Effects of cortical spreading depression on blood-brain barrier permeability in a mouse model of familiar hemiplegic migraine

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Introduction: Familial hemiplegic migraine type 1 (FHM-1) is a migraine subtype with aura that is associated with mutations in the *CACNA1A* gene, which encodes for the α_{1A} subunit of $Ca_V2.1$ channels. FHM-1 mutation S218L causes a severe, sometimes lethal phenotype in patients. It was shown that transgenic knock-in mice expressing this mutation have increased CSD frequency and propagation speed of cortical spreading depression,[1] a slowly propagating neuronal and glial cell depolarization that leads to depression of neuronal activity, is thought to be the main cause of migraine aura. The CSD properties in S218L mice are in line with the human FHM1 phenotype with severe and prolonged post-CSD neurological deficits. Cortical spreading depression (CSD), a slowly propagating neuronal and glial cell depolarization that leads to depression of neuronal activity, is thought to be the main cause of migraine aura.[2] The present study investigates the effect of experimentally induced CDSs on blood-brain barrier permeability of heterozygote S218L mice. Breakdown of the blood-brain barrier (BBB) was assessed using a combination of gadolinium-enhanced MRI and histology.

Methods: *CSD induction:* The skull was exposed and a square cranial window (1 mm x 1 mm) ~2 mm lateral and ~3.5 mm posterior to Bregma was created in the right hemisphere. Seven CSD waves were induced by applying 30 sec pulses of 1M KCl onto the exposed cortex. The contralateral side served as control.

MRI scans: In vivo T1W RARE MRI scans were taken using a vertical bore 9.4 T Bruker system. The mice were scanned under isoflurane anaesthesia. The dynamics of the BBB opening were followed on day 0, 1, 2, 3, and day 9 post-surgery. At each timepoint, a prescan was first taken, then 10 mmol/kg GdDOTA was administered i.p.. Immediately following the injection, 6 consecutive scans were recorded. Scanning parameters were: TE = 11.67ms, TR = 870ms, RARE factor = 2, FOV = 20x20mm^2, matrix = 256 x 256, no slices = 22, slice thickness = 0.5mm, 6 averages. The permeability of the BBB was estimated using a Patlak model [2].

Histology: Brains were collected from a separate group of mice which were also subjected to CSD and sacrificed at the same time-points as those used for the MRI scans. Brains were sectioned into 5-µm-thick slices and were stained for GFAP, IgG, CD31, Laminin, ZO-1, and GLUT-1.

Results: Contrast-enhanced MRI after CSD induction revealed blood-brain barrier leakage in the ipsilateral cortex, as well as the hippocampus (Figure 1). The leakage appeared strongest in the cortex (Figure 2), and the maximum was reached around 1 day after CSD induction (a 20-fold increase compared to the contralateral site). Nine days after CSD induction, the blood-brain barrier was found to be still open in the cortex ($p<0.02$). In the hippocampus, BBB leakage was observed up to 3 days after CSD induction; after 9 days the BBB appeared to be restored. Histological analysis revealed astrocyte and microglia activation in the ipsilateral site and profound leakage of the blood-brain barrier (data not shown).

Conclusions: CSD events in heterozygote S218L mice cause BBB break-down in the cortex, hippocampus and thalamus. Blood-brain barrier leakage peaks at one day after CSD induction. BBB integrity and function do recover, but the recovery takes more than 9 days in this mouse model. These results indicate that in the susceptible brain, BBB disruption is a severe and long-lasting complication of spreading depression.

1. Eikermann-Haerter K, *et al* (2009) Genetic and hormonal factors modulate spreading depression and transient hemiparesis in mouse models of familial hemiplegic migraine type 1. J Clin Invest 119 (1): 99-109.
2. Kors EE, *et al* (2001) Delayed cerebral edema and fatal coma after minor head trauma: role of the *CACNA1A* calcium channel subunit gene and relationship with familial hemiplegic migraine. Ann Neurol 49(6):753-60.
3. Abo-Ramadan U, *et al* (2009) Post-ischemic leakiness of the blood-brain barrier: A quantitative and systematic assessment by Patlak plots. Experimental Neurology 219: 328-333

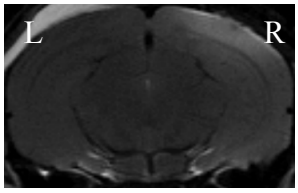

Figure 1: T1W image of a mouse brain positioned at ~ -3.0mm Bregma, 1 day after CSD induction. The right hemisphere (R) served as induction site for the CSD session.

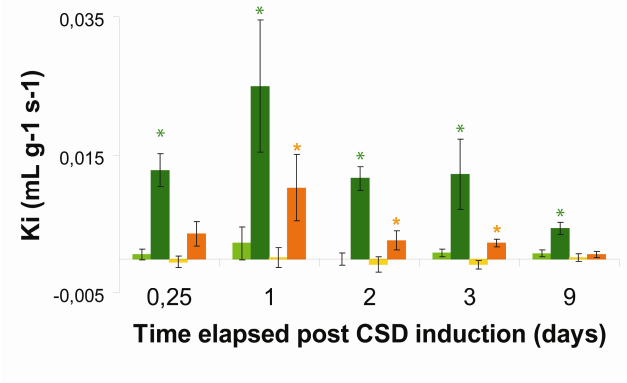

Figure 2: Blood to brain transfer rate constant estimated by Patlak plots for the ipsilateral (darker shade) and contralateral (lighter shade) of the cortex (green) and hippocampus (orange) after CSD induction, at ~-3.0 Bregma. The error bars represent the standard deviation. The stars indicate a statistically significant difference between the ipsilateral and contralateral site ($p<0.05$, 2-tail paired Student's T test).