LDL Nanoparticles as Magnetic Resonance Imaging Contrast Agents

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Background. The recent development of targeted paramagnetic contrast agents promises to greatly expand the diagnostic specificity of MRI. By targeting specific cell surface epitopes, these agents will permit imaging of specific cell populations *in vivo*. The use of nanoplatforms (nanoscale structures typically smaller than 100 nm) to simultaneously deliver multiple Gd-chelates is particularly useful for MRI because of the low inherent sensitivity of this technique. Most of the existing targeted nanoplatforms consist of synthetic nanostructures. While many of these particles have shown good targeting with high payloads of Gd, their large size confined them to the vascular compartment. The utility of these synthetic nanoparticles may also be limited by biocompatibility, biodegradability and toxicity problems. Low-density lipoproteins (LDL) constitute a naturally occurring endogenous nanoplatform in mammals. These nanoparticles (22nm) specifically transport cholesterol to cells expressing the LDL receptor (LDLR). After binding to the LDLR, LDL is internalized by endocytosis and degraded within lysosomes while the receptor is recycled to the cell surface. With a recycle time of 10 min and a lifetime of ~24 hr, this receptor pathway efficiently delivers many LDL molecules and their payloads to LDLR expressing cells. In addition to a number of normal tissues such as the liver, adrenal glands and ovaries which utilize the LDLR system, several tumors also over-express the LDLR, presumably to provide cholesterol to sustain a high rate of membrane synthesis. LDL can be modified to incorporate contrast agents for MRI. Here we evaluate Gd-labeled LDL as a receptor targeted contrast agent for tumor detection.

Methods. Lipophilic DTPA chelates (DTPA-Bis(sterylamide) were synthesized by conjugating stearylamine and DTPA at a molar ratio of 2:1.[1] Then under alkaline conditions the lipophilic chelate was incorporated into LDL at different molar ratios (200-500:1).[1] Following chelation with Gd(III), a series of Gd-labeled LDL particles were prepared ranging between 150 and 496 Gd(III) per particle. The Gd-labeled LDL particles were characterized by several *in vitro* tests which included: particle size measurements by light scattering (zetasizer 300HS, 10 mW He-Ne laser, wavelength=633nm, detector angle=90º) and electron microscopy (Joel JEM 1010 electron microscope with Hamamatsu CCD camera operating at 80 kv); surface charge properties by agarose gel electrophoresis (0.5% agarose); and LDLR binding specificity using Gd-LDL co-labeled with an NIR dye for confocal microscopy (Leica TCS SPII laser scanning confocal microscope, filter setting ex. 633nm and em. 638-800nm).[2] For *in vivo* studies nude mice bearing LDLR over-expressing tumor xenographs ($HepG_2$) were used. Gd-labeled LDL was administered i.v. at a dose of 0.04 mmol/kg, and T1-weighted images (TR/TE=500/15 ms, matrix=256 x 128, FOV=4 x 2 cm, slice thk=1 mm, signal average=4) were obtained with a 4.7 T/50 cm bore Varian INOVA spectrometer/imager prior to and at various times following injection of the contrast agent. Contrast enhancement values were calculated by relating the pixel intensity of the target tissue (liver or tumor) to an unaffected tissue (skeletal muscle) prior to and following injection of Gd-LDL

Results and Discussion. *In vitro* experiments revealed that the physical dimensions of Gd-labeled LDL (up to payloads of 300:1) were similar to those of native LDL At higher payloads of Gd (496:1) the particle displayed an altered size distribution pattern. Electron microscopy yielded similar results. Agarose gel electrophoresis indicates that incorporating up to 245 Gd-DTPA moieties into LDL does not alter the valence or surface charge density of LDL. Similarly at higher payloads (450:1) the LDL particles exhibit a slight increase in electrophoretic mobility. Confocal microscopy indicated that Gd-Labeled LDL (160:1) still displayed selective binding to and uptake through the LDLR. Representative T1 weighted coronal images are displayed in Figure 1. In pre-contrast images, there was little intrinsic signal contrast between the liver parenchyma/ muscle and tumor/leg muscle. At 5 hr post-contrast administration, marked signal enhancement (55.40%) was apparent in the liver while markedly less contrast enhancement was exhibited by the tumor (<10%). By 24 hr signal enhancement within the liver began to decrease (25%). In contrast, the signal enhancement within the tumor showed a striking significant increase (25%). These levels of tissue contrast were sustained through 36 hr post-contrast. Collectively the results from these studies demonstrate the utility of receptor targeted MRI LDL-based contrast agents.

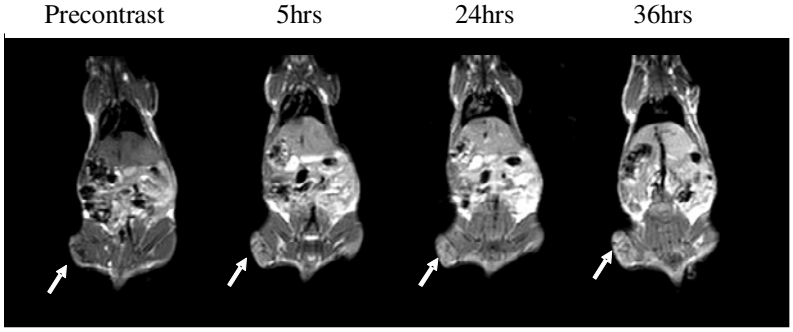

Figure 1. T1-weighted coronal spin-echo images of a nude mouse with a subcutaneous implanted Hep-G_2 tumor in its thigh. Images are of the mouse prior to administration of Gd-DTPA-SA-LDL (pre-contrast) and at various times following the intravenous administration of MR contrast agent (5, 24 and 36 hours). Arrow indicates tumor.

References: 1: Jansanada et *al., Bioconjug Chem*, 7, 72, 1996; 2: Li *et al., J Biomed Opt*, 10, 41203, 2005.
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