MR Angiography and Vascular Corrosion Casting as Tools in Biomedical Research: Application to Transgenic Mice Modeling Alzheimer's Disease

T. Krucker[1], S. Zurbruegg[2], M. Staufenbiel[3], N. Beckmann[2]

[1]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California, United States, [2]Discovery Technologies, Novartis Institutes for BioMedical Research, Basel, Switzerland, [3]Nervous System Area, Novartis Institutes for BioMedical Research, Basel, Switzerland

Neurodegenerative diseases other than vascular dementia or stroke, have been associated with vascular alterations. Reduced blood flow has been reported as a consistent physiological deficit in Alzheimer's disease in particular and might also be an important factor in mental illness. Here we discuss the use of two techniques, namely magnetic resonance angiography (MRA) and vascular corrosion casting, to characterize local alterations in flow and in the vascular architecture of transgenic mice modeling Alzheimer's disease.

Methods:

Animals: The generation of APP23 mice containing the murine Thy-1 promoter driving neuron specific expression of human mutated APP_{751} is described in detail in (1). Studies were carried out on age-matched APP23 mice and control, non-transgenic littermates.

MRA: Mice were anaesthetized with forene (1.5%) in a mixture of O_2/N_2O (1:2), administered via a face mask. Measurements were carried out with a Bruker Biospec 47/40 system. Angiograms were obtained using a 3D gradient-echo sequence with the following imaging parameters: TR/TE 40/1.6 ms; matrix 96x192x64; FOV 1.44x1.92x0.64 cm^3, 2 averages. The RF pulse was frequency-selective, thereby exciting a coronal slice 0.64 cm thick. Magnetization transfer contrast was attained by a frequency-selective gaussian pulse of 3500 µs duration, with B_1 = 2 µT and a frequency offset of 2500 Hz with respect to the water resonance, preceding the 3D gradient-echo sequence by 2.4 ms.

Casts: Mice were deeply anaesthetized; the heart was exposed and the left ventricle punctured with a 19-gauge needle. The right atrium was punctured to provide outflow and the perfusate was injected under a pressure of about 80-100 mm Hg. A prefixation solution (20 ml, 4% performaldehyde in PBS) was infused at the same rate immediately followed by 10 ml of resin. The resin-filled tissue was kept at room temperature overnight to complete resin curing. Soft tissue was removed from the head by maceration in 20% KOH during 24 h followed by decalcification in 5% formic acid (12 h).

Results and Discussion:

The overview in fig. 1 shows a vascular corrosion cast of a complete mouse brain. At the base of the brain cast, the prominent shape of the CoW is readily recognizable. On the cast surface of larger vessels, details of the endothelium including imprints of endothelial nuclei and cell borders, allowed to distinguish arteries from veins. In arteries endothelial cells are elongated and aligned parallel with the long axis of the vessel while those of veins are more rounded and less oriented. Capillaries, the location of the blood-brain barrier, have diameters ranging from 5-7 µm. In normal mice they form a dense array often spaced less than 50 µm apart.

High resolution MRA demonstrated flow perturbations in principal arteries at the CoW in old APP23 mice (2). Flow voids were detected at the internal carotid artery of 11-month-old APP23 mice. At the age of 20 months, additional flow disturbances were observed in large arteries at the CoW (fig. 2). Vascular corrosion casts revealed that, at sites where flow voids were detected *in vivo*, vessel elimination, substitution and/or deformation had taken place. The detailed architecture of the vasculature visible in the casts assisted the identification of smaller vessels most likely formed as substitution or anastomosis within the CoW (fig. 2).

Although MRA in mice is primarily limited to depict larger vessels in region of the Circle of Willis (CoW), it can provide a global view of flow alterations under *in vivo* conditions. The method is sensitive to detect changes in the vascular structure before the net flow through a vessel is affected. Measurements may be easily repeated on the same animals since besides anesthesia no other invasive intervention is necessary. The technique of vascular corrosion casting requires the animal to be perfused first with a resin. After maceration of the surrounding tissue, imaging the stained casts with scanning electron microscopy (SEM) and micro-computed tomography (microCT) can provide further information at high spatial resolution on the disturbed cerebrovascular architecture and morphology at the level of smaller arteries and capillaries.

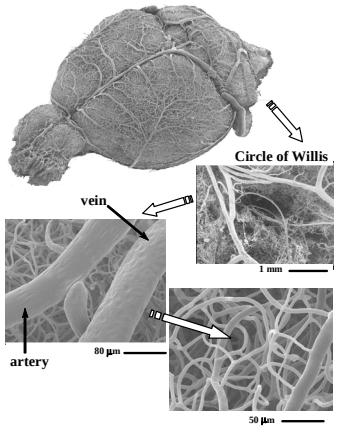

Fig. 1 (Cast)

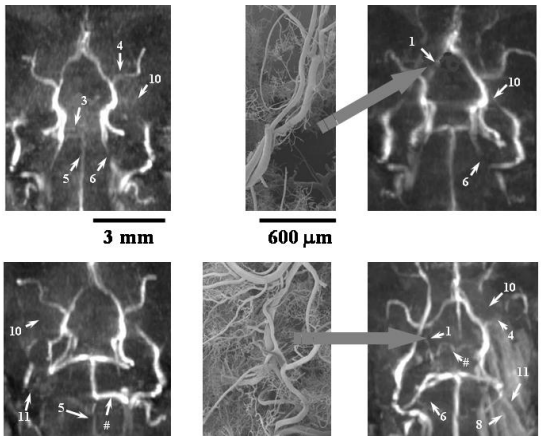

Fig. 2 (MRA+cast)

1. Sturchler-Pierrat C, et al. *PNAS* 1997;94:13287-13292.

2. Beckmann N, et al. . *J Neurosci* 2003; **23:** 8453-8459.