ASL-BASED CEREBRAL PERFUSION WITH OR WITHOUT PERMANENT UNILATERAL COMMON CAROTID ARTERY OCCLUSION: TOWARDS AN OPTIMIZED MOUSE STROKE MODEL.

Tom Dresselaers[1], Wouter Oosterlinck[2], Tom Struys[1,3], Kristof Govaerts[1], Ivo Lambrichts[3], Paul Herijgers[2], and Uwe Himmelreich[1]

[1]*Department of Imaging & Pathology, KU Leuven, Leuven, Vl.Brabant, Belgium,* [2]*Department of Experimental Cardiac Surgery, KU Leuven, Leuven, Vl.Brabant, Belgium,* [3]*Department of Functional Morphology, Hasselt University, Diepenbeek, Limburg, Belgium*

Target audience

This abstract is relevant to scientists with an interest in stroke, specifically animal models of stroke, and potentially to those focusing on vessel occlusion models (in relation to neurodegeneration).

Purpose

Ischemic stroke is the third leading cause of death and the leading cause of long-term disability. In mice, the transient middle cerebral artery occlusion (tMCAO) model is the most widely used animal model to mimic this devastating neurologic disease (1). However, a common procedure in this tMCAO model is the permanent unilateral ligation of the common carotid artery (ulCCA) in which the ipsilateral perfusion becomes highly dependent on collateral flow. The impact of these deviating flow values on the development of the stroke lesion is however unclear. In this study, we firstly mapped the regional perfusion differences resulting from a permanent ulCCA *in vivo*. Secondly, an adapted surgery protocol was developed which allows full reperfusion of the assaulted brain region by applying a suture of the CCA after preparation of a puncture in the CCA that would allow insertion of an occluding filament. Differences in cerebral blood flow (CBF) levels between both approaches were monitored by arterial spin labeling (ASL).

Materials & Methods

Male C57Jbl-6 mice (8 weeks) were used in this study. In group 1, a typical sham operation for stroke experiments of the tMCAO model was performed which involved a puncture of the common carotid artery (CCA), large enough to allow filament insertion, followed by a permanent unilateral ligation of the CCA, all under isoflurane anesthesia. In group 2, an identical procedure was performed but the CCA was sutured (10.0 or 11.0 suture). Group 3 had no surgery. Mice were scanned 8h, 1 day and 1 week post surgery (isoflurane 1.2-1.4%, in O_2).

MR images were recorded on a 9.4 T Biospec small animal MR system (20cm horizontal bore, Bruker Biospin, Ettlingen, Germany) using a 7 cm linearly polarized resonator for transmission and an actively-decoupled mouse brain surface coil for receiving (Rapid Biomedical, Rimpar, Germany). ASL data were acquired using a FAIR approach (2-3) and a RARE readout with the following specific parameters: TR 10s, TE 5.2 ms, rare factor 72, FOV 2.5x2.5 cm, matrix 128x128 with partial FT acceleration to 128x72, fourteen inversion times from 300-4000ms, using a inversion hyperbolic secant of 14ms, (Paravison 5.1, Bruker). CBF values were calculated using the T1 difference method (5). ADC values from the same slice were determined using echo planar readout (3segments, b=2500 s/mm^2, 30 directions, gradient echo). For MR angiography a 3D TOF FLASH sequence was used (TR/TE=20/2.4ms; flip angle=20deg, FOV=2.56x2.56x1.6 cm, isotropic resolution of 50um). T_2 values were determined using a multi-slice multi-echo sequence (TR=2856ms , 10 TE's from 12 to 120ms). Mean and standard deviations are reported.

Results

In vivo data confirm that permanent ulCCA causes a significant drop in the vascular perfusion of the ipsilateral hemisphere of about 50% under isoflurane anesthesia within 1 day with partial recovery at 1 week (fig. 1), as previously reported (4). However, no ischemic lesion is created as shown by unaltered apparent diffusion constants (thalamic ADC ratio of ipsi- to contralateral hemisphere 0.97±0.01 and 0.99±0.01, for respectively ligated and sutured at 8h) or T_2 (thalamic T_2 ratio of ipsi- to contralateral hemisphere 1.00±0.01 and 1.00±0.02, for respectively ligated and sutured at 1 day). Inter-hemispheric differences in CBF values tend to normalize after one month following surgery (4). In animals which underwent the adapted surgery protocol, characterized by suturing of the CCA, CBF was immediately restored to normal values post surgery (fig.2). MR angiography showed an open carotid throughout the investigated time frame.

Fig. 1

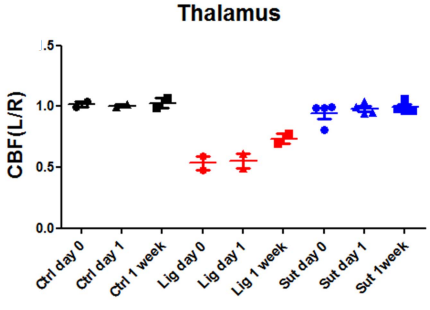
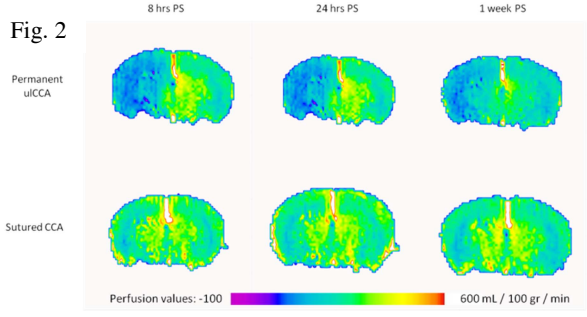


Fig. 2



Discussion

Since perfusion levels and blood pressure are known to influence stroke lesions, an animal model with limited impact on perfusion, other than those induced by the tMCAO, seems preferable. Furthermore collateral circulation is strain dependent making the ligation of the CCA undesirable in several strains. Although the CBF differences seen here are potentially enhanced due to the well documented vasodilatory effect of isoflurane. Our data does show that, with suture of the CCA, perfusion differences can be avoided.

Conclusion

Suture of the CCA in the tMCAO model is feasible and avoids dependency on collateral blood flow which may affect the CBF levels as seen using the conventional approach of ligation of the CCA. We therefore hypothesize that this new suture protocol might enhance the outcome of tMCAO stroke mice, which is known to be characterized by a high mortality rate.

References

(1) Liu et al., J Biomed Biotechnol. 2011 (2) Kim et al., MRM 1995 (3) Kwong et al.; MRM 1995 (4) Struys et al., ISMRM 2012 (5) Williams DS, et al., PNAS, 89: p.212-216 (1992).