Enzymatic conversion of [1-13C]pyruvate to [1-13C]lactate in glioma rat model evaluated by 11.7T NMR correlated with DCE-MRI at 3T.

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Introduction

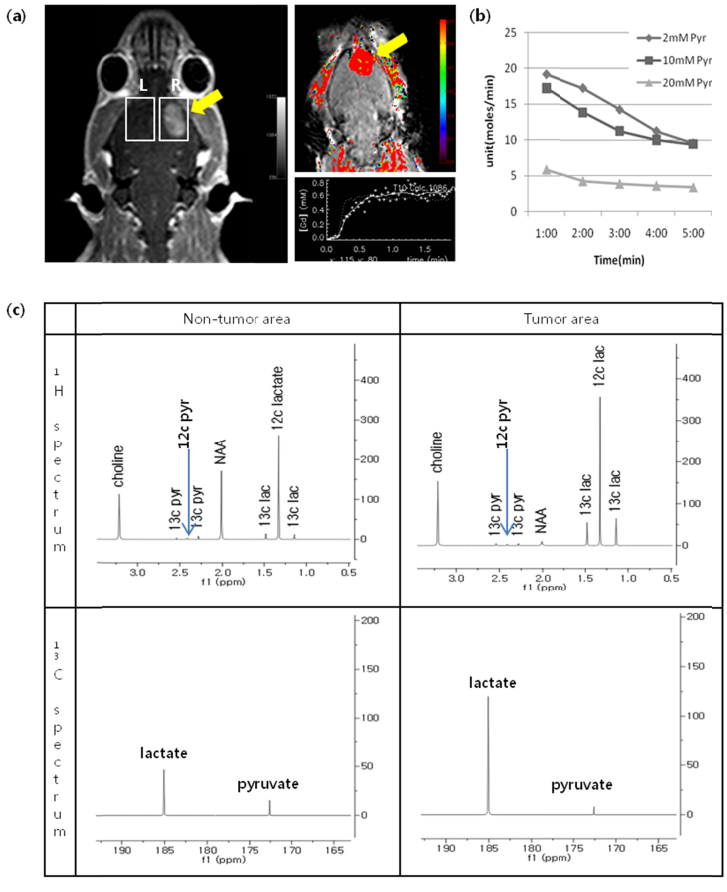
Nuclear magnetic resonance (NMR) spectroscopy has been extensively used in the field of biochemistry, primarily to investigate biological macromolecules. Recently, NMR study has been extended to live cells and tissues. Among its many applications, metabolic tracking by using hyperpolarized [1-^{13}C]pyruvate has been a robust tool to detect the in-vivo tumor metabolism.(1,2) IV injection of hyperpolarized [1-^{13}C]pyruvate gives us a new understanding to investigate the Warburg effect on the tumor metabolism. However interpretation of in-vivo tumor glucose metabolism may be confounded by enzymatic conversion from pyruvate to lactate versus magnetization transfer from the hyperpolarized ^{13}C labeled substrate to non-hyperpolarized ^{13}C labeled substrate. In this study we investigated enzymatic conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate in glioma rat model with exclusion of magnetization transfer effect. Total amount of lactate pool was measured by ^{1}H NMR.

Materials and methods

C6 glioma brain tumor animal model (three6-week-oldfemale SD rats) was used. MRI scanning was carried out using a whole-body 3T scanner (Signa, Philips Medical System). Gd-DTPA (Magnevist, Schering, Germany) was administrated at a dose of 281mg/kg as a bolus dose via tail-vein injection. DCE-MRI was performed using a perfusion-weighted spin echo sequence with the following acquisition parameters: FOV (mm) = 50 x 35, RFOV (%) = 71, Matrix scan = 112, reconstruction = 224, TR/TE (ms) = 12 / 4.0, slice number = 11, dynamic scans = 60, 1mm slice thickness with a total acquisition time of 2min 6s. To evaluate the enzymatic conversion in vivo, the animals were injected by 100mM [1-^{13}C]pyruvate for 15 seconds by tail vein. And then rats were euthanized by cervical dislocation at5min after injection. The brain tumor samples were promptly obtained and preserved in LN_2 until NMR study performed. NMR data acquisition: All one-dimensional HR-MAS MR spectra of the brain samples were measured with a500 MHz (11.7 T) NMR spectrometer (Agilent, VNMRS 500) operated at a proton and carbon NMR frequency. Temperature was set to 25°C. Proton and carbon NMR experiment took 30 minutes and 38 hours, respectively. Frozen samples were thawed in NMR laboratory, weighed, and placed into 4-mm NMR tube using an HR-MAS nanoprobe (Agilent, Walnut Creek, CA). Cellular enzyme assay was performed to measure the LDH activity of the C6 glioma cells (4.5 x 10^{7}) by lysed in RIPA buffer.

Results and Discussion

Figure (a) shows DCE-MRI image of rat brain glioma. The left box (L) indicates non-tumor area and the right box (R) indicates tumor area injected by C6 glioma cells. Perfusion map (right upper) shows increased permeability by red color in the tumor. Fitting graphs (right lower) shows enhancement pattern in the tumor. Increased rate constant indicates increased vascular permeability in metabolically active highly malignant tumor (K_{ep}: 3.12 ± 0.84/min, n=3). Figure (b) shows the LDH enzyme activity dependent on the substrate (pyruvate) concentration in C6 glioma cell lysate. The specific activity of LDH is 0.10 unit/mg with 2mM pyruvate, 0.19 unit/mg with 10mM pyruvate and 0.10 unit/mg with 20mM pyruvate. Figure (c) shows ^{1}H and ^{13}C NMR spectrums of normal brain tissue and glioma. Depicted p.p.m. of each metabolite In ^{1}H NMR is as follows. ^{12}C-lactate:1.3 p.p.m.,^{13}C–lactate: 1.5, 1.1p.p.m.,^{12}C-pyrvate:2.4p.p.m., ^{13}C–pyruvate:2.3, 2.5p.p.m., choline: 3.2p.p.m., NAA: 2.0 p.p.m. Depicted p.p.m. of each metabolite in ^{13}C NMR is as follows. ^{13}C-lactate:185.1 p.p.m., ^{13}C-pyruvate:172.7p.p.m. The N-acetyl aspartate/Choline(NAA/Cho) ratio is 1.13 in normal brain and 0.16 in glioma. Delivery of pyruvate to the glioma would be abundant analogized from the K_{ep} value. But the pyruvate in the glioma was low (26.06; arbitrary unit, area under the peak, AUP) than the normal brain (52.44$_{AUP}$).The enzymatic conversion ratio (lactate/pyruvate) in ^{13}C spectrum in normal brain was 5.2~5.5%and 34.0% in glioma. Total lactate pool measured ^{1}H NMR was 1471$_{AUP}$ (^{12}C) and 750$_{AUP}$ (^{13}C) in glioma, 1370$_{AUP}$ (^{12}C) and 200$_{AUP}$ (^{13}C) in normal brain. This explains the high metabolic status in the glioma expressed as high level of LDH enzyme activity and high enzymatic conversion ratio of pyruvate.



Conclusion

DCE-MRI evidenced abundant [1-^{13}C]pyruvate supply to the glioma. In ^{1}H NMR, NAA/choline peak clearly demonstrated the malignancy of the tumor. Total lactate pool was significantly abundant in glioma than normal brain. This study would suggest that the enzymatic conversion is the one of the important factor to evaluate metabolism of the tumor. Correlation analysis of signals from NMR spectroscopy and DCE-MRI could be useful to evaluate the tumor metabolism and validate the characteristics of malignant tumor for novel the rapeutics.

References: (1) Shulman RG et al., Science 1979;13;160-6 (2) Day SE et al., Nat Med 2007;13:1382-7