MRI Investigation of Angiogenesis Enhanced by Sildenafil Treatment of Embolic Stroke in Rat

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Introduction Administration of sildenafil by inhibiting of cGMP breakdown increases brain levels of cGMP[1,2]. An elevated cGMP levels in cerebral tissues may be involved in enhancing the improvement of functional outcome, angiogenesis and synaptogenesis after embolic stroke in rats[2]. A vascular permeability related MRI parameter, K_i, provides a sensitive index of the temporal profile of angiogenesis after stroke[3]. And the directionality of diffusion, known as fractional anisotropy (FA), is correlated directly with histological markers of myelination.

Materials and Methods Male Wistar rats (300~350g) subjected to embolic stroke at middle cerebral artery (MCA) were randomly assigned to treatment (n=11) and control (n=10) groups. Sildenafil was administered at a dose of 10mg/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 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 SWI, DWI, T2WI, CBF, K_i and DTI, was performed before ischemia, repeatedly at 24 hours and weekly up to six weeks after stroke for all animals. Image analysis was performed with Eigentool, SPIN (for SWI) and DtiStudio (for DTI), respectively. All animals were performed functional tests once a week started from 24h after stroke. MCID image analysis system was used for histological measurements. Coronal sections examined under the light microscope were stained with H&E for the evaluation of infarction, with EBA for cerebral vessels and with Bielschowsky's silver & Luxol fast blue for presynaptic plasticity and synaptogenesis.

Results Ischemic infarction volumes measured histologically and by MRI showed no statistical difference ($p>0.05$) between the treated and the control groups. However, the averaged lesion volume of treated group was smaller than that of control group. Particularly, the low CBF area of the treated group was smaller and the mean value of the low CBF area of the treated group was higher than those of the control group. Neurological functional test demonstrated that the sildenafil treatment significantly ($p<0.05$) improved the mNSS score and performance on foot-fault test of stroke rats from 2 or 3 weeks up to 6 weeks after stroke. Fig.1a exhibits a typical pattern of K_i evolution for angiogenesis. Regional K_i apparently increased from 1 to 3 weeks post stroke, which indicated that angiogenesis in that region occur remarkably during the period. The localized K_i value returned to normal from 4 weeks, which indicated that the new vessels matured at 4 weeks after stroke. Another typical pattern of K_i evolution was shown in Fig.1b, where the regional K_i value kept elevated from 1 week after stroke, which indicated that angiogenesis in the area occurs incessantly. Distinguished from the patterns for angiogenesis, typical K_i temporal pattern for BBB disruption was presented in Fig.1c, where the elevated K_i value generally disappeared after 1 week of embolic stroke. Figs.1 d-e showed evolutions of SWI for a control rat (d) and a treated rat (e). Dark lines in SWI images (arrow) indicated the formation of venous vasculature. These dark lines formed clearly at 3 or 5 weeks after stroke for treated or control rats, respectively. It suggested that angiogenesis occurred earlier and developed faster in sildenafil treated animals compared with control rats. Histological measurements at 6 weeks post stroke using EBA staining were shown in figure 2. For both treated (Fig.2a) and control (Fig.2c) rats with embolic stroke, vessels were enlarged and vascular densities were increased in angiogenesis areas comparing to those in contralateral hemisphere (Fig.2b). Quantitatively, vascular density in angiogenesis area increased 53.8±21.1% for treated rats and 28.0±14.8% for control animals (Fig.2d) at 6 weeks after stroke. Angiogenesis was significantly enhanced by the sildenafil treatment ($p<0.03$). As shown in figure 3, at 24h post stroke, CBF map showed an ischemic area (Fig.3a) and T_2 map identified the lesion area (Fig.3b). The FA map at this time slightly decreased in part of ischemic area (Fig.3c). Six weeks later, CBF map detected an angiogenesis area (arrow in Fig.3d) and T_2 map identified the area in ischemic boundary region (Fig.3e). A remarkable increase of FA in the same region was detected at this time (Fig.3f). The elevated FA along the ischemic boundary area should be caused by the growth of axon and myelin, and confirmed by histology.

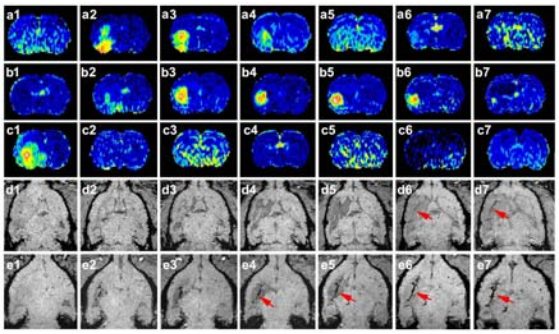

Fig.1 Ki patterns for angiogenesis (a, b) or BBB damage (c) from 1 day to 6 weeks after stroke. SWI detected angiogenesis after stroke for a control rat (d) or a sildenafil treated rat (e).

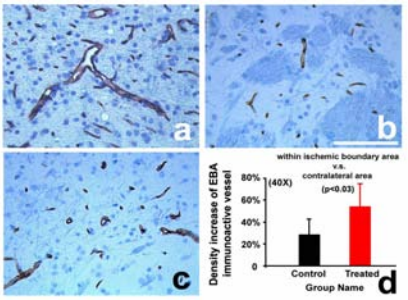

Fig.2 Vascular density in angiogenesis areas increased 53.8 % for treated rats (a) and 28.0% for control rats (c) compared with contralateral density (b) measured under 40x microscope (d).

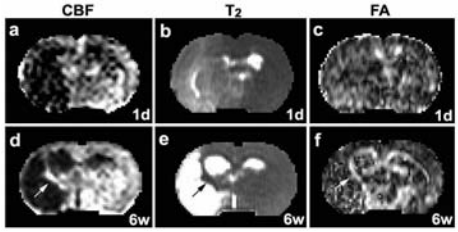

Fig.3 At 6 weeks after stroke, CBF map detected an angiogenesis area, an increase of FA in the same area was detected, which indicated the growth of axon and myelin.

Discussion Angiogenesis happened after stroke in either saline or sildenafil treated animals observed by K_i maps or SWI images and were significantly enhanced in sildenafil treated rats measured histologically. However, the K_i map was hard to localize the angiogenesis region since the elevated K_i area was obviously larger. SWI provided a complimentary tool to localize the angiogenesis region. Since SWI is sensitive to venous blood, the SWI enhancement occurred when the vasculature of angiogenesis was developed. Microvessel density (MVD) in angiogenesis area (boundary of ischemic lesion) identified histologically showed a significant difference between two groups. FA map of water diffusion detected the synaptogenesis with elevated FA value caused by the increased axon density and oriented along ischemic boundary. At 6 weeks post stroke, the elevated area in FA map matches not only with axonal density stained by Bielschowsky's silver & Luxol fast blue, but also with the areas of elevated MVD and CBF values measured by MRI. This result may indicate that elevated CBF caused by MVD increase, which was induced by angiogenesis, may provide a relative better environment for promoting neurogenesis and synaptogenesis in treated animals. All of these may contribute the significant improvement of functional recovery of stroke rat treated with sildenafil.

References

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