Detection of Hyperpolarized [5-13C]-Glutamine in Brain

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Introduction

Several *in vitro* studies were done for understanding the distribution of transport proteins mediating amino acid homeostasis in the brain[1, 2]. At physiological state the entry of glutamine and glutamate to the central nervous system is greatly restricted and the BBB is arranged in a manner to prevent the accumulation of nitrogen rich molecules in the brain to provide an optimal chemical environment for cerebral function. A change in neurotransmitter levels (glutamate, glutamine and GABA) was demonstrated recently in the striatum at Parkinson's disease models using ^{1}H MRS[3]. Glutamine is able to cross the BBB by facilitated diffusion, however compared to other neutral amino acids this process is slower[1]. Actually two different transport systems were defined in the BBB for glutamine: facilitative carriers in the luminal membrane (blood facing) and sodium-dependent transporters A and N in the abluminal membranes (brain facing) [1, 2]. The aim of this study is to use hyperpolarized [5-^{13}C]-glutamine for the detection of glutamine transport through the intact BBB *in vivo,* and to evaluate its distribution in the brain region.

Methods

[5-^{13}C]-glutamine (3M, Cambridge Isotope Labs, USA) was whirl mixed with cesium hydroxide monohydrate (0.5M, Sigma Aldrich, USA), and then dissolved in DMSO (Sigma Aldrich, USA) as glassing agent containing OX063 radical (35mM, GE Healthcare, USA) and ProHance® (4.0 mM, Bracco Imaging, Italy) until complete dissolution[4]. After polarizing this mixture in a 3.35 T Hypersense DNP polarizer (Oxford Instruments, UK) for 90 min, it was dissolved in 5 ml of phosphate buffer (100mM in D_2O, pH = 7.2 after dissolution). All *in vivo* experiments were performed on healthy Lewis rats (n=2) using 3.0 T GE HTX system equipped with a dual-tuned ^{1}H-^{13}C volume coil. Hyperpolarized [5-^{13}C]-glutamine was injected via tail vein (injected dose 5ml/kg; glutamine concentration 60 mM). The brain was localized using ^{1}H-GRE images. The dynamic acquisition of ^{13}C spectra was started with the beginning of injection. The spectra were recorded from one axial slice with thickness = 20mm; TR= 1s; flip angle = 15°; excitation frequency centered on glutamine. The acquisition of ^{13}C images started 5 seconds after the injection of hyperpolarized glutamine. For ^{13}C MRI a spiral read out was used with FOV 8cm; real pixel size 5 x 5 mm^2, slice thickness = 20mm, TR = 1 s and a flip angle of 30°.

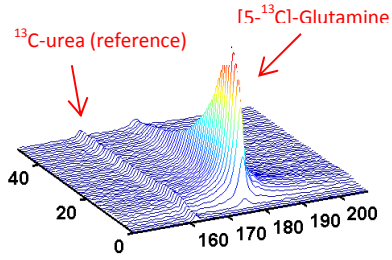

Figure 1: In vivo brain ^{13}C spectrum following the injection of hyperpolarized glutamine.

Results and discussion

The T1 of [5-^{13}C]-glutamine measured at 3T *in vitro* was 18s. The time evolution of one exemplary spectrum recorded in the brain slice after the injection of hyperpolarized [5-^{13}C]-glutamine is shown in Fig. 1. The main peak was assigned by its chemical shift (178 ppm) to [5-^{13}C]-glutamine. Increased [5-^{13}C]-glutamine signals in the brain region was detected by simple display of integrated [5-^{13}C]-glutamine images with corresponding GRE anatomical images in all animals (Fig.2). The distribution of hyperpolarized [5-^{13}C]-glutamine was imaged but the spatial resolution does not allow to differentiate between brain and blood. However even 30 s after the injection a sufficient glutamine signal was detected in different brain areas.

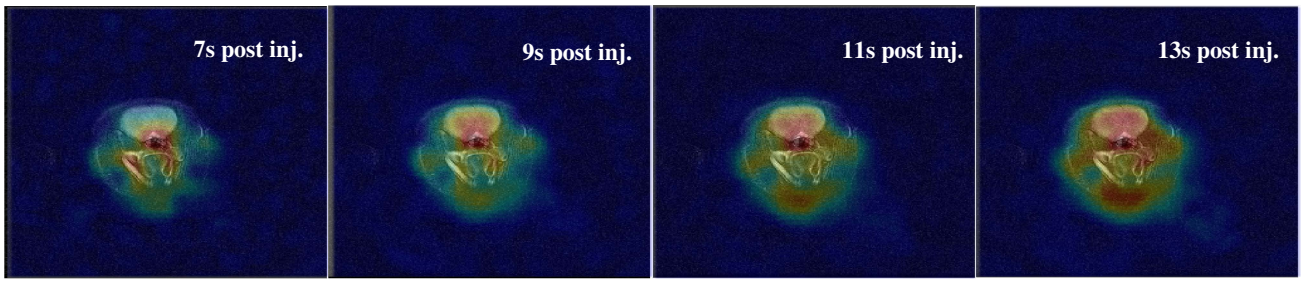

Figure 2: In vivo brain ^{13}C images over ^{1}H GRE at different time steps following the injection of hyperpolarized glutamine.

Conclusion

Glutamine can be considered as a promising candidate for future studies of neurodegenarative diseases connected with a change in neurotrasmitter levels. In the future ^{13}C-MRI might provide novel and more selective tools to study the transport systems and evaluate their regulation *in vivo*.

References

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