Magnetic Resonance Imaging of Gadolinium-Filled Neurophilic Lipid Nanoparticles

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Introduction

Effective diagnosis and staging of neural diseases represent a large area of unmet medical need. Magnetic resonance imaging (MRI) is routinely used to image the nervous system. However, even with the use of contrast agents, conventional MRI techniques are not sufficiently sensitive in detecting early signs of neural disorders. One major reason of MRI insufficient sensitivity is insufficient amounts of MRI contrast agents reaching the cells of the nervous system. Our long-term goal is to improve diagnosing and staging diseases of the nervous system. We have obtained preliminary data that our novel, biocompatible lipid nanoparticles, composed of Dioleoylphosphatidyl-choline (DOPC), Poloxamer 188 (P188) and cholesterol (chol), are taken up by neurons, glial cells, and brain microvascular endothelial cells *in vitro*, and when injected into mouse lateral tail veins, the nanoparticles accumulate in sciatic nerves and choroid plexus in the brain. We propose using "neurophilic" nanoparticles to deliver the novel experimental contrast agent gadofluorine M, a gadolinium-based contrast agent that provides the signal intensity in T1-weighted MR imaging, to the nervous system. Our hypothesis is that gadofluorine (Gf) can be efficiently delivered to the nervous system by the neurophilic nanoparticles.

Experimental

Gf-filled nanoparticles were prepared as a lyophilizate and stored at -20°C until use. The day of the experiment, the lyophilizate was reconstituted with 0.9% saline. Free Gf was separated from Gf-filled nanoparticles by 10-kDa filter spin columns (Millipore). Efficiency of Gf incorporation was determined by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS, UF Major Analytical Instrumentation Center). The Agilent 4.7T/200 MHz MRI spectrometer (UF AMRIS) was used to characterize the longitudinal and transverse relaxation MR imaging time constants, T1 and T2, of Gf-filled nanoparticles incubated in 0.9% saline.

Results and Discussion

Our NHMFL user proposal funding was approved in October 2012. Since then, we had prepared different Gf-filled nanoparticle formulations, with the eventual goal of performing MR imaging on mice injected with Gf-filled neurophilic nanoparticles. We had developed two different Gf-filled nanoparticle formulations. Formulations 1 and 2 were prepared by incorporating nanoparticles with 45 and 60 mol% Gf, respectively. ICP-MS showed that 67 and 72% of Gf was incorporated into formulations 1 and 2, respectively. With such high Gf incorporation, we felt that these two formulations would be suitable for MR imaging. The Agilent 4.7T/200 MHz MRI spectrometer (UF AMRIS) was used to determine the T1 and T2 of Gf-filled nanoparticles incubated in saline. **Table 1** showed that the r1 and r2 of the Gf-filled nanoparticles were very comparable with that of the standard. We are very encouraged by the MR data. We plan to proceed to the next stage of the plan, i.e. inject mice intravenously with Gf-filled nanoparticles and image them with the 4.7-Tesla MRI spectrometer.

Table 1. r1 and r2 of Gf-filled nanoparticles in saline.

Gf*	r1 ($mM^{-1}s^{-1}$)	r2 ($mM^{-1}s^{-1}$)	r1/r2
Standard	7.375	15.713	0.509
Gf-filled nanoparticles (formulation 1)	10.987	20.305	0.569
Gf-filled nanoparticles (formulation 2)	7.911	16.040	0.493

* T1 and T2 of the Gf standard and the Gf-filled nanoparticles were determined by the Agilent 4.7T/200 MHz MRI spectrometer (UF AMRIS) at room temperature.

Conclusions

Next year, we will begin collecting MRI data of mice injected with Gf-filled nanparticles. Mice will be imaged at baseline, 2 h, 4 h, 8 h, 24 h and 48 h post-nanoparticle administration. We plan to image mouse whole body first, and then focus imaging on mouse's sciatic nerve, spinal cord, brain, liver and/or kidney.