PERMANENT MCAO IN SHEEP

a new large animal model of focal cerebral ischemia

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Introduction

The efficiency of experimental umbilical cord blood (UCB) and bone marrow (BM) cell therapies has been shown in stroke models by using rodents (see also poster: **Correlation between Behavioural Improvement, Reactive Gliosis and Lesioned Area in a Stem Cell Therapy of Stroke**). However, transplantation to clinical application requires close-to-practice models, i.e. large animal models of focal cerebral ischemia. Existing primate models are cost intensive, restricted to highly specialized breeding facilities and limited by ethical considerations. Therefore, we developed a new, cost effective stroke model in sheep including reproducible neurological dysfunctions and reproducible lesion size, variable by proximal or distal occlusion of the middle cerebral artery (MCAO) according to its origin.

Summary

- MCAO in sheep results in **reproducible behavioral consequences** and reproducible size of ischemic lesion
- to our knowledge, this is **the first** described **model of focal cerebral ischemia in sheep**
- the model will allow to **study efficiency of autologous cell and pharmaceutical therapies** upon stroke

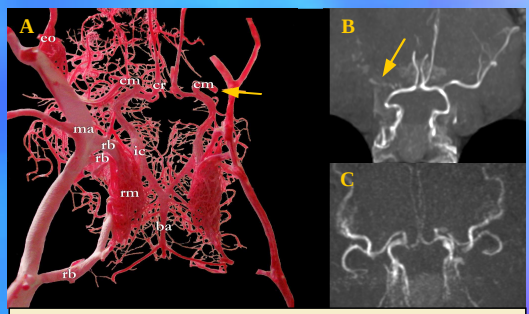

Fig. 1 Corrosion cast (A, basal view) and MRA images of cerebral arteries in sheep (B and C). Arrows in (A) and (B) indicate occlusion of the MCA. Cerebral arteries of a sham operated animal are shown in (C).

Material and Methods

Because of the rete mirabile (rm, **Fig. 1**), a network of small arteries supplied by afferent rete branches (rb) of the maxillary artery (ma), intraluminal occlusion of the MCAO via catheter blockage or artificial thrombus is not possible. Hence, we performed a transcranial exposure of middle cerebral artery (MCA).

17 Merino sheep weighting 35 to 48 kg were randomly assigned to one of four groups: proximal (n=5) and distal (n=2) MCAO, sham operation (n=5) and control (n=5). Animals undergoing the surgical procedure were deeply anaesthetised. Following exposure of the MCA, the vessel was occluded or touched (sham) by high frequency bipolar forceps. After suture, animals underwent an analgetic and antibiotic treatment. Control animals did not underwent a surgical procedure. Neurological investigation was performed before surgery and at days 1, 4, 7, 10, 16, 25, and 32 following MCAO (**Fig. 3**) according to a score point system (**table**). Further, effects of MCAO were evaluated by magnetic resonance imaging (MRI) including T1-, T2-, T2*-sequences, diffusion weighted (DW) imaging (**Fig. 2A-G**) and magnetic resonance angiography (MRA, **Fig. 1B/C**) using a Philips 1,5T clinical scanner. Animals were killed at day 35 post op. and brains were removed for neuropathological investigation (**Fig. 2H**).

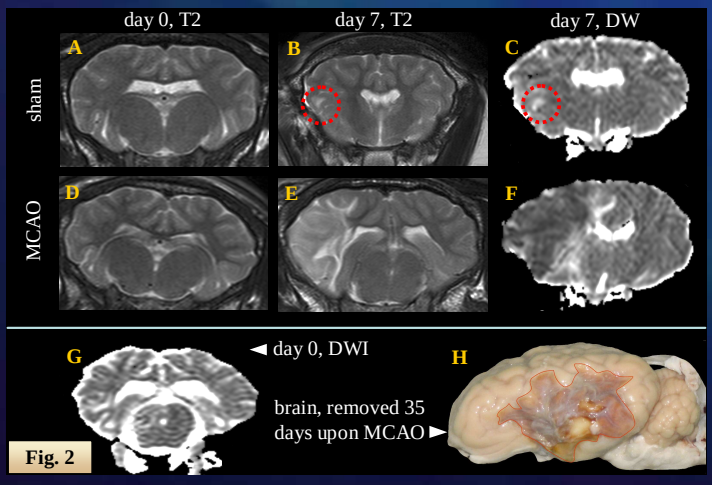

Fig. 2

Results

Proximal MCAO results in a large cortical lesion and neurological dysfunctions including absent/delayed startle reflexes, atactic movement, circling and torticollis. Distal MCAO causes a smaller cortical lesion and reduced functional deficits (data not shown). The MCA was clearly occluded as to be seen in MRA (**Fig. 1 B**). Further, sheep with occluded left MCA showed neurofunctional disabilities (**Fig. 3A/B**), as well as clear signs of ischemic brain tissue in MRI (**Fig. 2E/F**) and after brain removal (**Fig. 2H**).

Sham operated animals showed very mild, transient dysfunctions (**Fig. 3A**) while controls did not show sensomotoric disabilities. Indications of ischemia in sham operated animals was not observed using MRI (**Fig. 2B/C**). Although a small contusion damage could be observed in some cases (**Fig. 2B/C**, red dotted circle), this damage only results in slight, non permanent behavioral consequences.

In the ongoing study, we will investigate the efficiency of autologous UCB and BM cell therapy in sheep to prepare a clinical trial in humans.

Table neurological examination score point system

Item	score points (max. 14)
1. food debris remaining in mouth corner	
no	0
yes	1
2. torticollis	
no	0
yes	1
3. paretic right limbs / atactic movement	
no	0
yes	1
4. circlic movement	
no	0
occasionally	1
permanently	2
5. disturbed right hemistanding reaction	
no disturbance	0
immediate adjustment using left limbs	1
delayed adjustment using left limbs	2
delayed adjustment using right limbs	3
delayed using right hindlimb / no using right forelimb	4
no adjustment	5
6. disturbed right hopping reaction	
no disturbance	0
delayed medial, immediate lateral adjustment	1
delayed medial and lateral adjustment	2
no medial adjustment, delayed lateral adjustment	3
no adjustment	4

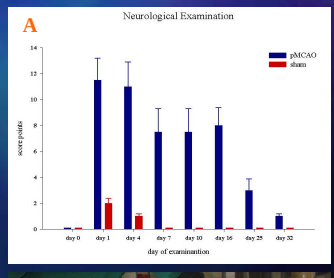

Fig. 3 Behavioral data of neurological examinations, presented as means ± SD (A). Absent medial hopping reaction of right forelimb after MCAO (B).

EXPERIMENTAL CELL THERAPY OF STROKE