Longitudinal Magnetic Resonance Imaging of Aged Rats with Sildenafil Treatment after Embolic Stroke

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Introduction Advanced age is an important risk factor and a predictor of poor outcome after treatment in stroke patients[1-2]. Aged rats subjected to stroke had a higher mortality rate and worse neurological deficits than young rats, and pharmacological effects after treatment of stroke in young rats may not necessarily be translated to older rats[3-4]. Clinical trials for neuroprotective therapy for stroke have failed. This may in part be attributed to the fact that preclinical studies of therapeutic agents have been primarily performed in young animals and not in aged animals, which reflect the target clinical population. Thus, using aged animals in experimental investigation of stroke and treatment would be more clinically relevant. We, therefore, tested the therapeutic effect of sildenafil treatment of stroke in aged animals and sought to determine whether MRI can identify therapeutically relevant restorative processes including angiogenesis, enhancement of local CBF, axonal remodeling and reduced cerebral tissue loss.

Materials and Methods Male Wistar rats 18 months of age and weighing 540 to 580g were subjected to embolic stroke and randomly assigned to either the treatment (n = 10) or control groups (n = 10). The model of embolic stroke, briefly, employs an aged white clot (blood of a rat was withdrawn into 20cm PE-50 tubing), which was prepared 24 hours (retained at 25ºC for 2h and at 4ºC for 22h) before ischemia and slowly injected into the internal carotid artery to the origin of the middle cerebral artery (MCA). In the treatment group, sildenafil (Viagra®, Pfizer Inc) was administered subcutaneously at a dose of 10 mg/kg daily for 7 days starting 24 hours after embolic MCA occlusion. The control group received an equal volume of saline. MRI, including DWI, T2WI, CBF and T2*WI, and functional tests were performed 24h and weekly to 6 weeks after stroke for all rats. Image analysis was performed with a home-developed software package, Eigentool.

Results MRI T_2^* maps showed evidence of ongoing angiogenesis after stroke, with low intensity regions on T_2^* map along the ischemic boundary within recovery area, in both saline treated (control) and delayed sildenafil treated aged rats. On a typical T_2^* map obtained 4w after stroke from a representative aged rat with sildenafil treatment, whose ischemic lesion area was demarcated by hyperintensity on a routine T_2 map acquired 24h after stroke (as shown in Fig. 1a), the hypointensity region, identified with two white arrows in figure 1b, was indicative of angiogenesis. The CBF map, obtained one week later (i.e., five weeks after stroke), demonstrated the elevated CBF values in brain tissue in the area with hypointensity on T_2^* map, indicated by arrows in figure 1c. The elevated CBF in that area is likely the direct result of angiogenesis, and is consistent with temporal and spatial profiles of angiogenesis. Six weeks after stroke, diffusion measurements of the sildenafil treated rat showed increases of diffusion anisotropy (DA) values in the same area as the elevated CBF did (identified by arrows in Fig.1d), which indicated enhanced axonal remodeling.

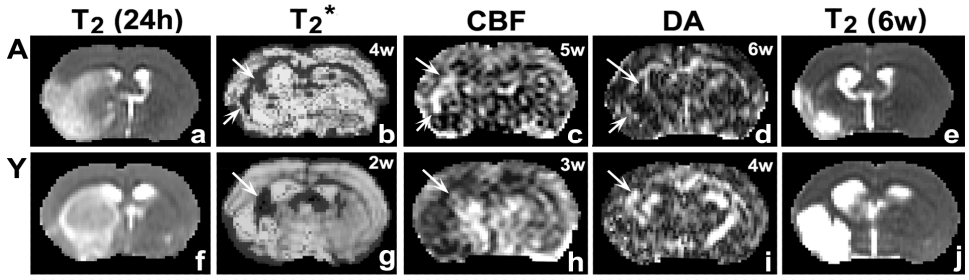

Fig.1 MRI of representative aged (A) and young (Y) rats with sildenafil treatment after stroke

Longitudinal MRI measurements demonstrated quantitative temporal features of T_2, CBF and DA for recovery cerebral tissue after stroke with or without sildenafil treatment in aged rats. The differences of T_2^* ratios (Fig.2a) between these two groups were significant at 4w and 5w after stroke ($p < 0.04$). Higher CBF ratios were observed in sildenafil treated aged animals, compared to control rats (Fig. 2b). At 6w after stroke, CBF was significantly higher in the sildenafil treated group than in the control group ($p < 0.05$). The increase of axonal density, including axonal outgrowth, remyelination and reorientation, in rat brain after stroke could cause elevation of DA. DA values monotonically increased starting from 1 week after stroke in the sildenafil treated group, but, 1 week delayed later in controls (Fig. 2c). Starting 4w after stroke, the DA ratios were significantly different between the two groups ($p < 0.03$). Temporal changes in ventricular volume in terms of the volume ratios (ipsilateral *vs* contralateral) for both treated and control groups of aged rats are presented in figure 2d. Ventricular volume ratios monotonically increased during 6 weeks after stroke for both treated and control groups. However, the rate of increase was slower in the treated group from 1 week after stroke. The mean ratio at 6 weeks after stroke was 1.27 ± 0.25 for the treated rats (n = 10) and 1.56 ± 0.29 for the controls (n = 10), which is significantly different ($p < 0.03$). Neurological test demonstrated a significant difference in group by time interaction ($p < 0.01$). The mNSS was improved at week 6 in sildenafil treated group, compared to controls ($p < 0.04$).

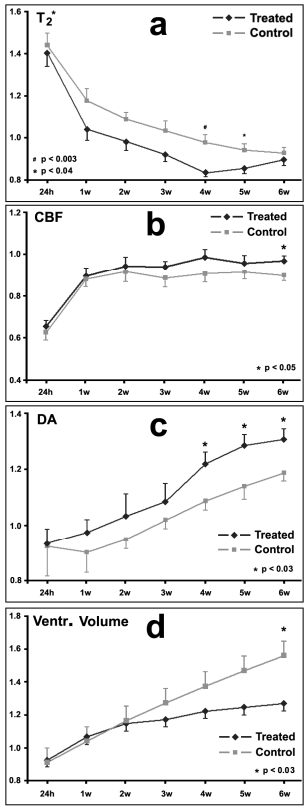

Fig.2 Quantitative MRI data

Discussion Our results demonstrated that treatment of embolic stroke with sildenafil in aged rats starting at 24 hours and continuing daily for 7 days significantly augmented angiogenesis and axonal remodeling, accompanied with increased of local blood flow and reduced expansion of the ipsilateral ventricle, in the ischemic recovery area observed weekly up to 6 weeks after stroke, compared to saline treated control aged rats. Neurological outcome measured by mNSS evaluation was significantly improved with sildenafil treatment of stroke in aged rats.

Recovery from ischemic stroke may be age associated. As shown in figure 1, features of angiogenesis and axonal remodeling on T_2^* and DA maps, with comparable lesion size, were much more visible and present two weeks earlier in sildenafil treated young animals (Y: the 2nd row of images) than in sildenafil treated aged rats (A: the 1st row of images). For control aged animals, T_2^* and DA features of angiogenesis and axonal remodeling after stroke, however, were hardly visible on the T_2^* and DA maps with naked-eye.

References

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