with a small but positive magnetic susceptibility that markedly increases signal intensity due to the shortening of T1

SUPERPARAMAGNETIC

A substance with a large positive magnetic susceptibility and one magnetic domain that becomes transiently magnetized in a magnetic field

NANOPARTICLES

Molecules <250 nm in diameter containing paramagnetic or superparamagnetic material that enable use of nanoparticles as contrast agents

POTENTIAL BIOLOGICAL TARGETS TO IDENTIFY ATHEROSCLEROSIS

Figure 1 provides an illustration of atherothrombotic plaque and some of the molecules that may serve as targets for noninvasive imaging. In selection of biological targets, it is important to remember that these molecules are often not unique to atherosclerosis. Therefore, we will focus on molecules that are present at increased levels in atherosclerosis but that are not found extensively throughout the vasculature. Additionally, molecular targets are often present in very small levels (10^{-9} to 10^{-13} M/g tissue)¹⁰ and pose a challenge to imaging with MRI due to inadequate signal intensity with standard contrast agents. Therefore, the development of contrast agents that carry a large payload of PARAMAGNETIC molecules and are linked to specific peptides or antibodies can enable increased signal intensity in regions containing the target of interest.¹¹ Table 1 provides a list of molecules covered in this review that may serve as candidates to non-invasively identify high-risk atherosclerotic plaques with MRI, and the pros and cons relating to their use.

Macrophages and foam cells

The macrophage scavenger receptors on the surface of mononuclear cells may serve as an excellent candidate for the identification of high-risk atherosclerotic lesions. Macrophage scavenger receptor A, an integral membrane glycoprotein, is important in the formation of foam cells⁵ and atherosclerosis.¹² While there are several other families of macrophage scavenger receptors, macrophage scavenger receptor A is widely expressed on macrophages in atheromas and developing lesions.¹³ Another candidate is glycoprotein CD36, a B family macrophage scavenger receptor, which is expressed on mononuclear cells, platelets, endothelial cells and adipose tissues. In an immunohistochemical study of the differential expression of macrophage scavenger receptor A types I and II and CD36 in human aorta, Nakata *et al.*¹⁴ found that macrophages surrounding the core region in atherosclerotic plaque stained strongly for macrophage scavenger receptor A but weakly for CD36. The core region, however, stained strongly for CD36 but mildly to moderately for macrophage scavenger receptor A.

Ultrasmall SUPERPARAMAGNETIC particles of iron oxide (USPIOs) are iron oxide NANOPARTICLES bound to a low-molecular-

weight compound, such as dextran or citrate, and have a mean diameter of 18–30 nm.¹⁵ In contrast to larger superparamagnetic iron oxide preparations (diameter of around 150 nm), USPIOs have a longer intravascular half-life and can better extravasate through tight capillary pores,¹⁵ allowing them to remain in the system for longer and be taken up by the mononuclear phagocytic system.^{16,17} These particles are valuable because of iron-associated T2-shortening and T2*-shortening effects, resulting in loss of signal from regions that had uptake of USPIOs. Although several studies have confirmed the ability of USPIOs to identify macrophages and atherosclerotic plaque in animal models,^{18–20} Kooi *et al.*²¹ demonstrated the ability of USPIOs to image human atherosclerosis *in vivo*. Interestingly, the histological analysis demonstrated uptake of USPIOs in 75% of ruptured or rupture-prone lesions but in only 7% of stable lesions. Therefore, USPIOs may be used to target inflamed atherosclerotic plaque that is rich in macrophages and which could be prone to rupture.

Modified lipoproteins

Since oxidized or modified lipoproteins serve as a stimulus for the formation of atherosclerotic plaque, monoclonal antibodies to oxidized low-density lipoprotein (oxLDL) may serve to non-invasively diagnose and follow the progression or regression of atherosclerosis.²² The importance of oxLDL is stressed by histopathological evidence of large amounts of oxLDL, cholesterol, cholesterol esters, phospholipids and their breakdown products in lesions responsible for acute coronary syndromes.²³ Tsimikas *et al.*^{24,25} revealed that radioactively labeled antibodies to oxLDL can identify atherosclerotic plaque and be used to assess plaque burden (Figure 2). Therefore, antibodies to oxLDL linked to MR contrast agents may be useful tools to evaluate lesions non-invasively and follow the regression of atherosclerosis in patients undergoing lipid-lowering therapy.

 $\alpha_v\beta_3$ Integrin

The $\alpha_v\beta_3$ integrin plays an important role in angiogenesis²⁶ and appears in elevated levels in atherosclerotic plaque due to the increased vascularization and increased growth of the vaso vasorum.²⁷ Lanza *et al.*^{28–30} have performed several studies evaluating the ability to image angiogenesis using a novel $\alpha_v\beta_3$ -targeted nanoparticle. In one such study in early-stage

Table 1 Potential molecular targets of imaging of high-risk atherosclerotic plaques.

Molecular target	Pros	Cons
Endothelial integrins (ICAM, VCAM, P-selectin, and E-selectin)	Expressed on dysfunctional endothelium and areas of atherosclerosis	Many people such as smokers and diabetics, frequently have a great deal of dysfunctional endothelium, and targeting these molecules would not be specific for lesions prone to rupture due to widespread endothelium expression
OxLDL	Lesions prone to rupture have been shown to contain high levels of OxLDL	OxLDL is present in early lesions that are not yet prone to rupture but to a lesser degree than in more mature lesions
Macrophage scavenger receptors	Expressed at elevated levels on foam cells and resident macrophages of atherosclerotic plaque; high-risk lesions also have high macrophage density	The majority of contrast agent that specifically targets macrophage scavenger receptors will probably be retained in the liver and reticuloendothelial system due to the large presence of macrophages
$\alpha_v\beta_3$ Integrin	Present in neovascularization and may be used to identify high-risk plaque and areas of restenosis	This integrin is found in areas of neovascularization and detection of atherosclerosis depends on the presence of neovascularization within the plaque
MMP-2 and MMP-9	MMP-2 and MMP-9 are expressed at elevated levels in high-risk atherosclerotic plaque	MMPs are expressed throughout the body and vasculature
Extracellular matrix proteins	Some extracellular matrix protein concentrations are elevated in lesions prone to rupture	The extracellular matrix proteins are found throughout the vasculature
Tissue factor	Elevated tissue factor in the blood and lesions can identify people at risk for rupture of atherothrombotic lesions	Tissue factor is found in the subendothelial space of all people

ICAM, intercellular adhesion molecule; MMP, matrix metalloproteinases; OxLDL, oxidized low-density lipoprotein; VCAM, vascular-cell adhesion molecule.

atherosclerosis, the use of $\alpha_v\beta_3$ -targeted nanoparticles enabled a 47% enhancement in MRI signal averaged throughout the abdominal aortic wall of New Zealand white rabbits after 2 h compared with nontargeted nanoparticles.²⁸ The presence of $\alpha_v\beta_3$ integrin in the aortic wall and the expansion of the aortic vasa vasorum was confirmed with immunohistochemistry and histology in the hyperlipidemic aortas, whereas sparse neovasculature was found in the same regions of the aortas of control rabbits.

Extracellular matrix and the fibrous cap

The fibrous cap of atheromas serves a vital purpose in separating the thrombogenic molecules of the necrotic lipid core from the circulation. Numerous studies have investigated differential

expression of extracellular matrix proteins, such as collagens, proteoglycans, hyaluronan, tenascin-C and many other molecules.^{31–33} Importantly, although the composition of the extracellular matrix in atherosclerotic plaque might differ from that of the extracellular matrix in normal vessel walls, the components are the same and, therefore, are likely to have poor specificity. One imaging agent, gadofluorine M, appears to have a strong affinity for the extracellular matrix of atherosclerotic plaque.³⁴ Barkhausen *et al.*³⁵ demonstrated that gadofluorine M enhances aortic-wall imaging in Watanabe heritable hyperlipidemic rabbits but not in control rabbits. Sirol *et al.*³⁶ have shown that gadofluorine M results in a 164% signal increase at 1 h after contrast administration and a 207% signal increase at 24 h after

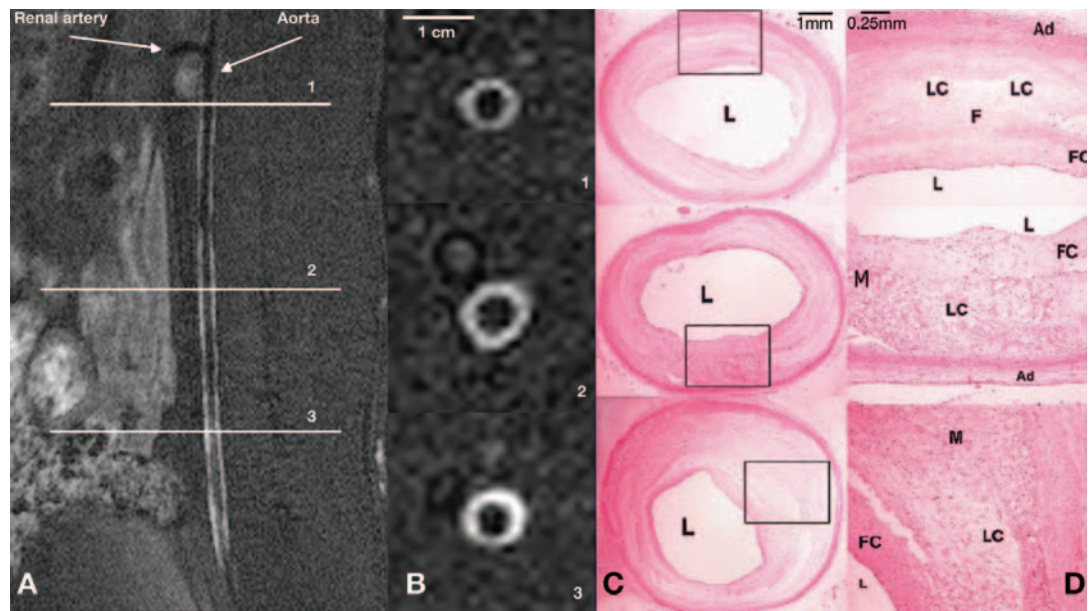


Figure 3 *In vivo* MR images and corresponding histopathological sections of atherosclerotic rabbit abdominal aorta. (A) Sagittal view of heterogeneous plaque enhancement along the aortic length with the use of IR-DIFF-TFL sequence 24 h after gadofluorine injection. Three slice locations (1, 2, 3) were chosen for transverse MR images (B) and histopathologic analysis (C). (B) Transverse view showing differences in signal intensity depending on the slice locations (1, 2, 3). (C) Magnifications are indicated by squares in areas of interest. (D) Corresponding histopathologic sections, stained with hematoxylin and eosin. At level 1, enhancement is heterogeneous because of accumulation of lipids within the fibrous area. At level 2, large lipid core corresponds to the highest enhancement within the plaque. Plaque at level 3 is mainly composed of lipids with perfect matching of the highest plaque enhancement. Ad, adventitia; F, loose fibrous; FC, fibrous cap; L, lumen; LC, lipid core; M, macrophages.

administration.³⁶ Gadofluorine M seems to have a high affinity for lipid-rich plaque (Figure 3).

Matrix metalloproteinases

Since the integrity of the fibrous cap is essential for separating the lumen from the thrombogenic core, identification of factors that lead to the disruption of the fibrous cap may aid in identifying plaque prone to rupture. The role of MMPs in atherosclerosis has been investigated.⁹ Concentrations of the gelatinases MMP-2 and MMP-9 appear to be raised in atherosclerotic plaque and unstable lesions.^{37,38} MMP-9 colocalizes with macrophages in the plaque, whereas MMP-2 has diffuse staining throughout the plaque.³⁹ Therefore, these two MMPs, and potentially others, may serve as candidates to identify high-risk lesions. Since MMPs are associated with all lesions, however, careful assessment of their targeting potential is needed.

Tissue factor

Tissue factor is a membrane-bound glycoprotein that binds to factor VII and activates the coagulation cascade when exposed to plasma proteins. In normal vessels, smooth-muscle cells in the tunica

media and fibroblasts in the adventitia synthesize tissue factor.³⁹ However, most cells in atherosclerotic plaque express tissue factor,⁴⁰ especially in the necrotic lipid core.⁴¹ Hember *et al.*⁴² showed inhibition of thrombus propagation by using antibodies specifically to tissue factor. Although this method was used to prevent the formation of clotting, the technology could be used to detect regions with elevated tissue factor, especially those exposed to blood during plaque rupture in acute coronary syndromes or stroke. Tissue-factor-specific MR contrast agents have been developed that demonstrate the ability to image regions of increased tissue factor expression.⁴³

Platelets, fibrin and other components of thrombus

The occlusion of a vessel's lumen with thrombus is often the ultimate cause of the myocardial ischemic injury. Therefore, targeting blood components that play an important role in thrombus formation may aid in the detection of thrombi and the accompanying pathologic diseases. Several MR contrast agents have been developed that specifically target thrombus components.⁴⁴⁻⁴⁸

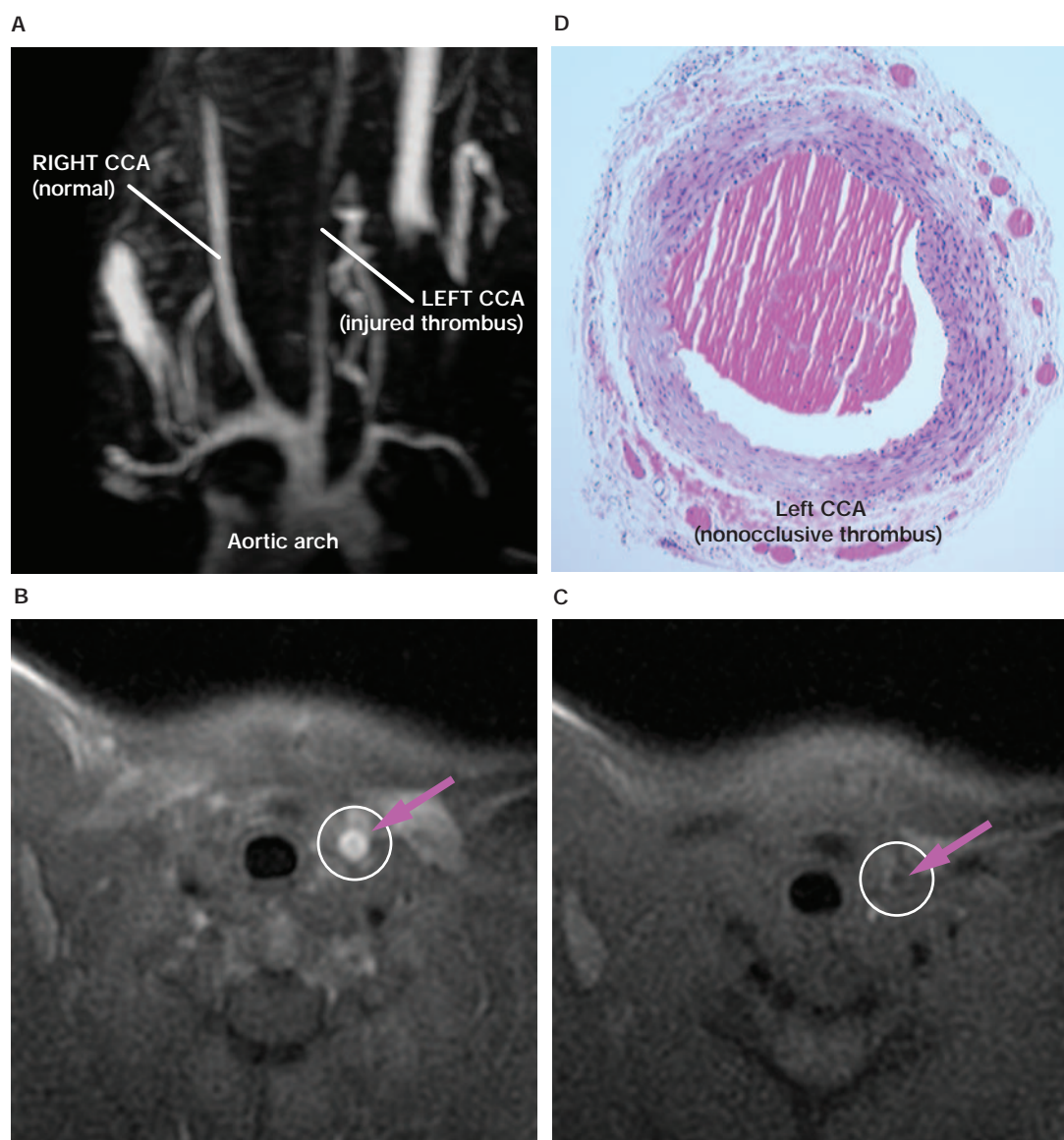


Figure 4 Blood flow in uninjured right and thrombosis-injured left common carotid artery of a guinea pig. (A) Contrast-enhanced MR angiogram using fibrin-specific contrast agent. (B) T1-weighted contrast enhancement of a thrombus in the injured left common carotid artery (arrow). (C) Precontrast imaging. (D) Corresponding histologic hematoxylin and eosin stained section of the left injured common carotid artery; the contralateral uninjured artery shows no enhancement (arrow).

Several *in-situ* studies have imaged human and canine thrombi with fibrin-specific GADOLINIUM (Gd)-loaded lipid-encapsulated perfluorocarbon nanoparticles.^{44–46} Activated platelets have been imaged using an USPIO-arginine-glycine-aspartic acid peptide construct that targets the glycoprotein α_{IIb}/β_3 receptor.⁴⁸ Similar to the Gd-loaded nanoparticles, contrast enhancement was limited to the thrombus surface. A factor XIIIa-sensitive MR contrast agent has been created that consists of dextran-coated caged iron oxide particles (30–50 nm diameter) conjugated to an α_2 -antiplasmin

peptide.⁴⁹ This contrast agent was studied *in vitro* and demonstrated the ability to cross-link factor XIIIa.⁴⁹ Finally, a small-molecule peptide derivative that binds to fibrin can penetrate thrombi and it appears that 4 Gd molecules per peptide at a low dose (2–5 $\mu\text{M}/\text{kg}$) was sufficient to create an MRI signal for visualization of thrombus.⁵⁰ Figure 4 illustrates the ability of a fibrin-specific contrast agent to noninvasively enhance thrombus with MRI. These studies reveal that MRI can improve imaging of thrombus by using contrast agents that specifically target components of the thrombus.

GLOSSARY

GADOLINIUM

Paramagnetic metallic element used as the active component of several MR contrast agents to decrease tissue T1 relaxation times

CONCLUSION

Advances in research and technologies are coming to fruition in clinical practice. The targeting of plaque components as well as the conjugation of ligands (antibodies, small peptides, antibody fragments etc.) that specifically target these components might enable assessment of the progress of therapy to reduce plaque volume and inflammation. With the merging of exciting research in the fields of molecular biology and MRI, major advances in the imaging of atherosclerosis may soon translate to the clinical setting.

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Competing interests

The authors declared they have no competing interests.