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 stroke model by evaluating tissue reorganization in perilesional areas using DTI technique.

Materials and methods: A total of sixteen male Sprague-Dawley rats weighing 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. 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 slices of the images with b=0 (b0), images with b=1000 s/mm² (b1000) and ADC maps. After normalizing signal from the perilesional region to the contralateral 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 (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 might 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 slices of the images with b=0 (b0), images with b=1000 s/mm² (b1000) and ADC maps. After normalizing signal from the perilesional region to the contralateral 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.

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 marker to reflect the level of neural regrowth and to monitor the progress of stroke recovery.