MRI metrics detected axonal outgrowth and plasticity in rat brain after embolic stroke

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Introduction 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 cerebral blood flow (CBF) caused by either recanalization of occluded arteries or high density of functional blood vessels postangiogenesis in the ischemic boundary after stroke improves the regional cerebral tissue microenvironment and may be beneficial for neurogenesis and axonal outgrowth, leading to white matter reorganization which may improve recovery of neurological function[3-6]. However, few studies to date have provided evidences of relations among angiogenesis, improved CBF and axonal outgrowth after stroke. In rats, administration of sildenafil inhibits breakdown of cGMP (cyclic guanosine monophosphate) and causes it to increase in the brain, which may improve angiogenesis, axonal plasticity and functional outcome after embolic stroke in rats[5,6].

Materials and Methods Male Wistar rats (300~350 g) were subjected to embolic stroke at the middle cerebral artery (MCA) and randomly assigned to treatment (n = 11) and control groups (n = 10). In the treated group, sildenafil was administered subcutaneously at a dose of 10 mg/kg 24 hours after MCA occlusion and then daily for 6 days. The control group was treated with saline. All rats were sacrificed 6 weeks after stroke. MRI measurements were performed using a 7T system with a Bruker console. A complete set of MRI images, including DWI, T2WI, CBF, T2*WI and DTI, was obtained before ischemia and repeated at 24 hours and then weekly for up to 6 weeks after stroke. Image analysis was performed with Eigentool and DtiStudio (for DTI). All animals underwent functional tests prior to stroke and once a week starting 24 hours after stroke. An MCID (MicroComputer Imaging Device) system was used for histological measurements. Coronal sections were stained with H&E (hematoxylin and eosin) to evaluate infarction, EBA (endothelial barrier antigen) for cerebral vessels, or BLFB (Bielschowsky's silver and Luxol fast blue) for axonal outgrowth and plasticity and examined under an optical microscope.

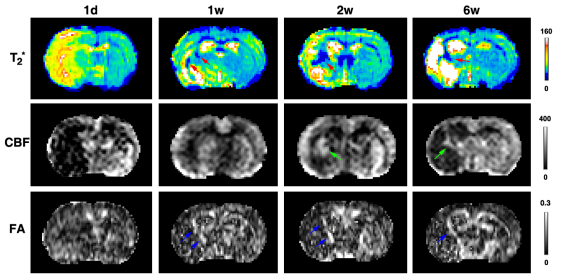

Fig.1 T2*WI detected angiogenesis (red arrows) and FA detected axonal outgrowth and plasticity (blue arrows) starting from 1 week after stroke in a sildenafiltreated rat. Local CBF was elevated starting 2 weeks after stroke (green arrows).

Results In the sildenafil treated rats, angiogenesis was detected starting from 1 week post embolic stroke using MRI T2*WI (1st row in Fig. 1). Over the same period, axonal outgrowth and plasticity were detected by the FA (Fractional Anisotropy) index of DWI (3rd row in Fig. 1), and regional CBF was elevated starting 2 weeks after stroke as measured by MRI (2nd row in Fig. 1). In the ischemic boundary demarcated by MRI T2WI (Fig. 2A), areas of angiogenesis and axonal outgrowth were co-localized with each other and with elevated CBF (Fig. 2B). Immunohistochemistry demonstrated that axonal and microvascular densities in the ischemic boundary were higher than in the homogeneous area (Fig. 2 D-I). Statistically, sildenafil treatment of embolic stroke enhanced axonal density in the boundary (46.8±18.8%) compared to the controls (24.3±14.7%, Fig. 2C) at 6 weeks after stroke. Axonal outgrowth was significantly enhanced by sildenafil (p < 0.04). DTI showed that axonal orientation at 6 weeks after stroke now paralleled the ischemic boundary whereas the homogeneous tissue in the contralateral hemisphere showed no change in orientation (Fig. 3). Tests of neurological functions demonstrated that sildenafil significantly (p < 0.05) improved the mNSS score and foot-fault performance of rats with stroke from 2 or 3 weeks up to 6 weeks after stroke, respectively.

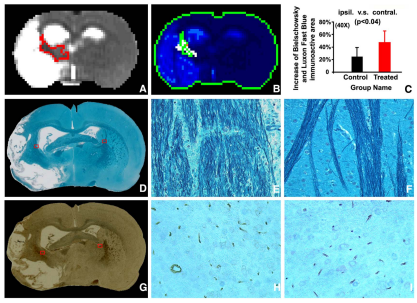

Fig.2 T2WI demarcated the ischemic boundary (A). Angiogenesis and axonal outgrowth were co-localized (B). BLFB- (D-F) and EBA-stained slices (G-I) demonstrated that axonal and microvascular densities were higher than in the homogeneous areas. Axonal density in sildenafiltreated rats was significantly higher than in controls measured histologically at 6 weeks (C).

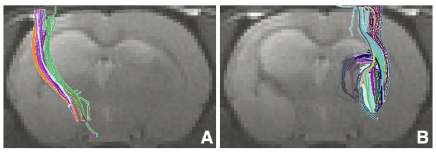

Fig.3 At 6 weeks after stroke, the DTI fiber track showed that axonal orientation now paralleled the ischemic boundary after brain tissue plasticity and reorganization (A), compared with the homogeneous cerebral tissue (B).

Discussion Angiogenesis and axonal outgrowth were detected in both sildenafil- and saline-treated (control) rats after stroke. However, they began earlier in the treated rats, starting 1 week v.s. 2 weeks after stroke. Angiogenesis and axonal outgrowth were co-localized with each other, both measured histologically and *in vivo* by MRI. This indicates that sildenafil treatment of stroke may simultaneously promote angiogenesis and axonal outgrowth after embolism in rats. Local CBF was increased after angiogenesis, which may facilitate white matter reorganization along the ischemic boundary detected by DTI. Both elevated CBF and re-organized white matter in ischemic areas may contribute to recovery of neuronal function after stroke in rats.

References

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