Temporal Evolution of 3-Nitropropionic Acid-Induced Neurodegeneration in the Rat Brain by $T_2$-Weighted, Diffusion-Weighted, and Perfusion MRI

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Introduction A hypothesized mechanism in neurodegenerative diseases is that impaired energy metabolism may underlie secondary excitotoxic neuronal death. Systemic administration of 3-nitropropionic acid (3NP), an irreversible mitochondrial toxin, produces striatal lesions in rats and non-human primates with symptoms mimicking Huntington's disease (HD) (1). Consequently, the in vivo characteristics of the temporal evolution and spatial distribution of the 3NP-induced lesions will be valuable for the evaluation of pathological stages and therapeutic efficiencies in HD. Thus, the first aim of the present study was to determine the time course of signal intensity changes on the diffusion-weighted image (DWI) and $T_2$-weighted image (T2WI) in different brain areas on the 3NP-induced HD model. A determination of the correlation of 3NP-induced structural alterations with regional cerebrovascular circulation was also desired for further pathophysiological insight of HD. Therefore, the second aim of this study was to measure the changes of regional cerebral blood volume by dynamic susceptibility contrast (DSC) imaging using the same animal model.

Materials and Methods Four to five-month-old Sprague-Dawley rats ($n = 5$) were anesthetized with sodium pentobarbital (Abbott, 60 mg/kg, i.p.). One femoral vein was cannulated with a catheter for drug administration and fluid supplement, and an endotracheal tube was set for artificial ventilation. The expiratory CO2 concentration was monitored by a capnometer and maintained at 3.5–4.5% by adjusting the tidal volume and ventilation rate. A muscle relaxant gallamine was given to avoid spontaneous ventilation and movement (Sigma, initial dose: 12 mg, maintaining dose: 6 mg/h, i.v.). A bolus injection of 3NP (Sigma, 30 mg/kg) was intravenously administered.

Experiments were performed on a 4.7 T spectrometer with an active shielding gradient (6.9 G/cm in 500 μs) and a 70 mm birdcage coil. The rat was placed in a prone position with a custom-designed head-holder. Images were obtained using a 5 cm FOV, 4-slice (2 mm thick with 0.5 mm skip), and 256 x 128 matrix. Fast spin-echo sequence was applied for $T_2$-weighted MRI with TR = 4000 ms, TE = 80 ms, and echo train length = 8. For diffusion-weighted MRI, the Stejskal-Tanner sequence was employed (TR = 2000 ms, TE = 59 ms, δ = 20 ms, Δ = 27 ms, and b-value = 1300 s/mm2). The control images of DWI and T2WI were acquired prior to 3NP administration. Two and half min post injection of 3NP, sequential DWI and T2WI was continuously performed for 8 hr. In a separate preparation ($n = 7$), the DSC MRI was performed 5 min before and 4 hr after 3NP administration. A series of 30 single-slice gradient-echo images with a 256 x 64 matrix, TR = 30 ms, TE = 10 ms, and $α = 15°$ were acquired. The bolus of susceptibility contrast agent gadopentetic acid (Scherin, 0.3 mmol/kg, i.v.) was injected 10 sec after the start of image acquisition. Data processing was performed with a commercial image analysis software (MRVision). The signal-to-noise ratios (SNR) in the striatum, hippocampus, and cortex were assessed. The relative cerebral blood volume (rCBV) maps were generated by the integral under the $Δ^2$ transit curves. Statistical analyses were performed using paired Student t-test to compare control and 3NP-treated data. P < 0.01 was considered statistically significant.

Results Intravenous injection of 3NP consistently produced bilateral brain lesions. Fig. 1 shows representative images obtained from the DWI and T2WI at the indicated time points. Fig. 2 illustrates the signal intensity (SI) as a function of time post systemic 3NP administration. The SIs on the DWI in the striatum and hippocampus were elevated by 30% at 195 min after 3NP administration and progressively increased to 80% at 270 min at which point they reached plateau. On the T2WI, 30% SI were observed in the same areas as DWI but not until 295 min, and reached 80% at 450 min. The results demonstrated that DWI is superior to T2WI in detecting early onset of excitotoxic injuries. SI in the cortex did not change in either the DWI or T2WI throughout the experimental period (Figs. 1 and 2). Increases in perfusion were seen following 3NP administration. Striatal rCBV was significantly increases by 77 ± 21% (p < 0.01) 4 hr post 3NP injection. Cortical rCBV was changes by 28 ± 22% without significance (p > 0.5).

Discussion We observed significant increases in SIs in the striatum and hippocampus after injection of 3NP but not in the cortex. The results are consistent with the another report that morphological injuries were found in the striatum and hippocampus but not in the cortex of 3NP-intoxicated rats (2). The early detection of striatal and hippocampal lesions on the DWI is believed to reflect a shift of water from the mobile extracellular environment to the immobile intracellular region. This shift may occur when trans-membrane ion gradients are disturbed by energy failure and/or excitotoxic injury. The results indicate that the DWI is useful for the early detection of excitotoxic lesions such as those present in ischemic, hypoglycemic, and kainate-induced brain injuries.

As a result of 3NP-intoxication, the striatal rCBVs from perfusion MRI were significantly increased to 77%. It has been reported that the effects of 3NP administration not only reduce the arterial bicarbonate and pH (3) but also increase the striatal lactate (4). Reduction of regional or systemic pH causes cerebrovasodilatation. We found that cortical rCBV was not significantly altered by 3NP administration which matches well with the findings of our cortical investigations using diffusion-weighted and $T_2$-weighted MRI. Thus, the combined structural and functional information performed by $T_2$-weighted, diffusion-weighted, and DSC MRI in the present study may provide new insights and therapeutic strategies in neurodegenerative diseases.

References