Characterization of a Novel Chronic Photothermotic Ring Stroke Model in Rats by Magnetic Resonance Imaging, Biochemical Imaging and Histology

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Synopsis
A novel photothermotic ring stroke model in the rat was characterized by magnetic resonance imaging (MRI), biochemical imaging, and histology. The reproducible lesion comprised the primarily damaged ring ("ring-lesion"), which degenerated into necrosis at 14 days after lesion induction, and the ring-encircled interior "region-at-risk" showing spontaneous recovery after 3 days, as documented by perfusion, ADC, and T₂ relaxation time normalization. Therefore, a considerable part of cells was salvaged in the region-at-risk, as reflected by partially maintained brain metabolism. Furthermore, this first noninvasive study on the ring stroke model demonstrates individual outcome prognosis by early MR tissue signature.

Methods
Male Wistar rats were exposed to photothermotic ring lesion by infusion of the photosensitizing dye erythrosin B and simultaneous irradiation of the rat’s cortex through the intact skull bone by a circular profile laser beam [1,2]. At 5 hours, 2, 3, 7, and 14 days after lesion induction animals were investigated by multiparametric MR imaging, using a 4.7T horizontal magnet (Bruker Biospec 47/50) with a Helmholz coil for RF transmission and a surface receiver coil. Perfusion-weighted imaging (PWI) was performed by the arterial spin labeling technique and a snapshot FLASH sequence (TR = 8.9 ms, TE = 5.3 ms, slice thickness = 2 mm, FOV = 4 x 4 cm²). Diffusion-weighted imaging was recorded by a Stejskal-Tanner spinecho sequence (TR = 2325 ms, TE = 35.2 ms, slice thickness = 1.21 mm, FOV = 4 x 4 cm²) with 2 diffusion-encoding gradient strengths (b = 30 and 1500 s/mm²), and T₂ images were acquired by a CPMG sequence (TR = 3000 ms, TE = 12.5 ms, slice thickness = 1.21 mm, FOV = 4 x 4 cm²) using 16 echoes. Regional evaluation of MR parameters was done by region-of-interest analysis ("ring-lesion" and "region-at-risk", see Fig. 1). At the end of the experiment (14 days), CBF-autoradiography was performed and regional distribution of adenosine triphosphate (ATP) and pH was evaluated by biochemical imaging. Frozen brain material was further analyzed by hematoxylin/eosin (HE) and silver impregnated staining (SIS).

Results
The chronic changes in MR parameters are shown for a representative animal in Fig. 1. The primary ring-lesion was easily detected by a strong ADC decrease and intense T₂ increase in horizontal superficial slices (Fig. 1, first column). PWI signal declined on average to 37±5% of contralateral control in this region (Fig. 1, PWI, 2d). The simultaneous intense ADC decrease to 55±13% (cytotoxic edema) and T₂ increase to 175±11% (vasogenic edema) was followed by necrosis as shown by low relative ATP (18±2%), alkalosis (pH = 7.65±0.44), and tissue damage within the primarily lesioned cones in coronal sections (HE + SIS, Fig. 2). In the secondarily affected region-at-risk, a PWI decrease at 2 days (36±5%) was followed by spontaneous partial reperfusion as measured at 3 and 7 days after ring stroke (83±11%) in 4/6 animals, resulting in secondary deterioration at 14 days (60±17%), resulting in secondary deterioration at 14 days (60±17%). T₂ decrease was moderate (78±9%), but reperfusion was obviously inhibited by a strong vasogenic edema (T₂ = 166±12%; Fig. 1, 2d). The incomplete and late restoration of tissue perfusion resulted in reduced ATP levels (ATP = 46±11%) and alkalosis (pH = 7.34±0.55), (Fig. 2, ATP + pH). The impaired brain metabolism in the region-at-risk of these animals indicates that only part of the tissue was salvaged (Fig. 2, HE + SIS). In two animals, minor ischemic changes in the region-at-risk were followed by an intense hyperperfusion (PWI = 162±15%) leading to preservation of brain metabolism (ATP = 94±2%) and only slightly acidic pH (6.76±0.02), indicating complete tissue survival in the region-at-risk of these animals. Early MR changes enabled prediction of outcome (14d) by tissue signature (2d); a) initial ADC drop to below 60% of control resulted in very low ATP levels, indicating breakdown of energy metabolism, b) moderate ADC decrease (70-90%) in combination with a strong vasogenic edema (T₂ > 160%) led to impaired energy metabolism (35% < ATP < 60%), c) minor early ADC changes (90-100%) and moderate T₂ increase (100-125%) were followed by strong hyperperfusion, resulting in almost normal ATP (> 92%). The complete preservation of energy metabolism in the latter case suggest that the tissue was not seriously affected.

Conclusions
We have shown that multiparametric MRI, biochemical imaging and histology are able to provide a detailed characterization and differentiation of both relevant regions, i.e., the ring-lesion, and the region-at-risk, in this novel stroke model. With these parameters, we have demonstrated that the primary damaged ring-lesion is transferred to necrosis. In the region-at-risk, moderate ischemia up to 48 hours after ischemic onset is followed by spontaneous reperfusion and the capability of at least partial recovery. We found good agreement between final non-invasive measurements and histology as well as biochemical imaging, respectively. Furthermore, we demonstrated the prognostic value of MRI by early tissue signature.

References