6931

l Spreading Depolarization Serve as a Marker for Tissue Plasminogen Activator (tPA) Toxicity for Stroke Treatment?

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TARGET AUDIENCE Basic and Clinical Scientists in Stroke, Neuroimaging, Electrophysiology, and Neuropharmacology.

INTRODUCTION Thrombolysis by intravenous recombinant tissue plasminogen activator (tPA) is the only proven therapy for stroke. tPA treatment unfortunately is limited to only a small subset of patients because it has serious risk of causing hemorrhage and is useful only within ~4.5 hours after stroke onset.[1] A recent report showed that even for patients approved for tPA treatment, tPA still increases the chance of having intracerebral hemorrhage by 6% and raises fatality rate by 4% within 7 days.[2] As tPA is known to be neurotoxic, **identifying a marker to monitor tPA effect during the treatment is of great clinical importance to prevent tPA-induced damage**. We recently established a novel animal model that allows stroke induction during MRI scans.[3] This model has been shown useful to study cortical spreading depolarization (CSD) using high resolution MRI and has potential to create controllable ischemic lesions. CSDs are a series of electrical potential changes that usually appear spontaneously during the hyperacute phase of stroke. **CSD is known to exacerbate ischemic damage**, as the number of CSDs correlates with final infarct volumes and suppressing CSDs improves functional outcomes.[4,5] During CSDs, a continued cation influx occurs through glutamate-gated ion channels, which further depolarizes adjacent cells and contributes to the "spreading" phenomenon.[5] As tPA is known to stimulate glutamatergic receptors[6,7], we hypothesize that **CSD can serve as a real-time marker for tPA toxicity**. In this study, we aimed to demonstrate that: (i) **controllable ischemic lesions can be generated in our model inside the MRI**, and (ii) **tPA will attenuate CSDs in mild ischemic injury and exacerbate CSDs in more severe injury**.

METHODS Forty-four male Sprague Dawley rats (250–300 g) were anesthetized with 1.5% isoflurane, ventilated and paralyzed. A home-made MR-compatible optic fiber (200 µm) was placed above the primary somatosensory cortex of forelimb (**Fig. A**). Ischemic stroke was generated by using guided green laser in rats receiving i.v. Rose Bengal. Five groups of animals were used to study the effect of changing induction parameters. Group 1 used standard induction parameters: 10 mg/kg rose bengal with 10 mW laser illuminated for 10 min (n=14). While keeping the rest of the parameters fixed, Group 2 used 20 mg/kg rose bengal (n=6), Group 3 used 20 mW laser (n=5), Group 4 used 30 mW laser (n=5), Group 5 used 20 min illumination duration (n=6). MRI was performed on a Bruker 9.4T Biospec scanner with a home-made surface coil (ID=2 cm) and a separate neck coil for arterial spin labeling (ASL). CBF and ADC were alternatively acquired every 2.5 min for 200 min including 20 min baseline before stroke induction. CBF was measured by continuous ASL using single shot gradient-echo EPI. ADC was acquired with the same geometry using single shot spin-echo EPI. Imaging parameters were identical to that described previously.[3] T2-weighted images and TTC staining were performed at 24 h after stroke. In Group 6, both cortices were simultaneously lesioned by 8 and 30 mW laser, respectively, creating mild and severe ischemic lesion (n=3). Continuous tPA infusion was performed for 30 min. CBF was continuously acquired for more than 1 h and permeability was measured at the end of the study using dynamic contrast enhanced (DCE) MRI with Gd-DTPA. Evans blue staining was performed at 3 h after stroke onset. Image data were processed similarly as described elsewhere.[8] Statistical analysis was performed by ANOVA with Turkey's HSD post-hoc test, paired and independent t-tests. The number of CSDs was tabulated. Significant level was set at $P<0.05$.

RESULT & DISCUSSION This study demonstrated that by modulating stroke induction parameters, the lesion severity, diffusion-perfusion mismatch, final infarct volume, and the number of CSDs can be manipulated. Compared to Group 1 (10 mg/kg), faster ADC and CBF reduction and larger infarct volume was observed in Group 2 using a higher rose bengal dose (20 mg/kg) ($P<0.05$) **(Fig. B)**. Elevating laser power in Group 3 and 4 (20 and 30 mW, respectively) also significantly accelerated stroke progression ($P<0.05$) and increased final infarct volumes ($P<0.05$) **(Fig. C&D)**. We also demonstrated that with prolonged laser illumination duration in Group 5, no significant changes in lesion size, ADC/CBF time courses and mismatch volumes were observed, possibly due to rapid wash-out of rose bengal **(Fig. E)**. In Group 4, the number of CSDs increased significantly (~1.9 fold) ($P<0.05$) **(Fig F)**, in which larger mismatch volumes (~3.1 fold) ($P<0.05$) were observed compared to Group 1 **(Fig G)**. **These data suggest that lesion severity can be controlled in our model.**

Interestingly, our data in Group 6 showed that infusing tPA evoked 3-fold more CSDs in severe ischemic lesion, but instead suppressed CSDs in mild ischemic lesion in the same subject (both $P<0.05$) **(Fig I)**. DCE MRI and Evans blue staining in these animals showed a significant difference in permeability between two ischemic conditions, indicating that tPA-induced CSD changes might be related to blood-brain-barrier integrity **(Fig J)**. Our future studies will address the question that whether glutamatergic antagonist (e.g., Topiramate) modifies CSD signatures during tPA treatment in the hyperacute phase of stroke. Ultimately, this study should provide strong implications for clinical decision-making because clinicians can determine whether or not to terminate tPA infusion (usually ~1 h) for a stroke patient when multiple CSDs occur or co-administer a glutamate antagonist/anti-epileptic drug to reduce tPA tissue toxicity.

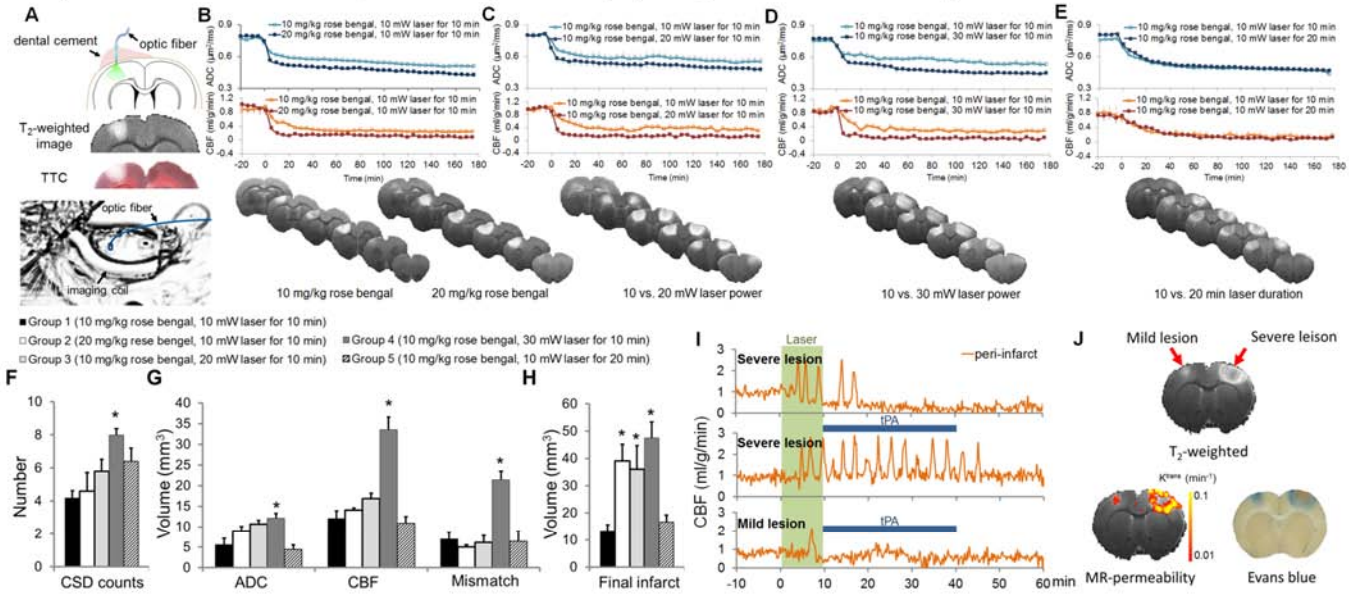

Figure (A) The schematic plot of the proposed model. Ischemic lesion was confirmed with T_2-weighted image and TTC stain in the same subject. Temporal evolution of ADC and CBF **(upper panel)** and T2w-images at 24 h **(lower panel)** in Group 1 and 2 **(B),** Group 3 **(C),** Group 4 **(D)** and Group 5 **(E).** The number of CSDs **(F)**, diffusion-perfusion mismatch **(G)** and final infarct volume **(H)** were also calculated in different stroke induction groups. The error bars are s.e.m. *indicates significant difference from Group 1 ($P<0.05$). **(I)** tPA induced nearly 3-fold more CSDs in severe ischemic lesion and suppressed CSDs in mild ischemic lesion. **(J)** T_2-weighted MRI, permeability MRI, and Evans blue staining in a representative subject with mild and severe ischemic lesion.

REFERENCE

[1]. Davis et al., Stroke, 2009;40:2266. [2] Sandercock et al., Lancet, 2012;379:2352. [3] Kao et al., ISMRM, 2013, #0335 (Oral). [4] Ayata et al,. Cephalalgia, 2009;29:1095. [5] Dreier, Nat Med, 2011; 17:439. [6] Casse et al., J Neurosci 2012;32:5186. [7] Kaur et al., JCBFM, 2004;24:945. [8] Shih et al., JCBFM, 2011, 31:832.