Effects of Reperfusion on Tissue Fates in Acute Stroke Rats: Pixel-by-Pixel Analysis of Quantitative Perfusion and Diffusion Imaging

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Introduction Diffusion-weighted imaging (DWI) is widely recognized as a powerful tool for the early detection and evaluation of stroke in both animal models and humans. Hyperintense regions on DWI correspond to tissues with a reduced apparent diffusion coefficient (ADC) of water. Similarly, perfusion-weighted imaging (PWI) provides information about the hemodynamic status of brain tissue and detects regions with impaired cerebral perfusion. Clinical studies had demonstrated that the impaired perfusion region is typically larger than the lesion detected by DWI early after stroke onset [1]. The difference between the PWI and DWI abnormalities was termed the diffusion/perfusion mismatch. The DWI lesion usually enlarges and the mismatch disappears over time if left untreated. The mismatch region may represent potentially salvageable brain tissue with timely and appropriate therapy [2]. Although the "perfusion-diffusion mismatch" is widely observed in acute human stroke, similar observations in animal stroke models have been limited and the temporal evolution of the perfusion-diffusion mismatch in animal models has yet to be systematically investigated. In this study, we utilized quantitative perfusion and diffusion imaging at reasonably high spatiotemporal resolution to investigate, on a pixel-by-pixel basis, the effects of reperfusion on the "perfusion-diffusion mismatch" and the "ischemic core" in transient focal ischemia in rats during the acute phase.

Method Six male SD rats (300-350g) were initially anesthetized with chloral hydrate (400mg/kg, ip). Focal ischemia was induced using the intraluminal middle cerebral artery occlusion (MCAO) method; reperfusion was performed at 60 mins post-occlusion by remotely withdrawing the occluder while the rats were in the magnet. 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 kept at 37.0±0.5°C. MRI data were acquired at 30, 60, immediately after reperfusion (labeled as REP), 90, 120, 180 mins, and followed by TTC staining at ~24 hrs post-occlusion. MRI was performed on a 4.7T/40cm magnet. ADC(trace) was measured using spin-echo EPI with matrix = 64x64, FOV = 2.5x1.9cm^2, seven 1.5-mm slices, TE = 43ms, TR = 2s, 16 averages, b = 10, 1504 s/mm^2 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 = 15ms. ADC(trace) and CBF images were calculated at each time point. Lesion volumes (LV's) were derived using the CBF (57±11 % reduction, 0.30±0.09 ml/g/min, n = 5) and the ADC (30±2% reduction, 0.53±0.03 x10^{-3}mm^2/s) viability thresholds established previously [3]. Pixel-by-pixel CBF-ADC scatter plots were analyzed at each time point.

Results Fig. 1 shows a representative data set. The CBF LV's at 30 mins (246±31 mm^3, mean±SD, n = 6) was large, whereas the small ADC LV grew from 30 mins (141±24 mm^3) to 60 mins (167±22mm^3). Following reperfusion, CBF LV markedly decreased as most of the hypoperfused regions returned to normal. ADC LV also decreased immediately after reperfusion (104±35mm^3) and then increased slightly as ischemia progressed (112±53 mm^3 at 3 hrs), but did not reach the CBF-defined LV at 60 mins ($P < 0.05$). The TTC infract volume at 24 hrs was 140±31 mm^3, slightly higher than, but not statistically different from, the ADC LV at 3hr.

Fig. 2 shows representative CBF-ADC scatterplots from one animal subdivided into four quadrants, the boundaries of which are defined by the CBF and ADC thresholds. Four clusters were identified: *i)* a "normal" cluster with normal CBF & ADC; *ii)* an "ischemic core" cluster with reduced CBF & ADC, *iii)* a "mismatch" cluster with reduced CBF but normal ADC, and *iv)* a "non-nourishing perfusion" cluster with abnormal ADC but normal CBF. In contrast to the normal left hemisphere which exhibited a single cluster, the right ischemic hemisphere pixels showed multiple distinct clusters and they evolved dynamically with time. There was a large pixel population in the "mismatch" zone at 60 mins prior to reperfusion that largely disappeared at 180 mins (**Fig. 2a**). To evaluate the effect of reperfusion in the spatial domain, evolution of different pixel populations in the ADC-CBF space were mapped onto the image space. After reperfusion, a substantial number of pixels in the core (red) zone at 60 mins migrated to the normal (green) and the mismatch (yellow) zone, while some remained in the core zone (red) or moved to the non-nourishing reperfusion zone (blue) (**Fig. 2b**). Similarly, after reperfusion, a substantial number of mismatch (yellow) pixels at 60 mins migrated to the normal zone, while some remained in the mismatch zone, but essentially none (< 1%) migrated to the core or "non-nourishing reperfusion" zone (**Fig. 2c**).

The fates of the core and mismatch pixels at 60 mins were evaluated by quantifying their tissue volumes, ADC and CBF on a pixel-by-pixel basis. **Fig.3** shows the group-average tissue volumes of the 60-min core and mismatch pixels as they subsequently migrated to different zones at 180 mins. The volume of the core pixels (**Fig. 3a**) markedly decreased after reperfusion and then increased slightly over time. At 180 mins, 25% of the core pixels migrated to the normal zone, 3% migrated to the mismatch zone. After reperfusion, the mismatch volume decreased with majority of the pixels (83%) migrating to the normal zone (**Fig. 3b**). The ADC and CBF of mismatch pixels subsequently salvaged tissues were higher than those became infracted (ADC: 0.72±.0.10x10^{-3} *vs* 0.60±.0.06x10^{-3} mm^2/s; CBF: 0.10±.0.10 *vs* 0.05±.0.11 ml/g/min, $P<0.05$).

Discussion & Conclusion Reperfusion salvaged substantial amount of at-risk tissues, which can be tracked on a pixel-by-pixel basis. In brief, following reperfusion, 28% of the "core" pixels and 90% of the "mismatch" pixels were salvaged at 3hrs. Our results showed that the salvaged tissues arose from both the mismatch and core zones. Salvaging "core" pixels suggested that ADC reduction is not synonymous with irreversible injury. Duration of exposure to reduced ADC is likely critical. The ADC decline in turn is expected to be dependent on the degree and duration of CBF deficit.

This study established a quantitative imaging and a pixel-by-pixel analysis protocol to evaluate the spatio-temporal evolution in the CBF-ADC and image spaces of ischemic brain injury during the acute phase. This analysis approach is expected to provide a powerful tool to evaluate drug efficacy, and potentially offer a means to make statistical prediction of ischemic tissue fates.

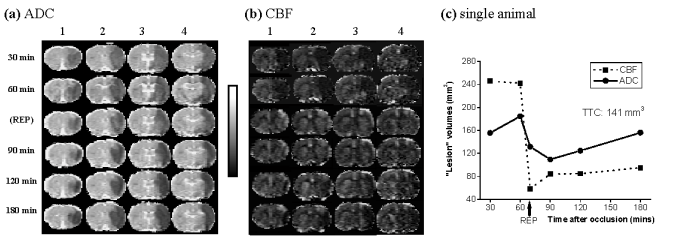

Figure 1 Grayscale bar = ADC: 0 – 0.001 mm^2/s, CBF = -1 – 2 ml/g/min.

References [1] Neumann HT, Stroke 1999; 30:1591; [2] Barber PA, Neurology 1998; 5:418; [3] Shen Q, JCBFM 2003, 23:1479.

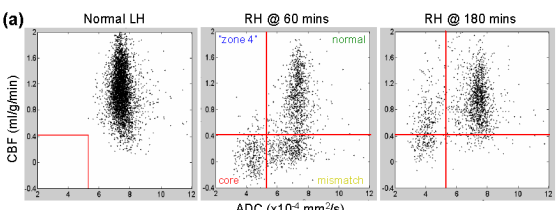

Figure 2 (a) CBF-ADC scatterplots of the contralateral normal left hemisphere and ipsilateral ischemic right hemisphere at 60 and 180 mins. The spatial evolution of **(b)** "core" and **(c)** "mismatch" pixels defined at 60 mins.

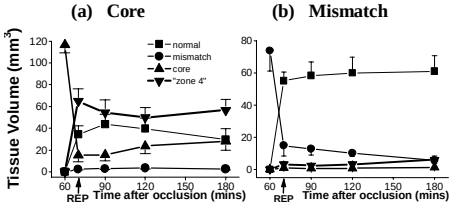

Figure 3 Group-average evolutions of **(a)** core pixels, **(b)** mismatch pixels before and after migration to different zones.