Parametric MRI Reveals Vascular Effects of Antibodies to the α1-Adrenergic Receptor by Demonstrating a Reduction in Relative Cerebral Blood Volume (rCBV)

Andreas Pohlmann1, Babette Dieringer1, Peter Karczewski2, Natali Wisbrun1, Irina Palatnik1, Christina Eichhorn3, Petra Hempel1, Rudolf Kunze1, Marion Bimmer4, and Thoralf Niendorf1,2
1Berlin Ultrahigh Field Facility (B.U.F.F.), Max-Delbrück Center for Molecular Medicine, Berlin, Germany, 2E.R.D.E.-AAK-Diagnostik GmbH, Berlin, Germany, 3Animal Facilities, Max Delbrück Center for Molecular Medicine, Berlin, Germany, 4IT Department, Max-Delbrück Center for Molecular Medicine, Berlin, Germany, 5E.R.D.E. e.V., Berlin, Germany, 6Autoimmunity and G Protein-Coupled Receptors, Max Delbrück Center for Molecular Medicine, Berlin, Germany, 7Experimental and Clinical Research Center, a cooperation between the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine, Berlin, Germany

Introduction:
Brain vasculature is critical for the development of different types of dementia such as Alzheimer’s and vascular dementia. There is evidence that dementia of the Alzheimer’s type may be primarily a vascular disease [1]. Agonistic autoantibodies to the α1-adrenergic receptor (α1-AR) were found to be associated with widespread diseases such as different types of hypertension and type 2 diabetes [2–5]. The occurrence of these antibodies in diseases with significant vascular involvements suggests their importance in vascular pathology [6]. Rats immunized with α1-AR peptides developed receptor-specific antibodies and damages in the aorta and mesenteric artery [7]. Recently, a study employing time-of-flight magnetic resonance angiography (TOF-MRA) showed that antibodies to the α1-AR cause impairments of blood flow in larger vessels in the brain region of rats [8]. We aimed to investigate whether these impairments are accompanied by vascular impairments of the smaller vasculature within the brain. To this end this work examines the long-term effects of α1-AR antibodies on relative cerebral blood volume (rCBV) in rats. For this purpose R1 and R2 changes induced by an i.v. administered intravascular contrast agent (ferumoxytol [9]) were quantified.

Materials and Methods:
Animal Model: Forty male Wistar rats were obtained at 10–13 age of weeks (280-350g) and allocated to random four groups. One group (A) received monthly i.v. injections of α1-AR antibody (700 mg/kg body weight). The corresponding control group (I) received the same dose of control IgG. A third group (P) was immunized by s.c. injection of 300 mg α1-AR peptide coupled to bovine serum albumin (BSA) and emulsified in incomplete Freund’s adjuvance at 0, 2 and 4 weeks and then monthly. The respective control animals (C) were subcutaneously injected with BSA. MR imaging: Multi-echo spin-echo images (MSME, TR = 2000ms, TE = 10-70ms) for T1 mapping were acquired on a 1.4T Bruker Biospec (Ettlingen, Germany) using a four-element rat head optimized surface coil (RX) combined with a volume resonator (TX). For T2* mapping a multi-echo gradient-echo (MGE, TR = 620ms, TE = 2.14-17.2ms, FA = 40°) was applied. The entire brain was covered using 21 coronal slices (thickness = 1mm, FOV = (35x35) mm2, matrix = 256x256). Experimental protocol: Experiments were conducted in-vivo using isoflurane anesthesia (1.8-2.2% in 100% air) after 9 months and 11 months of treatment. Following baseline T1/T2* mapping, 10 mg of Fe/kg ferumoxytol (Feraheme, AMAG Pharmaceuticals, Inc) was administered using a power injector at a rate of 15 ml/h via a tail vein catheter. 3 minutes after end of injection T1/T2* mapping was repeated. Analysis: Parameter maps were calculated from denoised images (SANLM filter, VBM8 toolbox, SPM8, www.fil.ion.ucl.ac.uk/spm), converted to R1/R2 maps, and subtraction of pre-contrast maps from post-contrast maps yielded ∆R1*/∆R2* maps (Fig. 1) from which the cerebrum was extracted by manual segmentation, followed by an automated division of each brain map into a dorsal and ventral half. Quantitative histogram analysis was then applied to the dorsal brain half only to exclude unwanted susceptibility effects of nearby cavities.

Results:
Histogram analysis was carried out for all 16 data sets (2 parameters, 4 groups, 2 time points). Both α1-AR groups were compared with their respective control group (A vs I, P vs C). Differences between these groups in all eight comparisons was statistically significant (Kolmogorov-Smirnov test, p < 0.05). Both parameters, ∆R1* and ∆R2*, were reduced in the antibody group compared to their controls, which was reflected in the histograms as a shift towards lower values. This shift was larger for ∆R1* than for ∆R2* as illustrated in Fig. 2 and Fig. 3a. ∆R1* and ∆R2* were found to be larger for the antibody group versus the peptide group as highlighted in Fig. 3a and Fig. 3b. For the peptide a marked reduction was observed only in ∆R2* at 11 months (Fig. 3b). ∆R1* and ∆R2* effects were similar at 9 months and 11 months after treatment. Since ∆R1* and ∆R2* are surrogates for rCBV a reduction in these parameters indicates a decrease in rCBV. The largest effect was observed in the ∆R1*-based rCBV estimate for the antibody group (Fig. 3a).

Discussion and Conclusions:
Estimation of relative cerebral blood volume (rCBV) by means of ∆R1* and ∆R2* mapping in conjunction with an intravascular contrast agent demonstrated a significant vascular effect of α1-AR antibody and α1-AR peptide treatment. A reduction in rCBV was found, which supports the theory of vascular impairment caused by the α1-AR antibody and confirms previous TOF-MRA findings in larger vessels [8]. The treatment effect of the antibody was much more pronounced than that of the peptide, which is also consistent with MR angiography data [8]. Notwithstanding the relatively small size of the effects, these observations are underscored by the fact that both parameters were reduced in both treatment groups and at all time points included in this study. Conceivably magnitude differences in treatment effects for ∆R1* and ∆R2* might be related to their respective sensitivity to smaller/larger vessels, but such inferences would require backup by histological analyses, which are still pending. In conclusion, in the present work we provide further evidence for the potential of α1-AR antibodies to cause vascular impairments of brain vessels in a rat model. For the first time a link between vascular effects of α1-AR antibody/peptide treatment on a macroscopic scale (angiography) and microscopic scale (rCBV) could be established. This data underpins the suggested pathogenic significance of autoimmunity to the α1-AR for diseases of the central nervous system such as Alzheimer’s and vascular dementia [10].


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Figure 1: ∆R1* map (top) and ∆R2* map (bottom) at 11 months for a rat (group A).

Figure 2: Averaged histograms of ∆R1* at 11 months for the antibody group and control (IgG) group.

Figure 3a: Averaged histograms of ∆R1* at 11 months for the antibody group and control (IgG) group.

Figure 3b: Averaged histograms of ∆R1* at 11 months for the peptide group and control group.