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Effects of mild chronic cerebral hypoperfusion and early amyloid pathology on spatial learning and the cellular innate immune response in mice

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Abstract

Understanding the contribution of cerebrovascular factors in the progression of cognitive decline in Alzheimer's disease (AD) is a key step for the development of preventive therapies. Among these factors, chronic cerebral hypoperfusion is an early component of AD pathogenesis that can predict the progression from mild cognitive impairment to AD. Here, we investigated the effects of a protocol of mild chronic cerebral hypoperfusion in the APPswe/PS1 transgenic mouse model of AD. We observed that the permanent occlusion of the right common carotid artery induced spatial learning impairments in young APPswe/PS1 mice, but not in their wild type littermates. Furthermore, the extent of learning deficits strongly correlated with the number of cortical β-amyloid plaques, with the mobilization of monocytes into the blood and with the number of bone marrow-derived microglia in the brain. These results indicate that a mild reduction of cerebral blood flow can selectively induce cognitive deficits at an early stage of amyloid pathology, eliciting a cellular innate immune response, even without causing neuronal death.

Keywords: Oligoemia; Cerebral ischemia; Vascular dysfunction; Cerebrovascular disease; Chronic cerebral hypoperfusion; Cerebral blood flow; Alzheimer's disease; Vascular risk factors

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. With the recent advances in neuroimaging methods and cerebrospinal fluid biomarkers, it is becoming possible to identify patients at high risk of developing AD and to diagnose patients in the early symptomatic, predementia (or prodromal) phase of AD (Dubois et al., 2010). The early identification of these individuals will allow the development of novel preventive therapies that could delay or avoid the progression to AD dementia, significantly reducing the global burden of this disease. Accordingly, one interesting study has demonstrated that the lifetime risk of dementia in the population can be drastically reduced by delaying the age at onset of clinical dementia by only 5 years (Seshadri and Wolf, 2007).

In the past decade, a large number of studies have demonstrated that midlife vascular risk factors, such as hypertension, blood homocysteine and cholesterol levels, obesity, cigarette smoking, physical inactivity, and diabetes, are strongly associated with the risk of developing AD (Barnes and Yaffe, 2011; Kalaria, 2010). Moreover, a striking study has estimated that 1.1 million AD cases worldwide could be prevented if the prevalence of 7 potentially modifiable risk factors (including at least 5 vascular risk factors) was 10% lower (Barnes and Yaffe, 2011). These vascular risk factors can trigger several downstream events that converge on vascular dysfunction, potentially resulting in a chronic decrease in cerebral blood flow (CBF; de la Torre, 2012;

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Kalaria, 2010). Indeed, a growing body of evidence has suggested that chronic cerebral hypoperfusion develops several years before the onset of dementia (Luckhaus et al., 2008; Ruitenberg et al., 2005) and that the pattern of decreased CBF can be used to identify patients with amnestic mild cognitive impairment that will rapidly progress to AD in up to 3 years of follow-up (Borroni et al., 2006; Encinas et al., 2003; Habert et al., 2011; Hirao et al., 2005). Similarly, individuals carrying a mutation in the presenilin-1 gene (the most common cause of early-onset familial cases of AD) and subjects carrying the apolipoprotein E ε4 allele (a strong risk factor for AD) exhibit a decreased CBF in several brain regions preceding by many years the onset of AD cognitive deficits (Johnson et al., 2001; Thambisetty et al., 2010).

Such accumulating evidence indicates that chronic cerebral hypoperfusion is an early component of AD pathogenesis and that understanding the contribution of this factor to the progression of neuropathological alterations and to the clinical expression of AD is an important step for the development of novel preventive therapies. In this regard, transgenic animal models of AD can give new insights about the mechanisms that are involved in the interaction between chronic cerebral hypoperfusion and AD. For instance, 1 interesting study has demonstrated that bilateral common carotid artery stenosis synergistically induces learning impairments in the Barnes maze test in J20 transgenic mice, which carry a mutant form of the human amyloid precursor protein (APP), but not in wild type (WT) mice, an effect that was mediated by the induction of hippocampal neuronal loss (Yamada et al., 2011). Furthermore, even a mild reduction of CBF, obtained through the occlusion of the right common carotid artery (1 vessel occlusion or 1VO), synergistically induced learning deficits in 14-month-old female Tg2576 APP transgenic mice (Lee et al., 2011), which already develop other cognitive deficits by 9–10 months of age (Hsiao et al., 1996).

In this study, we sought to investigate the effects of a well-established protocol of chronic mild cerebral hypoperfusion (Kitagawa et al., 2005; Yoshizaki et al., 2008) on 4-month-old male APPswe/PS1 transgenic mice (from now on referred as to APP mice), i.e., at an early age when these mice still exhibit a very low number of β-amyloid (Aβ) parenchymal plaques in the brain and do not show cognitive impairments (Michaud et al., 2011; Naert and Rivest, 2011). Our results indicate that the permanent occlusion of the right common carotid artery induces spatial learning deficits in young APP mice, but not in their WT littermates, even without inducing neuronal death, and that this effect is accompanied by an acute cellular innate immune response.

2. Methods

2.1. Animals

Adult male APPswe/PS1 transgenic mice harboring the human presenilin 1 (A246E variant) and the chimeric mouse/human Aβ precursor protein (APP695swe) under the control of independent mouse prion protein promoter elements (B6C3-Tg(APP695) 3Dbo Tg(PSEN1) 5Dbo/J; Jackson Laboratories, Sacramento, CA, USA) were used in the experiments. All newborn pups were genotyped as described in the Jackson Laboratory protocol, and the WT littermates were used as controls. We also used transgenic mice expressing green fluorescent protein (GFP) under the control of a chicken β-actin promoter and cytomegalovirus enhancer (CByJ.B6-Tg(CAG-EGFP) 1Osb/J; Jackson Laboratories) as cell donors in the bone marrow transplantation experiment. All mouse strains were maintained in a C57BL/6J background.

Mice were housed 2–4 per cage and acclimated to standard laboratory conditions (12-hour light/dark cycle; lights on at 7:00 AM and off at 7:00 PM) with ad libitum access to mouse chow and water. All protocols were conducted according to the Canadian Council on Animal Care guidelines, as administered by the Laval University Animal Welfare Committee. All efforts were made to reduce the number of animals used and to avoid their suffering. The number of animals used in each experiment is shown in Table 1.

2.2. Unilateral common carotid artery occlusion and sham surgery

Four-month-old heterozygous APPswe/PS1 transgenic mice and their WT littermates were subjected to either sham surgery or to right common carotid artery permanent occlusion, under isoflurane anesthesia (2%). Briefly, a midline cervical incision was made and the right common carotid artery was exposed and double-ligated with 6-0 silk suture thread (1 vessel occlusion; 1VO group). The sham surgery consisted of a midline cervical incision under isoflurane

Table 1
Number of animals used in each experiment

	APP sham	APP 1VO	WT sham	WT 1VO	Euthanized at (time after surgery)
Behavior and histology (Figs. 1, 2, and 3)	16	18	17	21	6 wk
Behavior and FACS analysis (Fig. 4)	5	5	5	5	6 wk
Behavior and histology (chimeric mice; Fig. 5)	3	9	4	9	6 wk
Histology (Fig. 2)	3	6	3	7	3 d

We have also used 3 WT animals subjected to cerebral hypoxia-ischemia (as described in the Methods section), as a positive control for the experiments in Fig. 2.
Key: 1VO, 1 vessel occlusion; FACS, fluorescence-activated cell sorting; WT, wild type.