Investigation of Tissue Plasticity following Low-Dose Amphetamine Treatment in Transient Ischemic Rat Stroke Model Using Diffusion Tensor Imaging

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Introduction: Amphetamine is a potent psychomotor stimulant that induces neuronal release of norepinephrine, dopamine and serotonin [1]. Considerable evidences have suggested that amphetamine can improve functional outcome in animal models of stroke, which is involved in the mechanisms of induced axonal growth and reinnervation of brain tissues [2]. Although enhanced neural sprouting, synaptogenesis and behavioral recovery following amphetamine therapy have been shown in rat stroke models using histomolecular and behavioral analysis [3], information regarding the changes in noninvasive diffusion tensor imaging (DTI) after amphetamine treatment is limited. DTI has been increasingly used in animal studies to evaluate brain plasticity after treatment of stroke [4] due to its high sensitivity in detecting microstructural changes in white matter. In the present study, we investigate the effects of amphetamine on a rat stoke model by evaluating tissue reorganization in perilesional areas using DTI technique.

Materials and methods: A total of sixteen male Sprague-Dawley rats weighting 250-350 g were included in this study. Bilateral common carotids were ligated with nontraumatic arterial clips first and the right MCA was ligated with a 10-O suture to generate focal infarction in the cerebral cortex. The ligature and clips were then removed after either 60-min (8 rats) or 90-min (8 rats) ischemia to allow reperfusion. All animals received either amphetamine (2 mg/kg intraperitoneal, n=4 for 60-min and n=4 for 90-min ischemia) or vehicle (10 % DMSO in saline, n=4 for 60-min and n=4 for 90-min ischemia) injections at 3, 6, 9, 12, 16, 18, 21and 24 days, respectively, after stroke and were subjected to serial MRI measurements at 2, 10 and 25 days, respectively, after stroke using a Brucker 9.4T animal MRI scanner. Rats were imaged on day 2 using T2-weighted imaging (T2WI), day 10 and 25 using T2WI plus DTI, under isoflurane anesthesia (3% for induction and 1.8% for maintenance) in air / O_2 (80:20). T2WI were acquired with field of view (FOV) = 3.2 cm^2, matrix size = 128×128 (zero-filled to 256×256), repetition time (TR) = 2750 ms, echo time (TE) = 13.3 ms, and 23 slices with 1 mm thickness. DTI were acquired with a spin echo single-shot echo planar imaging sequence, 19 slices with 1mm thickness, FOV = 3.2 cm^2, matrix size = 96×96, TR = 9500 ms and TE =38 ms. Thirty diffusion-weighted images along independent orientations (b=1000 s/mm^2) and 1 baseline image (b=0) were acquired for each slice, and the acquisition was repeated three times to improve signal-to-noise ratio. The total acquisition time was approximately 40 min. Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were derived using dTV software (Image Computing and Analysis Laboratory, Department of Radiology, University of Tokyo Hospital, Tokyo, Japan). As the lesion size may be a confounding factor interfering with the treatment effects of amphetamine, we divided the rats into the treated and control groups according to similar lesion size measured from the T2WI acquired on day 2. A region-of-interest (ROI) was manually drawn within the perilesional hyperintense region on each of the lesion-containing slice shown on the FA map due to its relatively clear boundary (Fig. 1). For comparison, an additional ROI was placed in the area of contralateral white matter (external capsule, Fig. 1). The ROIs were then placed on identical sites on the images with b=0 (b0), images with b=1000 s/mm^2 (b1000) and ADC maps. After normalizing signal from the perilesional region to the contralteral white matter, the normalized signals were compared between the amphetamine-treated and vehicle-treated groups for 60-min and 90-min ischemia at day 10 and 25 using two-sample t-test. A p value < 0.05 was considered statistically significant. For Western analysis, detection of synaptophysin was performed using IRdye anti-mouse antibody (1:5000, IR800 Rockland, IR680 Odyssey) for 1 hour and scanned by the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE). The density of fluorescent was measured using Scion image analysis.

Results: The lesion volume (relative to the total brain volume) on day 2 after stroke determined from T2WI for the amphetamine-treated and control groups with 60 min ischemia were 8.93 ± 3.37 % and 7.85 ± 3.11 %, respectively. For groups with 90 min ischemia, the lesion volume on day 2 after stroke were 9.11 ± 1.72 % and 10.35 ± 2.99 % for amphetamine-treated and control groups, respectively. There was no significant difference in lesion volume among these groups. The normalized FA in the amphetamine-treated group on day 10 were 1.44 ± 0.24 (60-min ischemia) and 1.53 ± 0.21 (90-min ischemia), which were not significantly different than corresponding control groups (1.16 ± 0.17 and 1.19 ± 0.20 for 60-min and 90-min ischemia, respectively). However, on day 25 (Fig. 2 and 3), the normalized FA was significantly higher in the amphetamine-treated groups than the control groups for both 60-min and 90-min ischemia (1.60 ± 0.11 vs. 1.05 ± 0.06 for 60-min, P=0.0004 and 1.86 ± 0.40 vs. 1.11 ± 0.11 for 90-min ischemia, P=0.03). For both 60-min and 90-min ischemia, there is no significant difference of b0, b1000 and ADC between amphetamine-treated and control groups on day 10 and 25. In the Western analysis, synaptophysin expression was significantly higher in the amphetamine group (Fig. 4).

Discussion and conclusions: In this study we used DTI to assess plasticity of perilesional tissue following amphetamine treatment in a rat stroke model. For both groups with 60-min or 90-min ischemia, the amphetamine-treated animals showed higher FA values in areas surrounding the infarction, compared with the control groups. These findings suggest that amphetamine induces axonal growth and reinnervation of tissue around the lesion. We further showed that the synaptophysin expression was significantly higher in the amphetamine-treated group, consistent with the imaging findings. Previous studies have demonstrated that amphetamine administration increased synaptic connections and animals treated with amphetamine after stroke showed improvements in a skilled reaching test [3]. Taking together, FA might be used as a noninvasive maker to reflect the level of neural regrouth and to monitor the progress of stroke recovery.

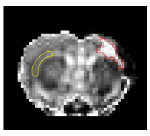

Fig. 1 ROI placement in regions of perilesion (red) and contralateral white matter (yellow).

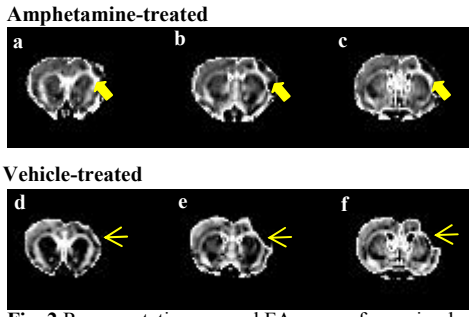

Fig. 2 Representative coronal FA maps of an animal from the amphetamine-treated group on day 25 (a-c) and an animal from the vehicle-treated group on day 25 (d-f). Animal from the amphetamine group demonstrated significantly higher FA values in perilesional areas (arrowhead) as compared to the vehicle group (arrow).

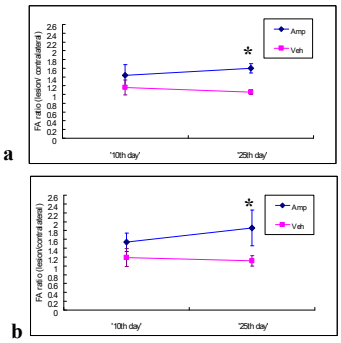

Fig. 3 Time course changes of normalized FA in animals with (a) 60-min and (b) 90-min ischemia (*P<0.05). Amp: amphetamine-treated; Veh: vehicle-treated.

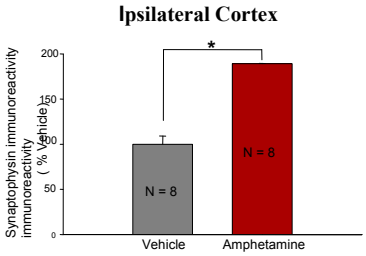

Fig. 4 Synaptophysin from all stroke animals was normalized to the vehicle-treated group. Synaptophysin expression was significantly higher in the Amphetamine-treated group. * P<0.05.

References: [1] Papadopoulos CM, et al., Stroke 2009;40: 294-302. [2] Stroemer RP, et al., Stroke 1998; 29: 2381-2395. [3] Ramic M, et al., Brain Res 2006; 1111: 176-186. [4] Li L, et al., Stroke 2009; 40:936-941.