Noninvasive detection of white matter reorganization enhanced by erythropoietin treatment in a rat model of focal ischemia using MRI

L. Li[1], Q. Jiang[1], G. Ding[1], L. Zhang[1], Z. Zhang[1], J. R. Ewing[1], and M. Chopp[1,2]

[1]Neuology, Henry Ford Health System, Detroit, MI, United States, [2]Physics, Oakland University, Rochester, MI, United States

Background and Purpose: Treatment of stroke with erythropoietin (EPO) promotes brain remodeling and improves neurological function[1-2]. However, the progress of white matter reorganization during brain repair after treatment, which is crucial for recovery of neurological function, has not been dynamically investigated to our knowledge. The objective of the present study was to noninvasively identify and monitor the progress of white matter reorganization within 6 weeks after the onset of stroke and correlate this structural change with improved neurological function using magnetic resonance imaging (MRI).

Materials and Methods: Male Wistar rats (300-350 g) were first subjected to middle cerebral artery occlusion (MCAo) and then treated with recombinant human erythropoietin (rhEPO) intraperitoneally at a dose of 5000 units/kg daily for 7 days (n = 11) or the same volume of saline (n = 7) starting 24 hours after MCAo. MRI measurements of T2- and diffusion-weighted images and cerebral blood flow (CBF) were performed and neurological severity score (NSS) was assessed at 1, 7, 14, 21, 28, 35 and 42 days after MCAo. Thirteen slices of T2 and fractional anisotropy (FA)[3] maps in identical locations were generated for each brain. All rats were euthanized 42 days after stroke and the processed brain tissue was stained with Luxol fast blue and Bielshowsky to demonstrate myelin (blue) and axons (black), respectively. Comparison of the histological sections and corresponding MRI images showed that reorganization of myelin and axons occurred in the area of ischemic recovery within a certain width along the boundary of the lesion (**Fig. 1f**), and the location of this structural change coincided with the elevated area on the FA map (**Fig. 1b**). To identify the areas of reorganization, the T2 map was used to detect the ischemic lesion and then a 6-pixel wide region of interest (ROI) was delineated on the identical slice of the FA map by expanding the rim of the lesion 6 pixels outwards (**Fig. 1b**). The mean value of FA plus 2 times the standard deviation, measured by symmetrically covering this ROI on the contralateral (non-ischemic) side (**Fig. 1c**), was used as a threshold to identify the increased pixels in the ROI on the ipsilateral side (**Fig. 1d**). For each rat, 13 FA slices were measured and the total number of identified pixels was calculated. To detect changes in CBF in the area of reorganization, the identified FA region obtained at 6 weeks was employed as a ROI to track evolution of CBF within the experimental period. Data were normalized to the contralateral side for each animal and averaged at the same time points for each experimental group.

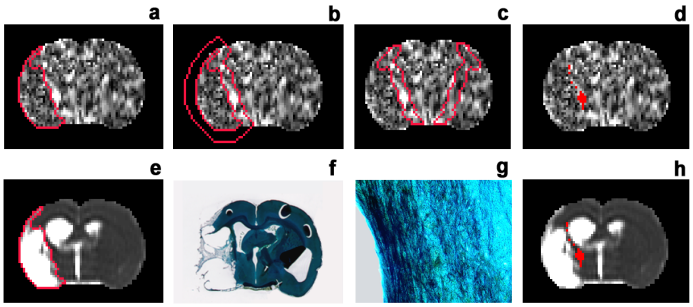

Fig. 1 Identification of areas of reorganization on an FA map. FA is relatively low (a) in the ischemic area determined by the T2 map (e). Reorganization of myelin and axons, confirmed histologically (f & g), takes place along the boundary of the lesion. This region of structural change appears bright on the FA map (a) and dark on the T2 map (e). A 6-pixel wide ROI along the boundary encompasses the region (b) and mean+2SD measured by symmetrically covering the ROI on the contralateral side (c) identifies the areas with elevated FA values (d & h).

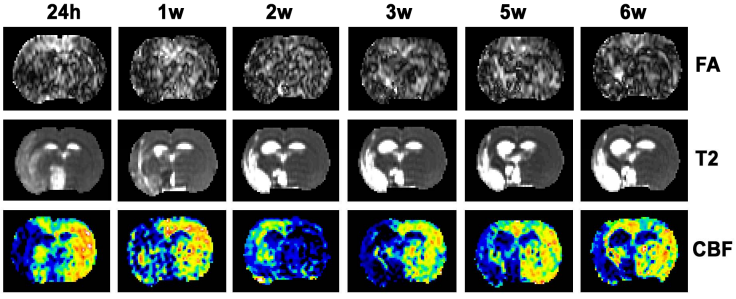

Fig. 2 MRI maps showing changes in FA, T2 and CBF after MCAo in a representative EPO-treated rat.

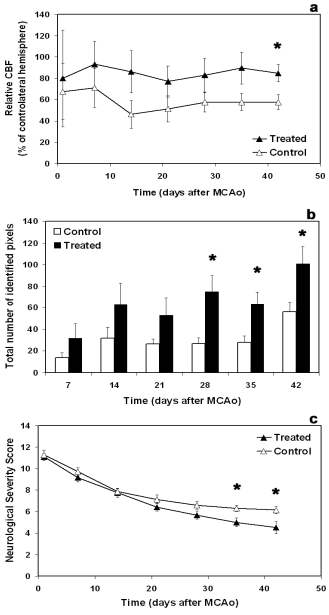

Fig. 3 Quantitative data showing relative CBF in the area of white matter reorganization (a); total number of identified pixels on the FA map (b); and neurological severity scores (c). Significance of difference: * = $p < 0.05$, comparing EPO-treated and non-treated groups at the same time points.

Results: Our data demonstrated that the area with an elevated FA value reflected white matter reorganization after stroke. This reorganization occurred earlier in the EPO-treated group than in the non-treated group. Elevated areas on the FA map appeared as early as 1 week after MCAo in the treated group (27%; 3 of 11) as opposed to only after 3 weeks in the control animals. In the treated group, the area of white matter reorganization characterized by the FA map coincided with the location of CBF restoration starting at 3 weeks (**Fig. 2**), whereas in the non-treated group we could observe no such obvious correlation. Relative CBF values in the area of white matter reorganization were higher in the treated group than in the non-treated group (**Fig. 3a**). Compared to the MCAo controls, treatment with EPO significantly increased the total number of identified pixels on the FA map from 3 to 6 weeks after stroke (**Fig. 3b**), concomitant with recovery of neurological function (**Fig. 3c**).

Conclusion: Our data in rats indicate that FA is a sensitive measure of white matter changes after stroke and provides an important noninvasive means for real-time evaluation of treatment efficacy and functional outcome. White matter reorganization during brain remodeling after stroke can be dynamically detected on the FA map. The increase in elevated pixels on the FA map characterizes the progress of the reorganization very well and therefore may be a good MRI index to predict recovery of neurological function. Treatment with EPO significantly enhances white matter reorganization, which correlates with local restoration of CBF and recovery of neurological function.

References:

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