

# Non-invasive detection of microvascular remodeling enhanced by erythropoietin treatment in a rat model of focal ischemia using MRI

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**Introduction:** In the clinic, patients with higher cerebral blood vessel density appear to make better progress and survive longer than patients with lower vascular density (1, 2). The elevated CBF, caused by high density of functional blood vessels post angiogenesis in ischemic boundary area after stroke, improves regional cerebral tissue microenvironment. Magnetic resonance imaging (MRI) can non-invasively assess several indices that characterize cerebral tissue evolution after stroke with or without treatment. However, the dynamic changes of microvasculature during brain repair after stroke, which is crucial for recovery of neurological function, has not been investigated (3). The main objective of the present study was to noninvasively monitor the changes of microvasculature parameters up to 6 weeks after the onset of stroke using magnetic resonance imaging (MRI).

**Methods:** Male Wistar rats (300~350g) subjected to embolic stroke at middle cerebral artery (MCA) were randomly assigned to treatment (n=8) and control (n=10) groups. Recombinant human erythropoietin (rhEPO) was administered at a dose of 5000units/kg subcutaneously in the treated group 24 hours after MCA occlusion and daily for an additional 6 days. Rats in the control group were treated with the same volume of saline. All rats were sacrificed at 6 weeks after stroke. MRI measurements were performed using a 7T system with Bruker console. A complete set of MRI images, including DWI, CBF, T<sub>2</sub>WI, and T<sub>2</sub>\*WI were performed before ischemia, repeatedly at 24hours and weekly up to 6 weeks after stroke for all animals. The T<sub>2</sub>- and T<sub>2</sub>\*- maps were obtained before and after intravascular injection of P904 (Guerbet Group Company, France). The derived MRI expressions for the mean vessel density (MVD), mean vessel size (VSI) and the mean segment length (MSL) to calculate the parameters at each time point (4, 5, 6). Ischemic areas were determined using the threshold T<sub>2</sub> value of mean + 2 standard deviations from the T<sub>2</sub> value measured in the contralateral hemisphere on T<sub>2</sub> maps after stroke. Regions of ischemic recovery were identified by subtracting the ischemic core areas obtained 6 weeks after stroke from the ischemic area on the T<sub>2</sub> maps obtained 1 day after stroke. A 5 x 5 pixel region of interest (ROI) was selected from the center of each area.

**Results and Discussion:** The results of the present study demonstrate that MRI MVD-, MRI MSL- and MRI VSI- map measurements, based on CBV, ADC,  $\Delta R_2$  and  $\Delta R_2^*$ , can be used to quantitatively investigate changes in the cerebral microvasculature induced by ischemia. Figure 1 shows dynamic evolution of T<sub>2</sub> maps at 24 hours, 2, 4 and 6 weeks after stroke (T, treated and C, control). MRI and histological measurements in this study demonstrated that neurorestorative treatment with EPO promotes vascular remodeling after embolic stroke. Compared to the MCAo controls, treatment with erythropoietin significantly increases the total number of Figure 2(a) MVD from 1-week to 3-week after stroke in the recovery area. The relative MSL gradually increased from week 1 to 4 weeks and peaked at 4 weeks then decreased for the non-treated control rats whereas the EPO treated rats increases fast and peaks early at 3 weeks for as shown in Figure 2b. The relative VSI for both treated and control stabilized decreased 4 weeks after stroke. The mean relative VSI in treated group is higher than that in the control group from 1 to 6 weeks after stroke as shown figure 2(c). A statistical significant difference was detected between two groups at 3 weeks.

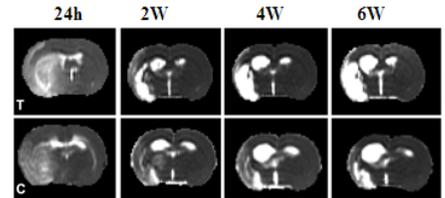


Figure 1: Evolution of T<sub>2</sub> maps at 24 hours, 2, 4 and 6 weeks after stroke

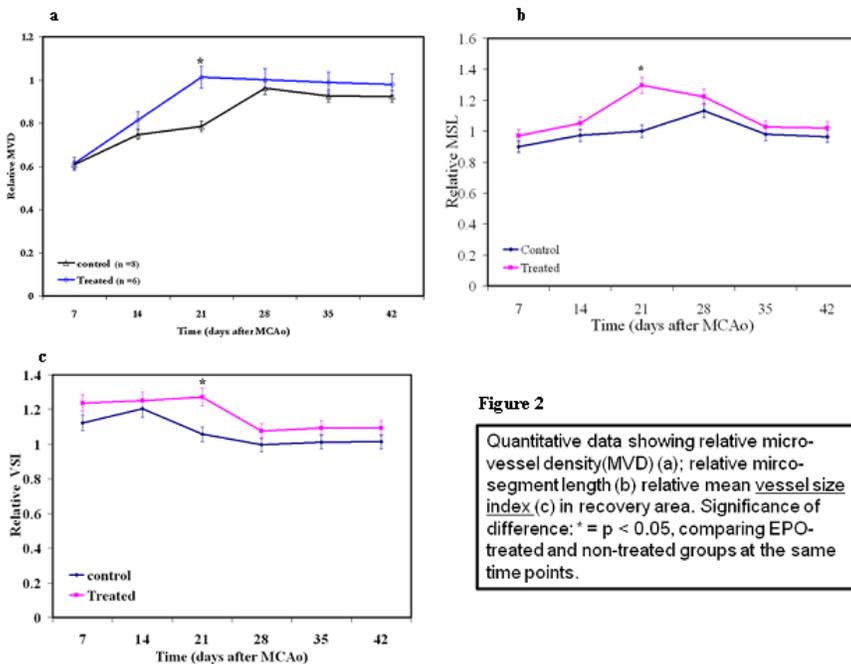


Figure 2

Quantitative data showing relative microvessel density(MVD) (a); relative microsegment length (b) relative mean vessel size index (c) in recovery area. Significance of difference: \* = p < 0.05, comparing EPO-treated and non-treated groups at the same time points.

**Conclusion:** Our data indicate that MVD and MSL are a sensitive measure of structural changes after stroke and provide an important non-invasive means for real-time evaluation of treatment efficacy and possible functional outcome. Microvascular changes during the brain remodeling after stroke can be dynamically detected by using MRI MVD-, MSL- and VSI- map. Treatment with EPO after stroke significantly enhances vascular remodeling, which associates with increased in MVD and improved recovery of neurological function.

**References:**

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