# Cerebral amyloid angiopathy in APP23 mice modelling Alzheimer's disease studied non-invasively by MRI: Application to passive amyloid-beta immunotherapy

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## *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 have been employed to detect by MRI CAA-related cerebrovascular changes in several transgenic mouse lines [5].

Treatment of plaque-bearing APP23 mice [6] displaying CAA with Aβ antibody β1 was demonstrated earlier to increase microhemorrhages [7]. A similar passive immunization protocol has been used here to determine whether SPIO-enhanced MRI would be sensitive to detect these microvascular lesions in vivo.

## *Materials and Methods:*

**Animals:** The generation of APP23 mice (human APP with the K670N/M671L mutation; [6]) has been described earlier. Male APP23 and age-matched wildtype mice have been used. Animals were 17 months old at the beginning of the study.

**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.

**Passive Immunization:** Animals were passively immunized weekly by intraperitoneal (i.p.) injections of 0.5 mg of β1 mouse monoclonal IgG2a antibody that recognizes amino acids 3 to 6 of human Aβ [8]. Age-matched control mice received weekly i.p. injections of 0.5 mg of a control antibody (monoclonal mouse anti-wheat auxin IgG2a antibody; AMS Biotechnology, Bioggio-Lugano, Switzerland). Four groups of mice were formed: (i) APP23 mice immunized with the β1 antibody (n=9 at the beginning of the immunization); (ii) APP23 mice immunized with the control antibody (n=10 at the beginning of the immunization); (iii) wildtype mice immunized with the β1 antibody (n=10 at the beginning of the immunization); (iv) wildtype mice immunized with the control antibody (n=10 at the beginning of the immunization). Animals were 18 months old at the beginning of the immunization.

## *Results and Discussion:*

Figure 1 shows coronal MR images extracted from 3D data sets acquired from two APP23 mice at baseline (17 months of age) and at 2 months after beginning of immunization (20 months of age). Both animals had received SPIO 24 h before being imaged. Additional sites of signal attenuation were detected only in the brain cortex of the mouse receiving the β1 antibody (arrows). The number of signal attenuation foci (means±sem, n=8-10 mice in each group and time point) in cerebral cortex, 24 h after SPIO administration to immunized mice, is represented in figure 2. Treatment with the β1 antibody increased the number of MRI signal attenuation foci. In contrast, there was only a small and non-significant trend towards an increase in the control antibody treated animals. The arrow indicates the beginning of the immunization, at 18 months of age. The significance levels p correspond to Mann-Whitney comparisons at the specified time points and, in parenthesis, to the longitudinal comparisons of the log10 of the individual counts using ANOVA with random effects. No sites of signal attenuation were detected in the brains of age-matched wild-type mice that received either the β1 or the control antibody. Histology performed at the end of the study demonstrated a significant increase of CAA-associated iron-containing macrophages blood vessels in the brain cortex of β1 antibody-treated APP23 mice. This confirms the described increase in CAA-related microvascular lesions in the cerebral cortex of APP23 mice following β1 antibody treatment [7].

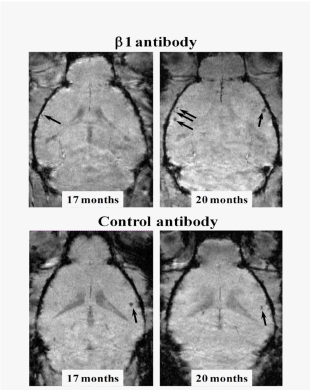


**Figure 1**

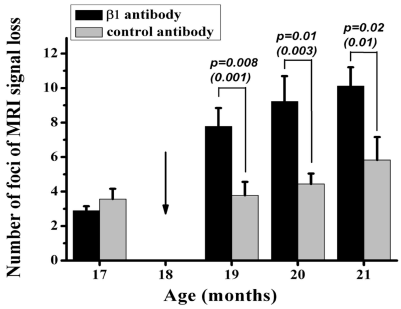


**Figure 2**

The present investigation illustrates that SPIO-enhanced MRI is well suited to detect the treatment-induced elevation of microhemorrhage in mice as observed in some studies of Aβ immunotherapy [7,9]. Lack of a change in control antibody-treated APP23 mice and β1-treated wild-type animals demonstrated the specificity of the measurements. These results are in agreement with previous studies of β1-treated aged APP23 mice demonstrating a twofold increase in the frequency and severity of microhemorrhages [7,10]. Overall, our in vivo and post-mortem observations show an increase in CAA-related microvascular pathology in the cerebral cortex of APP23 mice as a consequence of passive Aβ immunotherapy. They demonstrate the sensitivity of the MRI method used to detect this increase in living animals. As SPIO is approved for clinical use, the approach has translational potential and can be used to screen or to monitor patients for microvascular lesions.

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