Selective hyperpolarized 13C metabolic signal suppression from a liver-specific gadolinium contrast agent measured using an NMR-compatible perfusion bioreactor

Ting Sun, Renuka Sriram, Kathy Giacomini, James Chien, Sook Wah Yee, Shubhangi Agarwal, Joao Piraquive Agudelo, Cornelius Von Morze, John Kurhanewicz, Huadan Xue, Michael A. Ohliger

Highlights of the abstract

The transgenic cell culture HEK293-1B1 and an NMR-compatible perfusion bioreactor to investigate the effects of intracellular gadoxetic acid on the observed signals arising from intracellular and extracellular ^{13}C metabolites.

Introduction:

Hyperpolarized (HP) ^{13}C MR is a powerful technology for monitoring real-time metabolism in tumors. Elevated production of [1-^{13}C]lactate from [1-^{13}C]pyruvate is near-universally observed in many tumors, including those in the liver[(1)]. Recently, the hepatocyte-specific gadolinium-based contrast agent gadoxetate has been proposed as a method to selectively suppress hyperpolarized signal arising from normal liver cells, while isolating the signals arising from tumor cells[(2)]. Although this strategy has been successfully implemented in vivo, little is known about the detailed interactions of hyperpolarized metabolites with intracellular and extracellular gadolinium.

Methods:

Embryonic kidney HEK293 cells were stably transfected with human organic anion-transporting polypepetide 1B1(OATP1B1), which is the transporter that is chiefly responsible for importing gadoxetate into cells[(3,4)]. These cells were prepared for bioreactor NMR experiments by electrostatic encapsulation into alginate microspheres[(5)]. The cell-laden microspheres were maintained at physiological conditions by circulating DMEM media at 37℃ with continuing oxygen in the 5mm NMR tubes used in bioreactor. Experiments were conducted on a 500 MHz Varian Inova with a 5mm probe at 37℃. [1-^{13}C]pyruvic acid was hyperpolarized using a HyperSense DNP polarizer operating at 3T and subsequently dissolved and neutralized for a final concentration of 50mM. 900 uL of this solution was infused into the bioreactor system and NMR experiments were performed by application of a 30° degree pulse every 3 s and for a total acquisition time of 300 s, using a sweep width of 100ppm. In order to determine the effects of intracellular gadoxetate on the HP metabolite signal, HP ^{13}C experiments were performed prior to and after infusion of 0.5 mM gadoxetate (figure 1a). Control experiments were performed using the same protocol with gadopentetate dimeglumine, which is a strictly extracellular agent.

Results:

For all experiments, extracellular lactate was higher than intracellular lactate, suggesting a rapid lactate export in these cell lines. Following infusion with gadoxetate, total production of intracellular and extracellular lactate was reduced by 41% and 43.2%, respectively. Alanine and bicarbonate signals were completely suppressed. This reflects the expected selective

suppression of intracellular metabolite polarization due to gadoxetate residing in cells. Because all extracellular lactate was generated within the cells, decreased intracellular lactate signal lead to a similar decrease in the extracellular lactate signal. By contrast, when the strictly extracellular gadolinium agent (gadopentetate) was used, total production of intracellular and extracellular lactate was only reduced by 5.6% and 6.4%, respectively. Alanine and bicarbonate signals were preserved (Figure 1b-c).

Conclusion:

Using a perfusion bioreactor system and isolated cells, we were able to observe the selective effect of the intracellular gadolinium agent, gadoxetate, on the hyperpolarized signal generated within cells. In the future, this system will enable better understanding of how targeted relaxation agents affect observed HP ^{13}C signals, expanding the way these agents can be used to isolate specific cell types in vivo.

References:

1. Kurhanewicz J, et al. Neoplasia, 2019;21(1):1-16.
2. Ohliger, M. A. et al. Magn Reson Med 77, 2356–2363 (2017).
3. Shu Y, et al. The Journal of clinical investigation 2007;117(5):1422-31.
4. Leonhardt M, et al. Drug metabolism and disposition, 2010;38(7):1024-8.
5. Keshari KR, et al. Cancer research 2013;73(2):529-38.