MR Imaging of Low Density Lipoprotein and Folate Receptors

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Introduction

Liver is the primary organ that expresses a high level of low density lipoprotein receptor (LDLr), however, under certain pathological conditions, e.g., familial hypercholesteremia (FH) functional LDLr is deficient on the surface of hepatocytes. A gene therapy strategy that restores the functional LDLr on hepatocytes holds great promise for correcting this deadly genetic disorder that leads to premature atherosclerosis (1).

The folate receptor (Fr) is a glycosylphosphatidylinositol-anchored, high affinity folate binding protein over-expressed in various types of human tumors (2) whereas expression of Fr is tightly regulated in normal cells (3). In order to utilize high-resolution MR imaging for quantification of these receptors, strategies were examined in which a Gd-based *paramagnetic* agent and an iron oxide based *superparamagnetic* agent was conjugated to LDL (taken up by LDLr) and folate (taken up by Fr), respectively.

Materials and Methods

Two prototypes of contrast agents were developed; one is a Gd-based compound (PTIR267) and the other a folate-conjugated iron oxide nanoparticle (IO-folate). LDL is pre-labeled by PTIR267 that contains a GdDTPA molecule attached to a long lipophilic anchor that intercalates into the phospholipid monolayer on the surface of the LDL particle. Both agents contain a fluorophore that enables their detection by confocal fluorescent microscopy once they are taken up by cells expressing LDLrs or Frs. For *in vivo* experiments, mice were sedated with 1% isoflurane and their rectal temperatures and ECGs were monitored. MR imaging was performed on a 4.7 T horizontal bore Varian INOVA spectrometer using a 42 mm bird cage coil. GdDTPA-labeled LDL (in 0.3 ml PBS containing 0.045 µmole of labeled LDL protein) was infused through the tail vein of a C57/Black mouse in 30 minutes. A LDLr knockout mouse model was established in the same strain. For estimation of T_1 changes in response to uptake of PTIR267-labeled LDL into liver, T_1 maps were generated using the TOMROP sequence (4) with the following parameters: TE=2.2 ms, TI =120 ms, number of TI intervals = 50, flip angle =20°, FOV= 6 cm, matrix=256^2 and 1mm slice thickness. IO-folate solution (0.8 Fe mg/ml) in 0.15 ml was infused intravenously (i.v.) into a nude mouse bearing on its hind limb a human nasopharyngeal carcinoma (KB) that expresses high levels of Fr. The mouse remained in the magnet during the experiment, and T_2-weighted images were acquired before and after IO-folate administration using the following parameters: TR/TE=1/0.01s, flip=20°, FOV=3.5 cm, matrix=128^2, 2 mm slice thickness.

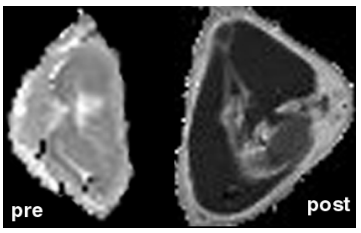

Fig.1 T1 maps of mouse liver pre and 6-hr post PTIR267 labeled LDL administration.

Results

The ratio of Gd molecules to LDL particles is ~100:1. The receptor-mediated specific uptake of PTIR267-labeled LDL into hepatocytes and IO-folate into Fr-expressed KB cells were confirmed by confocal fluorescent microscopy. Uptake of labeled LDL and IO-folate can be competitively inhibited by unlabeled LDL and folic acid, respectively. T_1 maps of normal liver before (a) and 6 hours after i.v. injection of PTIR267 labeled LDL (b) are shown in Fig.1. Previous experiments suggested that accumulation of labeled LDL takes over 5 hours. Therefore, injection was performed on the bench (not in the magnet) and pre and post-contrast T_1 values (instead of T_1-weighted images) were used for detection of uptake. The T_1 values (mean ± stdev) measured from ROIs placed on liver parenchyma are 1.05 ± 0.01s (n=3 slices) for pre-contrast liver and 0.28±0.02s for post-contrast liver (n=3 slices).

Figure 2 shows the T_2-weighted images acquired before and 2.5 hours after i.v. administration of IO-folate. The tumor is outlined by a dotted line and a leg muscle by a dashed line. A phantom placed adjacent to the mouse is also shown in the lower left corner of the image. ROIs placed within the tumor indicated an intensity decrease of 38% from the pre- to post-contrast image, whereas the muscle intensity decreased less than 13%, and no intensity change was detected in the phantom from the corresponding images.

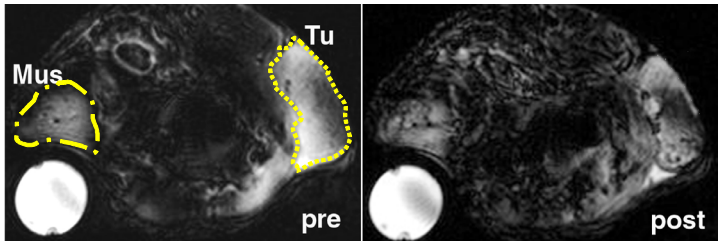

Fig. 2 T_2-weighted image of KB tumor pre and 2.5 hour post contrast

Conclusions

For labeled LDL, over 70% shortening of normal liver T_1 in response to the uptake was achieved and is consistent with the fact that liver is the primary organ expressing LDLr. The observed T_1- shortening is also consistent with what we previously observed in LDLr over-expressed tumor after injection of labeled LDL (5) but is appreciably greater primarily because the labeling ratio between PTIR and LDL has been increased. This technique would allow MR imaging detection of functional LDLrs that are restored by gene therapy targeted to the LDLr-deficient liver of a FH animal model and may be applicable to the clinic. For IO-folate, a 38% decrease in intensity in T_2-weighted images was detected in Fr over-expressed KB tumor. Some non-specific uptake in normal muscle was also observed. Receptor mediated uptake of labeled-LDL and IO-folate is an amplifying process because the ligands (labeled LDL or IO-folate) are trapped inside the cell while the receptors are recycled back to the cell surface. Intracellular accumulation may be slow because of the limited number of receptors per cell and their slow turn-over (e.g., it takes an LDL receptor about 10 minutes from endocytosis to recycling back to the cell surface) (6). The strategy of using paramagnetic or superparamagnetic contrast agents to image receptors has been validated; the latter agents exhibit higher sensitivity.

References

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