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Research article

Cortical hypoperfusion and reduced cerebral metabolic rate of oxygen in the arcAβ mouse model of Alzheimer's disease

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ABSTRACT

The effect of cerebral amyloidosis on cerebral hemodynamics was investigated with photoacoustic tomography (PAT) and magnetic resonance imaging (MRI). First, the sensitivity and robustness of PAT for deriving metrics of vascular and tissue oxygenation in the murine brain was assessed in wild-type mice with a hyperoxia-normoxia challenge. Secondly, cerebral oxygenation was assessed in young and aged arcAβ mice and wild-type controls with PAT, while cerebral blood flow (CBF) was determined by perfusion MRI. The investigations revealed that PAT can sensitively and robustly detect physiological changes in vascular and tissue oxygenation. An advanced stage of cerebral amyloidosis in arcAβ mice is accompanied by a decreases in cortical CBF and the cerebral metabolic rate of oxygen ($CMRO_2$), as oxygen extraction fraction (OEF) has been found unaffected. Thus, PAT constitutes a robust non-invasive tool for deriving metrics of tissue oxygenation, extraction and metabolism in the mouse brain under physiological and disease states.

1. Introduction

The brain has a very high demand for oxygen compared to other organs (it utilizes approximately 20% of the body's total oxygen consumption), making tight regulation of cerebral blood flow (CBF) and oxygen delivery critical for brain function [1]. The regional quantification of oxygen saturation (SO_2), brain oxygen extraction fraction (OEF) and, in conjunction with perfusion imaging for CBF, the cerebral metabolic rate of oxygen ($CMRO_2$) are key measures of brain hemodynamic function. Quantification of these parameters has helped to elucidate brain functional physiology and holds translational potential as a clinical tool for evaluating neurological disorders such as stroke, brain tumors and Alzheimer's disease (AD) and other age-related pathologies.

AD is associated with vascular dysfunction which partakes in the pathogenesis of the disease [2]. Patients with AD show regional hypoperfusion, decreased levels of oxygenated hemoglobin (HbO_2) and tissue oxygenation [3], as well as capillary dysfunction at an early disease stage, reported to be associated with cognitive symptom severity and neurodegeneration [4]. ^{15}O PET study in AD patients showed reduced $CMRO_2$ compare to healthy controls [5]. Transgenic mice overexpressing human amyloid precursor protein (APP) are widely used models of AD. These mice display progressive amyloidosis in the brain and enable studying the influence of amyloid-beta (Aβ) accumulation on vascular function [6]. In APP mice hypoperfusion and capillary dysfunction [7] have been described, but the relationships to oxygen transport and metabolism have not yet been investigated. Moreover, methods which are non-invasive possess a high sensitivity and spatial resolution, cover a large field-of-view and are easy to apply are highly desired.

In recent years, a variety of biological and medical imaging techniques have been developed to assess cerebral oxygenation in animal models and in humans. Positron emission tomography (PET) with ^{15}O is considered the gold standard for the whole-brain oxygenation measurement in clinical setting [8]. While the method has been applied to mice [9], the method suffers from low spatial resolution which limits its usefulness for studying small species Moreover, the short half-life of [^{15}O] requires an on-site cyclotron, which restricts the widespread use of this approach. Alternatively, MRI-based techniques such as blood oxygenation level-dependent (BOLD), quantitative BOLD, susceptometry, T_2-relaxation-under-spin-tagging and ^{17}O MR spectroscopy among others have been proposed for mapping oxygenation [10]. Measurements of BOLD and CBF in conjunction with hypercapnic or hyperoxic respiratory challenges have been proposed for measuring relative changes and absolute value of $CMRO_2$ [11]. However MR methods that infer the regional concentration of oxygen by measuring tissue R_2, R_2' or R_2* relaxation rates (such as BOLD) or bulk susceptibility, which is

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sensitive to paramagnetic compounds such as Hb, are prone to confounder as non-vascular tissue compartments invariably contribute to the signals or are sensitive to changes in vessel caliber and orientation. This limits their applicability for characterizing brain diseases that involve significant remodeling of the vasculature such as in arcAβ mouse model [12–14].

Optical imaging such as Doppler optical coherence tomography [15], laser Doppler spectroscopy [16], two-photon [17], near-infrared spectroscopy [18], photoacoustic microscopy [19,20], and single-impulse panoramic photoacoustic computed tomography [21] have been used for deriving metrics of regional tissue oxygenation in small animals; yet these techniques, are often invasive, or suffer from a small field-of-view and limited depth penetration. By using multiple wavelengths for illuminating and spectral unmixing algorithms, photoacoustic tomography (PAT) resolves signal contribution from different photoabsorbing molecules in tissue simultaneously based on their spectral properties [22]. In biological tissue major absorbers such as hemoglobin serves as an endogenous contrast agent for PAT [23,24]. As hemoglobins display significant absorption values in the red and near-infrared spectral domain, PAT allows the non-invasive assessment of hemodynamic parameters relatively deep in tissue (mm range) [25] in the brains of rats and mice [26].

Here we describe an approach to estimate OEF and $CMRO_2$ in mouse brain based on PAT estimates of arterial oxygen saturation (S_aO_2) in the middle cerebral artery (MCA) and venous oxygen saturation (S_vO_2) in the superior sagittal sinus (SSS) as well as in cortical tissue oxygenation (S_tO_2) of mouse in combination with CBF measurements by using perfusion magnetic resonance imaging (MRI). We found reduced cortical CBF in aged transgenic arcAβ mice, which displays abundant parenchymal and vascular amyloid deposition [24,27]. Due to the inability of the vascular system to compensate the decrease in oxygen delivery by increasing the OEF, $CMRO_2$ values were found to be significantly decreased.

2. Methods

2.1. Animal model

Ten female non-transgenic C57BL/6 mice (12 weeks) were purchased from Janvier (France) and used in the oxygen-challenge PAT validation experiment for deriving S_aO_2 in the MCA, S_vO_2 in the SSS and S_tO_2 in the cortical tissue. Eighteen transgenic arcAβ mice overexpressing the human APP695 transgene containing the Swedish (K670N/M671L) and arctic (E693G) mutation under the control of the prion protein promoter and eighteen age-matched non-transgenic C57BL/6 littermates of both sexes were bred at Phenomic Center ETH Zurich (Switzerland) and were assessed at 6- and 24-months of age. ArcAβ mice are characterized by Aβ plaque formation at 6-months of age together with a pronounced cerebral amyloid angiopathy [27]. Animals were housed in ventilated cages inside a temperature-controlled room, under a 12-h dark/light cycle. Pelleted food (3437PXL15, CARGILL) and water were provided *ad-libitum*. Using StatMate (Graphpad prism 7.0, USA) a sample size of $n = 6$ per group was calculated a priory for the primary end point CBF, a fixed effect omnibus, one-way ANOVA with four groups, and an effect size $f = 0.78$, $\alpha = 0.05$ and $\beta = 0.2$. Consequently, group sizes $n > 6$ were used.

2.2. Perfusion MRI protocol

MRI was performed on a 7/16 small animal MR Pharmascan (Bruker Biospin GmbH, Ettlingen, Germany) equipped with an actively shielded gradient set of 760 mT/m with a 80 μs rise time and operated by a Paravision 6.0 software platform (Bruker Biospin GmbH, Germany). We used a circular polarized volume resonator for signal transmission and an actively decoupled mouse brain quadrature surface coil with integrated combiner and preamplifier for signal receiving (Bruker BioSpin, Germany). Thirty-three mice were anesthetized with an initial dose of 4% isoflurane (Abbott, Cham, Switzerland) in oxygen/air (200:800 mL/min) mixture in the induction box and were maintained at 1.5% isoflurane in oxygen/air (100:400 mL/min) mixture supplied via a nose cone. Mice were placed in prone position on a water-heated support to keep body temperature at 36.5 ± 0.5 °C monitored with a rectal temperature probe. T_2-weighted anatomical reference images were acquired in coronal and sagittal orientations and served for accurate positioning of the arterial spin labeling (ASL) slice (Fig. 3A). A spin-echo sequence was used with rapid acquisition relaxation enhancement, echo time = 33 ms; relaxation time = 2500 ms; rapid acquisition relaxation enhancement factor = 8; flip angle = 90°; fifteen sagittal slices of 1 mm thickness; field-of-view = 20 × 20 mm; image matrix = 256 × 256; spatial resolution = 78 × 78 μm; resulting in an acquisition time of 2 min 40 s.

Perfusion was measured under resting conditions using an ASL method with a flow sensitive alternating inversion recovery technique. Fieldmap-based shimming was performed using the automated MAPshim routine to improve the homogeneity of the magnetic field. A spin-echo planar imaging sequence preceded by a 180° hyperbolic secant RF inversion pulse was used with echo time = 12.47 ms; relaxation time = 12000 ms; flip angle = 90°. One axial slide of 1 mm thickness was acquired approximately at Bregma −1.46 mm with a field-of-view = 20 × 20 mm; image matrix = 128 × 96, with a spatial resolution = 156 μm × 208 μm. Inversion parameters were: inversion slab thickness = 4 mm, slice margin = 1.5 mm. Sixteen images with increasing inversion times (10 s, 50–3000 ms) were obtained for T_1 calculations, with a total scan time of 11 min and 55 s. Inversion recovery data from the imaging slices was acquired after selective inversion interleaved with non-selective inversion. For each mouse brain in the current study, a T_2-weighted anatomical image was acquired at the same position as the ASL slice for drawing regions of interest (ROI). Paxinos mouse brain atlas was used as the anatomical reference for scan and analysis.

2.3. PAT and image paradigm

For PAT the inVision 128 small animal multi-spectral optoacoustic tomography system (iThera Medical GmbH, Munich, Germany) was used as described before [28]. We first tested the sensitivity and repeatability of PAT for detecting physiological changes in blood and tissue oxygenation by applying an oxygen challenge. Longitudinal PAT measurements were performed in 10 wild-type mice for 15 min. Mice were anesthetized with an oxygen/air mixture (200:800 mL/min) in the induction box and anesthesia was maintained at 1.5% isoflurane supplied via a nose cone under normal air supply (oxygen/air 100:400 mL/min). The hyperoxia challenge consists of two cycles: normoxia (oxygen/air 0:500 mL/min, fraction of inspired oxygen $FiO_2 = 20\%$, 3 min) – 100% hyperoxia (oxygen/air 500:0 mL/min, $FiO_2 = 100\%$, 3 min) during continuous imaging were performed based on previous photoacoustic microscopy setting [21] as illustrated in Fig. 1 and Supplementary Fig. 1. The paradigm allowed us to compare the repeatability of the approach (comparing test and retest values). The body temperature of the mouse was kept at 36.5 °C while imaging by adjusting the temperature of the water chamber. Laser excitation pulses of 9 ns were delivered at five wavelengths (715, 730, 760, 800, 850 nm); one coronal slice was examined, 1 average was collected [28]. Data were acquired continuously for 15 min resulting in 360 frames for each mouse corresponding to an acquisition time of 2.5 s per image frame.

In the second part of the study we assessed the capability of PAT to detect alteration in oxygen saturation, extraction and