Long-Term Observations after Transient Middle Cerebral Artery Occlusion (tMCAO) in Mice

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Background

The challenge of translating preclinical into clinical advances in stroke research is partly due to limitations of the established in-vivo models¹⁾. tMCAO in mice models closely the ischemia / reperfusion (I/R) injury of patients with acute large vessel occlusion and recanalization. Due to the high mortality of this model long-term observations were hitherto impossible. A new care protocol comprising close clinical monitoring and individual care increases the survival rate > 70%²⁾ and was used to get first representative insights into the dynamics of post-reperfusion inflammation and regeneration. Special focus was on the contribution of S1P to those dynamics as it is known to be a crucial regulator of barrier integrity and immune cell migration.

Post-Stroke Monitoring and Care

A1 – A Stroke-Unit Concept for Experimental Work

A2 – Body Weight Monitoring

A3 – Mortality after tMCAO

A1: In analogy to stroke patients mice need close monitoring and scoring to receive tailored treatment comprising feeding support and warming

A2: Maximum of metabolic crisis is reached at day 5 after tMCAO and at day 3 after sham surgery (replication of 2), own data

A3: Establishment group displays mortality at still insufficient support, experimental group shows mortality reduction by optimal post-stroke care (replication of 2), own data

1) Dirnagl et al., Stroke 2013

2) Loubopoulos et al., JCBFM 2016

mRNA-Sequencing of Microvessels After tMCAO

B1 – experimental design

B2 – tMCAO model

B3 – Sample Isolation

B1: tMCAO vs. sham surgery on male C57Bl/6, brain removal after 24h or 7d, pooling of 3 hemispheres to 1 sample, isolation of microvessels, isolation of mRNA; 4 samples per group; bioinformatical analysis by Stefan Guenther et al.

B2: insertion of silicon coated suture (Doccol) via common carotid artery (CCA) until middle cerebral artery (MCA), reperfusion after 60min

B3: homogenisation of brain tissue, centrifugation (10min, 400g), resuspension of pellet in 25% BSA, centrifugation (30min, 2000g), resuspension of pellet in RLT lysis buffer, isolation of fragments (70 – 100 µm) by cell strainers (Falcon), mRNA isolation (Quiagen); all steps at 4°C

Expression Dynamics after Stroke

C1 – Expression Dynamics after tMCAO

C2 – Astrogliosis

C3 – mRNA Sequencing Analysis

C2: IHC staining of periinfarct cortex 24h vs. 7d after tMCAO (DAPI, **rbαGFAP** **Abcam**), PFA-fixed cryosections

C3: Massive outburst of inflammatory cytokines 24h post I/R injury, accompanied by massive upregulation of S1P generating enzyme sphingosine kinase 1; striking increase of proliferation markers 7d post tMCAO