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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

It is now well-established that plaque vulnerability is mainly linked to plaque composition and not necessarily to the degree of luminal narrowing (1). Diagnostic tools that can accurately characterize plaque composition, particularly components that mediate the transition of stable plaques to vulnerable/high-risk plaques, are needed to monitor disease and predict cardiovascular events (2). Oxidized low-density lipoprotein (OxLDL) has been identified as a key factor in the initiation, progression, and destabilization of vulnerable atherosclerotic plaques in animals and humans (3). OxLDL is a heterogeneous entity that contains a variety of oxidation-specific epitopes that mediate immunological and inflammatory pathways leading to atherogenesis (4). Recent

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Abbreviations and Acronyms

apoE$^{-/-}$ = apolipoprotein E deficient
Gd = gadolinium
GRASP = gradient echo acquisition for superparamagnetic particles with positive contrast
GRE = gradient echo
ICP-MS = inductively coupled plasma mass spectrometry
LDL = low-density lipoprotein
LSPIO = lipid-coated superparamagnetic iron oxide particle
LUSPIO = lipid-coated ultra-small superparamagnetic iron particle
MDA = malondialdehyde
MR = magnetic resonance
MRI = magnetic resonance imaging
OxLDL = oxidized low-density lipoprotein
PEG = pegylated
RES = reticuloendothelial system
USPIO = ultrasmall iron oxide particle

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 (LSPIOs). Although the LUSPIOs are expected to exhibit better wall penetration, LSPIOs 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 LSPIO particle surface via S-acetylthioglycolic 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 LSPIOs were prepared according to previously reported techniques (see

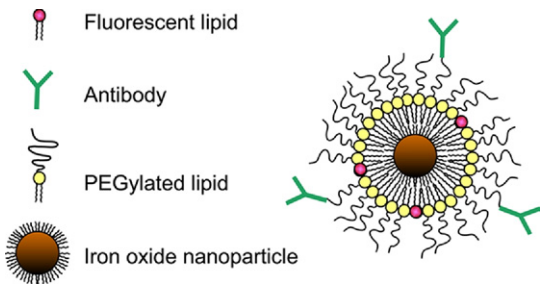

Figure 1 Diagram

Schematic diagram of the lipid-coated iron oxide particles formed.