Neuroprotective benefit in a neurorestorative treatment of embolic stroke with EPO in rats detected by MRI

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Introduction Erythropoietin (EPO) is a hematopoietic cytokine.[1] Acute treatment of embolic stroke with EPO, even at 6 h after middle cerebral artery occlusion (MCAo), has been shown to reduce infarct volume after stroke in rats.[2,3] Moreover, chronic treatment with EPO starting 24 h after MCAo promoted angiogenesis, neurogenesis[4] and white matter plasticity while increasing vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF).[5] However, no significant differences in infarct volume were observed in the neurorestorative treatment of stroke with EPO in rats. Based on these findings, investigations of neuroprotective effects during early EPO treatment of stroke or neurorestorative effects during chronic EPO treatment have been performed separately.[3-5] Our data derived from dynamic MRI measurements demonstrate that when rats with embolic stroke were given neurorestorative EPO treatment, they exhibited a neuroprotective benefit as well: EPO significantly reduced loss of brain tissue by restraining the expansion of ipsilateral ventricle inside of rat brain.

Materials and Methods The middle cerebral artery (MCA) of adult male Wistar rats (300~350 g) was occluded by placing an embolus at its origin. Animals were randomly assigned to treatment (n = 11) and control groups (n = 11). In the treated group, EPO was administered intraperitoneally at a dose of 5,000 IU/kg daily for 7 days starting 24 hours after MCAo. The control group was treated with the same volume of saline. All rats were killed 6 weeks after stroke. MRI was performed using a 7T system with a Bruker console. A complete set of MRI images, including DWI, T2WI, CBF, SWI and T2*WI, was obtained before ischemia and repeated at 24 hours, then weekly for up to 6 weeks after stroke. Image analysis was performed with an Eigentool software package. All animals underwent functional testing prior to MCAo and once a week for 6 weeks starting 24 hours after stroke. A MicroComputer Imaging Device (MCID) system and laser scanning confocal microscopy (LSCM) were used for histological measurements. Coronal sections were stained with hematoxylin and eosin (H&E) to evaluate infarction or Bielschowsky's silver and Luxol fast blue (BLFB) for axonal outgrowth and plasticity and examined under an optical microscope. Cerebral microvessels were perfused with fluorescein isothiocyanate (FITC)-dextran and measured under LSCM.

Results MRI and histological measurements in this study demonstrated that neurorestorative treatment with EPO enhanced brain plasticity after embolic stroke in the treated animals compared to the controls, consistent with our published histological findings.[4] In EPO-treated rats, angiogenesis can be earlier detected by T2*WI or SWI (Fig.1 A-B, red arrows) than in controls, and elevated CBF was detectable later in the same area on MRI CBF map (Fig.1 C, red arrow). Diffusion anisotropy (DA) also showed promising results; increased DA values (Fig.1 E, red arrow) were located in the same area that demonstrated enhanced white matter plasticity on immuno-chemical staining. FITC- and BLFB-stained slices showed increased numbers of microvessels and axons in this region (Fig.1 D, F). Chronic EPO therapy resulted in not only neurorestoration but also a neuroprotective benefit based on the T2WI data (Fig.2: T, treated; C, control). Dynamic T2WI images demonstrated that ventricular volume in the ipsilateral hemisphere increased after stroke, but this expansion was restrained in EPO-treated rats. Two representative H&E-stained sections are shown in Fig.3 (A, B). Using the T2WI data, ventricular volume ratios (ipsilateral *vs* contralateral) 6 weeks after stroke were 1.99 ± 0.15 for the treated rats (n = 8) and 2.35 ± 0.26 for the controls (n = 6) (Fig.3 C), which is a significant difference (p < 0.01).

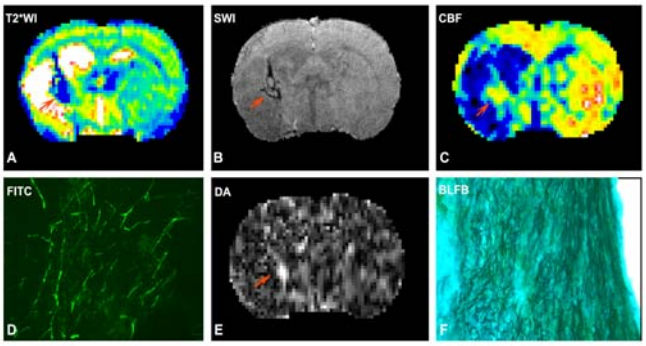

Fig.1 T2*WI (A) and SWI (B) detected angiogenesis after stroke treated with EPO, CBF (C) and DA measured elevated values afterwards. FITC and BLFB sections show microvessels and axons using microscopy.

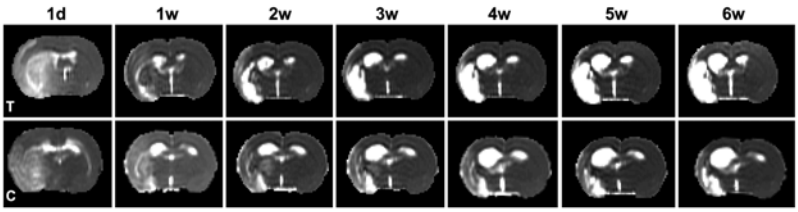

Fig.2 T2WI images demonstrate that the volume expansion of ipsilateral ventricle was restrained in EPO treated rats (T) compared to control rats (C).

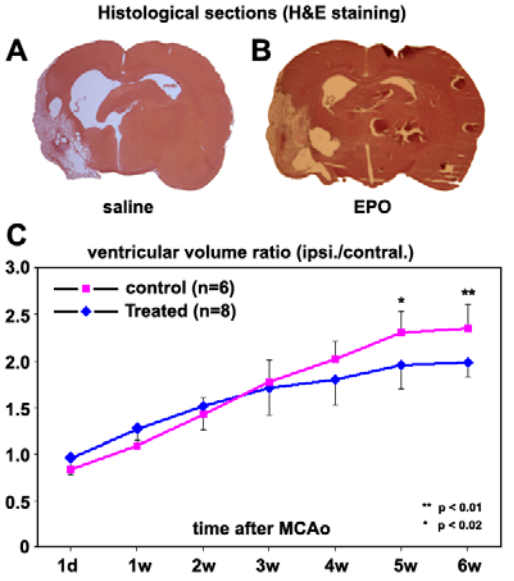

Fig.3 Representative H&E sections for treated (A) and control (B) rats showed different ventricular expansion. Quantitative ventricle volume measurements from T2WI demonstrated that EPO treatment restrained the ventricular expansion compared with control rats. The differences are significant at 5 and 6 weeks after stroke.

Discussion When the ipsilateral ventricle expands, the volume of the cerebral parenchyma in that hemisphere decreases, which indicates loss of cerebral tissue. Chronic treatment of stroke with EPO protects against further loss of cerebral tissue by slowing the increase in ventricular volume. Thus treatment with EPO starting 24 h after embolic stroke for 7 days can have both neurorestorative and neuroprotective benefits. Presently neuroprotective and neurorestorative treatments are carried out separately, while restorative treatment has traditionally been considered to have no protective effect because it does not reduce infarct volume. Our results demonstrate that in fact chronic treatment of embolic stroke with EPO reduced the expansion of ventricular volume that ultimately protects against loss of cerebral tissue, though it did not reduce lesion volume.

References

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