Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

The effect of ageing on neurogenesis and oxidative stress in the $APP_{swe}/PS1_{deltaE9}$ mouse model of Alzheimer's disease

*Alison Hamilton, Christian Holscher**

School of Biomedical Science, University of Ulster, Coleraine, Northern Ireland, UK

ARTICLE INFO

Article history:
Accepted 6 February 2012
Available online 13 February 2012

Keywords:
Stem cells
Amyloid
Presenilin-1
Brain

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by memory loss and impaired cognitive function. One of the hallmarks of AD is the formation of beta amyloid (Aβ) plaques. Aβ has neurodegenerative properties and aggregates in the brain, causing inflammation, oxidative stress and eventually neuronal loss. In AD, adult neurogenesis in the dentate gyrus (DG) of the hippocampus is known to be impaired. We tested how ageing affects neurogenesis and oxidative stress in the commonly used $APP_{SWE}/PS1_{\Delta E9}$ mouse model of AD and their wild type (wt) littermate controls aged 3, 5, 10 and 15 months. Progenitor cell proliferation in the DG of APP/PS1 was lower at 3, 5 and 10 months compared to controls, while oxidative stress in APP/PS1 mice was increased in the cortex at 3 and 5 months of age compared to controls. The numbers of new neurons in the DG were decreased in APP/PS1 mice at 10 and 15 months. In APP/PS1 mice, Aβ plaques were evident in the cortex from 3 months onward; however these were small and few. Plaque size and number consistently increased with age in APP/PS1 mice. These results show that the damage to the brain occurs already very early in the brain, and although neurogenesis is impaired, it is still active even in late stage AD. Therefore, therapies would have the best effects if started early, but promoting neurogenesis may act in a protective and reconstructive way even in later stages of AD.

1. Introduction

Alzheimer's disease (AD) is characterised by neurodegeneration, attributed to the production and accumulation of beta amyloid (Aβ) in the brain (Selkoe, 2000). Accumulation of Aβ is associated with progression of AD pathogenesis, including onset of inflammation and oxidative stress, and the progression of neurodegeneration and cognitive decline (Bondolfi et al., 2002; Cirrito et al., 2008; Ramirez et al., 2005; Sasaki et al., 2001). Interest in understanding the role of Aβ in AD pathogenesis has led to the production of a variety of AD mouse models, which express various combinations of amyloid precursor protein (APP) and/or presenilin 1 (PS1) mutations.

The $APP_{SWE}/PS1_{\Delta E9}$ mouse model is a widely used model of AD, co-expressing Swedish, mutated human APP_{695} and human mutated PS1 in which exon 9 is deleted. Studies using $APP_{SWE}/PS1_{\Delta E9}$ mice have found that animals exhibit impaired exploratory behaviour, spatial memory and synaptic function (Lalonde et al., 2005; Malm et al., 2011; O'Leary and Brown,

* *Corresponding author at:* School of Biomedical Sciences, Centre for Molecular Biosciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, Northern Ireland, UK. Fax: +44 28 70124965.
E-mail address: c.holscher@ulster.ac.uk (C. Holscher).

0006-8993/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2012.02.015

2009). Memory impairments were seen as early as 7 months of age, while synaptic transmission is affected at 3 months (Goto et al., 2008; Volianskis et al., 2010). Production of Aβ has been shown to occur as early as 3 months in the form of both $A\beta_{1-40}$ and $A\beta_{1-42}$; however, plaque formation occurs later, at around 5–6 months (Goto et al., 2008; Volianskis et al., 2010).

There is increasing interest in the relationship between adult hippocampal neurogenesis and learning. Evidence suggests that learning and memory stimulate progenitor cell proliferation and that the rate of hippocampal neurogenesis directly correlates with changes in LTP (Bruel-Jungermann et al., 2006; Gould et al., 1999). However, it is well established that the rate of neurogenesis decreases as part of the natural ageing process (Kempermann et al., 2006; Kuhn et al., 2007; van Praag et al., 2005). Furthermore, the development of AD has been shown to exacerbate the decline in hippocampal neurogenesis and this impairment is believed to be the result of Aβ pathology (Faure et al., 2011; Wang et al., 2004; Wen et al., 2004). LTP and learning are impaired early in the $APP_{SWE}/PS1_{\Delta E9}$ mouse model of AD; based on the belief that LTP correlates with neurogenesis, we analysed whether or not neurogenesis is impaired in this mouse model and how early this change occurs.

In addition to the affect of Aβ on neurogenesis, it is possible that neurogenesis is further impaired by the onset of oxidative stress induced by Aβ and by plaque formation (Varadarajan et al., 2000). We measured the nucleotide oxidative stress product 8-oxoguanine in the hippocampus, which is a good marker as there is little ability to remove the 8-oxoguanine produced over time (Iida et al., 2002). The onset of oxidative stress in the AD brain has been found to exacerbate Aβ production, promote AD pathology and contribute to neurodegeneration (Guglielmotto et al., 2010; Lovell and Markesbery, 2007).

Here we show how age affects plaques formation in this mouse model and how these plaques correlate with oxidative stress and neurogenesis.

2. Results

2.1. Beta amyloid: cortex

Plaques were observed as early as 3 months; these plaques were rare and very small in size. One-way ANOVA over all groups showed there to be a significant affect of ageing ($F=72.87$, $P<0.0001$) (Fig. 1). As age increases plaque number increased significantly (Fig. 1), from 3 to 5 months (300% increase; $P<0.005$), from 5 to 10 months (200% increase; $P<0.001$) and from 10 to 15 months (91.7% increase; $P<0.001$).

2.2. Beta amyloid: hippocampus

As age increased, plaque load increased also; first plaques were apparent at 3 months of age, like in the cortex these plaques were small in size and very rare. One-way ANOVA over all groups showed a significant effect of ageing on plaque formation ($F=72.87$, $P<0.0001$) (Fig. 2). While the increase from 3 to 5 months was not significant (400% increase, $P>0.05$), however the increases in plaque number from 5 to 10 months and from 10 to 15 months were found to be significant (160% increase, $P<0.01$ and 231% increase, $P<0.001$ respectively).

2.3. Oxidative stress assessment (8-oxoguanine stain): cortex

In the cortex, the amount of 8-oxoguanine (8-oxog) expressed in the tissue increased for both the APP/PS1 mice and for the wild type littermate controls, as mice got older. Two-way ANOVA revealed that the effect of ageing and genotype was highly significant ($P<0.0001$, df 3, $F=38.73$ and $P=0.0015$, df 1, $F=10.46$ respectively); interaction was not significant ($P=0.7643$, df 1, $F=0.3845$) (Fig. 3).

In wild type mice, two-way ANOVA and Bonferroni post tests show that from 3 ($n=6$) to 5 ($n=6$) months and from 5 to 10 ($n=5$) months the increases in 8-oxog expression were not significant (100% increase, $P>0.05$ and 83.3% increase, $P>0.05$ respectively); however the increase in 8-oxog from 10 to 15 ($n=4$) months was found to be significant (172.5% increase, $P<0.001$). In APP/PS1 mice, two-way ANOVA and Bonferroni post tests showed that there was no significant increase in the amount of 8-oxog present from 3 ($n=6$) to 5 ($n=5$) months, though there was a trend (50% increase, $P>0.05$). No difference in the amount of 8-oxog was evident from 5 to 10 months (0% change, $P>0.05$). From 10 ($n=5$) to 15 ($n=5$) months, there was a substantial increase in 8-oxog levels, which was found to be significant (153.3% increase, $P<0.001$).

Unpaired T tests were performed to assess the difference in the amount of 8-oxog expression between genotypes. At 3 and 5 months (Fig. 3) the difference between the APP/PS1 mice and their controls was found to be of significance (233% increase, $P<0.0001$, $F=9.457$ and 150% increase, $P<0.0001$, $F=4.773$ respectively). While the differences between APP/PS1 and wild type mice at 10 and 15 months (Fig. 3) were not found to be significant (36.36% more, $P=0.5532$, $F=3.310$ and 26.67% more, $P=0.2437$, $F=1.222$ respectively).

2.4. Oxidative stress assessment (8-oxoguanine stain): hippocampus

In the hippocampus, 8-oxog expression increased with ageing in both APP/PS1 mice and wild type controls. Two-way ANOVA showed that only ageing was significant ($P<0.0001$, df 3, $F=19.06$); neither genotype ($P=0.0625$, df 1, $F=521$) nor interaction was of significance ($P=0.6569$, df 3, $F=0.5381$) (Fig. 4).

Two-way ANOVA and Bonferroni post tests revealed that 8-oxog increased from 3 to 5 months (45.5% increase, $P>0.05$), 5 to 10 months (26.67% increase, $P>0.05$) and from 10 to 15 months (113.3% increase, $P<0.001$); however only the increase from 10 to 15 months was of significance. Unlike wild type mice, the APP/PS1 mice exhibited increased 8-oxog expression from 3 to 5 months and from 10 to 15 months. Two-way ANOVA and Bonferroni post tests found that neither the increase from 3 to 5 months (58.3% increase, $P>0.05$), nor the decrease from 5 to 10 months (10.5% decrease, $P>0.05$), was of significance. Only the increase in 8-oxog from 10 to 15 months (94.12% increase, $P<0.01$) was of significance.

To assess the differences in 8-oxog expression between APP/PS1 mice and wild type mice, unpaired T tests were