Quantitative Assessment of Temporal Changes in the “Perfusion/Diffusion Mismatch” Following Focal Cerebral Ischemia in the Rat Brain

X. Meng1, Q. Shen1, F. Li1, M. Fisher2, C. H. Sotak1, T. Q. Duong1

1Center for Comparative NeuroImaging, University of Massachusetts Medical School, Worcester, MA, United States, 2Dept. of Neurology, Umass Memorial Medical Center, University of Massachusetts Medical School, Worcester, MA, United States, 3Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, United States

Synopsis
The goals of this study were to: i) establish the absolute and percent ADC and CBF thresholds for quantifying the ischemic lesion volume in association with focal cerebral ischemia in the rat brain during the acute phase; and ii) employ these thresholds to assess the perfusion/diffusion mismatch in the ischemic lesion volume as a function of time. These results were validated by using histology.

Introduction
Diffusion-weighted magnetic resonance imaging (DWI) is an effective technique for the detection of acute cerebral ischemia within minutes following the onset of stroke. Hyperintense regions on the DWI correspond to tissues experiencing a reduction in the apparent diffusion coefficient (ADC) of water, which correlate with ischemia. In a permanent middle cerebral artery occlusion (MCAO) rat model, the region of reduced ADC values is generally smaller than the region of perfusion deficit during the early acute phase (i.e., the so-called “perfusion/diffusion mismatch”). During the first hours following stroke onset, however, the region of reduced CBF gradually expands until it defines the entire ischemic territory (as defined by post-mortem histology) and coincides with the region of reduced perfusion (which generally portends a poor outcome). Although the perfusion/diffusion mismatch is readily observed in acute human stroke, similar observations in animal models of stroke have been limited and the temporal evolution of the perfusion/diffusion mismatch in a rat permanent MCAO model has yet to be investigated. Such studies would be important for assessing the extent and severity of ischemic injury as well as evaluating the efficacy of drug treatment that may retard or inhibit the temporal progression of the ischemic insult. In this study, we have developed quantitative perfusion and ADC imaging thresholds by correlating the ischemic lesion volumes obtained by the two techniques with histological infarct volumes obtained by TTC (2,3,5-triphenyltetrazolium chloride) staining. These thresholds were then used to characterize the temporal evolution of the perfusion/diffusion mismatch following permanent MCAO in the rat brain.

Method
Sprague-Dawley rats (300-350g) were initially anesthetized with chloral hydrate (400mg/kg). Surgery was performed to permanently occlude the middle cerebral artery via intraluminal occlusion. The right femoral artery was catheterized for blood gas sampling and continuous monitoring of blood pressure and heart rate. Anesthesia was switched to 1% isoflurane during MRI. The rectal temperature was maintained at 37.0±0.5°C. Imaging was performed at 0.5, 1.0, 1.5, 2, 3, and 24 hours after stroke, followed by histological TTC staining at 24 hrs. Two groups of animals were studied. In group I (n = 5, training set), ADC and CBF thresholds at 3hrs were derived by correlating the abnormal ADC and CBF volumes with TTC infarct volume. In group II (n = 6), the ADC and CBF lesion volume in a separate group of animals were evaluated as a functional of time by using the thresholds derived from group I. These results were also compared with TTC staining.

MRI was performed on a 4.7T/40cm magnet. An actively-decoupled surface coil was used for brain imaging and a neck coil for CBF labeling. Anatomical (RARE) images were acquired with TR=2s, 8 echo trains, TE=6.0ms, matrix = 256x256, FOV=2.5x1.9cm2, seven 1.5-mm slices and 4 averages. ADC(trace) was measured using spin-echo EPI with matrix=64x64, FOV=2.5x1.9cm2, and six 1.5-mm slices, TE=60ms, TR=1.5s, 4 averages, b = 5, 1200 s/mm2 along each of the 3 principle axes. CBF was measured using the continuous arterial spin-labeling technique with single-shot, gradient-echo EPI, with parameters similar to the ADC measurement except TE=17ms, and TR=2s, and 100 pairs of images were acquired for averaging. ADC(trace) and CBF images were calculated at each time point. Both quantitative volume changes with edema correction as well as percent hemispheric lesion volume (%HLV, with intrinsic edema correction) were analyzed; there was no statistical difference between the two methods, and thus only the quantitative volume method was presented.

Results
From group I, the ADC and CBF thresholds that yielded the abnormal volumes that eventually led to infarction were established and are summarized in Table 1. The normal ADC and CBF values in the left hemisphere were 0.76±0.03 x10−3 mm2/s and 0.75±0.20 ml/g/minute, respectively, consistent with those reported in the literature. The average TTC infarct volume was 199±31 mm3. The ADC and CBF thresholds that yielded the same infarct volume as TTC were 0.53±0.02 x10−3 mm2/s (30±1% reduction) and 0.3±0.04 ml/g/minute (57±5% reduction), respectively. Note that the standard deviation of CBF of the left hemisphere was substantially higher than the CBF threshold that defined the lesion volume, suggesting that there is a critical CBF value below which the tissues will become ischemic.

Thresholds derived from group I were used to predict the lesion volumes in a separate group of animals (group II). ADC and CBF lesion volumes were plotted against TTC infarct volume (Fig. 1). Excellent correlations of the ADC-defined lesion volume (r=0.93) and CBF-defined volume (r=0.95) with TTC infarct volume were observed. Excellent one-to-one correspondence (with the y=x unity line) was also observed (r=0.99 and 0.92 for ADC- and CBF-defined lesion volume, respectively). The normalized lesion volumes from all animals in group II are summarized in Table 1 (p < 0.05 at 30 and 60 min). The CBF lesion volumes were relatively constant over time. At the early time point, there was a large mismatch between the ADC- and CBF-defined lesion volumes. Such mismatch became smaller with time, indicating that the lesion volume grew over time. The spatial progression of ADC-defined lesion volume is shown in Fig 3. The regions that show progressive ADC deterioration were medial and peripheral to the ischemic core. The CBF-defined volume did not change over time (last row of Fig 3) as expected.

Discussion
As observed in acute human stroke, the rat MCAO model also demonstrates a significant perfusion/diffusion mismatch during the early acute phase. As ischemia proceeds, the ADC lesion volume increases until it converges with the CBF lesion volume at 3 hrs. The data in Fig 2 suggest that there is a therapeutic window in this model where some ischemic damage may be reversed if the intervention (e.g., reperfusion) is administered within the first 30-60 min. The ADC and CBF thresholds herein were established at the 3hr. These thresholds are likely to be smaller at earlier time points and need to be evaluated. Similarly, these thresholds may also be different in a reperfusion model.

Conclusion
Quantitative CBF and ADC thresholds were established and successfully applied to evaluate the temporal evolution of the perfusion/diffusion mismatch in a permanent rat MCAO model. The ADC and CBF-derived lesion volumes showed excellent correlation and correspondence with histology. Such studies should be important for assessing the extent and severity of ischemic injury as well as evaluating the efficacy of therapeutic interventions that may retard or inhibit the temporal progression of the acute insult.

Table 1. (Group I, mean ± SEM, n=5)

<table>
<thead>
<tr>
<th>Normal ADC</th>
<th>Normal CBF</th>
<th>TTC (mm3)</th>
<th>Abnormal ADC</th>
<th>Abnormal CBF</th>
<th>ADC threshold</th>
<th>CBF threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.76±0.03</td>
<td>0.75±0.20</td>
<td>199±31</td>
<td>0.53±0.02</td>
<td>0.30±0.04</td>
<td>30±1%</td>
<td>57±5%</td>
</tr>
</tbody>
</table>

Fig. 1 (group II)

Fig. 2 (group II)

Fig. 3