An MRI-based method to quantify apoptosis in vivo

Chenchen Liu[1], Nuri B Farber[2], Joel R Garbow[3], and Joseph JH Ackerman[4]

[1]Chemistry, Washington University in St.Louis, St. Louis, MO, United States, [2]Psychiatry, Washington University in St.Louis, St. Louis, MO, United States, [3]Radiology, Washington University in St.Louis, St. Louis, MO, United States, [4]Chemistry and Radiology, Washington University in St.Louis, St. Louis, MO, United States

Introduction: Apoptosis, also known as programmed cell death, is a cell-eliminating process in which unhealthy or unneeded cells are culled in a controlled manner. About 60 billion cells (~1% of all cells in the human body) die through apoptosis in the human body every day. Apoptosis is therefore a major aspect of human biology. Currently, histology (caspase-3 and silver staining) is the gold-standard method for qualifying / quantifying apoptosis. However, histological techniques require resection and destruction of the tissue, thus longitudinal pre-clinical and clinical studies are infeasible. MRI may offer a non-invasive / non-destructive and translatable means to detect and quantify apoptosis.

A novel rodent model of pure apoptosis has been reported by Ikonomidou and Olney.[1,2] In this model, ethanol administration to neonates results in histology-validated apoptosis in the brain, predominantly in the cingulate cortex and hippocampus regions. While challenging to employ, given the small size and fragility of rodent pups, this rodent model of pure apoptosis offers the opportunity to test the apoptosis-identifying / -differentiating ability of various MRI contrasts. We have initiated such studies by taking advantage of the high sensitivity afforded by pre-clinical MRI at 11.74 T.

Methods: Apoptosis was induced by administering 20% ethanol (EtOH) in normal saline to post-natal day 7 (P7) Sprague-Dawley rats in two 2.5 g EtOH/kg doses spaced two hours apart. The subjects were anesthetized with isoflurane/O_2 (2%) and monitored with temperature/respiration probes throughout the experiments. A birdcage coil (~4 cm diameter) was used as transmit and receive coil. T_1-weighted, gradient echo multi-slice transaxial images were collected (TR = 0.25 s, TE = 0.004 s, FOV = 20.0 × 20.0 mm^2, slice thickness = 0.5 mm) on an 11.74 T scanner 4h, 6h, 8h, and 10h after the first EtOH dose was administered. A control group was administered saline instead of EtOH but was otherwise treated with the same procedure.

Results: We observed hyper-intensity at the cingulate cortex region on T_1-weighted images 4h after the first injection (Fig. 1), which is consistent with apoptotic regions observed by histology (Fig. 2). No such signal enhancement was seen under control (saline) conditions. ROI analyses (in which cortical signal was normalized to unaffected muscle signal) showed that the T_1-weighted cortical MRI signal was 23% greater following EtOH administration than observed for saline-treated control.

Discussion and Conclusion: In this pilot study, we observed a 23% signal enhancement in apoptotic cingulate cortex with T_1-weighted imaging 4h after injection. We speculate that this signal enhancement is due to macromolecular and microstructural changes (cell shrinkage and macrophage congregation), which are known to be associated with apoptosis.[3, 4] These initial data are encouraging, as detection of the early onset of apoptosis by MRI would be a significant achievement. Ongoing experiments are employing multiple-contrasts, including magnetization-transfer and diffusion-weighted MRI, to provide greater sensitivity to the immediate downstream effects of apoptosis. The need for greater signal-to-noise sensitivity with such small rodent subjects (rat pups) has motivated development of an actively decoupled surface-coil receive / volume-coil transmit system in place of the single-coil mode volume coil used in examining initial subject cohorts.

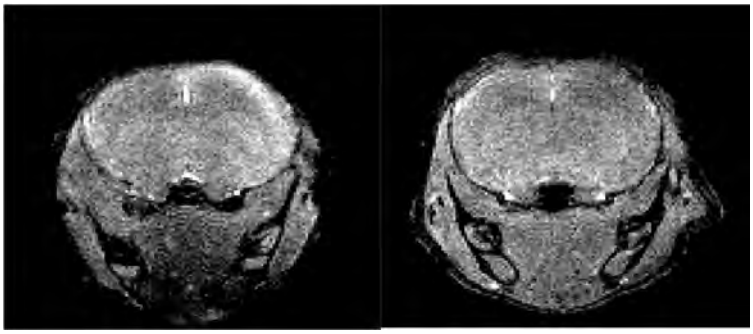

Figure 1 - T_1-weighted images at 4h following the first EtOH injection (left) or saline injection (right)

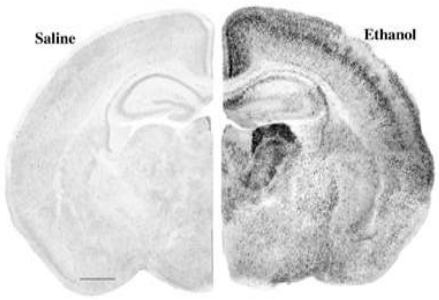

Figure 2 - Histological sections from 8-day-old mouse brain 24h following subcutaneous treatment with saline (left) or ethanol (right)

References

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