Survival from Brain Metastasis of Breast Cancer is Inversely Correlated with Hyperpolarized 1-13C Lactate/Pyruvate ratio: Preliminary Study in Nude Mouse Model.

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Introduction Since tumor cells actively metabolize pyruvate to lactate regardless of oxygen availability, the conversion ratio of pyruvate to lactate has been investigated as a robust imaging biomarker to evaluate the status of cancer. Metabolic alteration in cancer cells preceded the morphologic or phenotype change. Thus, the ability of real time *in-vivo* imaging of cancer metabolism using hyperpolarized 13C MRS opens the way to monitor the efficacy of anti-cancer therapeutics and predict the prognosis even before the conventional imaging can detect the morphologic change. In this respect, hyperpolarized 13C MRS imaging can be a useful tool to evaluate the alteration of metabolic flux in cancer cells by a medication regulates glucose metabolism. Metformin is a medication reducing the levels of circulating glucose and plasma insulin for type II diabetes patients. According to the clinical epidemiological research, metformin reduces cancer incidence or mortality in type II diabetes patients. Metformin inhibits the growth and proliferation of breast cancer cells by activation of AMPK. In this study, we evaluated the survival rate and lactate/pyruvate ratio using hyperpolarized [1-13C] pyruvate MRS after metformin treatment in the brain metastasis of breast cancer mouse model.

Materials and Methods All MRI experiments were performed on a 9.4T Bruker BioSpec 94/20 USR small animal imaging system (Bruker BioSpin MRI GmbH, Ettlingen, Germany) equipped with 1H-13C dual-tuned surface transmit/receive coil (20mm diameter). Six weeks old BALB/C nude mice were used for animal study. 2×10^5 MDA-MB-231 breast cancer cells were stereotactically implanted in the striatum of brain. Four animals were treated with 300 mg/kg oral metformin for 5 times a week from 1 week after intracranial cancer cell injection until the animals die. Four animals were treated with saline. We performed the T2-weighted MRI scan and measured tumor volumes using OsiriX DICOM viewer at 2 weeks after intracranial injection. For *in-vivo* dynamic 13C MRS experiments, 75mM [1-13C] pyruvic acid doped with 15mM Trityl radical and 1.5M Dotarem was polarized using HyperSense DNP polarizer (Oxford Instruments, Molecular Biotools, Oxford, UK). After dissolution into aqueous state, approximately 350ul of the hyperpolarized pyruvate solution was injected through a tail vain catheter in BALB/C nude mouse at 3 weeks after implantation. All procedures were approved by the Animal Care and Use Committees at Yonsei University College of Medicine. The analysis of the 13C MRS data was performed on the homebuilt software. Quantification of individual spectra performed after automatic phasing, baseline subtraction, and frequency correction. The stacks of spectral peaks were used to calculate the conversion ratio of [1-13C] lactate from [1-13C] pyruvate.

Figure 1: Axial T2-weighted MR image at 3 weeks after tumor cell implantation at the left striatum. (upper). Dynamic curves were obtained from the stacks plots of [1-13C] lactate and [1-13C] pyruvate (bottom).

Results and Discussion Brain tumor was identified and measured by T2-weighted MR images (Figure 1 upper) and the pyruvate to lactate flux of the tumor was calculated from the dynamic 13C MRS (Figure 1 bottom). Because the growth of the tumor is variable in individual mice, the growth rate of tumor was calculated by measuring tumor volume in T2-weighted image at week 2 and week 3 for each animal. Although the metformin treated group seemed retard in growth than the sham treated group, there was no statistical significance in difference (Figure 2 left). Also, hyperpolarized 13C MRSI at week 3 showed no difference in the Lactate/pyruvate ratio between the two groups (Figure 2 right). And the pyruvate to lactate conversion ratio for the unit volume of tumor also showed no difference between the two groups. The survival analysis showed that there was no difference in the survival between the metformin treated group and the sham group (Figure 3). However, regardless of experimental group, the individual survival and the lactate/pyruvate ratio was inversely correlated (Figure 4). By these results we can infer the fact that the prognosis of the brain metastasis form breast cancer is related with the lactate/pyruvate ratio. It has been reported that the metformin inhibit cell growth, induce apoptosis, and decrease the cancer stem cell population. However in our model system, anti-cancer therapeutic effect of metformin was not proved. There are limitations of this study. The interval from baseline imaging before treatment to follow up 13C MRS imaging was only one week. The metabolic alteration induced by metformin might be not enough to be monitored by 13C MRS with only one week treatment in this model sltudy. The number of animal is small to validate the statistical significance. More large number of model study would worth to validate the significance.

Conclusion In brain metastasis from breast cancer mouse model, we evaluate the pyruvate to lactate conversion ratio to monitor the alteration of metabolism by metformin by hyperpolarized 13C metabolic MRS imaging. The inhibition of tumor growth by metformin was not proved by conventional imaging and hyperpolarized 13C MRSI in one week oral metformin treatment. Individual conversion ratio of 13C pyruvate to 13C lactate showed inverse correlation with survival regardless of experimental group. Hyperpolarized 13C MRSI may be useful tool to predict the prognosis of cancer patients

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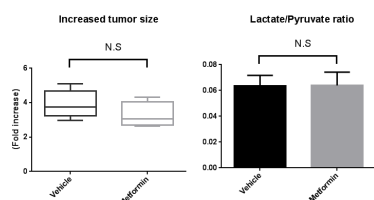
Figure 2: Difference was not shown in growth rate (left) and conversion ratio of 13C pyruvate to lactate (right) between the metformin treated group and the sham treated group
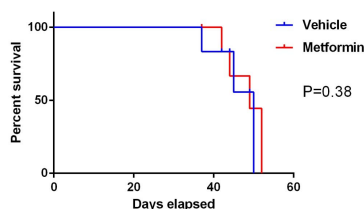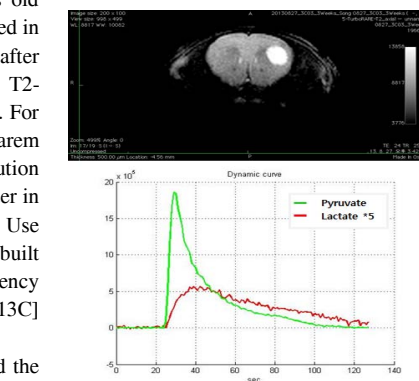

Figure 3: Survival curves were calculated using Kaplan-Meier method from 4 animals of each group. No difference between the sham treated group and the metformin treated group was shown..
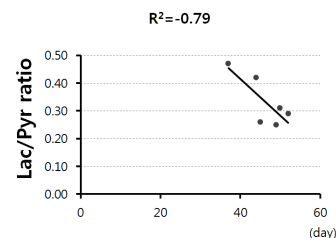

Figure 4: Individual survival and lactate/pyruvate ratio was inversely correlated from 6 animals showing reliable 13C signal to noise ratio.