Cerebral amyloid angiopathy in transgenic mice modelling Alzheimer's disease studied non-invasively by MRI

N. Beckmann[1], C. Cannet[1], C. Gerard[1], D. Abramowski[2], and M. Staufenbiel[2]

[1]Global Imaging Group, Novartis Institutes for BioMedical Research, Basel, BS, Switzerland, [2]Nervous System Department, Novartis Institutes for BioMedical Research, Basel, BS, Switzerland

Introduction:

Vascular abnormalities coexist commonly with the histological features of AD. About 80% of AD cases exhibit cerebral amyloid angiopathy (CAA) [1], characterized by deposition of amyloid-β (Aβ) peptide in the walls of cerebral vessels. The presence of macrophages close to or in the walls of CAA-affected vessels has been related to the progression of CAA-related vasculopathy in AD patients [2]. Microglia and macrophages surrounding CAA-affected microvessels in the brain parenchyma [3], or circulating monocytes that migrate from the lumen into the vessel wall [2,4] have been shown to contribute to the vascular thickening. Superparamagnetic iron oxide (SPIO) particles were used to detect by MRI CAA-related cerebrovascular changes in several transgenic mouse lines, characterized by different Aß40/Aß42 ratios.

Materials and Methods:

Animals: The generation of APP23 (human APP with the K670N/M671L mutation; [5]), APP51 (human wild-type APP; [6]) and PS45 mice (human presenilin-1 with the G384A mutation; [7]) has been described earlier. APP24 mice were generated in the same way as APP23 but in addition to the Swedish K670N/M671L mutation they contained the London V717L mutation. APP23xPS45 were obtained by crossbreeding the respective lines [8].

MRI: Mice were anesthetized with 1.3 % isoflurane (Abbott, Cham, Switzerland) in a mixture of O_2/N_2O (1:2) administered via a face mask. No stereotactic holding was used. Measurements were carried out with a Biospec 47/40 spectrometer (Bruker, Karlsruhe, Germany) operating at 4.7 T. Images were obtained using a three-dimensional (3D) gradient-echo sequence with the following imaging parameters: TR 40 ms; TE 8 ms; matrix 256x192x48; FOV 2.8x1.44x1.44 cm^3, 2 averages. The data sets were reconstructed to $(256)^3$. Acquisitions were performed 24 h after intravenous Endorem (Guerbet, Aulnay-sur-Bois, France) injection (0.2 ml). Foci in the cortex presenting signal attenuation and a minimum diameter of 150 μm were counted throughout the whole brain. To ensure that the same site was not counted multiple times, its presence was carefully controlled over several consecutive slices from the 3D data set.

Histology: Performed at the levels of lesions observed by MRI. Hematoxylin and eosin staining was employed to assess the general morphology. The Perls/Prussian blue reaction, the periodic acid-Schiff (PAS) reaction and Congo red were used to visualize ferric iron, pathologic deposits and amyloid, respectively.

Quantification of Aß levels: Forebrain hemispheres, excluding olfactory bulb, brain stem and cerebellum, were homogenized by sonication. Supernatants of extracts were analyzed by electrochemoluminescence linked immunoassays (Human Aß40 and Aß42 Ultrasensitive Kits, Meso Scale Discovery, Gaithersburg, MD).

Results and Discussion:

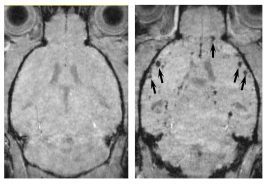

Figure 1

Figure 1 shows coronal MR images extracted from 3D data sets acquired from 28-month-old mice, 24 h after SPIO. Signal attenuations were apparent in multiple foci throughout the brain cortex of the APP23 mouse, as indicated by the arrows, as well as in thalamic regions. No contrast change was detected in the brain of the wildtype control 24 h following SPIO (fig. 1). Histology revealed that iron was localized in or around damaged vessels at sites of signal loss detected in vivo by MRI in APP23 mice. Iron was encountered in the vessel wall, at the level of amyloid deposits or in the vicinity of pathologic blood vessels, mainly entrapped in microglia cells/macrophages. Since iron was mainly found entrapped in macrophages, it is conceivable that SPIO nanoparticles were absorbed in the blood circulation by monocytes, which then infiltrated the walls of pathologic vessels.

To further evaluate the specificity of the method for macrophage-associated vascular alterations, the vascular pathology in APP23 was reduced by crossbreeding with mutated presenilin transgenic mice (APP23xPS45, 15-17 months). Transgenic lines forming less CAA were also used, namely APP51 (28-30 months) and APP24 (24 months). Histological analysis of the neocortex confirmed that vascular Aß was most pronounced in 24-28-month-old APP23 mice. In contrast, only scattered vascular amyloid was seen in APP23xPS45 mice although they contained as much compact and more diffuse Aβ deposits at an younger age. Biochemically this was associated with a shift of isoforms from Aβ40 to Aβ42 (fig. 2). APP51 mice showed a similar distribution of Aβ deposits but these were generally less abundant than in APP23 mice. This was reflected in the lower Aβ level but unchanged Aβ42/Aβ40 ratio. For APP24 mice, the amount of vascular Aβ was lower than that in APP23 animals. This is in agreement with a generally lower Aβ level than in APP23 mice and a shift from Aβ40 to Aβ42, which was moderate compared to APP23xPS45 mice.

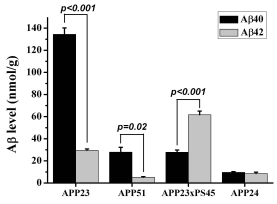

Figure 2 - Forebrain Aβ levels

In contrast to the MRI results concerning the APP23 animals, no significant increases in number of foci displaying MRI signal attenuation were encountered in the cortex or other brain regions of APP23xPS45, APP24 and APP51 transgenic mice (fig. 3). These observations are consistent with the histological results indicating a more pronounced presence of vascular Aß in APP23 than in the other three lines. Of note, APP23xPS45 mice having higher Aß42 levels than APP23 animals displayed significantly more parenchymal plaques. Our results are thus in agreement with the fact that the predominant Aß-peptide present in vascular amyloid related to CAA is Aß40, whereas in brain parenchymal plaques it is Aß42 [9]. A recent study suggests that the vascular and parenchymal lesions have an additive effect on cognitive impairment in AD patients [10]. In summary, MRI in combination with the administration of SPIO is an important tool to study CAA in transgenic mice modeling AD.

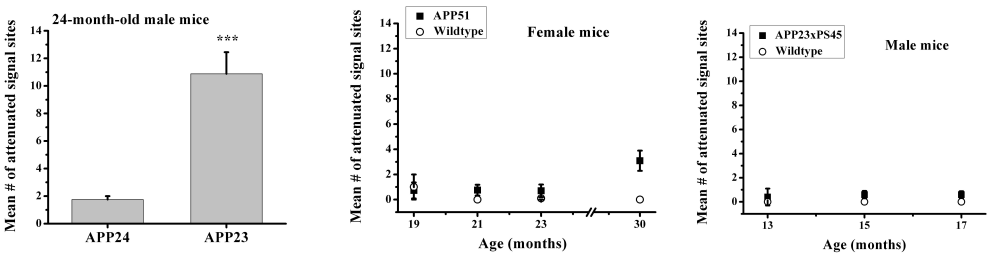

Figure 3

1. Jellinger KA. J Neural Transm 2002; 109:813-836.
2. Vinters HV. Stroke 1987; 18:311-324.
3. Maat-Schieman MLC et al. J Neuropathol Exp Neurol 1997; 56:273–284.
4. Vinters HV et al. Acta Neuropathol (Berl) 1998; 95:235–244.
5. Sturchler-Pierrat C et al. Proc Natl Acad Sci USA 1997; 94:13287-13292.
6. Bodendorf U et al. J Neurochem 2002; 80:799-806.
7. Herzig MC et al. Nat Neurosci 2004; 7:954-960.
8. Busche MA et al. Science 2008; 321:1686-1689.
9. McCarron MO et al. NeuroReport 2000; 11:937-940.
10. Launer LJ et al. Neurobiol Aging 2008; 29:1587-1590.