from which future ovulations are drawn. There thus exists the potential for premature loss of ovarian function. A leading theory for these ovarian changes is that dechelation of gadolinium following Omniscan injection leads to heavy metal toxicity with subsequent microvascularity effects. Further studies evaluating subchronic toxicity are warranted, examining in greater depth the toxicity observed with Omniscan and evaluating other chelates with similar instability (Optimark).[2]

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Molecular Imaging of Human Thrombus with Computed Tomography

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INTRODUCTION

The formation of thrombus on ruptured atherosclerotic plaques is well recognized as the precursor of myocardial infarction and stroke[1]. Although several techniques exist for the detection of lumenal stenoses associated with advanced carotid and coronary artery disease, such as angiography and duplex ultrasound imaging, they are insensitive to the mural abnormalities and disruptions that precipitate thromboembolic events. Emerging molecular imaging approaches use fibrin-specific contrast agents to improve the detection and localization of occult microthrombi with noninvasive imaging techniques[2–6]. These ligand-based biochemical imaging techniques require high-avidity, target-specific probes with robust signal amplification compatible with a sensitive, high-resolution imaging modality.

High-speed multi-slice computed tomography (CT) is gaining widespread visibility for detection of advanced vascular disease (i.e., angiography and calcium scoring). This expanding interest in CT for cardiac imaging is fueled by its unique ability to obtain high quality images (spatial resolution and contrast resolution and signal-to-noise ratio) with very short image acquisition times resulting from the development of multi-slice CT scanners, rapid gantry rotation, partial image reconstruction algorithms and ECG gating[7]. The addition of effective molecular imaging agents specific for fibrin associated with plaque rupture presents an attractive extension for CT, which may further support its use as an initial, noninvasive imaging modality for evaluation of coronary and carotid disease in high-risk patients. The development of a CT fibrin-targeted contrast agent should allow anatomical as well as functional assessment of atherosclerotic plaques with a single, convenient, noninvasive imaging modality. Moreover, in cases where luminal thrombus is detected, the radio-opaque fibrin biomarker could further guide follow-up evaluation and intervention under fluoroscopy in the cardiac catheterization lab.

We have previously reported a fibrin-targeted nanoparticle agent for molecular imaging of thrombi via ultrasound[5] and MRI[2]. This agent is a ligand-directed, lipid-encapsulated liquid perfluorocarbon nanoparticle (250 nm nominal diameter) that has high target avidity and a prolonged systemic half-life. Similar perfluorocarbon emulsions have exhibited adequate x-ray attenuation characteristics when used as volume-delineating bowel contrast agents. However, the perfluorocarbon core may not provide sufficient attenuation as a thin, single layer of particles when targeted to a clot or cellular surface.

The objective of the present study was to develop and characterize thrombus-specific molecular imaging agents appropriate for use with high-speed, multi-slice CT scanners. The x-ray attenuation characteristics of nanoparticles incorporating perfluorocarbon and iodinated-oil cores were prepared and examined in suspension and when targeted to human plasma clots using avidin-biotin interactions. A more clinically relevant thrombus-specific nanoparticle formulation was also created and characterized that used anti-fibrin F(ab) fragments covalently coupled to the lipid surface to provide a simple, single step system.

MATERIALS AND METHODS

Nanoparticle Formulation

Fibrin-targeted nanoparticles were produced with methods developed in our laboratory[2]. Emulsions comprised 20% (v/v) of a hydrophobic core material, 2% (w/v) safflower oil, 2% (w/v) of a surfactant commixture, 1.7% (w/v) glycerin and water representing the balance. For targeting experiments employing avidin-biotin interactions, the surfactant commixture included 63 mole% lecithin (Avanti Polar Lipids), 15 mole% cholesterol (Sigma Chemical Co.) and 2 mole% biotinylated dipalmitoylphosphatidylethanolamine (Avanti Polar Lipids). Anti-fibrin monoclonal antibody[8] (1H10) was produced, purified, and biotinylated by conventional methods. Covalently-coupled anti-fibrin nanoparticles were produced by incorporating 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-4-(p-maleimidophenyl)butyramide (MPB-PE; Avanti Polar Lipids) into the outer lipid monolayer of the emulsion to accommodate subsequent ligand conjugation[9]. Anti-fibrin $F_{(ab)}'$ fragments, produced by papain digestion and purified on Protein A columns, were combined with the MPB-PE-derivatized emulsion (1 to 2 mg $F_{(ab)}'$/ml of emulsion) at pH 6.7 under nitrogen overnight. The conjugated nanoparticles were dialyzed, placed into vials, and stored at 4°C.

Three nanoparticle formulations were produced with different hydrophobic core materials: ethiodized oil (Savage Laboratories), perfluorooctylbromide (PFOB; Minnesota Manufacturing and Mining) and safflower oil. Particle sizes were determined at 37°C with a laser light-scattering submicron particle analyzer (Malvern Instruments). Iodine and PFOB nanoparticle emulsions were serially diluted with de-ionized water and were imaged in the CT scanner as described below and the attenuation was calculated.

Preparation of Human Fibrin Clots

Fresh-frozen human plasma anti-coagulated with sodium citrate was used to form cylindrical clots by combining plasma, 100 mM calcium chloride (3:1 v/v), and 5 U thrombin (Dade Behring) around a 5–0 silk suture in a 5-mm-diameter plastic mold. The clots were suspended in sterile saline inside plastic snap-cap tubes. For avidin-biotin targeting, clots were serially incubated with 150 μg biotinylated anti-fibrin antibodies (1H10) overnight at 4°C, followed by 50 μg avidin for 1 hr at 37°C, and then 250 μl of biotinylated nanoparticles for 1 hr at 37°C to complete the binding. Clots were rinsed three times with sterile saline after each incubation step to remove any unbound reactants. Scanning electron microscopy has previously shown that a very dense layer of nanoparticles specifically binds to the fibrin fibrils of the clot using these methods[2]. Clots were incubated with iodine or PFOB nanoparticles mixed with safflower oil nanoparticles in ratios of 100%:0%, 75%:25%, 50%:50% and 0%:100%, respectively.

In experiments using anti-fibrin nanoparticles covalently coupled to F(ab) fragments, clots were incubated for two hours with 250 μl of the fibrin-specific iodine nanoparticles. To demonstrate the specificity of fibrin targeting, a sub-set of clots (n=2) were pre-treated for two hours with the fibrin-targeted safflower oil nanoparticles followed by the fibrin-targeted iodinated particles. Clots were thoroughly rinsed in triplicate after each incubation to remove unbound particles.

CT Imaging

Nanoparticle suspensions and human plasma clots were imaged on an AcQSim-CT scanner (Philips Medical Systems). Axial images (2 mm slice thickness) were collected over a 120 mm field of view, with 80 KeV and 400 mA. Regions of interest were manually drawn on an image analysis workstation (EasyVision, Philips Medical