

MRI Detection and Histological Correlates of the Dependence of Brain Damage on Recovery Time Between Recurrent Mild Strokes.

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Target Audience: Clinicians and Basic Scientists Interested in Imaging Mild Brain Damage.

Purpose: To determine whether recovery from an initial mild ischemic insult influences the susceptibility of the brain to a second mild insult.

Background: Clinically, patients with stroke often experience a transient ischemic attack (TIA) or minor stroke with a high recurrence within the first days to weeks¹. It is possible that recovery from an initial mild ischemic insult influences the susceptibility of the brain to a second insult but it is unknown how a first insult impacts the severity of a subsequent mild ischemic event. We hypothesized that total brain damage from recurrent stroke would be affected by the time between mild insults at different times during the first week. Thus we investigated the damage, as characterized by magnetic resonance imaging (MRI) and histological assessments, produced by a combination of two mild photothrombosis (PT) insults with a 1 day, 2 day, 3 day or 1 week interval between them.

Methods: The method of PT² was modified to induce a mild stroke in 38 Wistar rats. Rats were anesthetized, the skull was exposed and thinned and covered by a 3x3mm² open mask. Rose Bengal (10mg/kg) was administered IV followed by skull illumination (approx. 35000 lux) with white light. The PT procedure was repeated at 1d, 2d, 3d and 1wk following the first PT (n=5/group). At 24 hours after each PT, MR scans (matrix 256x256, FOV 3cm²) were acquired using a 9.4T Bruker MR system. A T₂ spin echo sequence (TR/TE (inter echo) of 7000ms/10ms, 32 echoes), an echoplanar diffusion sequence (5 b values) and a perfusion sequence using arterial spin labeling were acquired³. Two days after the last surgery, animals were perfusion fixed and paraffin embedded brain sections were stained with hematoxylin and eosin (H&E). Additional rats with a single PT were also perfused at 1d, 2d, 3d and 1wk to study histological changes over time. The cortex under the illuminated region was analyzed and the same region was used for histological scoring with a scale of: 0 for no damage, 1 for scattered necrosis, 2 for incomplete infarct and 3 for complete infarct. Comparisons between groups were made using an ANOVA and a Student Neuman-Keul's multiple comparison of means.

Results: The modified photothrombotic model usually produced a mild stroke consisting of modest increases (10-20%) in T₂ and scattered necrosis that was associated with transient ischemia within the illuminated area (Fig A). ADC changes were less sensitive for detecting mild injury. For each of the recurrent groups, rats with an initial mild injury had similar mean T₂ increase at 24hr after the first PT. A second mild ischemic insult enhanced the damage detected with MRI (Fig B,C). However, the T₂ increase varied depending on the interval between the two ischemic episodes and was significantly greater for 1, 2 or 3 d intervals than for a 1 wk recovery interval between insults (p<0.05) (Fig D). H&E sections showed scattered necrosis at 24hr after a single mild PT. Recurrent mild PT resulted in more severe tissue damage compared with single PT (p<0.05). The cumulative damage was more pronounced with shorter intervals (1d to 3d) than that with a longer recovery interval (1wk). Perfusion changes reflected the T₂ imaging, with ischemia being most severe in animals with recurrent 1 and 2 day PT compared to the 1w group.

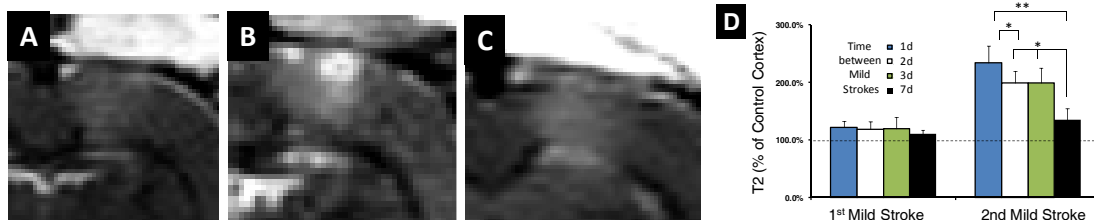


Fig. Recurrent Mild Photothrombosis (PT). T₂ weighted images at 24h after a mild transient ischemia (A) or after a recurrent PT either 1d after the 1st PT (B) or 7d after the 1st PT (C). T₂ increases after the recurrent PT were greatest if the recovery between insults was 1 to 3 days (D). *P<0.05; **P<0.001 difference between groups.

Discussion: The results demonstrate that the photothrombosis method can be modified to produce a transient ischemia associated with selective necrosis. This mild injury is detected as a modest increase in T₂ without significant changes in ADC. A recurrent mild PT enhanced the tissue damage detected as an augmentation in T₂. The enhancement is time-dependent being most pronounced if the recurrent stroke occurs at an acute time point (1d) versus a more chronic time point (1wk) after the initial insult. This suggests that brain is most susceptible to a second ischemic insult in the first few days after a mild ischemic insult and therapeutic intervention is urgent to help prevent a subsequent severe stroke in patients with mild stroke or TIA.

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References (1.) Chandratheva, A., Mehta, Z., Geraghty, O.C., Marquardt, L., & Rothwell, P.M. *Neurology* **72**, 1941-1947 (2009). (2) Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. *Ann Neurol* 17:497-504.1985. (3) J. Kaur, U.I. Tuor, Z. Zhao, P.A. Barber. *J Cereb Blood Flow Metabol* 31:1874-85. 2011.