MRI investigation of combination treatment of embolic stroke in rat with rt-PA and atorvastatin

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Introduction Acute thrombolytic therapy with recombinant tissue plasminogen activator (rt-PA) is a successful treatment for ischemic stroke when administered within three hours of stroke onset [1]. However, treatment with rt-PA is given to only approximately 2% of stroke patients, primarily, because of the narrow three-hour therapeutic window [2]. Treatment with rt-PA alone at four hours after stroke onset fails to produce therapeutic benefit, or increases the risk of developing hemorrhage [3]. Therefore, it would be desirable to improve the efficacy and safety, as well as to increase the therapeutic window for thrombolytic therapy of stroke. Statins reduce the incidence of stroke and have beneficial microvascular effects. Thus, statins are potential agents for co-treatment of stroke with rt-PA. Atorvastatin (Lipitor®, Pfizer) is a second-generation inhibitor of HMG-CoA reductase. We investigated the effects of combination treatment of atorvastatin and rt-PA including the measurements of MRI, as well as fluorescence microscopy and histology.

Materials and Methods Male Wistar rats (300~400g) subjected to embolic stroke were randomized into two groups with (n=9) combination treatment of rt-PA and atorvastatin, or receiving saline (n=10) at 4h after embolic embolic middle cerebral artery occlusion (MCAo). A total dose 10mg/kg of rt-PA was injected intravenously with 10% bolus and the remainder at a continuous infusion over a 30-minute interval using a syringe infusion pump. Atorvastatin was given i.p. at a dose of 20mg/kg and was followed by a second dose of 20mg/kg at 24h. All animals were sacrificed 48h after MCAo and the MRI measurements. MRI measurements were performed using a 7T system with a Bruker console. A complete set of sequences required approximately two hours of scan time.

Results Treatment of embolic stroke in rat with rt-PA and atorvastatin 4h after MCAo significantly ($p<0.01$) reduced ischemic lesion volumes (23.1±9.6%, percentage of ipsilateral hemisphere volume) compared to saline treated rats (38.8±13.3%) measured histologically at 48h after stroke. Our MRI data revealed a significant decrease in the average relative areas with low CBF (Fig.1A), high T_2 (Fig.1B) or T_1 (Fig.1C) at 24h and 48h in the treated group compared to the control group. The data from ADC_w map (Fig.1D) did not show any significant difference between the treated group and the control group after the acute period of stroke (24h $p>0.2$ and 48h $p>0.7$). Measurement of microvascular patency indicates that co-administration of rt-PA and atorvastatin therapy improved microvascular patency of rat brain. For the control rat, the CBF map acquired at 48h demonstrated no improvement of cerebral microcirculation perfusion in both cortex and subcortex of the ipsilateral hemisphere, although the rat had a patent right MCA. In the rat treated with rt-PA and atorvastatin, with a patent right MCA, the microcirculation perfusion of the ipsilateral hemisphere at the 48h was elevated compared with the contralateral hemisphere. Fluorescent microscopy revealed that cerebral microvessels in the MCA territory were well perfused by FITC-dextran, which matches areas with elevated CBF on CBF map measured at 48h after MCAo (Fig.2). Quantitative microvascular patency showed of 16.3±5.5 (percentage of FOV) for treated rats and 12.4±3.5 for control rats ($p<0.05$).

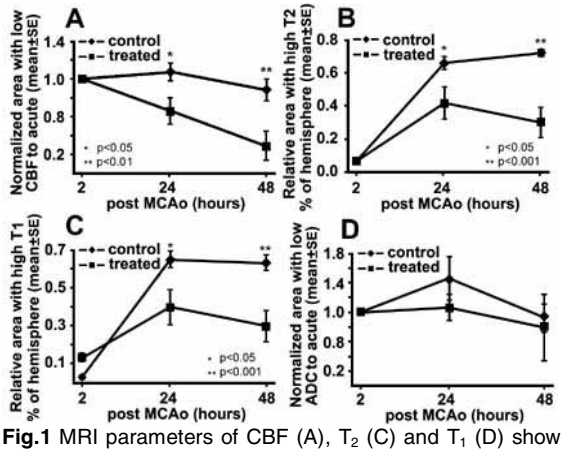

Fig.1 MRI parameters of CBF (A), T_2 (C) and T_1 (D) show significant reduction of lesion area in stroke rats treated with rt-PA and atorvastatin, compared with control animals receiving saline. The ADC_w does not show the difference.

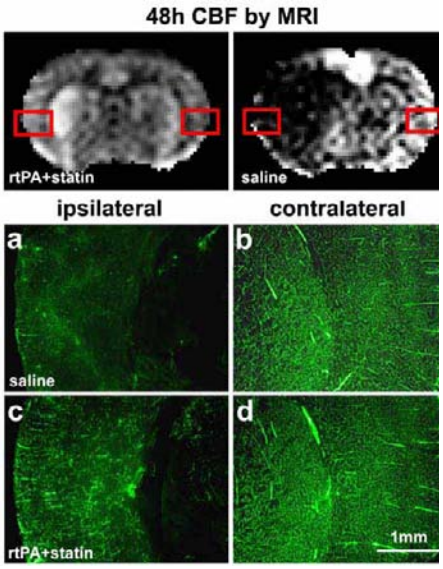

Fig.2 Measurements of microvascular patency by FITC-dextran matched the CBF maps. The treated rat with patent MCA shows recovered CBF and reduced microvascular perfusion deficit at 48h after stroke.

Discussion Our data indicate that the combined rt-PA and atorvastatin treatment at 4h after the embolic MCAo significantly reduced microvascular perfusion deficits compared with the control animals. In the combination treated rats, MRA and CBF images show MCA recanalization and recovery of CBF, respectively, which match the cerebral microvascular patency data obtained from fluorescence microscopy. This may be attributed to atorvastatin, which increases the efficacy of fibrinolytic therapy with rt-PA and protects microvessels downstream of the occluded MCA from secondary occlusion. The MRI CBF, T_1 and T_2 maps reflect the enhanced tissue perfusion or the reduction of ischemic lesion after the combination treatment of embolic stroke with rt-PA and atorvastatin.

References

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