Gadolinium Distribution Around Blood Brain Barrier Pre-Exosome-Mediated MRI Contrast Agent Delivery

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Introduction

Magnetic resonance imaging (MRI) contrast agents (MCA) that are used for detecting tissue pathologies are largely incapable of crossing blood brain barrier (BBB). In comparison, the lipophilic and non-immunogenic nature of exosomes allows for drug delivery past the highly restrictive BBB. Exosomes have been shown to deliver pharmaceutical agents to specific tissue targets such as cancer tumor, heart muscles etc. Hence, we aimed to generate MCA loaded BBB permeable exosomes. The properties of Gadolinium allow it to effect the relaxation times of certain materials in the body and allow contrast that enables the visualization of certain pathologies. Before injecting the exosomes we show the distribution of gadolinium around the blood brain barrier and its limitations in studying brain pathology utilizing an experimental autoimmune encephalitis (EAE)/ mouse model of multiple sclerosis in C57BL/6 mice.

Methods

C57BL/6 mice were injected once with 300 μg of MOG (35-55) (Mimotopes, Australia) emulsified with complete Freund’s Adjuvant in the flanks and inter peritoneal (IP) injection of 500ng pertussis toxin per animal. Two days later the animals were IP injected with 500ng of pertussis toxin. Four Balb-c57 mice immunized with MOG peptide fragment (35-55 amino acid) and 2saline injected animals were scanned for LMCE at pre-induction and at 0.5, 1, 2 and 4 weeks post induction (PI).

For the acquisition of the gadolinium around the BBB 2D T1 weighted RARE-FLAIR sequence was applied (Acquisition Time = 31m 30s, Repetition time = 3500 ms, Echo time = 5.44, Averages = 3, rare factor = 1, Field of view = 18 x 19 mm2, Slice thickness = 0.7 mm and 0.1mm gap, Resolution = 0.1x0.1 mm2, bandwidth = 89 kHz, Inversion time = 1162 ms).

A tail vein injection of contrast agent (Gadovist 0.6 micromol/gm body weight, Bayer) was applied to the animals. A sequence was used for data acquisition 20 minutes post Gadovist injection to generate images for detecting meningeal contrast.

We measured mean intensity of the gadolinium signal within the ROIs placed on the forebrain dorsal meninges.

Results

Figure 1: Clinical monitoring of the clinical scores and the body weight of the animals.

![Figure 1](image1.png)

EAE was induced in the animals (n=4) with MOG 35-55. The control animals were injected with saline (n=2). A. The animals were daily monitored for assessment of their disability on the ISSG scale. We observed a sudden increase in the disability starting day 10 for EAE induced animals, following the the disability score stabilized to its peak value. B. The body weight showed a non-significant declining trend in the weights starting at day 5 but recovered to normal control levels.

Figure 2: The peak of gadolinium was associated with weight loss starting at 1 week PI. Importantly, peak gadolinium preceded the clinical disability score increase and clinical symptoms starting at 2 weeks PI (Figure 3).

Figure 3: To understand the relationship between the different stages of the disease we calculated correlation between the three different stages of the disease A. Early stage, B. Acute stage, and C. Chronic stage in the chronic phase of the disease. The Pearson correlation H2 value is reported in the graph for the EAE animals.

Figure 4: EAE mice exhibit inflammatory cells at the meninges;

EAE mice exhibit an increased density of late expressing inflammatory cells at the meninges. Mice were induced for EAE and scanned at one week after the EAE induction. Following the scan, the animals were sacrificed and stained for monospecific and monoclonal markers. B)) We noted that there was a significantly higher density of microglial cells at the meninges of EAE animals (A) when compared with saline animals (B, C and D) and stage matched images from the corresponding EAE and the saline animal respectively.

Summary

• The EAE induced animals exhibited a steep increase in the clinical disability score, starting at day 10 post induction (PI) and stabilized to a peak value at day 20 PI (Figure 1).

• All 4 EAE-MOG animals showed presence of gadolinium, but none of the control saline mice (Figure 2A & B) showed the same. The peak signal intensity of gadolinium was evidenced at one week PI in the meninges (Figure 2D).

• The gadolinium intensity decreased through 2-4-week PI, but it never returned to the baseline or saline injected normality levels (Figure 2D).

• Histological analysis of the brain tissue showed a higher density of immune response cells in the meninges of the EAE-MOG animals.

Conclusion

Serial MRI demonstrated that distribution of gadolinium at peak intensity in the meninges corresponds with the acute inflammatory phase of EAE-MOG disease progression. The peak meningeal intensity observation was followed by development of clinical disease symptoms and associated with higher inflammatory cell density. After 10 days PI the gadolinium intensity decreases whereas clinical scores of the animals deteriorate. This demonstrates that gadolinium limits our study of pathology due to its inability to effectively cross the BBB.

Future Developments

We plan to isolate exosomes from HEK293T cells which over-express rabies virus signaling peptide conjugated Lamp2b exosomal protein. Modified Lamp2b can now target the exosome to brain neuronal receptors. We will next use sonication to load the exosomes with a MCA (Gadovist).

Following this, we aim to use experimental autoimmune encephalomyelitis mouse model test our exosomes. Our objective is to demonstrate that exosomes are capable of delivering Gadovist into the brain and successfully map pathological developments using MRI scans.

References