Evaluation of the Early Metabolic Response induced by 5-Fluorouracil on Mouse Mammary Cancer Cells under fasting conditions using Hyperpolarized 13C-labeled Pyruvate.

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Introduction

The application of nuclear magnetic resonance (NMR) for metabolic analysis has been limited by the intrinsically low sensitivity of ^{13}C nuclei. Recently, dynamic nuclear polarization (DNP), which is a technique for increasing NMR signal intensity, has demonstrated the ability to enable to detect *in vivo* metabolism and pathological processes. This technique may allow noninvasive, quantitative visualization of tumor metabolic processes. The purpose of this study was 1) to detect the early metabolic response induced by 5-fluorouracil (5FU) and 2) to evaluate the effect of the nutritional status on mouse mammary cancer cells using hyperpolarized 1-^{13}C-pyruvate.

Materials and Methods

FM3A cancer cells of mammary tumors derived from C3H mice were cultured 48 hours in RPMI1640 medium supplemented with or without glucose (2g/L) and evaluated in this study. They were divided two groups of no treatment and treatment with 5FU for 22 hours at 5 μM followed by 1 hour at 200 μM before NMR measurement. There were four Glc(+)+5FU(-) cases, three Glc(+)+5FU(+) cases, four Glc(-)+5FU(-) cases and three Glc(-)+5FU(+) cases. The number of cells and their viability were assessed by microscopic observation at the end of each experiment with an equal volume of Trypan blue (0.3% in 0.81% sodium chloride and 0.06% potassium phosphate). 1-^{13}C-pyruvate (99% enriched) was dissolved in D_2O/Glycerol solution and Ox63 radicals were dissolved as polarization samples. The sample was submerged in liquid helium in a DNP polarizer magnet (3.35T; HyperSense, Oxford Instruments). The sample temperature was reduced to 1.4K and microwave irradiation at 94.110GHz and 60mW was used for polarization for 1 hour. The dissolution was then carried out with 3 mL phosphate buffered saline with 0.01% EDTA. The substance solution was injected via a capillary line into the vials to be mixed with the centrifuged cells and then transferred into the NMR tube, which was placed in an NMR scanner (DRX600, Bruker BioSpin) with a 5 mm broadband probe tuned to 150.9MHz. The whole procedure, from the end of polarization until the NMR measurement, was performed within 10 sec[1]. The spectra of the hyperpolarized ^{13}C label were acquired by proton decoupling, using 15 flip angle pulses. The measurements were conducted over 120 sec and then were processed with 5 Hz exponential line broadening. The peaks and the integrals of 1-^{13}C-puruvate and 1-^{13}C-lactate were analyzed using the MestreNova version 5.3.0-4469 software package (Mestrelab Research S.L.). The peak integrals of the spectra were fit to an equation that has been published previously[2]. The fitting calculation was done using the solver function of Microsoft Excel 2007. The rate constant kP, kL per cell (kP/cell, kL/cell), their ratio (kP/kL) and lactate production ratio, which was the ratio of the area under the curve of pyruvate and lactate were evaluated. Lactate dehydrogenase (LDH) activity was measured following the methods of Bregmeyer and Bernt[3], and of Takada et al[4] and then the findings were compared with the results from the NMR measurements. The reaction of FM3A cell lysate was initiated by the addition of 30 mM pyruvate acid. The change of absorbance at 340 nm was monitored with a double beam spectrophotometer (UV-1600, Shimadzu, Japan).

Results and Discussions

The spectrum and fitting curves of NMR measurements are shown in **Figure1**. The peaks of lactate were found in Glc(+) though they were not visible in Glc(-). The results of kP/kL and LDH activity measured by absorption spectroscopy were shown in **Figure 2 and 3**. The kP/kLs under fasting conditions with or without 5FU were significantly lower in comparison to normal conditions. The same trends were shown in the LDH activity which was measured by absorption spectroscopy. These results confirmed that the nutrition status affected the cell metabolism, especially in the presence of 5FU treatment. The survival rate of the cells is shown in **Figure 4**. No difference was observed with 5FU treatment, although there was a significant difference associated with the nutrition status without 5FU treatment. These results suggested that this technique is able to detect the early metabolic response before morphological cell death under 5FU treatment.

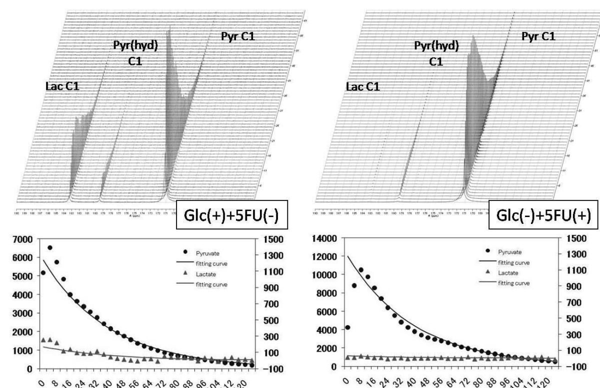

Fig.1 The spectrum and fitting curves of NMR measurements.

Conclusions

The NMR measurements using hyperpolarized 1-^{13}C-pyruvate allowed for the detection of the early metabolic response induced by an anticancer agent. These results indicated the potential usefulness of utilizing molecular imaging to evaluate the tumor metabolic process.

References

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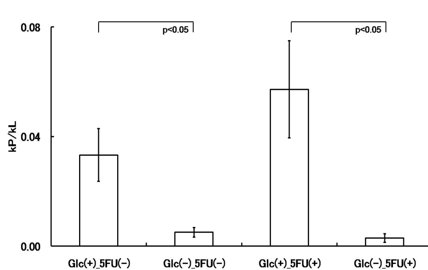

Fig. 2 kP/kL with or without 5FU under normal and fasting conditions.

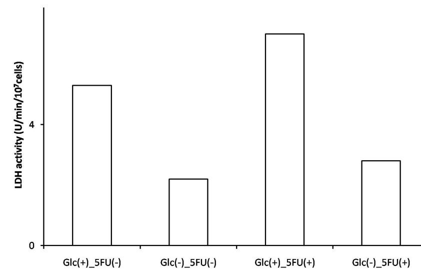

Fig. 3 LDH activity with or without 5FU under normal and fasting conditions measured by absorption spectroscopy.

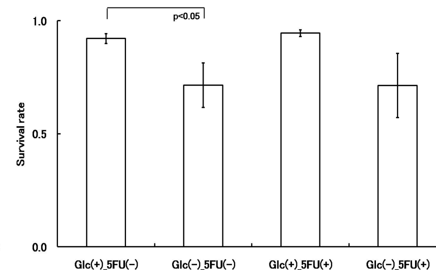

Fig. 4 Survival rate of the cells.