Effect of Combined Treatment with rt-PA and GPIIb/IIIa inhibitor on Embolic Stroke in Rat Measured by MRI

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ABSTRACT

Rt-PA in combination with 7E3 F(ab')$_2$ was employed to treat rats at 4 hours after the onset of embolic MCA occlusion in order to suppress platelet aggregation and thereby improve the efficacy of thrombolytic therapy. Our data indicate that the combined treatment significantly improved tissue microcirculation downstream of the embolus site and reduced infarct volume.

INTRODUCTION

Studies on experimental and human stroke suggest that platelets play a role in the development and progression of cell damage after focal cerebral ischemia [1]. Suppression of platelet deposition at the initial site of obstruction and downstream microvessels may improve the efficacy of thrombolytic therapy. Combination therapy with rt-PA and a GPIIb/IIIa inhibitor in humans suffering myocardial infarction improves cardiac microcirculatory function [2]. In this study we tested the hypothesis that the combination treatment with rt-PA and 7E3 F(ab')$_2$ improves the efficacy of thrombolytic therapy in embolic stoke, which can be detected by MRI.

EXPERIMENT

Male Wistar rats, weighting 300 - 350 g, were subjected to MCA occlusion. Two groups were investigated using a 7-Tesla MRI system. Rats were randomized into two groups: Group 1 (n=10) with saline treatment at 4h; Group 2 (n=12) with treatment of rt-PA and 7E3 F(ab')$_2$, a GPIIb/IIIa inhibitor. The 7E3 F(ab')$_2$ was given intravenously at a bolus dose of 6.0 mg/kg 4h after embolic MCA occlusion and was followed by a second i.p. dose of 6.0 mg/kg 12 hours after the first. Recombinant human t-PA was infused intravenously at a total dose of 10mg/kg (10% bolus 4h after ischemia, and the remainder at a continuous infusion over 30 minutes). All animals were sacrificed at 48 hours after MCA occlusion. During MRI measurements, the animals were anesthetized (N_2O 69%, O_2 30%, and halothane 1.0%). Rectal temperature was kept at 37ºC ± 1.0ºC. Three complete sets of MR images were acquired at 2, 24 and 48 hours after the onset of embolization for all animals. MRI sequences including DWI, T1WI, T2WI, PWI, MRA, MT (magnetization transfer) and Look-Locker T_1 measurements were employed.

RESULTS

The MRI experiments showed that CBF in the ipsilateral tissue of rats treated with rt-PA and 7E3 F(ab')$_2$ recovered at 24h and 48h (see Fig.1a) compared to of rats receiving saline (Fig.1b). Measurements of microvascular patency by quantitative immunohistochemistry, shown in Fig.1c and Fig.1d, are consistent with the MRI results. The MRA images indicated that the treatment of embolic stroke in rat at four hours after MCA occlusion with rt-PA and 7E3 F(ab')$_2$ increased the probability of the embolus being dissolved (50% for treated group and 20% for control group at 24h). Treatment with rt-PA and 7E3 F(ab')$_2$ significantly reduced ischemic lesion volumes compared to saline treated rats (Fig.2). Significant decreases in the average relative area with low CBF (percentage area containing CBF<50 ml/100g•min to area of ipsilateral hemisphere) were detected at 24h and 48h ($p<0.01$) in the treated group compared to control group. The decrease of ischemic lesion size from the T_2 map for treated group was significantly lower than in the control group (24h, $p<0.02$ and 48h, $p<0.01$). The histological infarction volume for treated group was significantly reduced compared with control group ($p<0.002$).

CONCLUSION

Our data indicate that the treatment of rats after 4h of embolic stroke with rt-PA and 7E3 F(ab')$_2$ is effective in improving the ipsilateral cerebral blood flow and the microvascular patency of rat brain and significantly reducing the infarction volume as compared to control rats. Our data suggest that the combined treatment may be potentially useful for extending the therapy time window for treatment of embolic stroke with rt-PA.

REFERENCE

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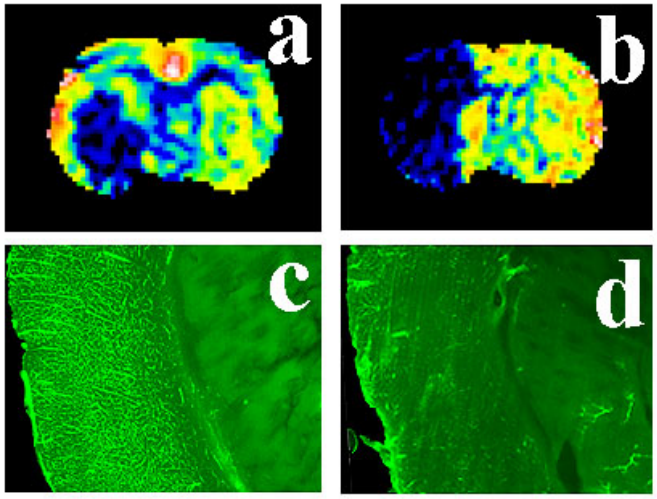

Fig.1 CBF (a) and patency (c) of microvessel recovery observed at 48h in a rat treated with rt-PA and 7E3 F(ab')$_2$ at 4 hours after embolization. No recovery in CBF (b) and histological picture (d) was found for control rat at 48h.

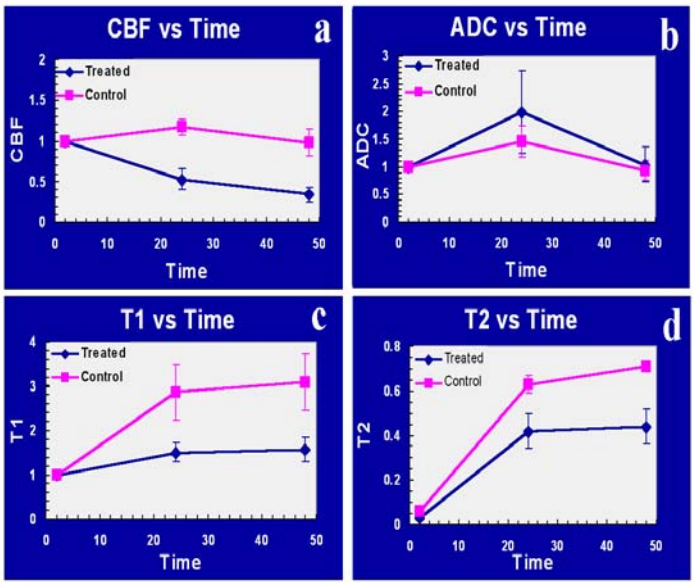

Fig.2 Normalized group average of lesion areas in MRI parameter maps for two animal groups, indicating that treatment with rt-PA and 7E3 F(ab')$_2$ at 4 hours after embolization reduce the infarction volume.