^{1}H MRS assessment of spermatogenic activity in experimentally injured rat testes

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Introduction: Magnetic resonance spectroscopy (MRS) measurements in vivo by stimulated echo acquisition mode (STEAM) sequence with short echo time (TE) allow sensitive detection of creatine, choline, glycine and glutamate signals in the healthy rat testis [1]. To determine if the signal intensity of these metabolites is a useful indicator of spermatogenic activity in vivo, MRS study was carried out in the rat testis with normal or impaired spermatogenesis by low-dose glycerol administration. The anti-spermatogenic effect of glycerol is attributed to the hyperosmotic effect and the disruption of blood testis barrier, and it yields spermatogenic impairment at various levels in severity depend on the concentration of glycerol and the duration after the administration [2].

Materials and methods: Twenty-one adult male Wister rats were administered a single intra-testicular injection of 10-20 % glycerol solution or purified water (sham) in the unilateral testis. The contralateral one was treated as a control. After 1 to 12 weeks MR measurements by STEAM sequence (repetition time / mixing time / TE, in msec = 5000 / 30 / 15; a volume of interest (VOI), 4x4x4-6x6x6 mm^3, number of accumulations, 128-256) were performed using INOVA spectrometer (Varian, Inc. CA. USA) equipped with a 4.7 Tesla (T) magnet. An in-house-built quadrature surface coil was used to transmit radio-frequency (RF) pulses and receive signals. The signal intensities of creatine $N(CH_3)$, choline, glycine, and glutamate (C2-H) were determined by a cut-and-weigh method, and the obtained values were normalized with the signal intensity of water in the same VOI. Spermatogenic activity in 100-200 seminiferous tubules in the central part of the testis was histologically evaluated and classified into 10-point scale reported by Johnsen (Johnsen score: the tubules with complete spermatogenesis is scored as 10, and those with complete disappearance of germ cells and Sertoli cells as 1) [3]. The correlation between the signal intensity of metabolites and the average values of Johnsen score was evaluated by Spearman rank-order correlation coefficient test.

Results and discussions: The signal intensities of creatine, choline, glycine, and glutamate in the glycerol-treated testis were significantly reduced compared with those in the sham and the control testis (Fig. 1). The average values of Johnsen score in the glycerol treated testis were significantly lower than those in the sham and control. The signal intensities of creatine and choline were strongly correlated with Johnsen score (rs = 0.85 for creatine, and rs = 0.80 for choline) (Fig. 2). The signal intensities of glycine and glutamate did not show strong correlation as those of creatine and choline. Since germ cells are actively proliferated for producing new sperms in healthy testicular tissue, the amount of the precursor and degradated products of cell membrane may increase leading high concentration of choline metabolites. In contrast, the amount of these metabolites could decrease if spermatogenesis was suppressed and the number of germ cells decreased. Normal testicular tissue also contains the large amount of creatine in the seminiferous tubular lumen [4]. The reduced signal of creatine in the damaged testis presumably resulted from the dysfunction of creatine synthesis and of its retention mechanism in the seminiferous tubule.

Conclusion: The signal intensities of creatine and choline in ^{1}H MRS are suggested as a good indicator of spermatogenic activity in vivo.

References: [1] Yamaguchi M, et al. Magn Reson Med 2006;55:749-754. [2] Igdoura SA, et al. J Androl 1994; 5:234-243.
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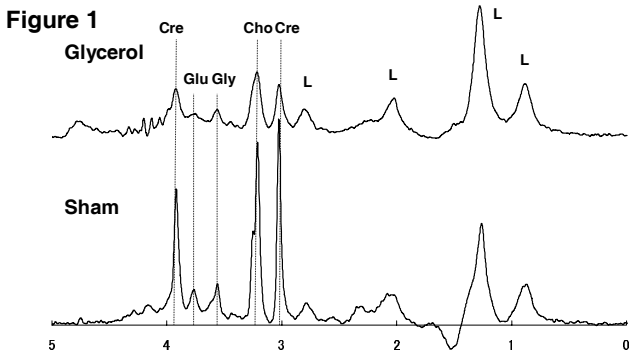

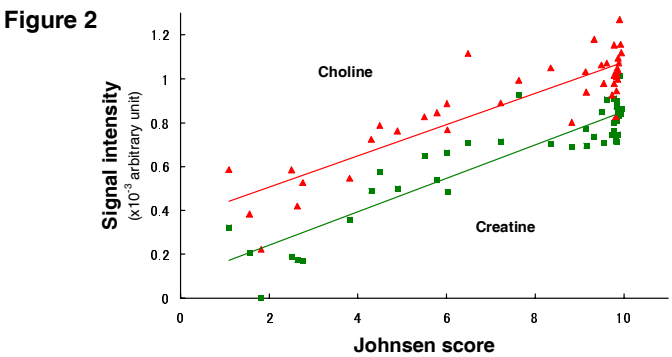

Fig.1 ^{1}H MR spectra in the rat testis with 10% glycerol administration (top) shows the reduction in signal intensities of creatine (Cre), choline (Cho), glycine (Gly), and glutamate C2-H (Glu) compared with those in a sham testis (bottom). Large lipid signals (L) are present in 0.89-2.78 ppm.
Fig.2 The scatter plot shows strong correlations between Johnsen score and the signal intensities of creatine (dots in green), and choline (dots in red), respectively.