Novel combination therapy of recombinant annexin 2 and low dose recombinant tissue plasminogen activator in embolic rat stroke model: A magnetic resonance imaging study

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Introduction. In the treatment of acute phase ischemic stroke, tPA has high risk of hemorrhage which is usually fatal, and the treatment time window is very narrow. Therefore, the search for alternatives to tPA that can reduce hemorrhagic risk and lengthen treatment time window is an urgent priority. The recent studies report recombinant annexin 2 (rAN2) amplifies tPA converted plasmin generation in vitro (1), and suggest rAN2 as an alternative novel fibrinolytic agent with a significantly lower rate of hemorrhagic transformation (2). The ability to visualize ischemic tissue status longitudinally by MRI could provide vital data for understanding drug action on different ischemic tissue types, determining the optimal treatment time window, and improving experimental designs to reach the maximum efficacy, and avoiding fatal hemorrhagic transformation. In this study, we hypothesized the combination therapy of tPA and rAN2 reduces infarct size and significantly lowers the risk of hemorrhagic transformation.

Methods. 4 groups of embolic stroke rats (3) were intravenously injected with 1) saline (N=6), 2) low dose (2.5 mg/kg) recombinant tPA (rtPA) (N=4), 3) standard dose (10 mg/kg) rtPA (N=6), and 4) combination of low dose (2.5 mg/kg) rtPA and rAN2 (0.25 mg/kg) (N=5) at 1hr after stroke induction, respectively. MRI was performed to evaluate temporal evolution of lesion volume in acute phase until 4 hrs after stroke onset. The final infarct volume was evaluated by TTC staining at 48 hrs after stroke, and then cryostat sections were stained with hematoxylin and eosin and inspected by light microscopy to evaluate the risk of hemorrhagic transformation. The severity of hemorrhages was scaled (4).

MRI was performed on Bruker 7T using a 2.3-cm diameter surface coil. For each MRI modality, 7 coronal image slices was acquired with a field of view (FOV) of 2.56 cm x 2.56 cm, and a slice thickness of 1.5 mm. DWI in three orthogonal gradient directions was acquired using single-shot, spin-echo, echo-planar imaging (EPI) with the parameters as follows: TR = 2 s (90º flip angle), TE = 40 ms, b = 0 and 1100 s/mm^2, 16 transients for signal averaging, Δ = 20 ms and δ = 6.5 ms. CBF was measured using continuous ASL technique with single shot, gradient-echo EPI with the parameters of TE = 13 ms, TR = 2.8 s, labeling duration = 2.3 s, and postlabeling delay = 500ms. The 2D time-of-flight MR angiography (MRA) was acquired to identify large vessels at 1 hr and 4 hrs after stroke, using TR= 20 ms, flip angle= 80°, TE= 6.3 ms, slice thickness= 0.9 mm, FOV= 2.56 × 2.56 cm, matrix= 128 × 128, 20 slices, and 4 averages.

Results. **Figure 1** shows ADC images, CBF images, and MRA images acquired at 1 hr (pre-treatment) and 4 hrs (post-treatment) after stroke onset in each group. The standard dose rtPA-treated group and combination therapy group show ADC lesion decrease and CBF improvement after drug administration. The MRA images show recanalization of right middle cerebral artery in 5 of 6 animals of the standard dose rtPA-treated group and 4 of 5 of the combination therapy group. On the other hand, the saline group showed lesion development and no improvement of CBF and MRA. In the low dose rtPA-treated group, the ADC lesion did not change significantly until 4 hrs. **Figure 2** shows the temporal profiles of lesion volume (A) and CBF in the ischemic core (B). The combination therapy group shows the significant lesion reduction and CBF restoration, similar to the standard dose rtPA-treated group, immediately after drug administration, while the saline group and the low dose rtPA-treated groups show no significant improvement. Note that the difference of lesion volume between 4 hrs after stroke and 48 hrs was smaller in the combination therapy group (4.99% at 4 hrs vs. 8.71% at 48 hrs) than in the standard rtPA-treated group (3.4% at 4 hrs vs. 11.52 % at 48 hrs). **Table 1** shows the incidence of hemorrhagic transformation in each group. The differences among groups were statistically not significant (Mann-Whitney U test).

Discussion and Conclusion. The major findings of this study are: 1) The combination therapy has the similar beneficial effect as treatment with the standard dose rtPA treatment. 2) The lesion development from 4 to 48 hrs after stroke is smaller in the combination group than that in the standard dose rtPA group, though the incidence of hemorrhagic transformation was not significantly different among groups. rtPA also has harmful effect in stroke treatment by promoting brain matrix metalloproteinase level, which disrupt blood brain barrier (5). Therefore, the combination therapy has a possibility to reduce the harmful side effect of rtPA without reducing its benefits as treatment.

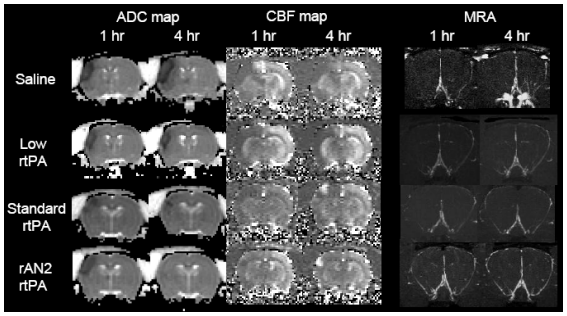

Figure 1. ADC images, CBF images, MRA images obtained at 1 hr (pre-treatment) and 4 hrs (post-treatment) after stroke in each group. The standard dose rtPA-treated group and combination therapy group show ADC lesion reduction, CBF restoration, and MCA recanalization.

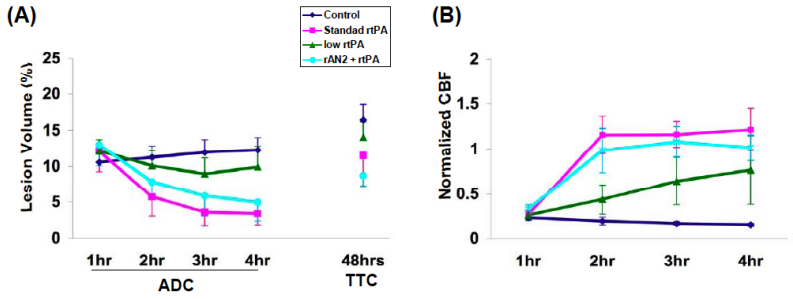

Figure 2. The temporal profiles of lesion volume (A) and normalized CBF in the ischemic core (B) in each group. The standard dose rtPA-treated group and the combination therapy group show significant reduction in lesion volume and significant restoration of CBF after treatment than that in the saline group.

	Histological score				
Group	0	1	2	3	4
Saline (n=6)	5	0	0	1	0
low rtPA (n=4)	3	1	0	0	0
standard rtPA (n=6)	2	1	0	2	1
Combination (n=5)	3	1	0	1	0

Table 1. Brain sections were graded for hemorrhage as follows: 0, no hemorrhage; 1, single microscopically visible hemorrhage; 2, multiple microscopically visible hemorrhages; 3, macroscopically visible non–space-occupying hemorrhage; 4, macroscopically visible space-occupying hemorrhage.

References 1) Hajjar et al. J. Biol. Chem. 269:21191, 1994. 2) Tanaka et al. Brain Res. 1165:135, 2007. 3) Tanaka et al. Acta Neurochir. Suppl. 86:141, 2003 4) Nissen et al. Stroke. 34: 2019, 2003. 5) Tsuji et al. Stroke. 36:1954, 2005.