In vivo studies on a hyperpolarized choline contrast agent: design and implementation of a new biomarker

H. Allouche-Arnon[1], A. K. Grant[2], E. Vinogradov[2], X. Wang[2], R. E. Lenkinski[2], A. Gamliel[1], R. Nalbandian[1], L. Frydman[3], J. M. Gomori[1], C. M. Barzilay[4], and R. Katz-Brull[1,5]

[1]Department of Radiology, Hadassah Hebrew University Medical Center, Jerusalem, Israel, [2]Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [3]Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel, [4]Medicinal Chemistry-School of Pharmacy, Hebrew University of Jerusalem, Jerusalem, Israel, [5]BrainWatch Ltd., Tel-Aviv, Israel

Introduction

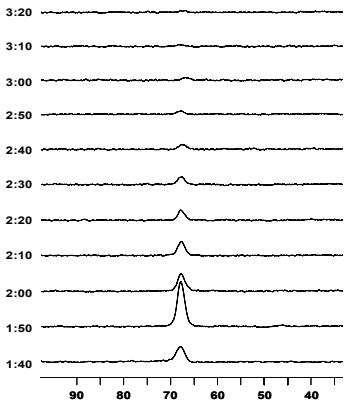
Choline is an essential nutrient that is involved in multiple cellular processes in multiple organs[1]. Choline metabolism is altered in pathological conditions such as malignancy (breast, brain, and prostate cancer)[2-4] and neurodegenerative processes such as Alzheimer's Disease where either an accumulation (of phosphocholine)[5, 6] or diminution (of acetylcholine)[7] is detected, respectively. Therefore, the ability to monitor the metabolism of choline to its metabolites holds promise to serve as a direct diagnostic tool and to provide important biomarkers for several indications. The low background signal of carbon-13 MRS and its wide spectral window (200 ppm) makes carbon-13 spectroscopy an attractive strategy for *in vivo* detection of choline metabolism. However, the *in vivo* detection of this metabolism with a high temporal resolution remains impractical in most cases due to the low sensitivity of ^{13}C MRS combined with the low concentration of choline metabolites. For this reason, the technology for *ex-vivo* hyperpolarization of ^{13}C, which has been shown to provide more than 10,000 fold enhancement in ^{13}C signal[8] is likely to aid in the case of choline metabolism. However, the visibility of the hyperpolarized signal is limited by the decay of the hyperpolarization from the moment of its generation due to spin-lattice relaxation (T_1 relaxation time). A previous study surmounted this limitation by substituting the proton positions with deuterons[9]. This strategy resulted in a 7 fold increase of the ^{13}C T_1 in the respective choline analog. In addition, it was shown that the deuteration of the choline molecule did not interfere with the DNP hyperpolarization process[9]. This promising results led to a study of a new biomarker namely, [1,1,2,2-D_4,2-^{13}C]-choline. Here we report on the first *in vivo* studies of carbon-13 hyperpolarized [1,1,2,2-D_4,2-^{13}C]-choline.

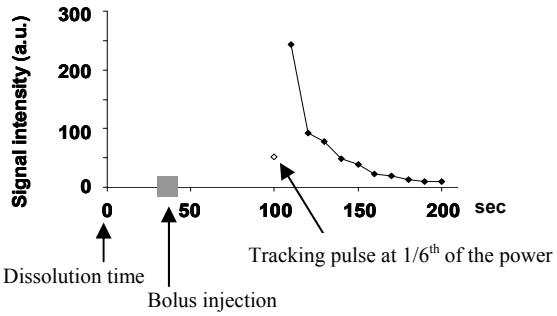
Materials and Methods

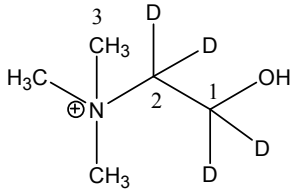
The double-labeled choline analog [1,1,2,2-D_4,2-^{13}C]-choline Cl^- (BW-42, BrainWatch Ltd., Tel-Aviv, Israel) was dissolved in 1:1 D_2O:DMSO-d_6 containing 15 mM trityl free radical (OX063, GE Healthcare, London, UK) and polarized in a HyperSense DNP polarizer (Oxford Instruments Molecular Biotools, Abindgdon, UK) at 1.4°K. Mice (n = 2, male, FVB) and rats (n = 3, male, Wistar) were anesthetized using isoflurane. Anesthesia was induced with 3% isoflurane and maintained for up to two hours with 1.5% isoflurane. Hyperpolarized [1,1,2,2-D_4,2-^{13}C]-choline was bolus injected to the tail vein of the animals. A 4.7 T scanner was used for imaging and hyperpolarized spectroscopy. ^{13}C spectra of the animal's head were recorded using home-built ^{13}C surface coils (8 mm and 16 mm diameter).

Results

The enhancement factor of [1,1,2,2-D_4,2-^{13}C]-choline (see structure below) was found to be ~12,000, in solution, about 20 sec after dissolution, at 4.7 T. The stack plot below shows consecutive ^{13}C spectra that were recorded from a rat's brain region after an injection of hyperpolarized [1,1,2,2-D_4,2-^{13}C]-choline. The time course of this study is shown on the right. In all of the studies (in 2 mice and 3 rats) the signal of hyperpolarized choline was clearly detectable using a surface coil placed over the animal's head. This signal increased with the dose and decayed with time. In the study below: the hyperpolarized choline signal was clearly detectable in the brain region for about 3 minutes.







Discussion

The *in vivo* results of hyperpolarized [1,1,2,2-D_4,2-^{13}C]-choline showed the presence of the hyperpolarized compound in the animal's head in five of the five studies, thereby proving the feasibility of using this contrast agent *in vivo*. The decay of the polarization level was in agreement with the previous T_1 measurement of ~ 35 sec [9], assuming arrival of non-excited polarized agent into the detection region prior to each RF excitation. We attribute this effect to a much larger pool of hyperpolarized choline circulating in the body which continues to perfuse the brain with high intensity choline signal.

In summary, these results are encouraging and demonstrate the potential of the hyperpolarized [1,1,2,2-D_4,2-^{13}C]-choline as a contrast agent for monitoring metabolism *in vivo* over several minutes. Further studies with this agent will focus on improving the spectral resolution, optimizing the temporal window for detection of metabolism, providing spatial localization of the data, and the use of animal models of diseases such as cancer and Alzheimer's disease.

Acknowledgment

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