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

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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 R_2* and R_2 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 weeks of age (280-350g) and allocated at random to 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 T_2 mapping were acquired on a 9.4T Bruker Biospec (Ettlingen, Germany) using a four-element rat head optimized surface coil (RX) combined with a volume resonator (TX). For T_2* 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) mm^2, matrix = 256x256). ***Experimental protocol:*** Experiments were conducted *in-vivo* under isoflurane anesthesia (1.8-2.2% in 100% air) after 9 months and 11 months of treatment. Following baseline T_2/T_2* 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 T_2/T_2* 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 R_2*/R_2 maps, and subtraction of pre-contrast maps from post-contrast maps yielded ΔR_2*/ΔR_2 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 were statistically significant (Kolmogorov-Smirnov test, $p < 0.05$). Both parameters, ΔR_2* and ΔR_2, 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 ΔR_2* than for ΔR_2 as illustrated in Fig. 2 and Fig. 3a. ΔR_2* and ΔR_2 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 ΔR_2* at 11 months (Fig. 3b). ΔR_2* and ΔR_2 effects were similar at 9 months and 11 months after treatment. Since ΔR_2* and ΔR_2 are surrogates for rCBV a reduction in these parameters indicates a decrease in rCBV. The largest effect was observed in the ΔR_2*-based rCBV estimate for the antibody group (Fig. 3a).

Discussion and Conclusions:

Estimation of relative cerebral blood volume (rCBV) by means of ΔR_2* and ΔR_2 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 ΔR_2* and ΔR_2 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].

References: [1] de la Torre, Stroke 2002, 2005 [2] Wenzel, PLoS ONE 2008, [3] Fu, Lancet 1994, [4] Luther, Hypertension 1997, [5] Hempel, Scand J Immunol 2009, [6] Piascik, PLoS ONE 2010, [7] Zhou, Cell Mol Immunol, [8] Karczewski, PLoS ONE 2012, [9] Pohlmann, ISMRM 2012, [10] Karczewski, Scand J Immunol 2012.

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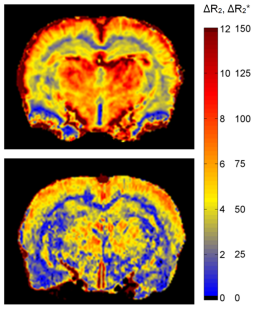

Figure 1: ΔR_2* map (top) and ΔR_2 map (bottom) at 11 months for a rat (group A).

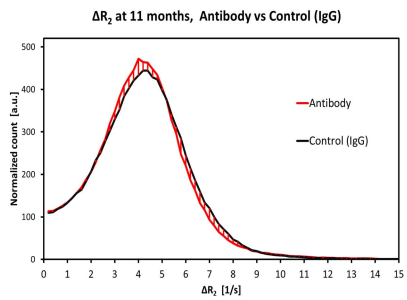

Figure 2: Averaged histograms of ΔR_2 at 11 months for the antibody group and control (IgG) group.

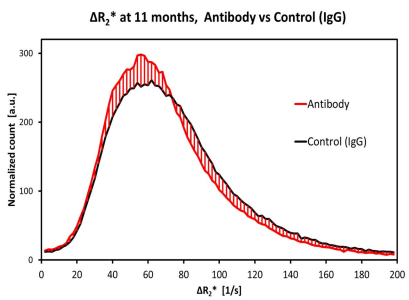

Figure 3a: Averaged histograms of ΔR_2* at 11 months for the antibody group and control (IgG) group.

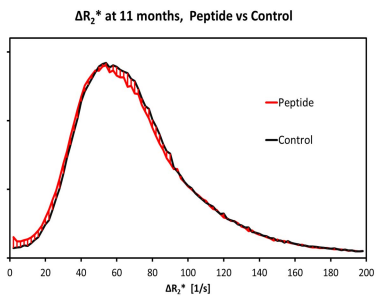

Figure 3b: Averaged histograms of ΔR_2* at 11 months for the peptide group and control group.