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Featured Article

Capillary dysfunction is associated with symptom severity and neurodegeneration in Alzheimer's disease

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Abstract

Introduction: We examined whether cortical microvascular blood volume and hemodynamics in Alzheimer's disease (AD) are consistent with tissue hypoxia and whether they correlate with cognitive performance and the degree of cortical thinning.

Methods: Thirty-two AD patients underwent cognitive testing, structural magnetic resonance imaging (MRI), and perfusion MRI at baseline and after 6 months. We measured cortical thickness, microvascular cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT), and capillary transit time heterogeneity (CTH) and estimated tissue oxygen tension (P_tO_2).

Results: At baseline, poor cognitive performance and regional cortical thinning correlated with lower CBF and CBV, with higher MTT and CTH and with low P_tO_2 across the cortex. Cognitive decline over time was associated with increasing whole brain relative transit time heterogeneity (RTH = CTH/MTT).

Discussion: Our results confirm the importance of microvascular pathology in AD. Deteriorating microvascular hemodynamics may cause hypoxia, which is known to precipitate amyloid retention.

Keywords: Alzheimer's disease; Magnetic resonance imaging (MRI); Perfusion imaging; Capillary dysfunction; Microvascular dysfunction; Neurovascular dysfunction; Capillary transit time heterogeneity (CTH); Relative transit time heterogeneity (RTH); Cerebral blood flow (CBF); Cerebral blood volume (CBV); Hypoxia; Tissue oxygen tension (P_tO_2); Cortical thinning; Neurodegeneration

1. Introduction

Amyloid β (Aβ) peptide accumulates in the brain parenchyma of patients with Alzheimer's disease (AD), forming oligomers and plaques, which disrupts neuronal function, activates inflammatory processes, and stimulates the build-up of phosphorylated tau-protein, leading to the formation of neurofibrillary tangles (NFTs) and neurotoxic tau species [1]. Aβ levels do not, however, correlate well with cognitive decline and neurodegeneration [2], and therapeutic reduction of Aβ plaque burden has so far failed to prevent or reverse cognitive decline in AD [3].

AD is associated with changes in vascular function [4,5] and shares risk factors with cardiovascular diseases [6]. Clearly, vascular risk factors affect organ oxygen supply and indeed, hypoxia stimulates inflammation [7], the formation of Aβ and NFTs, and impairs Aβ degradation and clearance across the blood-brain barrier (BBB) [7,8]. However,

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although cerebral perfusion deteriorates in the years before AD symptoms appear in humans [9,10], cerebral blood flow (CBF) remains above the ischemic threshold at which hypoperfusion gives rise to neurological symptoms and neuronal damage [11]. Furthermore, young carriers of the *APOE* ε4 allele, which is associated with increased risk of developing AD in later life, display abnormally high CBF values and CBF responses [12–14]. Altogether, it remains unclear whether compromised blood supply and subsequent hypoxia plays a role in the etiology of AD.

In addition to its blood supply, the brain's access to oxygen depends on the distribution of blood across its tissue capillaries [15]. In fact, complex signaling mechanisms within the capillary bed prevent blood from following the shortest capillary paths back to the heart [6,16]. Otherwise, excessive shunting would cause severe tissue hypoxia despite normal blood supply. Hence, we have extended the traditional relation between blood supply and oxygen availability in tissue to take the distribution of blood across the capillary bed into account [15]. To a first approximation, the oxygen availability in tissue can be inferred from the mean transit time (MTT) of erythrocytes as they pass through the capillary bed combined with the standard deviation (SD) of their transit times, the capillary transit time heterogeneity (CTH). MTT is the ratio between cerebral blood volume (CBV) and CBF and, therefore, reflects capillary density and blood supply, whereas CTH reflects capillary function, which limits the efficacy of oxygen extraction from blood. In normal brain, CTH is high during rest but decreases during functional hyperemia, facilitating efficient oxygen extraction during increased metabolic demands [15]. However, if capillary flows cannot be homogenized to reduce CTH, tissue hypoxia can ensue at CBF levels well above the classical ischemic CBF threshold [16].

Changes in capillary function are likely to develop slowly, for example, in relation to cardiovascular risk factors. Meanwhile, flow-metabolism coupling mechanisms are expected to adjust CBF to compensate for reductions in oxygen extraction efficacy. We have, therefore, proposed that the hyperemia in young, asymptomatic *APOE* ε4 carriers could serve to compensate for mild capillary dysfunction and thereby slightly reduced oxygen extraction efficacy, whereas the attenuated CBF responses in animal models of AD [17] and the observed hypoperfusion in presymptomatic AD [9] may reduce the net shunting of oxygenated blood through the microvasculature. Hypoperfusion thereby, paradoxically, secures sufficient oxygen to maintain brain function, albeit at the expense of downstream tissue hypoxia, inflammation, and ultimately neurodegeneration [6].

The aim of this study was to examine whether cerebral hemodynamics in AD reveal any signs of severe capillary dysfunction, consistent with tissue hypoxia. Perfusion-weighted imaging (PWI), by which CBV, CBF, and MTT are derived from the retention of bolus-injected contrast media (CM) in the vasculature [18], was recently extended to estimate CTH [19]. We, therefore, applied a dynamic susceptibility contrast (DSC) magnetic resonance imaging (MRI) technique, particularly sensitized to CM in capillary-sized vessels, to obtain MTT and CTH estimates in AD patients. We then asked how oxygen utilization, similar in size to that of normal, resting human brain, would affect tissue oxygen tension (P_tO_2) given these hemodynamic conditions. We hypothesized that (i) microvascular hemodynamics would be more affected (CTH and MTT be higher) and calculated P_tO_2 values be lower, in the cortex which is most affected by atrophy in AD compared with nonatrophic areas and (ii) patients' symptom severity would correlate with the degree of capillary dysfunction (CTH elevation) and (inversely) with P_tO_2.

2. Materials and methods

This study is part of a placebo-controlled clinical trial testing the effect of liraglutide, a glucagon-like peptide-1 receptor agonist, in AD patients [20], registered at ClinicalTrials.gov: NCT01469351 and approved by relevant authorities [20]. Subjects underwent MRI, cognitive tests, and Pittsburgh compound B positron emission tomography (PiB-PET) to ascertain their amyloid status after inclusion (baseline) and after 6 months (follow-up), during which subjects were blindly randomized to receive liraglutide and placebo, respectively. Group-averaged cognitive scores did not change in either study group [21], but we controlled for any drug effect in our statistical models when using follow-up imaging data from the entire study group to identify neuroimaging correlates of long-term changes in individual cognitive scores. Otherwise, trial outcomes will not be addressed here. Patients gave written informed consent before inclusion.

2.1. Subjects

Patients aged 50–80 years and diagnosed with AD according to *International Classification of Diseases, Tenth Revision*, were recruited from dementia clinics in the Central and Northern Denmark Regions, as described elsewhere [20]. Thirty-two AD patients (12 females and 20 males, mean age 64.47 ± 6.94 years) who completed at least baseline structural MRI and neuropsychological testing were included in the study. Inclusion and exclusion criteria, subject demographics, cognitive scores, amyloid status (Aβ-positive PET was defined as a frontal, temporal, or parietal mean cortical gray matter PiB standard uptake value ratio $\geq$ 1.4 using cerebellum as reference region), and scan completion according to substudy are listed in Table 1.

2.2. Neuropsychological testing

A trained physician (L.E.) conducted neuropsychological assessment, using Brief Cognitive Status Examination (BCSE) [22] from the Wechsler Memory Scale IV, at baseline and follow-up. BCSE explores general cognitive abilities such as memory deficits, verbal reproduction, time estimation, and mental control, with a minimum of