Targeted Iron Oxide Particles for In Vivo Magnetic Resonance Detection of Atherosclerotic Lesions With Antibodies Directed to Oxidation-Specific Epitopes

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Objectives

The aim of this study was to determine whether iron oxide particles targeted to oxidation-specific epitopes image atherosclerotic lesions.

Background

Oxidized low-density lipoprotein plays a major role in atherosclerotic plaque progression and destabilization. Prior studies indicate that gadolinium micelles labeled with oxidation-specific antibodies allow for in vivo detection of vulnerable plaques with magnetic resonance imaging (MRI). However, issues related to biotransformation/retention of gadolinium might limit clinical translation. Iron oxides are recognized as safe and effective contrast agents for MRI. Because the efficacy of passively targeted iron particles remains variable, it was hypothesized that iron particles targeted to oxidation-specific epitopes might increase the utility of this platform.

Methods

Lipid-coated ultra-small superparamagnetic iron particles (LUSPIOs) (<20 nm) and superparamagnetic iron particles (<40 nm) were conjugated with antibodies targeted to either malondialdehyde-lysine or oxidized phospholipid epitopes. All formulations were characterized, and their in vivo efficacy evaluated in apolipoprotein E deficient mice 24 h after bolus administration of a 3.9-mg Fe/kg dose with MRI. In vivo imaging data were correlated with the presence of oxidation-specific epitopes with immunohistochemistry.

Results

MRI of atherosclerotic lesions, as manifested by signal loss, was observed after administration of targeted LUSPIOs. Immunohistochemistry confirmed the presence of malondialdehyde-epitopes and iron particles. Limited signal attenuation was observed for untargeted LUSPIOs. Additionally, no significant arterial wall uptake was observed for targeted or untargeted lipid-coated superparamagnetic iron oxide particles, due to their limited ability to penetrate the vessel wall.

Conclusions

This study demonstrates that LUSPIOs targeted to oxidation-specific epitopes image atherosclerotic lesions and suggests a clinically translatable platform for the detection of atherosclerotic plaque. (J Am Coll Cardiol 2011; 57:337–47) © 2011 by the American College of Cardiology Foundation
studies have demonstrated that elevated levels of circulating oxidized phospholipids on apolipoprotein B-100 particles predict the presence and extent of angiographically defined coronary artery disease; progression of carotid and femoral artery atherosclerosis; and death, myocardial infarction, and stroke in unselected populations from the general community (5–8). Therefore development of sensitive molecular imaging probes that target oxidation-specific epitopes in the vessel wall might allow for in vivo detection of rupture-prone plaques.

Magnetic resonance imaging (MRI) has emerged as a promising diagnostic modality, due to its sub–millimeter spatial-resolution, for both the direct assessment of plaque burden and the evaluation of plaque composition (9,10). The magnetic resonance (MR) efficacy of gadolinium (Gd) pegylated (PEG) micelles targeted to oxidation-specific epitopes in imaging aortic atherosclerosis in apolipoprotein E deficient (apoE−/−) mice was recently reported (11). Those studies also indicated that targeted Gd micelles accumulate in macrophages after binding OxLDL extracellularly and therefore might also be a sensitive imaging technique to identify intraplaque macrophages in vivo.

Although the efficacy of this platform has been demonstrated, the long circulation times (>14 h) and high liver uptake (approximately 20% of the injected dose) of such Gd micelles might limit clinical translation, due to safety-related issues. Reported studies have indicated that intracellular uptake of Gd chelates might result in de-metallation and subsequent cell apoptosis (12,13). Studies in mice using Gd micelles have also shown significant transmetallation due to the prolonged circulation times exhibited by lipid-based nanoparticles relative to low molecular weight Gd chelates (14). Additionally, it has been hypothesized that transmetallation induces the nephrogenic systemic fibrosis in renally impaired patients after injection of clinically available low molecular weight Gd chelates (15).

The primary aim of the current study was to evaluate the efficacy of biocompatible iron oxide particles targeted to oxidation-specific epitopes in imaging atherosclerotic lesions. Dextran-coated ultrasmall iron oxide particles (USPIOs) have been used to passively target intraplaque macrophages (16–18). These USPIOs are desirable from a safety point of view, because cells associated with the reticuloendothelial system (RES) are able to safely eliminate iron (19). However, this passive targeting strategy might be suboptimal, because these materials require slow infusion and long time-intervals between administration and MRI (>24 h) (17,20). Therefore, we hypothesized that lipid-coated iron oxide particles targeted to oxidation-specific epitopes might increase the clinical utility of the iron oxide platform for MRI of vulnerable atherosclerotic plaque.

**Methods**

To assess the effect of core size on the magnetization (and resultant MR signal) and hydrated particle size on the ability to penetrate the arterial wall, 2 types of iron oxide particles were evaluated: oxidation-specific, epitope-targeted, lipid-coated, ultrasmall superparamagnetic iron oxide particles (LUSPIOs), and larger lipid-coated superparamagnetic iron oxide particles (USPIOs). Although the USPIOs are expected to exhibit better wall penetration, LUSPIOs are expected to generate greater MR signal loss. It is currently unclear whether it is more advantageous to have a greater number of less-effective particles within the wall or a fewer number of more-effective particles within the arterial wall.

A full description of the Methods is given in the Online Appendix. A brief overview is given in the following text.

**Synthesis of oxidation-specific, epitope-targeted iron oxide particles.** Two murine (malondialdehyde [MDA]2 and E06) and 1 fully human single-chain (IK17) Fv antibody fragment targeted to oxidation-specific epitopes were covalently attached (0.35 mg/mg Fe) to the surface of monocrystalline and monodisperse LUSPIO and USPIO particle surface via S-acetyltioglycolic acid N-hydroxysuccinimide ester modification (Fig. 1). The lipid-coated LUSPIOs were prepared by first synthesizing the iron core, as previously reported for Clariscan (NC100150 Injection, Amersham). The LUSPIOs were prepared according to previously reported techniques (see
Online Appendix for details). These iron oxide particles contain PEG side chains rather than dextran.

**Characterization of iron oxide particles.** All samples were concentrated to 1 mg Fe/ml; sterile filtered; and characterized with respect to iron content, hydrated particle size, particle structure, longitudinal (r1) and transverse (r2) relaxation properties, and stability with respect to storage.

**In vitro macrophage uptake of iron oxide particles.** Previous in vitro studies have shown that oxidation-specific, epitope-targeted Gd micelles exhibit increased macrophage uptake, particularly when pre-incubated with the MDA-low-density lipoprotein (LDL) (21). Because iron oxide particles are known to also passively target macrophages, in vitro cell studies were performed to determine the extent of passive macrophage uptake. Macrophages were pre-exposed to MDA-LDL for 24 h. Then the following materials were added to the macrophages: 1) 1 mmol/l Fe of untargeted LUSPIO (n = 2); 2) 1 mmol/l Fe of MDA2-labeled LUSPIO (n = 2); 3) 1 mmol/l Fe of untargeted LSPIO (n = 2); and 4) 1 mmol/l Fe of MDA2-labeled LSPIO (n = 2). Feridex (Berlex, Inc., Wayne, New Jersey) (1 mmol/l Fe) and saline (0.15 ml) were used as controls. The rationale for using Feridex as a positive control is related to published studies that show strong macrophage uptake of dextran-coated iron oxide particles, such as Feridex, fractionated Feridex, and Combidx (Advanced Magnetics). Iron content within macrophages was determined with inductively coupled plasma mass spectrometry (ICP-MS). Laser scanning confocal microscopy and Perl's Prussian Blue staining (for determination of intracellular iron content within macrophages) were also performed using standard methods.

**Animal models and pharmacokinetics and RES uptake.** Eight- to ten-month-old apoE/−/− mice on a C57BL/6 background on a high-cholesterol diet (0.2% total cholesterol, Harlan Teklad, Madison, Wisconsin) were used for all studies. Age-matched C57BL/6 wild-type (WT) mice on a C57BL/6 background on a high-cholesterol diet (0.2% total cholesterol, Harlan Teklad, Madison, Wisconsin) were used as controls. The blood half-lives and liver uptake were determined with standard relaxometry methods after animals were administered 3.9 mg Fe/kg LUSPIO or LSPIO via retro-orbital injection. The percentage injected dose was determined on the basis of the iron concentration/g wet organ weight.

**MRI.** All MRIs were performed at 9.4-T with a 89-mm bore system operating at a proton frequency of 400 MHz (Bruker Instruments, Billerica, Massachusetts) before and 24 h after the administration of a 3.9-μg Fe/kg dose. To obtain in vivo R2* maps, multiple gradient echo (GRE) sequences with the following pulse sequence parameters were applied: repetition time = 29.1 ms, echo time = 5.1 to 10 ms (n = 5), flip angle = 30°, number of signal averages = 6, in-plane resolution = 0.098 mm², 100% z-rephasing gradient. Twenty slices were acquired from the level of the renal arteries to the iliac bifurcation. The R2*-maps were generated for the matched pre- and post-images on a pixel-by-pixel basis using a custom Matlab program (R2007b, Mathworks, Natick, Massachusetts). The signal intensity associated with each pixel was normalized to the SD of adjacent noise (placed above the spine of the mouse) before linear fitting of the signal-to-noise ratio versus echo time. The R2* values were then obtained with regions of interest drawn on the arterial wall on slices (n > 5) exhibiting either R2* modulation after contrast or arterial wall thickening, indicative of plaque deposition. The relative percentage change in the R2* values were determined as: % change = (R2*post−R2*pre)/R2*post · 100.

Immediately after GRE acquisition, a gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP) sequence was applied using 50% of the z-rephasing gradient. The GRASP sequence is extremely useful when trying to determine whether MR signal loss is due to iron oxide deposition or other endogenous artifacts (motion, partial voluming, and peri-vascular effects) that might also promote signal loss. The GRASP sequence cannot be used alone, however, because this sequence does not provide adequate anatomic information. In similarity to GRE sequences, GRASP signal might be observed in lymphatic tissue that might also sequester the iron oxide particles. However, this sequence is extremely useful to differentiate between iron oxide deposition and artifacts that are often present when imaging the arterial wall. Previously published studies at lower magnetic fields (1.5- to 3.0-T) have shown that the GRASP sequence is most effective when the rephrasing gradient is reduced from 100% to 25% to 30% (22,23). However, at 9.4-T, preliminary studies indicate that a reduction in the z-rephasing gradient from 100% to 50% allows for adequate susceptibility matching and signal gain. As a result, for the GRASP sequence all parameters were held constant (relative to the GRE sequence) except for the z-rephasing gradient that was reduced to 50%. Because equivalent imaging geometry was used for both GRE and GRASP, the imaging results obtained from these sequences were directly matched and compared.

**Competitive inhibition studies.** The specificity of the MDA2-labeled LUSPIOs for MDA-lysine epitopes within the vessel wall of apoE/−/− mice was evaluated with in vivo competitive inhibition. Age-matched apoE/−/− mice (n = 3) were coadministered 3 mg of free MDA2 antibody and 3.9 mg Fe/kg MDA2-labeled LUSPIOs via tail vein injection. The MR enhancement (as R2*) of arterial wall was determined and compared with the enhancement observed after the administration of MDA2-labeled LUSPIOs only (n = 9).

**Immunohistochemistry.** Immunohistochemistry in selected atherosclerotic plaques for macrophages, MDA-lysine epitopes, and iron oxide deposition (Perl's Prussian Blue) was performed.

**Statistical analysis.** One-way analysis of variance with Bonferroni post-hoc multiple comparison tests was used to compare the R2* values obtained as a function of time.
before and after injection between the iron oxide groups. One-way analysis of variance was used to compare the mean values obtained 24 h after injection. For all statistical analysis, p < 0.05 was considered significant.

Results

Characterization of iron oxide particles. The physical and chemical properties of the iron oxide formulations are summarized in Table 1. The LSPIOs exhibited significantly greater hydrated particle diameters, iron core sizes (Fig. 2), and dipolar r2 values relative to the LUSPIO. The negative transmission electron microscopy images show the iron core (dark) surrounded by lipids (light), thereby indicating effective lipid coating of the iron cores. Additionally, the transmission electron microscopy images show large polydisperse LSPIO iron cores that might comprise either multiple small cores or one large core. Although the presence of single, large iron cores (crystal sizes >10 nm) might lead to ferromagnetism at physiological temperatures, we were unable to obtain magnetic hysteresis loops (to confirm remanence) due to instrument limitations.

The r2/r1 ratio observed for the LUSPIO was within the reported range of monocrystalline superparamagnetic iron oxide particles (24). Compared with the NC100150 Injection, however, the relatively high LUSPIO r2/r1 values were likely due to variations in the iron core synthesis that resulted in the formation of a polydisperse LUSPIO material. This was confirmed by the LUSPIO r1 nuclear magnetic relaxation dispersion profile (Fig. 3) that does not exhibit a low field dispersion characteristic of monocrystalline and monodisperse USPIOs (25). At clinical imaging field strengths (60 MHz) no significant difference was observed between the r1 values obtained for LUSPIOs and LSPIOs. The dipolar r2 values of the LSPIO were significantly greater than the values obtained for the LUSPIO at all field strengths tested. This result was expected, because the iron core and hydrated particles sizes were larger for this material.

The batch-to-batch variability of the untargeted LUSPIO and LSPIO formulations, as represented by the relative SD,
was <10% with respect to both the hydrated particle size and $r_2/r_1$ values. Both formulations exhibited limited (<5%) variation in size and/or $r_2/r_1$ values after 30-day storage at 4°C. These data strongly suggest that the iron formulations might be stored for up to 1 month without significant change in the hydrated particle size and relaxation properties.

Figure 4 summarizes the in vitro uptake of the untargeted and MDA2-labeled LUSPIOs and LSPIOs. The results suggest that PEG coating of the iron particles significantly limits J774A.1 macrophage uptake. The ICP-MS confirmed only nominal uptake of untargeted LUSPIOs and LSPIOs (below detection limits). However, conjugation of the MDA2 antibody to the surface of the particles resulted in significant increases in the LSPIO (0.4 pg Fe/cell) and LUSPIO (0.31 pg Fe/cell) uptake, as confirmed by histology. Incubation with Feridex resulted in strong unspecific uptake (1.9 pg Fe/cell), as anticipated. Although the uptake of the MDA2-labeled LUSPIO/LSPIOs might seem limited, relative to Feridex, it should be emphasized that only 1 in every 16 LUSPIO/LSPIO contains a targeting MDA2 antibody. This ratio was chosen to ensure that no more than 1 antibody was associated with an individual particle (to prevent aggregation) and due to limited availability of some of the antibodies used. As a result, only 6% of the LUSPIO/LSPIO dose is expected to be mediated into the cell. If every LUSPIO/LSPIO particle was MDA2-labeled (1:1 ratio), a theoretical uptake of 5 to 6 pg Fe/cell is expected (assuming linear uptake).

The targeted formulations exhibited prolonged circulation times relative to the untargeted material in apoE<sup>−/−</sup> mice. In WT mice, no significant variations in the blood half-lives were observed between the untargeted and oxidation-specific targeted formulations. This is consistent with data reported for oxidation-specific epitope targeted Gd micelles and suggests that interactions between the targeted particles and circulating OxLDL might prolong circulation times (11). Although the LUSPIOs exhibited significantly longer circulation times relative to the LSPIOs ($p < 0.01$), no significant variation in liver uptake was observed between the targeted and untargeted formulations 24 h after injection (Table 1). When compared with historical NC100105 Injection data, the untargeted LUSPIOs exhibited significantly reduced liver uptake (80% injected dose vs. 25% injected dose) (Table 1), thereby suggesting that modification of the LUSPIO coating inhibited uptake by the RES (26).

**In Vivo MRI.** There were no clinical signs indicative of toxicity during or after iron oxide administration. Representative in vivo MR images obtained before and 24 h after injection of the LUSPIO formulations are shown in Figure 4.
Figure 5. As illustrated in the untargeted images, the GRASP sequence was useful for differentiation between lymphatic drain (dark on GRE images and dark on GRASP images) and iron deposition within the arterial wall (dark on GRE images and bright on GRASP). Although nominal signal loss was observed after administration of the untargeted LUSPIOs, significant loss ($p < 0.003$) was observed 24 h after administration of the oxidation-specific epitope targeted LUSPIOs in apoE$^{-/-}$ mice, as confirmed by histology. Perl's staining also suggests that most iron deposition occurs in lesions rich in both MDA epitopes and macrophages/foam cells. Both MDA2 and IK17 targeted LUSPIOs exhibited similar MR efficacy ($p = 0.61$), as reflected in the percentage change in the $R_2^*$ values associated with the arterial wall (Fig. 5). The MR efficacy was, however, significantly higher for E06 LUSPIOs ($p = 0.019$) that target oxidized phospholipids, relative to MDA2 and IK17 that target MDA or MDA-like epitopes. The in vivo
competitive inhibition studies showed a 74.9% (p = 0.0002) reduction in R2* for mice administered free MDA2 at the time of MDA2-labeled LUSPIO micelle injection (Fig. 5).

Limited signal attenuation was observed after administration of untargeted and oxidation-specific, epitope-targeted LSPIOs (Fig. 6) in mice with significant atherosclerotic lesions. These findings strongly suggest that the LSPIOs are too large to allow for optimal diffusion into the arterial vessel wall. Additionally, the faster blood clearance of the LSPIO (Table 1) formulation might limit the amount of time in which these particles might have to diffuse into the wall.

Additionally, no significant (p = 0.22) arterial wall uptake was observed in WT mice after injection of the various iron oxide formulations tested. Perl's staining confirmed nominal iron oxide deposition associated with oxidation-specific epitopes in age-matched WT mice. These findings are consistent with previously reported data that show no significant (p > 0.5) uptake of oxidation-specific, targeted nanoparticles in the arterial wall of WT mice (11).

Correlation with the presence of oxidation-specific epitopes and macrophages with immunohistochemistry. Immunohistochemistry performed on a random subgroup of apoE−/− mice administered the MDA2-labeled LUSPIO showed strong correlation between iron oxide (Perl's staining) and MDA-lysine epitope (MDA3 staining) deposition (Fig. 7). Limited LUSPIO and MDA-lysine was observed in age-matched WT mice. Additionally, confocal microscopy confirmed the uptake of the oxidation-specific, targeted LUSPIOs (rhodamine-labeled) within atherosclerotic lesions and specifically within intraplaque macrophages/foam cells (anti-CD68–labeled) 24 h after injection (Fig. 7). Rhodamine-labeled targeted LUSPIOs were not observed in the extracellular matrix. These results are consistent with the findings reported for MDA2-Gd micelles (11). Additionally, strong colocalization between MDA epitopes and Perl's Prussian Blue staining was observed, thereby confirming the presence of the targeted LUSPIOs in macrophages/foam cells enriched in oxidation-specific epitopes.

Discussion

The results of the current study demonstrate that it is feasible to noninvasively image atherosclerotic lesions enriched in oxidation-specific epitopes with biocompatible, targeted, iron oxide MR molecular imaging probes. Reflecting the robustness of this approach, three different antibodies recognizing unique oxidation-specific epitopes demonstrated strong imaging characteristics. Furthermore, targeted LUSPIOs were more efficacious in imaging atherosclerotic plaques, relative to larger targeted LSPIOs that exhibited restricted accumulation within the arterial wall.
Using iron oxide platforms targeted to oxidation-specific epitopes might provide significant advantages when compared with current passive iron-oxide targeting approaches. For example, a wealth of animal and clinical data has demonstrated that oxidation-specific epitopes closely reflect the presence of atherosclerosis, plaque vulnerability, and clinical events \(2,8,27,28\). Whereas passively targeted dextran-coated iron oxide particles are taken up by a variety of macrophages located within the arterial wall and adventitia, PEG nanoparticles (both LUSPIO and targeted Gd
micelles) do not seem to be selectively taken up by macrophages unless they also contain an oxidation-specific antibody. In this way, the epitope-targeted PEG nanoparticles seem to selectively accumulate primarily within lipid-rich macrophage foam cells, perhaps due to binding extracellular OxLDL at these sites or to OxLDL bound on macrophage scavenger receptors, which then mediates enhanced uptake. As a result, molecular imaging probes that actively target oxidation-specific epitopes might eventually allow for in vivo evaluation of plaque vulnerability. Furthermore, preclinical studies have shown that, during atherosclerosis regression and plaque stabilization, oxidation-specific epitopes are removed before any significant geometric plaque regression (3,29), suggesting that monitoring of disease activity in response to therapeutic interventions might be feasible. In the current study, the specific targeting of the targeted LUSPIOs was demonstrated by the following: 1) untargeted control LUSPIOs exhibited limited in vitro macrophage uptake and nominal in vivo lesion enhancement; 2) oxidation-specific targeted LUSPIOs exhibited in vitro macrophage uptake and strong in vivo arterial wall enhancement in apoE−/− mice; 3) no in vivo oxidation-specific targeted LUSPIO uptake was observed in the arterial wall of age-matched WT mice; and 4) cotreatment of animals with free MDA2 and MDA2-labeled LUSPIOs resulted in reduced MR signal enhancement due to blocking of available antibody binding sites. Additionally, in contrast to passively targeted iron oxide particles, targeted particles may be administered as a bolus at clinically relevant iron dosages (<5 mg Fe/kg) and MRI may be performed within 24 h.

The results of the current study also suggest that in vivo efficacy is modulated by the ability of the particles to penetrate the arterial wall and not by the in vitro MR efficacy (as determined by the dipolar r2 values). It was evident that, even though the 35-nm LSPIOs exhibited 3-fold greater r2 values (3 times more effective at inducing MR signal loss relative to the LUSPIOs), the size of these particles restricted uptake into the arterial wall. For iron particles to be taken up by intraplaque macrophages, they must either penetrate the endothelium or diffuse into the plaque via the neovasculature. Because apoE−/− mice exhibit limited plaque neo-vascularization, intraplaque macrophage uptake is primarily dependent upon arterial wall diffusion. Unlike other flexible lipid nanoparticles such as liposomes and lipoproteins, iron oxide particles are rigid spheres that are unable to modify their shape to migrate through smaller pore sizes (30). Convective flow studies predict that the aortic endothelial tight junction associated with plaque is approximately 25 nm (31). Because the targeted LUSPIOs were smaller than these gap junctions (<20 nm), they exhibited greater arterial wall diffusion and subsequent intraplaque macrophage uptake relative to equivalent LSPIOs. However, it is anticipated that the optimal targeted iron oxide particle would maintain high r2 values (large iron core size) and have a total hydrated size <25 nm.

The increased blood half-life of the targeted particles was likely beneficial from an imaging efficacy point of view, because this might allow for greater time for accumulation of the particles within the arterial wall. That the smaller targeted LUSPIOs exhibited significantly longer circulation times relative to the LSPIOs is consistent with other studies that show large iron particles are generally cleared much faster than equivalent smaller particles (32,33). All iron formulations, however, exhibited lower plasma half-lives relative to oxidation-specific, epitope-targeted Gd micelles (9 h vs. 14 h) (11). The reduced circulation times are likely related to variations in the surface charge (−6 mV for the Gd micelles, and −2 mV for the iron particles) and/or total number of particles injected. Although variations in blood clearance were observed, no significant difference in liver uptake was observed for any of the formulations tested at 24 h after injection. The limited liver uptake (<40%) is suggestive of high uptake into tissue associated with the mononuclear phagocytic system (spleen, bone, lymph, and so forth) (33,34). However, these tissues were not analyzed for iron oxide content, due to the amount of tissue required to perform iron assays with relaxometry. Europium-enriched iron oxide formulations will be used in the future to obtain accurate biodistribution data with neutron activation techniques.

For the LUSPIOs, no significant difference in the MR efficacy, pharmacokinetics, or liver uptake was observed between MDA2 and IK17 particles. Previous studies have shown that both radiolabeled MDA2 and IK17 localize to lipid-rich, oxidation-rich atherosclerotic lesions in mice (3,29,35). Additionally, studies using oxidation-specific, epitope-targeted Gd micelles show limited variation in the MR signal obtained 24 to 72 h after injection of the MDA2 or IK17 formations. In the current study, significantly greater MR enhancement (as R2*) was observed after administration of the E06-labeled LUSPIOs. Although the reasons for this are not clear, it might be possible that the lesions stored exhibited more enriched oxidized phospholipid epitopes relative to MDA epitopes. The actual mechanism by which the oxidation-specific targeted particles accumulate within the arterial wall and are sequestered by intraplaque macrophages is still under investigation. Furthermore, although the R2* values obtained within the arterial wall correlate qualitatively with the presence of oxidation-specific epitopes and macrophages, additional studies are needed to quantitatively determine whether the strength of the signal correlates with evidence of plaque vulnerability. Studies are currently underway to correlate the in vivo R2* signal with histology in LDL receptor−/− mice during dietary intervention. Furthermore, both MDA2 and E06 are murine antibodies, and although they demonstrate the principal of targeting oxidation-specific epitopes very well, they cannot be used in clinical applications without humanizing them. However, IK17 is a fully
human, stable single chain fragment and thus a viable candidate for further pre-clinical evaluation for imaging human atherosclerotic lesions. The relative potential safety of iron oxide particles compared with Gd-containing micelles suggests a shorter pathway to clinical translation of this platform.

Conclusions

The present study demonstrates a sensitive, specific, and biocompatible method for the noninvasive imaging of oxidation-specific epitopes within the arterial wall. Due to the limited toxicity issues associated with iron oxide particles, if validated in humans, this approach might provide valuable tools for the noninvasive detection and monitoring of atherosclerosis.

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REFERENCES


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APPENDIX

For an additional methods section, please see the online version of this article.

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