Temporal MRI Profile of Hermorrhagic Transformation after Embolic Stroke in Spontaneously Hypertensive Rats

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Introduction:

Currently the only effective and approved therapy against acute ischemic stroke is treatment with recombinant tissue plasminogen activator (rtPA). However, the treatment time-window is seriously limited because of increased risk of hemorrhagic transformation (HT). Elucidation of the pathophysiological basis of HT after stroke may lead to improvement of treatment strategies. MRI offers a unique tool that allows serial *in vivo* assessment of multiple pathophysiological processes that are involved in HT.[1] The goal of this study is to identify MRI profiles of regions that develop HT within an ischemic lesion, using a model of stroke in spontaneously hypertensive rats.

Materials and Methods:

Spontaneously hypertensive rats (n=17) were subjected to unilateral embolic ischemic stroke according to the model of Zhang *et al.*[2] These rats were part of a larger study to detect effects of combination treatment strategies. Researchers are blinded for treatment assignments. At 0-2 h, 24 h and 7 days after stroke, rats were scanned on a Varian horizontal bore 4.7T MR system. MRI included T_2-weighted imaging (multiple spin echo; TR 3600 ms; TE 12-144 ms; matrix size 256x128x19), diffusion-weighted imaging (spin echo 8-shot EPI; TR 3500; TE 38.5 ms; b-values 0 and 1428 s/mm^2; 6 diffusion-weighting directions; matrix size 128x128x19), T_2*-weighted imaging (multiple gradient echo; TR 1400 ms; TE 7-70 ms; matrix size 256x128x19). Dynamic susceptibility contrast-enhanced MRI (gradient echo EPI; TR 330 ms; TE 25 ms; matrix size 64x64x5) was conducted in combination with an i.v. injection of 0.35 mmol/kg gadobutrol (Gadovist®) for calculation of CBV and CBF index (CBF_i) maps.[3] Subsequently, post-contrast T_1-weighted images (gradient echo; TR 160 ms; TE 4 ms; matrix size 256x128x19) were acquired every 2.7 minutes for 35 minutes. For all acquisitions FOV was 32 x 32 mm, with 1 mm slice thickness. Treatment was given after the first MRI session at 2 h post-stroke.

After MRI on the 7th day, rats were euthanized by a pentobarbital overdose, followed directly by intracardial perfusion with saline. After brain extraction and sectioning (2 mm. slice thickness), regions with clear red blood accumulation within the ischemic lesion territory were identified. These regions were outlined on corresponding MRI slices and defined as HT areas. Regions within the lesion and without HT (non-HT areas), as well as contralateral (cl) counterparts of these HT and non-HT areas, were also outlined. The temporal profile of mean T_2, T_2*, ADC trace, CBF_i, CBV and contrast-induced T_1-weighted signal change were calculated in these regions. Data were statistically analyzed by repeated measures one-way ANOVA with post hoc Student-Newman-Keuls testing. $P < 0.05$ was considered significant.

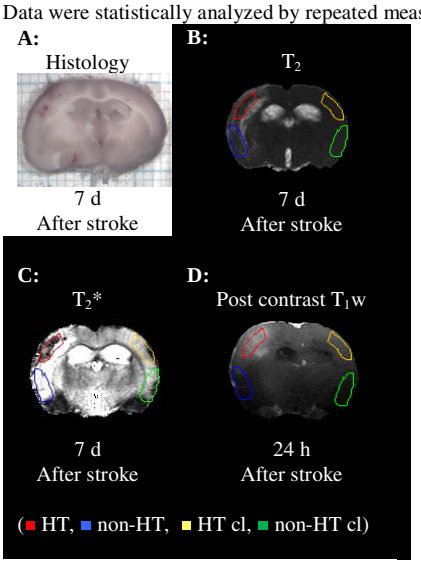

Figure 1: Different representative images of a rat brain slice displaying HT within the ischemic lesion. Regions selected for analysis are outlined on the MR images.

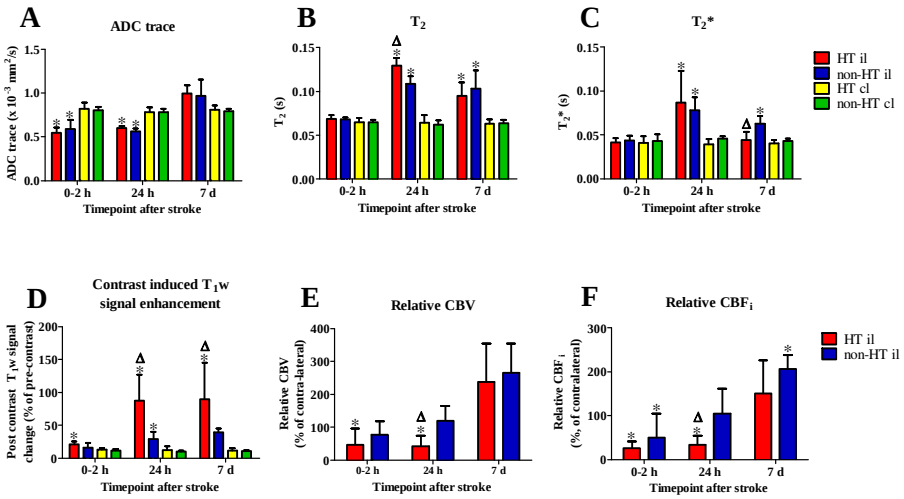

Figure 2: Graphs displaying ADC trace (A), T_2 (B), T_2 (C) and relative post-contrast T_1-weighted signal enhancement (D) in HT (■) and non-HT regions (■), and their contralateral counterparts HT cl (□) and non-HT cl (■). Also shown are CBV (E) and CBF_i (F) in HT (■) and non-HT regions (■), relative to contralateral values. Graphs show mean values (+ SD). *$P < 0.05$, vs. contralateral; $^{\Delta}P < 0.05$, vs. non-HT.*

Results:

Out of 17 rats, 11 animals completed the protocol, of which 5 showed a clear region of HT within the ischemic lesion. Fig. 1 shows intracerebral hemorrhage within the ischemic lesion on an extracted brain section (A) and corresponding T_2 (B) and T_2* maps (C) of a rat at 7 days post-stroke. Contrast leakage at 24 h after stroke in the same animal is evident on the post-gadobutrol T_1-weighted image in fig. 1D. Results from ROI analysis are shown in fig. 2. Acutely after stroke, ADC trace (fig. 2A) and CBF_i (fig. 2F) were reduced in ipsilateral HT and non-HT areas as compared to contralateral. Post-contrast T_1-weighted signal enhancement and CBV (fig. 2E) were significantly different from contralateral in HT but not in non-HT regions (fig. 2D). At 24 h, ADC trace was still decreased in the entire ischemic lesion, while T_2, T_2* and post-contrast T_1-weighted signal change were increased (fig. 2 A-D). In HT regions, T_2 elevation and gadobutrol enhancement were significantly larger compared to non-HT areas (fig. 2B, D). In addition, CBV and CBF_i were considerably lowered in HT regions at this stage, while hyperperfusion became evident after 7 days (fig. 2E, F). At day 7, when HT was identified histologically, T_2^* values were lower in HT than in non-HT regions (but not compared to contralateral) (fig. 2C). Also, contrast leakage was still markedly elevated in HT areas (fig. 2D).

Discussion:

We found that HT after unilateral stroke in spontaneously hypertensive rats is associated with locally reduced perfusion, largely increased leakage of the blood-brain barrier and elevated edema formation, particularly after 24 h, indicative of severe ischemic damage. HT may be directly detected by T_2^* shortening[4], however we found that the T_2^* in HT areas is influenced by T_2^* prolongation due to edema formation. Nevertheless, T_2^* dropped considerably between 24 h and 7 days in HT regions, which was not the case for the non-HT regions, suggesting that HT mainly developed between these time-points. Our study confirms that multiparametric MRI allows comprehensive assessment of diverse pathophysiological processes involved in HT. In particular MRI of perfusion status and blood-brain barrier integrity may provide prognostic information on risk of HT after stroke.

References:

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