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Stereological analysis of microvascular parameters in a double transgenic model of Alzheimer's disease

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Abstract

Morphological alterations in microvasculature occur as a common finding in the brains of non-demented aged persons and patients with Alzheimer's disease. Quantifying the extent of this vascular pathology, however, has been complicated by systematic error (bias) associated with the applications of assumption- and model-based morphometric techniques to human and animal tissues. The current study used novel assumption- and model-free stereological approaches to quantify capillary parameters in the corpus callosum of a double amyloid precursor protein/presenilin-1 transgenic murine model of Alzheimer's disease. The results revealed significant reductions in the total number of capillary segments in white matter of transgenic mice compared to non-transgenic littermates, with no differences in total capillary length. These findings support the view that the expression of mutant human genes for β-amyloid peptides alters the normal architecture of cerebral capillary vessels in the white matter of mouse brain, which may model microvasculature changes reported in Alzheimer's disease.
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Keywords: Microvasculature; Capillary segments; Total capillary length; Corpus callosum; Space balls; Unbiased stereology; Design-based

1. Introduction

Cerebral amyloid angiopathy (CAA) results from the accumulation of beta amyloid (Aß) deposits around capillaries, small arteries, and arterioles in the brain and meninges, and represents a significant cause of morbidity among the elderly. The range of vascular pathology attributable to CAA includes reduced cerebral perfusion, cortical microinfarction, and leukoencephalopathy [5,30]. At autopsy about 40% of cases with CAA show classical cortical neuropathology of Alzheimer's disease (AD), including Aß-containing plaques and neurofibrillary tangles, neuronal and synaptic degeneration, gliosis, and severe cortical and brain atrophy [15,16,29,32,33]. Findings from human studies suggest that CAA may accelerate the effects of mild AD-type neuropathology and promote the progression of early to severe dementia [5,14,15,29,32,33]. Electron microscopic studies in CAA, combined with double-immunostaining of Aß-containing vessels using vascular markers, confirmed the marked loss of smooth muscle in larger vessels and the absence or reduction of endothelium in capillary profiles [16,33]. Quantitative analyses confirm abundant Aß deposits in neocortical fields of vessels containing relatively thin arterial walls and significant clinicopathological correlations between CAA and dementia scores [33]. Since Aß leakage from capillaries into surrounding cortical parenchyma most likely occurs upstream to the deposition of amyloid-

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containing plaques and neurodegeneration in AD, CAA may represent one of the earliest markers of AD.

AD-type neuropathology associated with CAA is strongly linked to the deposition of Aß peptides [5,16,29]. Previous studies using double transgenic (dtg) mice that express familial AD mutations in amyloid precursor protein (APP) and presenilin-1 (PS1) show extensive Aß deposition around 7 months of age in brain regions affected in AD [22]. These findings were confirmed in a second line of dtg APP/PS1 mice in which CAA appears around months of age, coincident with behavioral disturbances (neophobia, aggression, impaired cognition), glutamate excitotoxicity, and decreased long-term potentiation [4,22]. Studies in single transgenic mouse models that overexpress the Swedish APP mutation and deposit Aß in cortical tissue have reported disturbances in normal patterns of vascular circulation, including impaired cerebrovascular responses and reduced blood flow [25,26]. Magnetic resonance angiography in single tg mice overexpressing mutant APP identified deficiencies in cerebral perfusion as a function of capillary deformation and loss [1]. Regarding possible mechanisms for this pathology, Aß may compromise the ability of cerebral endothelial cells to produce vascular relaxing factors, impair the ability of cerebral blood vessels to maintain adequate flow during hypotension, and/or block increases in blood flow evoked by enhanced brain activity [13].

Few studies have investigated whether the deposition of toxic Aß in transgenic mouse models of AD is associated with degenerative changes in microvasculature similar to those reported in aging and AD brains at autopsy. The current study used modern stereological approaches in conjunction with β-nicotinamide adenine di-nucleotide phosphate (NADPH)-diaphorase histochemistry to assess vascular changes in white matter of dtg mice resulting from the expression of transgenic Aß peptides [5,14–16,29,30,32,33]. NADPH-diaphorase histochemistry provides a specific histochemical marker for the production of nitric oxide (NO) for endothelial cells of capillary vessels [18,27]. Endothelial nitric oxide synthase (eNOS) is a 150-kDa NADPH-dependent enzyme that is constitutively expressed on endothelium cells of microvasculature in brain, and is essential to systemic and/or local vascular integrity. Like inducible nitric oxide synthase (iNOS), which is induced by cytokine-stimulated neuroinflammatory pathology [19], eNOS is competitively inhibited by the NADPH-diaphorase substrate, nitro blue tetrazolium (NBT) [12]. Experimental evidence indicates specific NADPH-diaphorase staining of angiogenic endothelial cells [18]. Thus, NADPH-diaphorase histochemistry provides an effective method of visualizing vascularization in the brain and other tissues.

Morphological and topological features of capillary vessels were quantified in white matter of 7-month-old dtg APP/PS1 mice that deposit relatively high levels of Aß at 4–5 months of age [2]. A computerized stereology system was used to estimate the total number of capillary segments, N_{cap}, with a single segment defined as the region between two capillary branch points in 3D, using the unbiased disector-based conneulor method [8]. Estimates of total capillary length, L_{cap}, and length density, L_V, were made using a novel and efficient technique based on virtual sphere probes [24].

2. Material and methods

2.1. Animals

Mice for this study were male $APP_{swe}/PS1_{\Delta E9}$ dtg or wild-type mice in a mixed strain background (primarily C57BL/6 and C3He/J) developed by Dr. David R. Borchelt and colleagues at the Johns Hopkins School of Medicine [2]. The mice were raised in a vivarium at the Gerontology Research Center in standard plastic cages (4 per cage) and bedding under controlled environmental conditions ($22 \pm 1\,^{\circ}C$, $70 \pm 10\%$ humidity) and artificial light (12 h light/dark), with ad libitum access to a conventional diet (NIH-07) and filtered water through an automated system. All procedures involving animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with the approval of the Gerontology Research Institute's committee on Animal Care of National Institute on Aging.

2.2. Tissue processing

At 7 months of age, mice were sacrificed by transcardial perfusion of 0.1 M phosphate buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in PBS. Prior to perfusion under isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL, USA) anesthesia, a blood sample was taken from the orbital sinus using a small capillary tube. This sample was spun in a centrifuge for 5 min, and the percentage of packed red blood cells measured as a fraction of whole blood (hematocrit).

After the perfusion, brains of the mice were removed and fixed overnight in buffered fixative, transferred to a 30% sucrose/PBS until sinking, then quickly frozen in cooled isopentane and stored in cryoprotectant at $-80\,^{\circ}C$ until sectioning. Frozen coronal sections were cut though the entire forebrain at a thickness of 50 μm. A systematic-random set of sections was sampled through the entire corpus callosum (CC), with every fifth section taken, starting with a random section in the first five sections. Sampled sections were stained by free-floating histochemistry for β-NADPH (Sigma, St. Louis, MO, USA). Sections were rinsed in PBS (pH 7.4) and incubated in the dark at $37\,^{\circ}C$ in PBS (pH 8.0) solution containing 0.5 mg/ml β-NADPH, 0.25 mg/ml NBT (Sigma), and 0.3% Triton X-100 (Research Products International Co, Elk Grove Village, IL, USA) for 4.5 h. Stained sections were mounted on subbed slides and dried for a minimum of 12 h. In preparation for counterstaining, sections were processed through a dehydrating ethanol series (70, 95, 100 95, 70% for 2 min each), followed by 1 min dH_2O rinse. Cresyl violet (Sigma) counterstaining for basophilic structures was applied for 2 min followed by color adjustment in 5% acetic