Neurochemical Changes in Rat Brain after 5-Fluorouracil Chemotherapy Assessed by ^{1}H MR Spectroscopy at 9.4 T

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Introduction: Chemotherapy (CTx) using 5-fluorouracil (5-FU) is applied as a standard regimen for breast, colorectal and gastric cancers, and reduces the risk of recurrence and prolongs the survival of patients. Previous studies have reported that cognitive impairment may occur in some patients treated with 5-FU [1, 2]. However, the underlying mechanisms of 5-FU-related cognitive impairment are unknown. The investigation of brain metabolite changes can help to understand the mechanism of this chemotherapy-induced cognitive impairment. Previous studies showed that NAA/Cr and Cho/Cr were reduced in human brain after combination CTx including 5-FU using ^{1}H MRS at 3 Tesla [3, 4]. At a higher magnetic field, a larger number of metabolites could be assessed. In this study, we assessed metabolite changes in 5-FU-administered rat brains using *in vivo* ^{1}H MR spectroscopy at 9.4 Tesla. In addition, hippocampal spatial memory was evaluated in 5-FU-administered rats using a behavioral test.

Materials and Methods

Animals: All animal experiments followed a protocol approved by the institutional animal experimental committee. Adult male Wistar rats (n = 26; Japan SLC, Inc., Hamamatsu, Japan), weighing 186-230 g, were used. The rats were randomly assigned to control (n = 13) or 5-FU-treated (n = 13) groups. The rats received intravenous injections of 25 mg/kg 5-FU (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) or an equivalent volume of saline via the tail vein over four consecutive days (total dose of 100 mg/kg).

MRI experiments: All MR measurements were performed with a 9.4 Tesla MRI scanner (BioSpec 94/20 USR, Bruker BioSpin, Germany) equipped with a phased-array brain coil. Rapid acquisition with relaxation enhancement (RARE) images (repetition time (TR) = 2500 ms, echo time (TE) = 33 ms, field-of-view = 40 mm x 40 mm, 256 x 256 matrix, 1 average) were acquired. *In vivo* ^{1}H MR spectra were acquired in the left hippocampus (Fig. a, b) using a single-voxel point-resolved spectroscopy (PRESS) sequence (TR = 2500 ms, TE = 20 ms, VOI = 3 x 3 x 3 mm^3, 256 averages, total scan time = 10 min 50 s) on day 9 (n = 22) after the first 5-FU injection. LCModel (LA Systems Inc., Japan) was used to quantify metabolite concentrations.

Behavioral test: Nine days after the first 5-FU injection, hippocampal spatial memory was assessed using a fear-conditioning test, which consists of training and contextual test sessions, in the 5-FU-treated (n = 6) and control (n = 6) groups using a contextual NIR video fear-conditioning system for rats (Med Associates, Inc., Vermont, USA). Of these, 4 5-FU-treated and 4 control rats also underwent MRS measurements. As a training session, the rats were placed in a chamber and given tone signals (10 s, 80 dB, 5000 Hz) and electric foot-shocks (2 s, 1.0 mA) three times at 60 s intervals. The next day, the contextual test was carried out in the same chamber as used for training without electric foot-shocks and tone signals. The length of freezing behavior, an indicator of hippocampal spatial memory, was measured over an 8 min period. Percentage freezing (the total time of freezing behavior [min] / 8 [min] x 100) was calculated.

Statistical analysis: Differences in metabolite concentrations as well as percentage freezing on day 9 between the 5-FU-treated and control groups were assessed by Student's *t*-test using SPSS (version 20, IBM Japan Inc., Tokyo, Japan). $P < 0.05$ was judged as statistically significant.

Results and Discussion: Glutamine concentration in the hippocampus (Fig. c) was significantly lower in the 5-FU-treated group (4.07 ± 0.44 μmol/g, mean ± SD) than in the control group (5.09 ± 0.69 μmol/g). Glutamine concentration in 73% of 5-FU-treated rats was lower than the minimum glutamine concentration in control rats (Fig. d). There was no statistically significant difference in other metabolite concentrations. In the contextual test, percentage freezing were 68 ± 19% (mean ± SD) and 78 ± 10% in 5-FU-treated and control rats, respectively ($p = 0.275$, Student's *t*-test). The correlation between glutamine concentration in the hippocampus and percentage freezing was not significant.

The reduction in glutamine concentration may be related to the pathogenesis of 5-FU-induced brain damage. We suspect that fluoroacetic acid and fluorocitrate, both of which are metabolites of α-fluoro-β-alanine (FBAL) and 5-FU, may injure glial cells in the hippocampus or damage their ability to produce glutamine [5]. In this preliminary study, the correlation between glutamine concentration in the hippocampus and hippocampal memory was not observed, possibly owing to the small sample size.

Conclusions: ^{1}H MRS with a high field could detect the reduction in glutamine concentration in the hippocampus after 5-FU treatment; however, the reduction in hippocampal glutamine did not seem to be related to hippocampal memory damage.

References: [1] Breast Cancer 2005; 12: 279-287, [2] Nat Rev Cancer 2007; 7(3): 192-201, [3] Hum Brain Mapp 2011; DOI: 10.1002/hbm.21422
[4] Proc. ISMRM 2012; 20: 4447, [5] GLIA 1997; 21: 106-113

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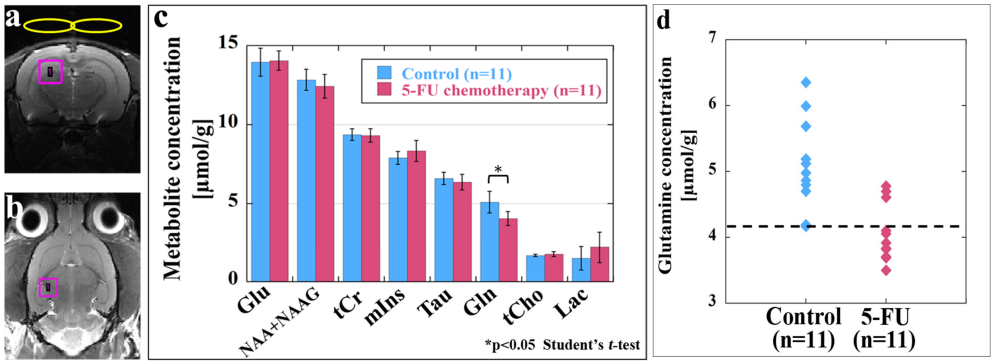

Figure a, b Examples of axial (a) and coronal (b) images of the rat brain. A 27-mm^3 voxel is placed in the hippocampus. Yellow circles indicate the position of the coil.
Figure c Bar chart showing metabolite concentration of the left hippocampus in control and 5-FU chemotherapy groups (Glu, glutamate; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; tCr, total creatine; mIns, myo-inositol; Tau, taurine; Gln, glutamine; tCho, total choline; Lac, lactate). Data are mean ± SD.
Figure d Box showing glutamine concentration in all cases.