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Research Report

Glio-vascular changes during ageing in wild-type and Alzheimer's disease-like APP/PS1 mice

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ABSTRACT

Vascular and glial involvement in the development of neurodegenerative disorders, such as Alzheimer's disease (AD), and age-related brain vulnerabilities have been suggested. Therefore, we sought to: (i) investigate which vascular and glial events are evident in ageing and/or AD, (ii) to establish the temporal evolution of vascular and glial changes in AD-like and wild-type (WT) mice and (iii) to relate them to amyloid-β (Aβ) peptide accumulation. We examined immunohistochemically hippocampi and cortex from APP/PS1dE9 and WT C57BL/6 mice along ageing and disease progression (young-adulthood, middle- and old-age). Ageing resulted in the increase in receptor for advanced glycation endproducts expression, as well as the entrance of thrombin and albumin in hippocampal parenchyma. In contrast, the loss of platelet-derived growth factor receptor-β (PDGFR-β) positive cells, in both regions, was only related to AD pathogenesis. Hypovascularization was affected by both ageing and AD in the hippocampus, but resulted from the interaction between both factors in the cortex. Astrogliosis was a result of AD in hippocampus and of both factors in cortex, while microgliosis was associated with fibrillar amyloid plaques in AD-like mice and with the interaction between both factors in each of the studied regions. In sum, these data show that senile plaques precede vascular and glial alterations only in hippocampus, whereas in cortex, vascular and glial alterations, namely the loss of PDGFR-β-positive cells and astrogliosis, accompanied the first senile plaques. Hence, this study points to vascular and glial events that co-exist in AD pathogenesis and age-related brain vulnerabilities.

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid precursor protein; BBB, blood-brain barrier; CD31, cluster of differentiation 31; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adapter molecule 1; PDGFR-β, platelet-derived growth factor receptor-β; RAGE, receptor for advanced glycation end products; Tg, transgenic.

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1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by intracellular neurofibrillary tangles of hyperphosphorylated tau (tauopathy), amyloid-β (Aβ) peptide extracellular aggregates (amyloidopathy), oxidative stress, inflammation and premature neuronal apoptosis (Serrano-Pozo et al., 2011). In the last few years, the relationship between the accumulation of Aβ, neuronal atrophy, glial activation and vascular dysfunction has been the target of multiple studies, as evidence grows to suggest that all of these events may possibly contribute to development of AD (Armstrong, 2013; Zlokovic, 2011). In fact, mice overexpressing amyloid precursor protein (APP) and presenting high levels of Aβ have a profound disruption of cerebrovascular autoregulation, an essential mechanism to maintain relatively constant cerebral blood flow, suggesting that cerebrovascular alterations may play a role in the mechanisms of AD (Niwa et al., 2002). Multiple studies have shown that cerebrovascular dysfunction, namely blood-brain barrier (BBB) disruption, and cerebral blood flow and capillary density alterations, may lead to faulty Aβ clearance from the brain (Deane et al., 2004a, 2004b), augmented influx of peripheral Aβ through the BBB (Eisele et al., 2010) and overexpression of APP (Kumar-Singh et al., 2005; Weller et al., 2008). Thus, more studies are required to understand the temporal relationship between vascular damage and glial activation, and whether neuronal dysfunction begins before or after the accumulation of Aβ and vascular damage. Furthermore, ageing is known to contribute to the compromise of BBB properties and to alterations of the microvasculature, which in turn contribute to age-related cognitive decline possibly due to their influence on neuronal and glial activity (Marques et al., 2013). Recently, a study of elderly individuals with normal cognition to mild dementia and manifesting vascular disease showed that vascular brain injury was more influential than Aβ to mild cognitive dysfunction (Marchant et al., 2013), which is particularly interesting because AD is not a vascular dementia. Hence, considering that ageing is the greatest risk factor for AD (Akinyemi et al., 2013) it is important to understand which are the age-associated brain alterations, in order to modulate or prevent them from occurring.

In this study we sought to establish the glial and vascular events that are prevalent in ageing and/or AD. Moreover, we wanted to understand how glial and vascular profiles evolve during ageing and AD progression. The results presented here reveal vascular and glial alterations occurring along ageing, thus contributing to a better understanding of age-related brain vulnerabilities. Moreover, by establishing the temporal sequence of events occurring in AD and identifying the early ones, this study points to potential therapeutic targets to counteract AD development and progression.

2. Results

2.1. *Endothelial changes during ageing in WT and in AD-like APP/PS1 mice*

Based on previous studies suggesting that ageing of healthy individuals and AD patients are both accompanied by alterations in endothelial cells (Farkas and Luiten, 2001), we investigated the temporal evolution of blood vessel density (Fig. 1) and microvascular immunoreactivity for the receptor for advanced glycation end products (RAGE) (Fig. 2) in hippocampus and cortex of wild-type (WT) and APP/PS1 mice. Analysis of the microvessel density of WT animals showed a 35% ($P<0.05$) and 46% ($P<0.05$) decrease from middle-age to old age in hippocampus (Fig. 1A and C) and cortex (Fig. 1B and D), respectively. Regarding APP/PS1 animals, no significant loss of microvessel density in hippocampus was observed along time (Fig. 1C), while in cortex a significant reduction of 40% from young adulthood to middle-age was observed ($P<0.01$, Fig. 1D). Therefore, the results obtained in the cortex show that the loss of microvascularization occurs earlier in AD, where a decline was observed from young adulthood to middle-age ($P<0.01$), when compared to WT mice, where the decrease was only observed from middle to old age mice ($P<0.05$). This fact supports that the major difference between both genotype groups was observed by middle-age, where APP/PS1 mice had significantly less ($P<0.01$ in hippocampus and $P<0.05$ in cortex) microvessel density than WT mice. Remarkably, by old age both APP/PS1 and WT mice presented similar microvessel density in both regions (Fig. 1C and D). Thus, both ageing and AD contributed to hypovascularization in hippocampus (two-way ANOVA F 6.012, $P<0.05$ and F 13.86, $P<0.01$, respectively), whereas in cortex it was a result of the interaction between ageing and AD (two-way ANOVA interaction AD × ageing F 3.733, $P<0.05$).

Analysis of RAGE expression by endothelial cells of WT animals showed an increase of the immunoreactivity per μm^2 of blood vessel over time, and that this increase was already apparent at middle age in the hippocampus (24% vs. young adult, $P<0.01$, Fig. 2C), and only at old age in the cortex (21% vs. young adult, $P<0.05$, Fig. 2D). The APP/PS1 animals also showed a 13% increase in RAGE immunoreactivity in hippocampus ($P<0.05$ young adulthood vs. middle-age, Fig. 2C) and, even though not significant, a 14% increase was observed in cortex (young adult vs. middle-age, Fig. 2D). Interestingly, the comparison of endothelial RAGE immunoreactivity between WT and APP/PS1 animals showed no difference in both regions. Accordingly, ageing was the major contributor for the alterations in RAGE immunoreactivity by endothelial cells in hippocampus (two-way ANOVA F 23.13, $P<0.001$) and cortex (two-way ANOVA F 9.874, $P<0.001$). In summary, endothelial-related changes indicate that APP/PS1 mice have a premature loss of capillary density compared to WT mice, but similar immunoreactivity of RAGE by endothelial cells with ageing.

2.2. *Pericyte alterations during ageing in WT and in AD-like APP/PS1 mice*

We wanted to know if ageing and/or AD affect blood vessel integrity by altering pericyte vascular coverage. For that purpose, we analyzed the widely used marker of pericytes, platelet-derived growth factor receptor-β (PDGFR-β) (Bell et al., 2010; Sá-Pereira et al., 2012) along ageing and/or AD (Fig. 3). Analysis of PDGFR-β-positive cells in hippocampus (Fig. 3A and C) and cortex (Fig. 3B and D) showed that in cortex there was nearly a triple number of PDGFR-β-positive cells per field