Tau Hyperphosphorylation in the Hippocampus of Alzheimer-like Rat is Associated with Reduction of Total Choline

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Introduction Formation of neurofibrillary tangle from hyperphosphorylated tau protein is one of the most prominent pathologic characteristics in Alzheimer's disease (AD). Glycogen synthase kinase-3 (GSK-3) appears to one of the major kinases that regulate tau phosphorylation [1]. Rat hippocampus slices treated with wortmannin (WT), an inhibitor of phosphatidylinositol 3-kinase (PI3K), and GF-109203X (GFX), an inhibitor of protein kinase C (PKC), show increased GSK-3 activity and Alzheimer-like (AD-like) tau hyperphosphorylation [2-3]. In this study, an AD-like rat model was developed by intraventricular co-injection of WT and GFX, and characterized by magnetic resonance imaging (MRI) and in vivo ^{1}H magnetic resonance spectroscopy (MRS).

Materials and Methods Thirty male Sprague-Dawley rats (220-250 g) were used in the study, among which 17 underwent MRI/MRS examinations. The rats in the model group received co-injection of 100 μM WT and 100 μM GFX (saline solution, total volume 10 μl) into the left lateral ventricle of brain [2-3]. The rats in the sham group had injection of the same amount of saline. The level of tau phosphorylation in the hippocampus (Hip) was measured by Western blot and immunohistochemistry. Morris water Maze test was performed to evaluate the behavioral changes. MRI/MRS examinations were carried out on a Bruker Biospec 4.7 T/30 cm scanner at 48 hrs after injection. During the MR session, the rats were anesthetized by 1.5% isoflurane in a 70:30 N_2O/O_2 gas mixture delivered via a nose cone, and the rectal temperature was maintained at 37.0-37.5 ^oC using a warm water pad. For each rat, localized ^{1}H MR spectra were acquired from bilateral Hip with a PRESS sequence (Fig. 2), spectral width 1502.4 Hz, acquisition size 2048, TR 2500 ms, TE 35 ms and 256 averages. Snapshot FLASH imaging combined with one-coil continuous arterial spin tagging was used to acquire quantitative perfusion images [4], with labeling time 1.5 s, FOV 4 cm×4 cm, matrix size 128×128, slice thickness 2 mm, TR 7.8 ms, TE 2.9 ms and 192 averages. T_2-weighted imaging was performed with TR 3000 ms and 6 echoes having TE ranging from 20 ms to 120 ms. Diffusion weighted images were acquired with TR 3000 ms, TE 51 ms, and three b values (28.8, 399.5 and 1266.4 s/mm^2) with δ 25 ms and Δ 10 ms.

Results Co-injection of WT and GFX into the lateral ventricle induced AD-like tau hyperphosphorylation in hippocampal CA3 region at 48 hrs after injection (Fig. 1 A). The results of Morris water Maze test showed that the AD-like rats had an increased latency to find the hidden platform at 48 hrs (Fig. 1 B). No statistically significant differences were found in quantitative hippocampal T_2, apparent diffusion coefficient (ADC) and CBF (Fig. 3) values between the AD-like group and the sham group. Compared to the sham rats, the AD-like rats showed significantly reduced Cho/Cr and Cho/NAA ratios in the Hip (Fig. 2).

Discussion Co-injection WT and GFX into the lateral cerebroventricle induced tau hyperphosphorylation in rat hippocampus and spatial memory deficiency at 48 hrs after injection. Hippocampal tau hyperphosphorylation does not result in significant changes in T_2, ADC and CBF, but is associated with reduced Cho/Cr and Cho/NAA ratios in the region. Our findings are consistent with previous results showing that the Cho peak intensity, measured by in vivo ^{1}H MRS, is decreased in the medial temporal lobe of mild cognitive impairment (MCI) and AD patients [5-6]. However, it has also been reported that there was no significant change of the Cho level but a significant decreased NAA/Cr ratio in the hippocampus of MCI and AD patients [7-8]. It is likely that the changes of NAA/Cr and Cho/Cr ratios in the MCI/AD patients are specific to brain regions and influenced by the stage of dementia. In our study, WT and GFX induced reduction of Cho signal intensity in the hippocampus appears to be a result of acute and reversible tau hyperphosphorylation in the absence of extensive neuronal degeneration.

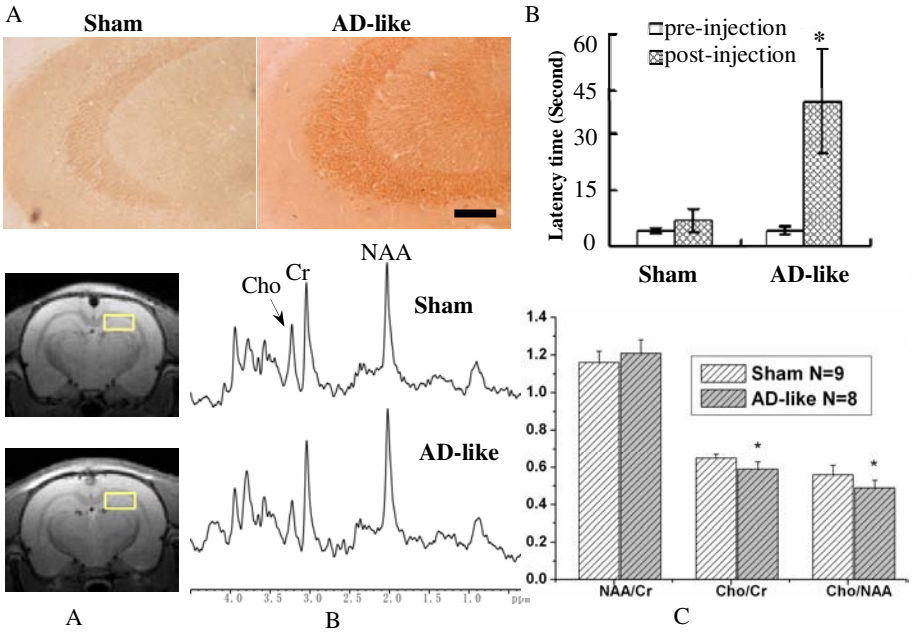

Figure 1 (A) The AD-like rats received co-jection of WT and GFX showed much stronger density of immunohisotlogical stain of phosphorylated tau in the CA3 regions of the hippocampus than the sham rats at 48 hrs after injection. The length of the black bar is 150 μm. (B) The rats were trained for 7+1 days in a Morris Water Maze pre-injection. The results of memory retention test showed the AD-like rats had an increased latency to find the hidden platform at 48 hrs after injection of WT+GFX, while no such significant difference was found in the sham-rats between pre-injection and post-injection. *p < 0.01 vs. the sham group.

Figure 2 (A) T_2-weighted images of brain slices at the level of hippocampus showing the locations of the voxels (3 mm×1.5mm×3 mm) used in ^{1}H MRS; (B) ^{1}H spectra acquired from the hippocampus of a sham rat and an AD-like rat; (C) Compared to the shame rats, the AD-like rats had significantly reduced Cho/Cr and Cho/NAA ratios (*p<0.005), but not the NAA/Cr ratio.

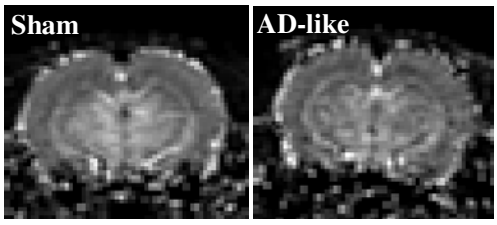

Figure 3 Quantitative perfusion images of brain slices at the level of hippocampus acquired from a shame rat and an AD-like rat.

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