Magnetic Resonance Imaging of Gadolinium-Filled Neurophilic Lipid Nanoparticles

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Introduction

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 demonstrated that our novel, biocompatible "neurophilic" lipid nanoparticles, composed of Dioleoylphosphatidyl-choline, Poloxamer 188 and cholesterol, are taken up by neurons, glial cells, and brain microvascular endothelial cells *in vitro*, and when injected into mice lateral tail veins, accumulate in sciatic nerves, and choroid plexus in the brain [1]. 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. Our preliminary data indicate that the r1 and r2 of the Gf-filled nanoparticles are similar to those of standard free Gf. We have begun collecting MRI data of mice injected with Gf-filled nanparticles.

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 by 10-kDa filter spin columns (Millipore). Mouse was injected intravenously (iv) with Gf-filled nanoparticles and imaged with the Agilent 4.7T/200 MHz MRI spectrometer (UF AMRIS) 24 h pre- and post-injection. Efficiency of manganese incorporation in nanoparticles was determined by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS, UF Major Analytical Instrumentation Center).

Results and Discussion

We focused on brain imaging. Both the Agilent 4.7T gradient echo (GEMS) and spin echo (SEMS) data indicate higher signal intensity in injected brain than in control brain. For example, the GEMS ratios of brain to back muscle are 1.15 and 0.97 in injected and control, respectively. The SEMS ratios of brain to back muscle are 1.41 and 1.02 in injected and control, respectively. However, we could not visualize brain structures clearly. In order to visualize brain structure more clearly, we would need to increase the Gf signals, for example by increasing injection doses, or optimizing postinjection imaging time points.

It is possible that Gf with a molecular weight of 1300 g/mol could not move efficiently from the choroid plexus into brain parenchyma for us to visualize the brain structure. We may have to use a different T1-weighted contrast agent, such as manganese (Mn), which has a much smaller molecular weight (55 g/mol). We have recruited Dr. Marcelo Febo, who is an expert in Mn-enhanced MRI, on our team. Using a similar protocol, we have developed Mn-filled nanoparticles. ICP-MS showed that 60% of Mn was incorporated into the nanoparticles. The Agilent 4.7T/200 MHz MRI spectrometer was used to determine the T1 of Mn-filled nanoparticles incubated in saline. At 1 mM concentration, the T1 of Mn-filled nanoparticles and free Mn are 1270 ms and 577 ms, respectively. The T1 of the Mn-filled nanoparticles is longer than that of free Mn probably because the Mn is inside the nanoparticles, and does not have access to water as easily as free Mn.

Conclusions

We plan to compare brain imaging of mice injected with Gf-filled nanoparticles versus that of Mn-filled nanoparticles. We will optimize the dosing and postinjection imaging time of these nanoparticles.

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References

[1] Lee, S., *et al.*, PLoS One, **8**, e78724 (2013).