MRI and MRSI characterization of the Quinolinic acid lesion model of Huntington's disease over 49 days: Persistence of low apparent diffusion coefficients and spontaneous recovery of N-Acetyl Aspartate levels

N. Shemesh[1], O. Sadan[2], E. Melamed[2], D. Offen[2], and Y. Cohen[1]

[1]School of Chemistry, Tel Aviv University, Tel Aviv, Israel, [2]Laboratory of Neurosciences, FMRC, Rabin Medical Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction. Huntington's disease (HD) is a hereditary neurodegenerative disease characterized by a progressive degeneration of the striatum. An important animal model for HD is the striatal injection of Quinolinic acid[1] (QA). QA induces neuronal damage via NMDA receptor activation and probably also by endogenous glutamate release that results in excitotoxicity of the affected neurons, mostly the medium spiny striatal neurons. Therefore, the striatal injection of QA in rodents leads to a slow striatal degeneration that mimics the earlier stages of HD[2]. Although this toxin-induced model does not carry the genetic background of HD, it is easily induced and highly reproducible, and is thus used in therapeutic oriented studies of HD. Comprehensive longitudinal MRI and MRSI studies have not been performed yet on the QA model, although a few MR studies showed the evolution of the lesion[3] while others showed NAA levels in a dose response manner in one time point[4]. In this study, we preformed *in-vivo* multiparametric MRI and MRSI studies that extended over 49 days to assess the spatial and temporal evolution of the neuropathology. We tested the relevance of our findings to the behavioral studies of the rats, and finally preformed the end-point histology analysis to validate the MRI findings.

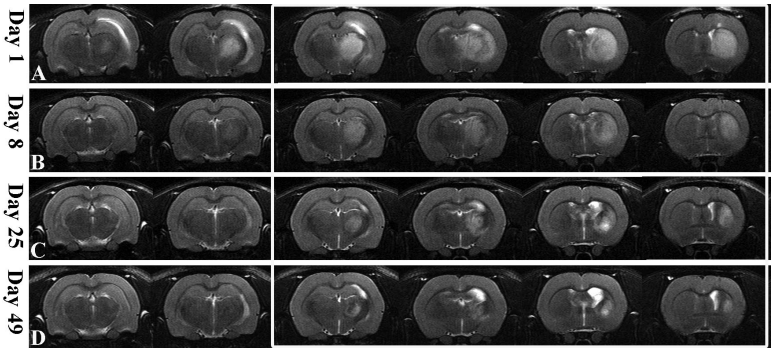

Figure 1

Materials and Methods. 150nmol of QA was injected to the left striatum of male Wistar rats weighing ~280gr (n=6). Controls (n=3) were treated with PBS. MR studies were performed on a 7.0T/30cm horizontal bore Bruker Biospec MRI scanner on days 1, 8, 25 and 49 post QA-injection. A body coil was used as the transmit coil, and a rat quadurature coil was used as the receiving coil. The MRI experiments consisted of 2D T_2 weighted images acquired with the following parameters: RARE8 TR/TE = 3500/75ms with an in-plane resolution of 100x100(μm^2) and a slice thickness of 1mm, and diffusion weighted images TR/TE=2800/54.5ms, Δ/δ=40/4.5ms, 2 points with b values of 0 and 1500s/mm^2, and a resolution of 200x200(μm^2) with a slice thickness of 1mm. The MRSI study was performed by a water suppressed 2D MRSI scan with TR/TE=2000/135ms, field of view of 2.56cm isotropic, the matrix size was 8x8 zero filled to 16x16, with a slice thickness of 4mm. Normalized N-Acetyl Aspartate (NAA) values were calculated by normalizing the NAA levels in the damaged side to a representative voxel in the same row in the contralateral side. Total striatum NAA levels were calculated by combining normalized NAA levels from all of the voxels in the striatum. Histology (CD68 staining) was performed on day 70.

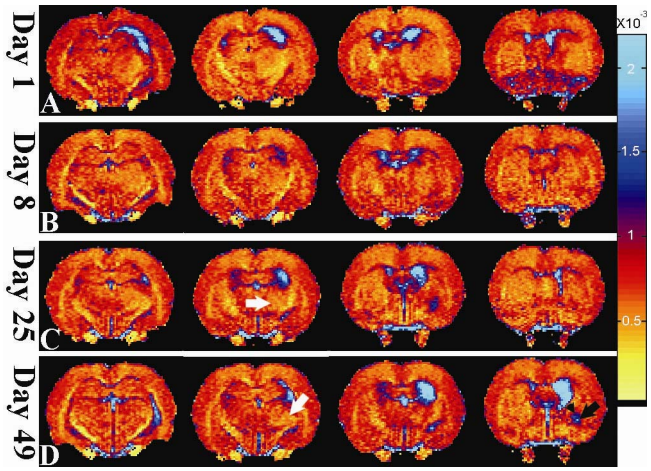

Figure 2

Results. Figure 1 shows the longitudinal coronal T_2 weighted images from one representative rat post-QA injection (only 6 of the 10 slices acquired are shown). In these images, the affected areas, found mostly in the striatum, appeared hyperintense. One day post QA injection (Figure 1A), a mild enlargement of the ipsilateral ventricle could be seen. The hyperintensity in the striatum slowly receded with time, and on day 25 the lesion appeared smaller and less intense (Figure 1C). Forty nine days after QA injection (Figure 1D), only 4 slices revealed some hyperintensity in the striatum. Comparing the apparent diffusion coefficients (ADC) maps (Figure 2) to the T_2 weighted images revealed a complex behavior. One day post QA injection the lesion was less apparent in the ADC maps as compared to the T_2 weighted images, and could be seen in only 3-4 slices as areas of low ADCs (Figure 2A). On day 8, the lesion was still distinguishable (Figure 2B), and after 25 and 49 days, two types of diffusion abnormalities were observed - a low ADC area (white arrow) and a high ADC area (black arrow) (Figure 2C and D respectively). A rim of intermediate ADC value can be seen around the region with high ADCs, indicating ongoing processes around the necrotic region (black arrowhead). The histological study revealed that the areas characterized by low ADCs corresponded to a dense positive stain for CD68, i.e. macrophages were detected. Areas characterized by high ADCs corresponded to necrotic regions. Figure 3 depicts the total-striatum normalized NAA levels between days 1 and 49 post QA injection. On day 1, total-striatum normalized NAA levels were reduced to 0.67±0.15 of the contralateral value. On day 8, the total-striatum normalized NAA levels did not show a statistically significant difference compared to day 1 and remained at 0.68±0.17. On day 25, a statistically significant partial recovery of total-striatum normalized NAA levels to 0.81±0.12 was observed. Forty nine days after QA injection, total-striatum normalized NAA levels further increased to 0.90±0.12, significantly different from total-striatum normalized NAA levels on days 25, 8 and 1 post QA injection. Total-striatum normalized NAA levels from the control group (injected with PBS) were found to be 1.00±0.06 (n=3), *P<0.0001 vs. day 1, $^{\$}$P<0.0001 vs. day 8 and $^{\&}$P<0.001 vs. day 25. We have also found differential improvement rates of NAA for voxels that were more severely impaired on day 1 (data not shown).

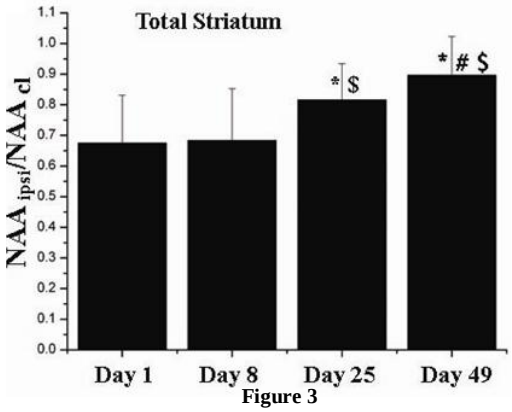

Figure 3

Conclusions. In this study, we have shown that the QA lesion model exhibits ongoing pathological processes over a period of at least 70 days post QA injection as deduced from MRI and MRSI parameters. We found both high and low ADCs in the striatum 49 days post QA injection, suggesting areas of different types of damage. These findings were corroborated by the histological findings, which showed that the necrotic regions were characterized by high ADCs, while the areas characterized by low ADCs were found to correlate with the high density of macrophages (CD68+). The MRSI study demonstrated the spontaneous regeneration of NAA levels in the striatum over 49 days. We therefore suggest that future studies in which the QA rat model is used for seeking new treatments for HD should take into consideration the spontaneous recovery that occurs in this model.

References: [1] Shwarcz R et al., Science (1983) 219: 316–318. [2] Alexi T et al., Prog. Neurobiol. (2000) 60: 409-470, [3] Guzman R et. al., Exp. Neurol. (1999) 156: 180-196 [4] Tkáč I et al., J. Neurosci. Res. (2001) 66: 891-898