Arterial spin labeling-based longitudinal monitoring of CBF and CVR following permanent unilateral ligation of the common carotid artery

Tom Struys[1,2], Tom Dresselaers[1], Sarah Caers[1], Wouter Oosterlinck[3], Ann Van Santvoort[1], Ivo Lambrichts[2], and Uwe Himmelreich[1]
[1]*Department of Medical Diagnostic Sciences, Katholieke Universiteit Leuven, Leuven, Belgium,* [2]*Department of Functional Morphology, Universiteit Hasselt, Diepenbeek, Belgium,* [3]*Cardiovascular Diseases, Katholieke Universiteit Leuven, Leuven, Belgium*

Introduction

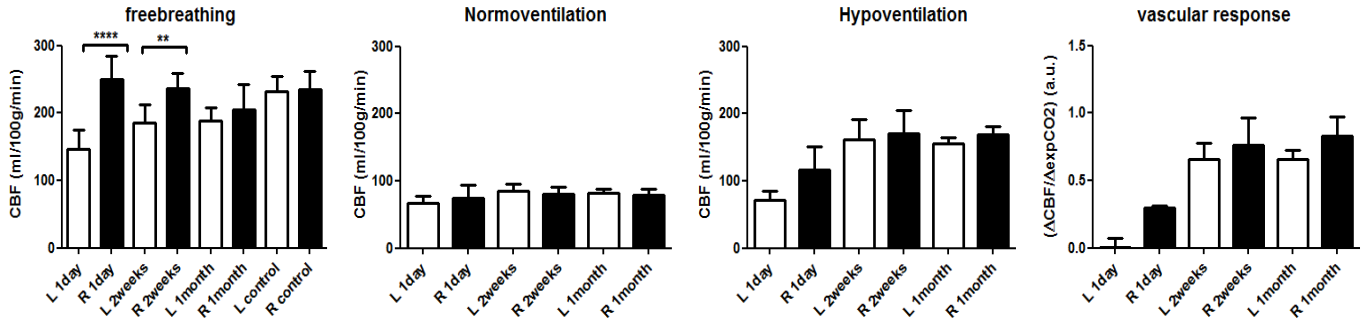
The most widely used and physiological relevant mouse model for ischemic stroke is the transient middle cerebral artery occlusion (tMCAO) model (1). Many clinical and preclinical MRI studies have focused on evaluation of the penumbra and potential recovery of this area which is characterized by a significant reduction in cerebral blood flow (CBF) (2-3). Inherent to the mouse tMCAO model is however the permanent unilateral ligation of the common carotid artery after removal of the occluding filament. Little attention has been paid to the effect of this permanent ligation, without application of a transient MCAO. Since in stroke studies the perfusion levels are often compared relative to the contralateral hemisphere, a deeper understanding of the ligation could contribute to the interpretation of the dynamics observed in the penumbra CBF in this mouse model. In this study, we report on longitudinal changes in absolute CBF and relative hemispheric variations observed in free breathing animals after a permanent ligation. Since it was recently shown that under ventilation also the cerebral vascular response (CVR) can be determined under monitoring of expired CO_2 levels (4), we implemented this approach in our study. Longitudinal variation was observed in the CVR for the ligated versus contralateral side while baseline CBF values seem similar.

Materials & Methods

Male C57Jbl-6 mice (8 weeks; body weight 27+/- 2.5g) were used in this study. At day 0, a unilateral permanent ligation of the common carotid artery was performed under isoflurane anesthesia. Animals were randomly subdivided in three groups which were respectively measured at 1, 12 and 30 days post-surgery. The amount of animals for the different time-points were for the free breathing group: day 1: n = 19; 2 weeks: n = 9; 1 month: n = 5, and for the ventilated group: day 1: n = 2; 2 weeks: n = 4 ; 1 month: n = 2. Animals were anaesthetized using either isoflurane for the free breathing animals or urethane and alpha-chloralose for the ventilated animals as previously described (4). MR images were recorded on the 9.4 T Biospec small animal MR system (Bruker Biospin, Ettlingen, Germany) using a 7 cm linearly polarized resonator for transmission and an actively-decoupled 2x2 phased array mouse brain surface coil for receiving (Rapid Biomedical, Rimpar, Germany). ASL data were acquired using a FAIR approach (5-6) 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, ten inversion times from 300-3000ms, using a inversion hyperbolic secant of 14ms, Paravison 5.1, Bruker. CBF values were calculated assuming an arterial $T_1$of 2.4s (7). ADC values from the same slice were determined using five b-values (0, 250, 500, 1000 and 1400 s/mm^2) and a gradient echo readout. For statistical analysis an ANOVA or Kruskal-Wallis test with multiple comparison correction was applied (Prism 5.04, GraphPad Soft.).

Results

Data from free breathing animals clearly indicate that a permanent unilateral ligation of the carotid artery results in a significant reduction in CBF in the affected hemisphere at 1 day and 12 days post surgery. Together with this response, CBF values in the contralateral hemisphere were found to be increased relative to the 1 month time-point. One month post surgery, differences in CBF values between both hemispheres leveled out. In a subgroup of animals, the experiment was repeated in ventilated animals, under hypo- and normoventilation and under monitoring of blood of pCO_2 values. Normoventilated animals however did not show any differences in CBF values between both hemispheres at all timepoints included in the study. When animals were challenged under hypoventilation, the vascular response in both hemispheres did reveal similar changes in CBF values as seen in the free breathing animals. Nevertheless, it should be noted that absolute CBF values did differ between free breathing and ventilated animals. No significant differences in ADC-values were found between both hemispheres at the different time-points evaluated in this study.



Discussion

The results from this study clearly indicate that a permanent unilateral ligation of the carotid artery, which is inherent to the mouse tMCAO model, has a profound effect on CBF values in the affected hemisphere. In both free breathing and ventilated animals, a gradual recovery of CBF values in the affected hemisphere occurred over time. These results indicate that a specific vascular response is triggered upon carotid artery ligation which could indicate a temporal limitation of the auto regulating capacity or might be mediated by means of angiogenesis (8). Taken together, the results reported here are of major importance for studies focusing on perfusion of the penumbra area in stroke animals because a vascular response elicited by a permanent ligation of the carotid artery might affect the recovery seen in stroke animals that would be reduced in case of a non-ligated carotid artery.

References

(1) Liu et al; J Biomed Biotechnol. 2011; (2) Farr and Wegener; J Cereb Blood Flow Metab. 2010; (3) Kallur and Hoehn; Method Mol Biol. 2011; (4) Oosterlinck et al MRM 2011; (5) Kim et al; MRM 1995; (6) Kwong et al; MRM 1995; (7) Kober et al; MRM 2004; (8) Seevinck et al; Angiogenesis 2010