Imaging carbonic anhydrase activity using hyperpolarized ^{13}C-labeled bicarbonate

F. A. Gallagher[1,2], M. I. Kettunen[2,3], R. L. McLaughlin[2,3], D-E. Hu[2,3], T. H. Witney[2,3], J. H. Ardenkjaer-Larsen[4], and K. M. Brindle[2,3]

[1]Radiology, University of Cambridge, Cambridge, United Kingdom, [2]CRUK Cambridge Research Institute, Cambridge, United Kingdom, [3]Biochemistry, University of Cambridge, Cambridge, United Kingdom, [4]GE Healthcare, Amersham, United Kingdom

Introduction

We have shown previously that ^{13}C-labeled bicarbonate ($H^{13}CO_3^-$) can be hyperpolarized using Dynamic Nuclear Polarisation (DNP) and that when this is injected *in vivo,* the enzyme carbonic anhydrase (CA) rapidly interconverts this with carbon dioxide ($^{13}CO_2$) [1]. Furthermore, we have also shown that selective saturation of the $^{13}CO_2$ signal *in vivo* will decrease the $H^{13}CO_3^-$ signal in the presence of the enzyme. The rate at which the $H^{13}CO_3^-$ signal decreases depends on the activity of the enzyme. CA is of biological importance because tumor-associated CAs (e.g. CAIX) have been shown to be strongly induced by hypoxia and controlled by hypoxia-inducible factor-1 (HIF-1) [2]. Therefore imaging CA may directly relate to hypoxia and HIF-1 activation.

Methods

^{13}C-labeled cesium bicarbonate was hyperpolarized as described previously [1]. A phantom was designed which consisted of 5 tubes each containing 0.4 ml of 500 mM phosphate buffer and 100 mg/l EDTA at pH 7.4. To these were added varying concentrations of the enzyme CA: 0-8 mg / ml. A ^{13}C-tuned surface coil was placed adjacent to the tubes. 300 µl of hyperpolarized bicarbonate was injected simultaneously into each tube and a series of echo-planar ^{13}C MR images (EPI) were acquired for the $H^{13}CO_3^-$ resonance with a nominal flip angle of 10°; field-of-view 6 x 3 cm; slice thickness 5 mm. Between image acquisitions, the $^{13}CO_2$ resonance was selectively saturated for a total of 670 ms. The CA activity map was derived from the $H^{13}CO_3^-$ signal intensity in the first image divided by that in the last image: the higher the CA concentration, the greater the decrease in the $H^{13}CO_3^-$ signal.

In vivo experiments were performed using a murine lymphoma model as previously described [1]. 0.2 ml of hyperpolarized $H^{13}CO_3^-$ was injected intravenously and chemical shift images (CSI) was acquired before and after 50 ms of saturation of the $^{13}CO_2$ resonance. The ratio of the two was used to estimate carbonic anhydrase activity; the lower the signal, the higher the CA activity.

Results

^{13}C-labeled cesium bicarbonate was hyperpolarized to 16% which represents a 20,000-fold increase above thermal polarization at 37ºC and 9.4 T. The signal intensity in the initial image was relatively homogeneous within the tubes. Following $^{13}CO_2$ saturation, the tubes with the most CA showed the greatest decrease in $H^{13}CO_3^-$ signal. The CA activity map was calculated and the rate of decrease in $H^{13}CO_3^-$ signal in these images showed a good correlation with the concentration of CA in each tube (Fig. 1)

In vivo, heterogeneity was demonstrated within the false-color ratio image although the areas of highest CA activity appeared to lie outside the tumor when compared to the black and white proton MR image (Fig. 2). This region may correspond to a blood vessel, since erythrocytes are know to have high levels of CA activity.

Discussion

The results show that the spatial distribution of CA activity can be imaged *in vitro* using DNP and $^{13}CO_2$ saturation. The *in vivo* image showed heterogeneity in CA activity but there was no clear difference in CA activity between the tumor and the surrounding tissue. This may be because CAIX expression is not high enough in this tumor model compared to blood CA activity. If other tumor models express sufficient CAIX to allow imaging, this technique could be used as a marker of hypoxia and HIF-1 activation.

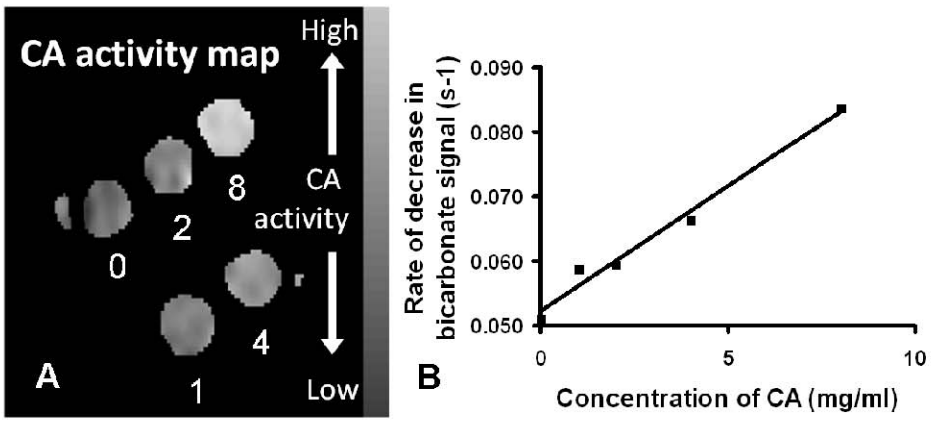

Figure 1. A: CA activity map of a phantom with the concentration of enzyme (mg/ml) shown adjacent to each tube. The brighter the image, the higher the CA activity.
B: Relationship between the signal intensity in this image and the corresponding concentration of CA in each tube.

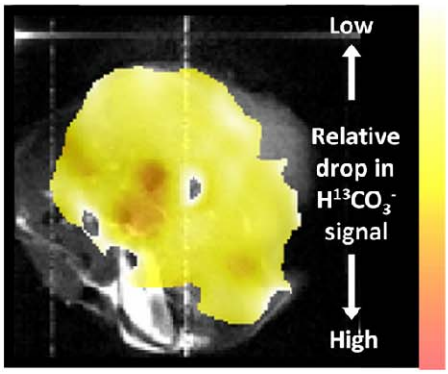

Figure 2. False-color $H^{13}CO_3^-$ ratio image acquired from two CSIs superimposed over a proton MR image. The darker the image, the higher the CA activity.

References

1. Gallagher FA *et al.* (2008), *Nature* 452:940.

2. Wykoff CC *et al.* (2000), *Cancer Research* 60:7075.