Inhibition of microglial activation in the treatment of Alzheimer's disease

Nicola Andrzejowska and Ian Wood. School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT

Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder characterised by progressive dementia and a build-up of amyloid protein plaques. There are 44 million people living with dementia worldwide resulting in significant burdens and an annual financial cost of £500 billion. Reducing the onset and progression of AD would make a significant impact on reducing these societal costs. Amyloid build-up stimulates the brain's immune cells, microglia, which become activated to remove the amyloid plaques. While this is thought to be initially beneficial, the prolonged chronic activation of these immune cells may contribute to disease progression (Fig 1). Recent research suggests that inhibiting microglial activation could provide therapeutic benefit in AD.

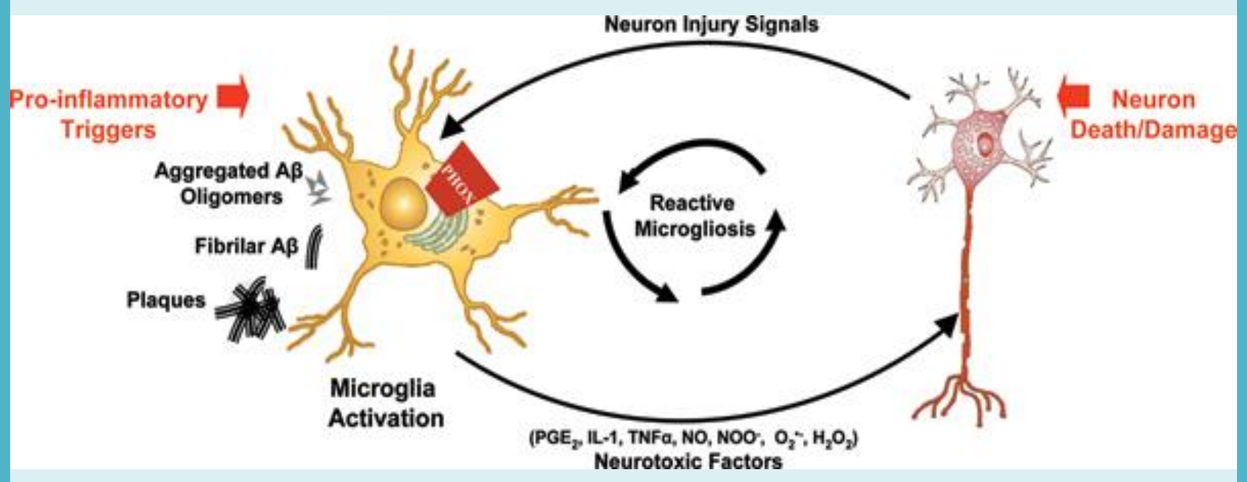

Fig1- Initially, amyloid plaques activate microglia to remove the amyloid. However prolonged microglia activation may contribute to neuronal death which maintains microglial activation. Image from Block, Michelle. (2008). NADPH oxidase as a therapeutic target in Alzheimer's disease. BMC neuroscience. 9 Suppl 2. S8. 10.1186/1471-2202-9-S2-S8.

In the nucleus of eukaryotic cells, negatively charged DNA is wrapped around positively charged histone proteins to allow sufficient compaction. Histone Deacetylase (HDAC) enzymes remove acetyl groups from such proteins, thus neutralising the charges to allow chromatin expansion, and thus gene transcription. Modification of non-histone proteins can also occur, including deacetylation of transcription factors that regulate gene expression.

Research at the University of Leeds has shown that inhibitors of HDACs reduce microglial activation, although their widespread effects make them unsuitable as a therapy. We are yet to determine how HDAC inhibitors reduce microglial activation but if the relevant cellular pathways could be identified, it would lead to specific targets for new drugs. One potential candidate protein is Nuclear factor kappa light-chain-enhancer of activated B cells (NF-kB), whose activity promotes microglia activation. Our aim was to determine if HDAC inhibitors reduce NF-kB function.

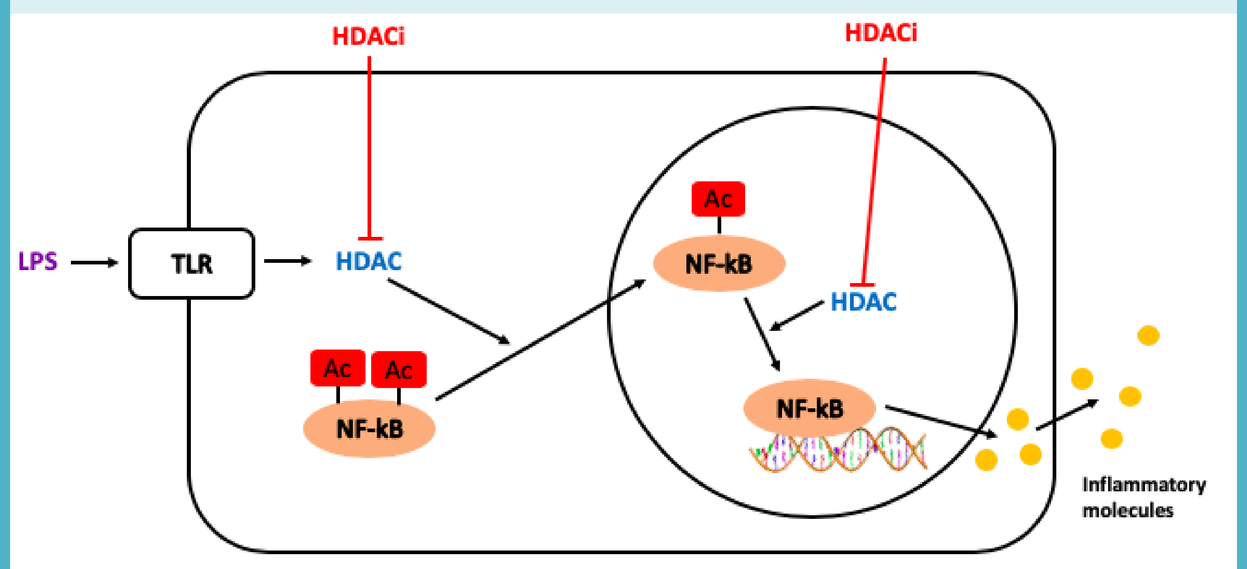

Fig2- In resting microglia inactive NF-κB is retained in the cytoplasm. Microglial stimulation results in acetylation of NF-κB causing it to move into the nucleus and promote gene expression, e.g.- inflammatory molecule interleukin-6 (IL-6).

Methods

Microglia extracted from mice (BV2 cells) were activated with lipopolysaccharide (LPS) in the presence of specific inhibitors and activation levels assessed by measuring the levels of IL-6.

The roles of specific cellular pathways in microglial activation were determined by incubation with specific inhibitors. The HDAC inhibitor SAHA was shown to significantly reduce microglial activity.

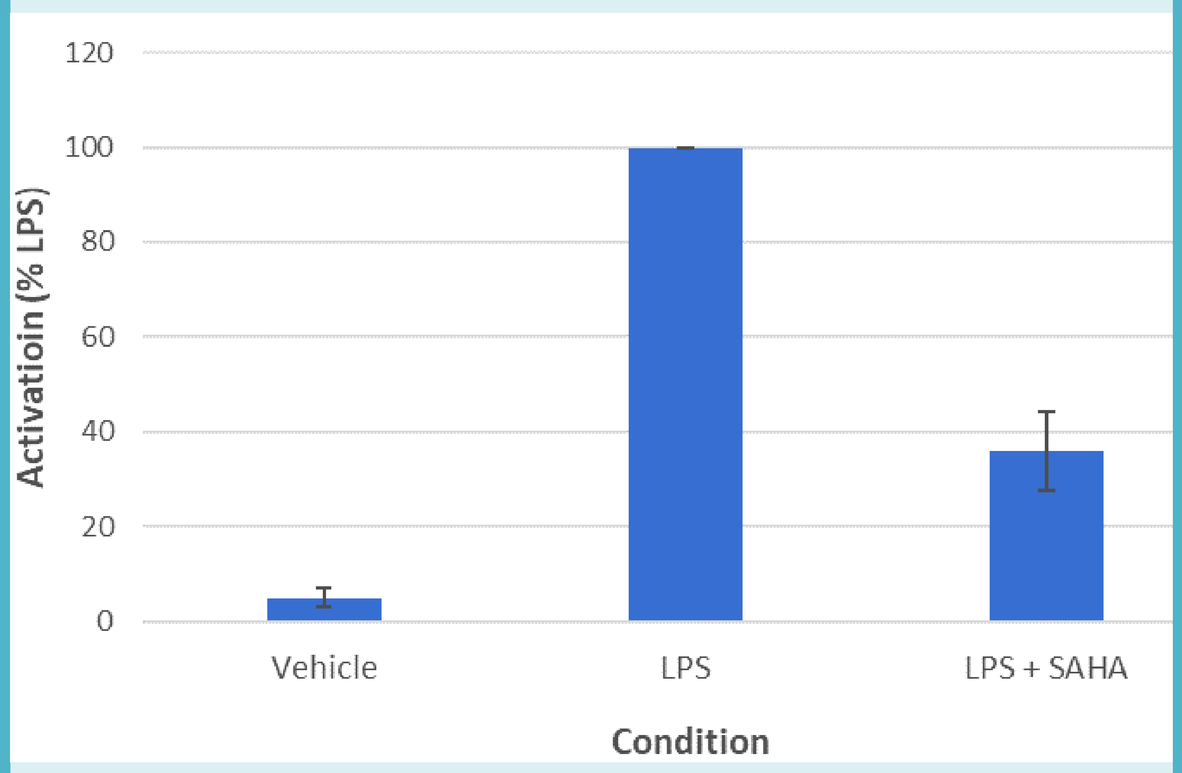

Fig3- Microglial activation expressed as a mean percentage of (positive control) LPS ±SEM (0.5µg/µl, 100%). Vehicle acting as the negative control (5±2%) and LPS+SAHA (1µM, 36±8%) testing the inhibitory effect of the HDAC inhibitor SAHA.

The effect of HDAC inhibition (SAHA) on NF-κB and its distribution in microglial cells was investigated using Western Blotting.

Cellular proteins were extracted, and the cytoplasmic and nuclear proteins were then separated for a Western Blot. These were transferred to a PVDF membrane and washed in primary (anti-NF-κB) then secondary (anti-mouse-horseradish peroxidase (HRP)) antibodies. The HRP antibodies allowed chemiluminescence imaging to determine protein concentration based on light intensity using Image-J software.

The concentrations of NF-κB were greater in the cytoplasm than the nucleus (Fig 4). Further optimisation is required to determine the effects of LPS and SAHA, as the low intensity signals have been hard to quantify using Image-J software.

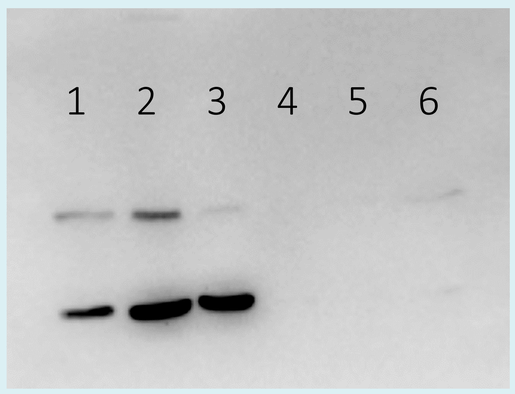

Fig4- Western Blot image depicting: cytoplasmic (1,2,3) and nuclear (4,5,6) concentrations of NFκB (top) and β-actin (bottom) in vehicle (1&4), LPS (2&5) and LPS+SAHA (3&6).

Summary

If the molecular mechanisms regulating microglial activity can be determined, it will allow the pursuit of novel therapeutic targets in the search for new treatments for AD. There are over 4000 potential HDAC targets and here we have identified that one of those candidates, NF-κB, is regulated by HDACs.