Probing cancer metabolism with hyperpolarized 5-13C-glutamine

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Target Audience
Oncologist, preclinical and DNP communities.

Purpose
Glutamine metabolism is a key target for alteration in cancer development. In particular, strong correlations have been reported between oncogene expression and activity of the glutaminase enzyme. This mitochondrial enzyme, responsible for the de-amidation of glutamine to form glutamate, is over-expressed in many tumour tissues1. In principle, hyperpolarized 13C-MR spectroscopy can provide insight to glutamine metabolism and should hence be a valuable tool to study changes in glutaminase activity as tumours progress. However, no such successful in vivo studies have been reported so far, even though several sound biological models have been tested2,3. The present work reports on a new and improved preparation of hyperpolarized [5-13C]glutamine, which provides a highly sensitive 13C-MR marker. The described CsOH/DMSO [5-13C]glutamine preparation yielded physiological tolerable solutions of the substrate which were hyperpolarized to a degree that allowed in vivo MR to be successfully performed on livers of healthy and Morris-tumour bearing rats. Moreover, an in vitro response to drug treatment in hepatoma cancer cells with the same formulation was also reported.

Methods
Two different [5-13C]glutamine formulations, a NaOH-[5-13C]glutamine and CsOH/DMSO-[5-13C]glutamine were prepared. Both formulations were characterized in terms of stability, impurities profile and hyperpolarization characteristics at the solid and liquid state. For the in vivo experiments, rat hepatoma cells McA-RH7777 were orthotopically injected under the hepatic capsula of the liver left lobe of Buffalo rats. CsOH/DMSO- [5-13C]glutamine was injected intravenously in the animals at the dose of 0.4 mmol/kg and a surface coil. A representative 13C-MR marker. The described CsOH/DMSO [5-13C]glutamine preparation yielded physiological tolerable solutions of the substrate which were hyperpolarized to a degree that allowed in vivo MR to be successfully performed on livers of healthy and Morris-tumour bearing rats. Moreover, an in vitro response to drug treatment in hepatoma cancer cells with the same formulation was also reported.

Results
A comparison between the NaOH and the CsOH/DMSO preparations of [5-13C]glutamine showed that by-products formation was much smaller in the latter one. Moreover, solid-state polarization levels of 34 ± 2 %, equalling a signal enhancement on the order of 103 to the equilibrium 13C spin polarization, were regularly obtained within 1.5 hours, allowing in vivo and in cell MR experiments. Representative coronal images of a tumour-bearing and a healthy rat at 3T are reported in Figure 1A and 1B, respectively. The sagittal view in Figure 1C better elucidates the selection strategy of the region of tumour bearing rat; B) healthy rat; C) sketch of the strategy for selection of the volume generating MR signal.

Discussion
With a new robust [5-13C]glutamine DNP preparation in CsOH/DMSO the liquid state polarization was improved 3-4 times relative to that used in reported studies and a strong reduction of by-products formation was achieved. The formulation allowed the first in vivo metabolic build-up from this hyperpolarized substrate in a tumour-bearing rat model. In vitro results suggest that it is also possible to monitor response to chemotherapy with the hyperpolarized metabolic marker [5-13C]glutamine.

Conclusion
Approaching in vivo metabolism with hyperpolarized [5-13C]glutamine has been made possible by a new robust DNP preparation of this marker. This new tool can be useful for many applications involving glutamine metabolism.

References