Detection of Apoptotic Neuronal Death in Live Animals using MR Imaging

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Introduction Apoptosis is a naturally occurring process in all multi-cellular organisms but can also be induced to occur earlier in cellular life span when there is a change in the environment, especially during oxidative stress or neurodegeneration. Apoptosis is characterized by chromatin condensation, nuclear DNA fragmentation, elevations of apoptosis-specific antigens, nuclear fragmentation and the appearance of apoptotic bodies without the disruption of cell membrane. Apoptotic neurons generally are detected using biopsy or autopsy samples in vitro. We have developed a contrast probe based on the autonomous uptake of short, single stranded DNA (ODN) by neural cells. This ODN has a complementary sequence to c-fos mRNA[1], and can be conjugated to a susceptibility MR contrast agent, MION[2] or CLIO via avidin-biotin linkage. The hypothesis is that live neurons will take up and retain MION-ODN conjugates; dying cells will not. We induced neuronal apoptosis in the brain using global stroke/heart attack in C57black6 mice; this model has been shown to induce neuronal apoptosis in the hippocampus[3-5].

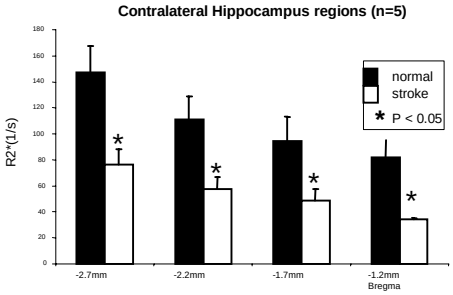

Fig 1.R_2* from hippocampus in live animals w/wo BCAO and infused with MION-ODN.

Methods Male C57black6 mice were anesthetized using katamine (100 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.). Cerebral ischemia was induced with the transient occlusion of the bilateral CAO for 60 minutes, after which the occlusion was released for blood reperfusion (≥ 7 days). Animals were infused with the contrast probe (Fe = 1.6 umoles/kg, ODN = 13 nmoles/kg in 2ul sodium citrate) via ICV route (LR:-1; AP:-0.2; DV:-3 mm, bregma). After 2 days uptake, we acquired MR images in live animals (with pure O_2 plus 2% halothane [800 ml/min flow rate]) using a 9.4Tesla magnet. We acquired T2* maps using serial GEFI sequences (TR/TE=500/2.3, 3, 4, 6, α=30) and T2-weighted RARE sequence (TR/TE=5000/15). The animals were sacrificed after MRI for histological confirmations of neuronal death using MR microscopy using a 14T magnet (3D FLASH, TR/TE=50/18, α=20), followed by Nissl or TUNEL (terminal UTP nick-end labeling) stains.

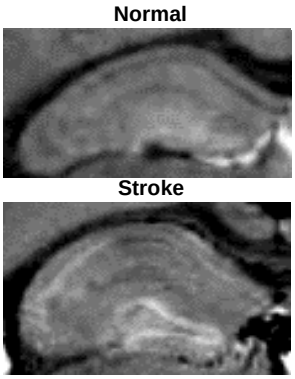

Fig 2.MR images of postmortem confirmation on MION-ODN retention in the hippocampus

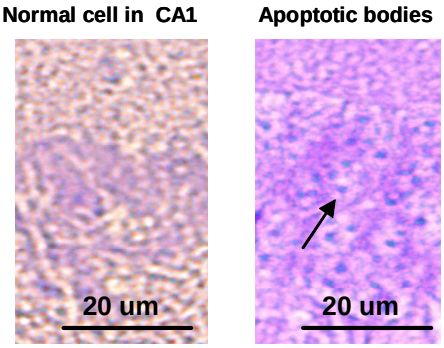

Fig 3 Nissl stains after MR image in Fig 2 showing the presence of the multiple nuclei in CA1 neurons 10 days after stroke (right) as compared to that of the normal brain (left)

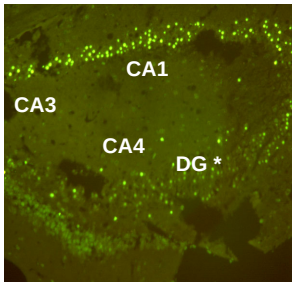

Fig 4.TUNEL stain in the hippocampus 2 days after BCAO

Results No evidence of necrosis was visible in T2 weighted images of the brain. Figure 1 shows R2* values (mean and SE) in the contra-lateral hippocampus of 5 animals 7 days after a global stroke. We observed a significantly reduced level ($p < 0.05$) of MION-ODN in the contra-lateral hippocampus after stroke (open bars) compared to the normal brains (filled bars). Figure 2 shows MR images of the *Cornu Ammonis* from the 14Tesla magnet. We observed the presence of MION in normal animals (n=4); we noted the absence of MION, particularly in the dentate gyrus and CA formations of the stroke brain. Figure 3 shows histological evidence of nuclear fragmentation in CA1 neurons after stroke. Figure 4 shows the location of neurons with DNA fragmentation during early apoptosis using a conventional postmortem TUNEL staining. These data are consistent with the hypothesis that apoptotic neurons do not retain MION-ODN.

Conclusion The absence of MION-retention in hippocampal neurons after stroke indicated (1) an inability to retain MION probe and/or (2) no intact target (mRNA transcript and genomic DNA) available for binding by the reporter probe in these apoptotic neurons. Our results suggest this contrast probe allows the MR detection of apoptosis in live animals (supported by R01NS45845 and MIND).

References cited 1. Cui et al., (1999) J. Neurosci 19:1335-44; 2. Shen et al., (1993) MRM 29:599-604; 3.Borane et al., (1993) J Cereb Blood Flow Metab 13:683-92. 4. Liu PK, et al., (1996) J. Neurosci 16:6795-806. 5. Fijii, et al., (1997) Stroke 28:1805-10.