The effect of amyloid beta on microglia in the aged compared to the young rat brain and the potential exacerbation of ageing in response to abeta infusion.

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"Alzheimer's is an irreversible progressive disease that slowly destroys memory and cognitive skills... Recent studies have shown it to involve the activation of microglia due to their interaction with Aβ plaques, as a component of the neural inflammatory response which characterises this neurological disease."

Abstract

Alzheimer's is an irreversible progressive disease that slowly destroys memory and cognitive skills. The decline in mental fitness associated with this disease is long established. Recent studies have shown it to involve the activation of microglia due to their interaction with Aβ plaques, as a component of the neural inflammatory response which characterises this neurological disease. It was postulated the exacerbating effects of both age and Aβ infusion, separately as well as combined, in this study. An increase in microglial activation was reported in the aged rat brain and in the brains of young rats chronically infused with Aβ infusion. However it was found that Aβ infusion in the aged brain attenuated the activation. This is in stark contrast to previous studies in this area and brings into question the relevance of Aβ infusion in the aged rat brain as a model of AD. However my study confirms the importance of age as a factor in the progression of this debilitating disease.

Introduction

The decline in mental fitness associated with Alzheimer disease is accompanied by physical changes in the brain, but the pathogenesis of those changes is not clear[1]. The major neuropathological hallmarks of Alzheimer's are extracellular β-amyloid (Aβ) plaques and neurofibrillary tangle formation. These plaques are insoluble fibres, protein aggregates sharing structural traits. Amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons and its proteolysis by β and γ secretases generates Aβ. Its fibrillar form is the primary component of amyloid plaques found in the brains of AD patients.

Gliosis is seen in AD; activated astrocytes and microglia are characteristically found in abundance near neurons and plaques, as first described by Alzheimer in 1911 [1]. Many of the microglia in pathologically affected areas of the brain have been found to express major histocompatability complex II (MHC II) suggesting a role for inflammation in AD [2]. When microglia interact with the deposited fibrillar forms of Aβ, it leads to the conversion of microglia into an activated cell and results in the synthesis and secretion of cytokines. Once activated, microglia are capable of producing a variety of pro-inflammatory mediators and potentially neurotoxic substances that could contribute to CNS injury[4].

To quantify the level of microglial activation, and therefore the extent of neurological insult, various markers can be used such asCD11b, MHC I and II.CD11b is a strong indicator of microglia cell activation in the presence of Aβ and/or in the aged brain. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a DNA/RNA addition product (adduct) which is formed to a greater degree when cells are in an oxidative environment. It is a marker of oxidative stress at the genetic level.

Various intracellular pathways including those involving activation of c-jun N-terminal Kinase (JNK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), are also activated in microglia resulting in the release of proinflammatory cytokines[3].

The aim of the project was to ascertain whether or not ageing exacerbates the response to the infusion of Aβ, while examining the effect of ageing and Aβ individually on neural inflammation. Changes in CD11b and 8-OHdG expression will be measured to look at microglial activation and oxidative damage, respectively. Intracellular signalling pathways JNK phosphorylation and NFkB were also examined.

Methods

Groups of young (3-4 months) and aged (22-24 months) rats were randomly divided and treated with a cocktail of $A\beta_{1-40}$ + $A\beta_{1-42}$, or the reverse peptide, $A\beta_{40-1}$. Animals were anaesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg).

Pumps delivered a cocktail of $A\beta_{1-40}$ (26.9 µM) and $A\beta_{1-42}$ (36.9 µM; aggregated for 24 h at 25 °C or 37 °C for 48 h retrospectively; Biosource, Belgium) or control peptide $A\beta_{40-1}$ (63.8 µM) intracerebroventricularly at the rate of 0.25 µl/h (±0.05 µl) for 28 days. All rats were housed in groups of 3 and kept under veterinary supervision in a controlled environment (12 h light schedule; ambient temperature 22-23°C) and all experiments were performed under a license issued by the Department of Health (Ireland) and in accordance with the guidelines laid down by the local ethical committee.

Immunohistochemical analysis of CD11b and 8-OHdG involved Cryostat sagittal brain sections (10µm thick) which were stained for CD11b and 8-OHdG and visualised by light microscopy. The sections to be stained for 8-OHdG were permeabilised in 0.1% Triton-X100 in Tris-buffered saline (TBS) pH7.4. Sections were incubated for 30 minutes at RT in 10% normal horse serum (Vector, UK), 4% bovine serum albumin (BSA) (Sigma, UK) in TBS, to block non-specific interactions and then overnight at 4°C in mouse anti-CD11b or mouse anti-8-OHdG antibody solution in 2% BSA in TBS. Negative controls were incubated for 2 hours at RT in 2% BSA in TBS alone. CD11b sections were counterstained with haematoxylin (RA Lamb, UK), and 8OHdG sections were counterstained with 0.1% Methyl Green, then dehydrated through a series of graded alcohols.

Western blotting was carried out and expression of JNK, pJNK and pIκB were analyzed by SDS-PAGE in homogenate prepared from hippocampus as previously described. After incubation with rabbit polyclonal pJNK antibody (1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA), rabbit polyclonal JNK antibody (1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA), or rabbit monoclonal pIκB antibody (1:1000; Cell Signaling Technology, Inc., MA 01923, USA), membranes were incubated with secondary antibody (1:5000; Jackson ImmunoResearch Europe Ltd.) and proteins detected using enhanced chemiluminescence (Millipore Ltd, Watford, UK). Loading controls were performed with mouse monoclonal anti-b-actin antibody (1:10,000; Sigma, Dorset, UK). Protein bands were quantified by densitometric analysis using FujiFilm dark box (LAS3000; FujiFilm, Dublin, Ireland).

Results

The age-related increase in hippocampal CD11b staining is down-regulated in Aβ-treated brains. In the progression of age, there is a clear and consistent increase in CD11b as well 8OHdG staining in the hippocampus of the rat brain indicating activation and proliferation of microglia. CD11b staining is identified by brown stains amongst purple counterstain which identifies the cell nucleus. The activated microglia display a branching appearance, bushy or 'rod-like', (see figure 1 (iii)). Whereas in their inactive states they are characterised by long branching processes with a small dense cell body (see Figure 1 (ii)). There is a significant increase in CD11b positive cells in aged in comparison to young controls, (see figure 1 (I) v (iii)). Upon chronic Aβ infusion in the young control, there is an increase in stained cells identifiable. However, there is a decrease in CD11b positive cells upon Aβ infusion, as seen in the comparison of figure 1 (iii) v (iv).

The age-related increase in 8OHdG staining was attenuated by chronic Aβ infusion. There is a clear increase in the number of cells positive for 8OHdG staining in aged compared to young control dentate gyrus, (see figure 2 (i) v (iii)). Upon chronic Aβ infusion, there was a consistent increase in staining of 8OHdG seen in young rats. However 8OHdG staining was markedly decreased upon Aβ infusion in the aged rats.

Chronic Aβ infusion decreased JNK expression but not phosphorylation in both young and aged hippocampus. Western blot analysis of JNK showed a marked decrease upon chronic Aβ infusion (Figure 3A; $p<0.05$, 2-way ANOVA). However the decrease in phosphorylated JNK was not statistically significant (Figure 3B).

Neither age nor Aβ infusion altered IkB phosphorylation in hippocampus. Western blot analysis of phosphorylated IkB did not show any change statistically significant as a result of either age or Aβ, or both (Figure 4).

Discussion

In this study, I investigated the increase in microglial activation in two different models of neuroinflammation: ageing and chronic infusion of Aβ, and how they interact. The results showed an age-related increase

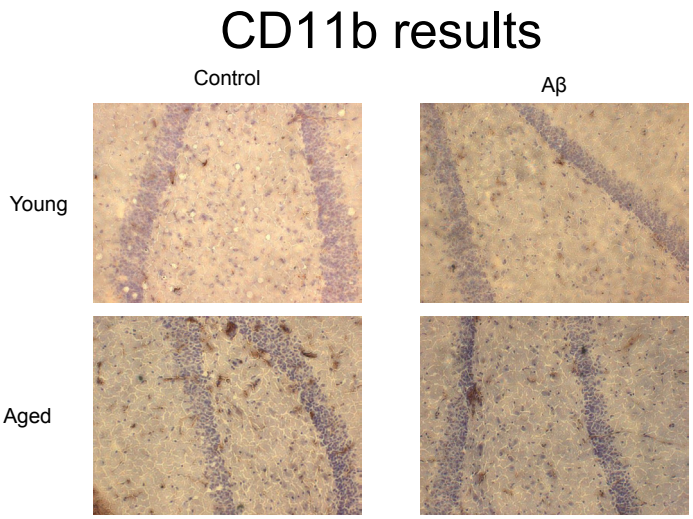


Figure 1. CD11b staining in the dentate gyrus by light microscopy. The arrow in Figure 1 (iii) indicates an example of a cell positive for CD11b by the ABC method. There is a marked increase in CD11b staining evident in aged (iii) compared with young control (i) hippocampus. Chronic Aβ infusion did not alter CD11b staining in young (ii) hippocampus, when compared to control (i).

8OHdG Results

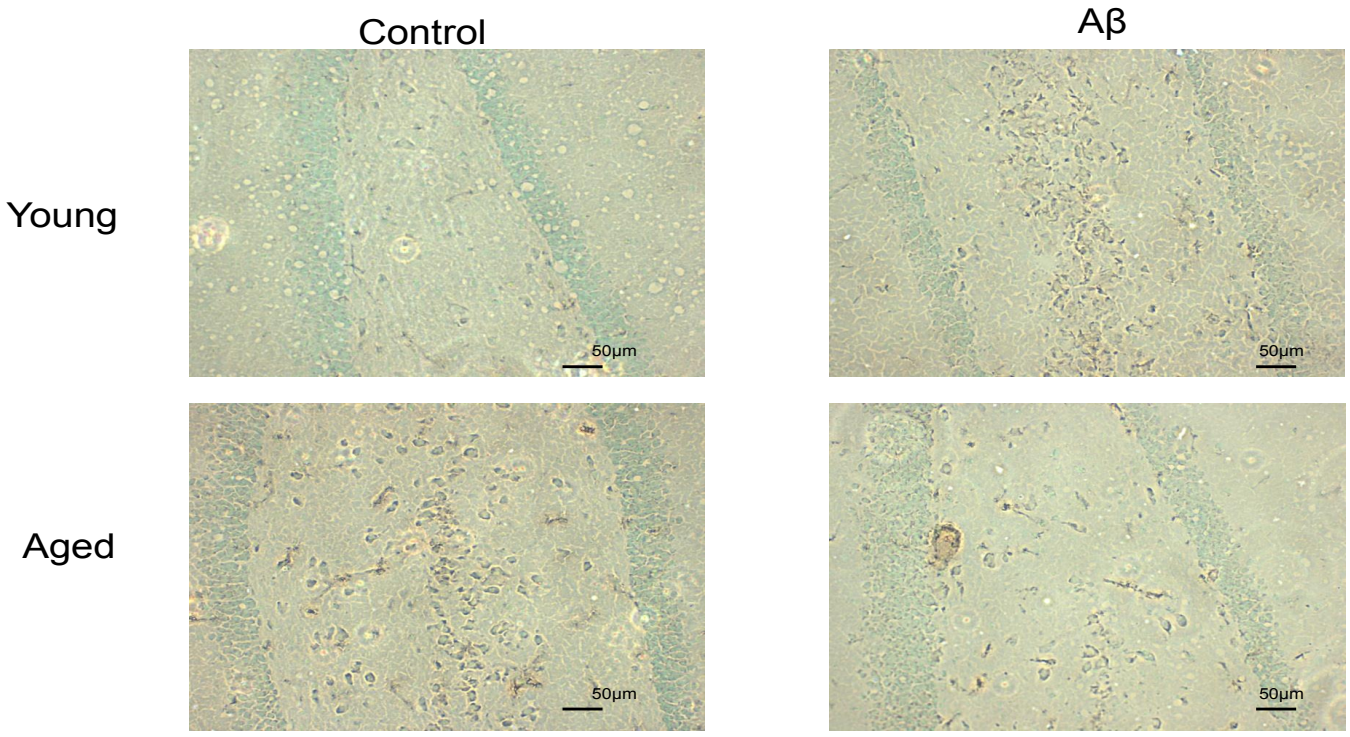
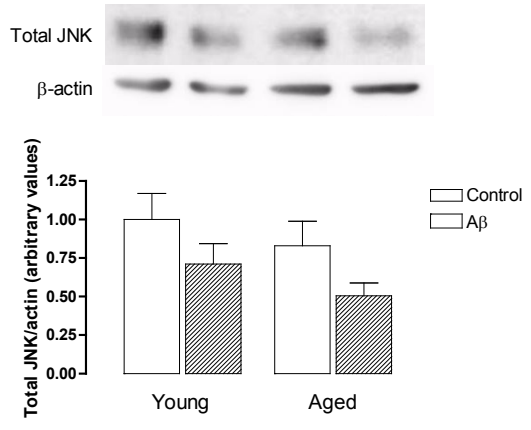


Figure 2. 8-OHdG staining in rat dentate gyrus visualised by light microscopy. The arrow in Figure 2(iii) indicates a cell positive for 8-OHdG. There was a significant increase in 8-OHdG staining in aged (iii) compared with young control (i) hippocampus. There was also an increase in staining as a result of chronic infusion of Aβ in young rats (ii) when compared to young control (i). However, there was a decreased level of 8-OHdG staining upon infusion of Aβ in aged rats (iv).



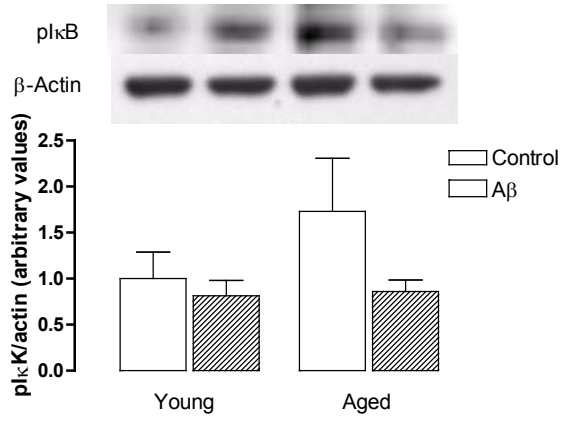


Figure 4 The change in phosphorylated IκB was not statistically significant.

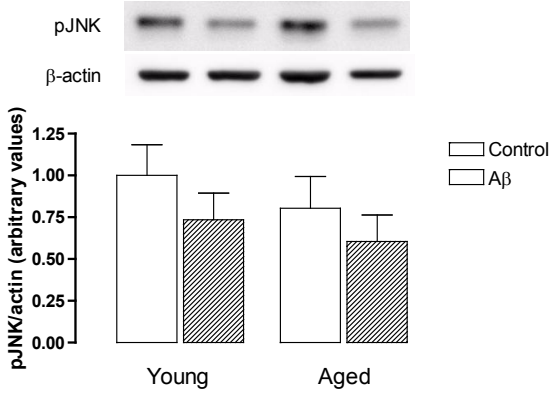


Figure 3 (Left - Top) There was a significant decrease in total JNK as a results of chronic Aβ infusion ($p<0.05$, 2-way ANOVA)

Left - Bottom)) but the decrease in phosphorylated JNK was not statistically significant.

in microglial activation as evidenced by increased CD11b and 8OHdG staining. The use of aged rats for 28 day chronic Aβ infusion is a novel experiment, but I hypothesized that microglial activation would increase in comparison to aged controls as well as young Aβ infused.

Age itself is a risk factor for the activation of microglial cells. The literature shows that the expression of immunologically important surface antigens, particularly MHCII, increase gradually with normal aging in rodent as well as human microglial cells[4]. However, in contrast to acute neuropathological conditions where microglial activation is fast and may involve proliferation, there is little direct evidence that the total number of microglial cells increase in the case of normal ageing[4].

Recent findings have shown CD11b mRNA to be increased in the hippocampus of aged rats in comparison with their young counterpart, which is indicative of neural insult[5]. The data presented here showing an age-related increase in CD11b staining in agreement with the literature, displays an increase in specific staining due to the upregulation of CD11b on the cell surface due to microglial activation.

Aβ infusion in young rats increased in CD11b as well as 8OHdG staining, indicating increased microglia activation. This is consistent with previous findings which have suggested that the inability of microglia to completely process fibrillar Aβ may result in activation of cells by the residual Aβ and therefore lead to a persistent neuroinflammatory state [6].

The chronic infusion of Aβ in aged rats has not been reported before to the best of my knowledge. The CD11b results displayed a decrease in staining, signifying a decrease in microglial activation from figure 1 (iii) to (iv). This is not in keeping with my hypothesis that ageing exacerbates neurodegeneration and inflammation. It also disagrees with findings which indicated that Aβ infusion induced inhibition of long term potentiation (LTP), coupled with increases in markers of neuroinflammation[7].8OHdG staining was attenuated upon chronic Aβ infusion in the aged rats.

Increased JNK activation has been previously reported in aged rats and was associated with a deficit in LTP [8]. Here we report a significant decrease in total-JNK expression in response to chronic Aβ infusion.

Conclusion

The purpose of this study was to assess the effect of ageing and chronic Aβ on microglial activation in young and aged rat brains. Results have shown that microglial activation play a hugely important role in the pathophysiology of AD. The hypothesized age-related increase in microglial activation was confirmed which further highlights the relevance of age as a factor in the progression of AD. The results for IkB were also statistically insignificant. Repeating these experiments to increase statistical power may clarify the findings. However, having hypothesized an exacerbation in the response to Aβ infusion, the results not only disprove this hypothesis but they also call into question the relevance of the infusion of Aβ into aged rats as a model for Alzheimer's, suggesting age and Aβ independently as the dominant factors involved in the aetiology of this complex disease, rather than their combination.

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