Characterization of a 60-minute MCAO using immunohistochemical evaluation in conjunction with MRI

Lora Talley Watts[1], Qiang Shen[1], and Timothy Duong[1]
[1]*Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States*

Target Audience: Researchers studying stroke.

Introduction: Perfusion and diffusion MRI has become the method of choice for early detection of acute stroke and for longitudinal evaluation of therapeutic efficacy. However, the underlying immunochemical changes associated with perfusion and diffusion abnormality remains poorly understood. The goal of this study was to investigate the expression of an early and late marker of the apoptotic cascade using antibodies against caspase-3 and neuronal cell death (TUNEL and Fluoro-Jade) associated with perfusion and diffusion abnormality at 3 and 24hrs after stroke.

Methods: Male Sprague-Dawley rats (250-300g) underwent 60-min middle cerebral artery occlusion (MCAO) under 1.5-2.0% isoflurane (n=2). MRI (CBF and ADC) was acquired at 3 and 24 hrs using a Bruker 11.7-T/16-cm scanner (1). Cryostat sections were stained from the same rat to determine the extent of induction of the apoptotic cascade (caspase 3) and resulting neuronal cell death (TUNEL and Fluoro-Jade staining) following MCAO at 3 or 24 hours using standard immunohistochemical methods. The expression of the three markers were quantified and correlated with ADC and CBF from carefully co-registered ROIs which were selected from different regions of the brain based on the 3 hour data to represent: i) the control left hemisphere, ii) the ischemic core, iii) a region with perfusion-diffusion mismatch. In addition, the expression of the three markers were also analyzed for the ischemic core, mismatch and normal pixels, defined at 3 hrs using the critical ADC threshold ($0.53\pm0.03\times10^{-3}$ mm^2/s) and CBF threshold (0.30±0.09 ml/g/min) below which is destined to infarct (1).

Results & Discussion: **Figure 1A** shows typical ADC and CBF maps 3 and 24 hrs post MCAO. Most of the mismatch infarcted at 24 hrs. Typical images of caspase-3, TUNEL and Fluro-Jade staining are shown for the core (red square), mismatch (green square) and normal (red square) ROIs (Figure 1B). Normal tissue demonstrates negligible expression of the three markers. The expression of caspase-3 progressively increased with time. The ischemic core showed greater expression compared to the mismatch. Fluro-Jade and TUNEL expression were slightly elevated at 3 hrs in both ischemic core and mismatch. Fluro-Jade and TUNEL expression increased by 24 hrs with the greatest expression found in the ischemic core.

Figure 2 shows the regression of ADC or CBF values versus the expression of the three markers. All correlations were negative, indicating that reduced CBF or ADC showed increased expression of stress and cell death markers. In addition, the slopes of the 3 and 24 hrs data sets were significantly different for TUNEL and Fluro-Jade ($p<0.05$, ANOVA), but not for caspase-3 ($p>0.05$). These findings suggest cell death increases with time although ADC and CBF were similar at the two time points, whereas the apoptotic markers were not different between the two time points.

Figure 3 shows the data binned into the three tissue types. Normal tissue showed negligible expression of apoptotic and cell death markers at both time points. Caspase-3 was elevated at 3hrs and reduced slightly at 24 hrs in the core and mismatch tissue. Both mismatch and ischemic core showed increased cell death and neuronal degeneration with time.

Conclusion: We found a strong correlation of ADC and CBF values with molecular markers of apoptosis, neuronal degeneration and cell death in the acute stroke phase and endpoint where final infarct was defined. The data demonstrates the progression of lesion into the mismatch area within twenty-four hours. These findings provide molecular underpinnings of quantitative prediction of ischemic tissue fates and functional reorganization based on acute MRI data.

References: 1) Shen Q, et al., JCBFM, 2003; 23:1479.

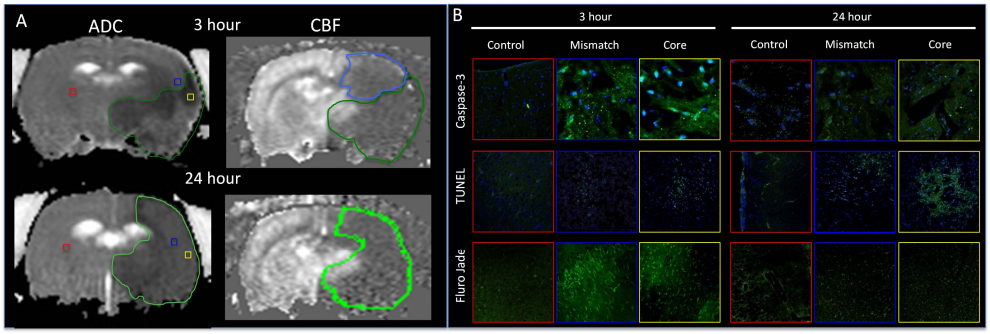

Figure 1. A) Representative ADC and CBF maps 3 and 24 hours post MCAO. ROI are outlined as core (green) and mismatch (blue) with the remaining region considered healthy. **B)** Within the selected ROIs images were taken in the area represented by the square boxes of Caspase-3, TUNEL and Fluro Jade immunostaining.

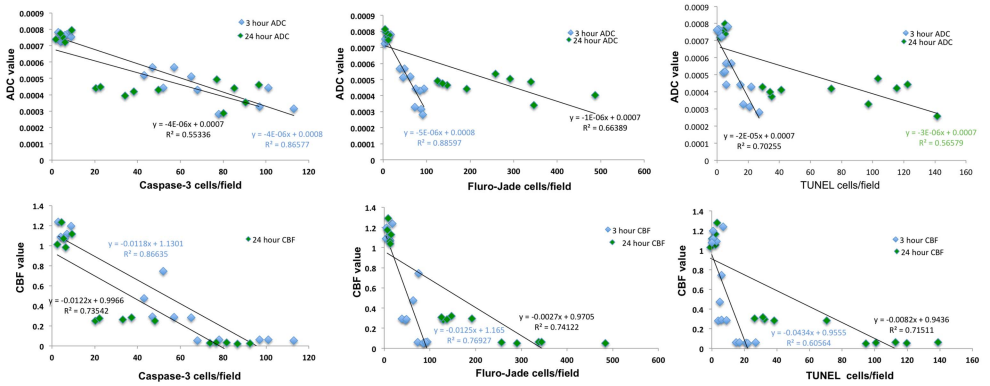

Figure 2. A) Regression analysis of CBF values and the average number of Caspase-3, TUNEL or Fluro Jade cells per field at 3 and 24 hours post stroke. **B)** Regression analysis of ADC values and the average number of cells positive for Caspase-3, TUNEL or Fluro Jade in each ROI 3 and 24 hours post MCAO.

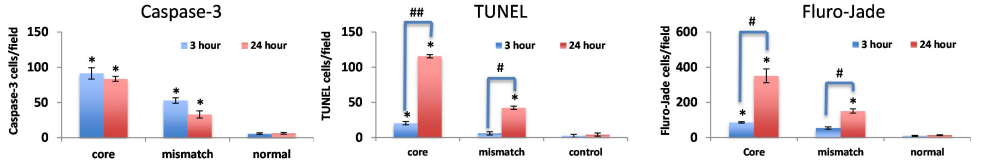

Figure 3. Quantification of immunomarkers (Caspase-3, TUNEL, and Fluro-Jade) defined by MRI signatures shown in Figure 1. Five regions in each ROI were imaged and averaged together. * = $p>0.05$ compared to control tissue; # = $p<0.05$ or ##= $p<0.001$ compared to 3 hours