CHAPTER I

INTRODUCTION

1. Background and Rationale

Iron accumulation in the brain progresses with age (Aquino et al. 2009). Because of its abundant lipid content and high oxygen consumption rate, this process increases brain vulnerability to free radical damage and subsequently brings about neurodegenerative changes. In degenerated brain regions of Alzheimer's disease (AD), an age-related neurological disease, abnormally high levels of iron have also been consistently reported (Connor et al. 1992; House et al. 2006). Although it has not yet known that iron accumulation in AD brain is an initial event that causes neurodegeneration or a consequence of the disease process, recent evidences have been reported that mutation in the gene involved in iron absorption such as HFE influence brain iron levels and also associate with AD (Sampietro et al. 2001; Pulliam et al. 2003; Berlin et al. 2004; Connor and Lee 2006; Bartzokis et al. 2010). These reports have opened the possibility that increased brain iron accumulation may involve in mechanisms that contribute to neurodegeneration in AD.

The major pathological features of AD are extracellular deposition of β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles, abnormal hyper-phosphorylation of tau protein. AD is also associated with alterations in mitochondrial function, increasing in oxidative stress, neuronal dying by apoptosis and neuroinflammation. All of these features have been reported to contribute to neuronal cells death. Growing body of evidence has been reported that neuroinflammation which mediated by activated microglia, a resident brain macrophage in central nervous system, may be a key factor that enhanced neuronal cells death. In this pathological setting, microglia become activated and infiltrated to Aβ plaques. Interestingly, activated microglia appears to enrich for iron (Connor et al. 1992; Zecca et al. 2004). However, its significance remains elusive. In AD, it has been reported that Aβ can

induced microglial activation resulting in the release of pro-inflammatory cytokines, inflammatory mediators and reactive oxygen/nitrogen intermediates, including interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), matrix metalloproteinase 9 (MMP-9) and nitric oxide (NO) (Block and Hong 2005) which has been reported to contribute to neuronal cell death. The transcription factor that plays a significant role in the inflammatory response of activated microglia includes an inducible nuclear factor-kappa B (NF-κB), which requires the activation of IκB kinase that phosphorylates inhibitor alpha of NF-κB leading to ubiquitination and degradation and resulting in the nuclear translocation of NF-κB. The transcription activity of NF-κB can be regulated by several kinases, including glycogen synthase kinase-3β (GSK-3β), a serine/threonine kinase originally identified as a key enzyme regulating glycogen synthesis (Hoeflich et al. 2000; Majumdar et al. 2004; Takada et al. 2004; Whittle et al. 2006). Recent evidence has implicated GSK-3β is involved in inflammatory processes (Cheng et al. 2009; Huang et al. 2009; Zhang et al. 2009). Moreover, GSK-3β has also been reported to be involved in others AD pathological features such as tau hyper-phosphorylation, Aβ production and neuronal apoptosis. Importantly, in the brain, GSK-3β activity has been reported to be increased with age (Lee et al. 2006). Similar findings have also been reported for brain iron levels, numbers of activated microglia and iron accumulation by microglia (Zecca et al. 2004). Furthermore, the activity of GSK-3β has been shown to be elevated in AD brain (Pei et al. 1999), where iron accumulation is pronounced (Connor et al. 1992). Accordingly, increased GSK-3β activity in the normal aged brain and in AD brain may be related to elevated levels of brain iron and may contribute to neurodegeneration in AD.

Therefore, the first part of this study, iron supplemented and lipopolysaccharide (LPS)-activated cultures of BV2 microglia was developed to mimic progressive iron accumulation by activated microglia and used to address the functional relationship between iron and microglial activation, which demonstrated to be partially mediated by GSK-3β. The influence of iron on NF-κB-mediated

microglial activation in association with GSK-3β activity levels was also investigated. The involvement of GSK-3β in the control of LPS-activated microglial cells was examined using lihium chloride (LiCl), a potent pharmacological inhibitor of GSK-3β. The cellular activation of microglial cells induced by LPS was determined by the levels of NF-κB nuclear translocation, the expressions of pro-inflammatory cytokines, the inflammatory mediators, including IL-1β, TNF-α, NO and MMP-9 and neurotoxicity respectively.

As mentioned earlier that mutation in the HFE gene lead to increase cellular iron uptake and increased brain iron accumulation in hemocromatosis, a disorder of iron overload (Nielsen *et al.*, 1995; Berg *et al.*, 2000). The two most common mutations of HFE are the C282Y and H63D. Several epidemiological studies have found an association of H36D HFE variant with AD (Sampietro *et al.*, 2001; Pulliam *et al.*, 2003; Berlin *et al.*, 2004; Connor & Lee, 2006). However, the mechanism has not yet known. There is increasing evidence that accumulation of iron in the brain can induce oxidative stress, alter mitochondrial function and neurodegeneration. Increased cellular iron uptake in HFE mutation might increase oxidative stress and alter mitochondrial function which may be contributing to neurodegeneration. Increased cellular iron uptake in HFE mutation may also alter GSK-3β activity leading to increase neuronal Aβ production and neuronal apoptosis. All of which are AD features. Thus, the second part, neuroblastoma cell lines expressing a mutation of H63D HFE variant was used to explore the mechanisms behind the associations between cellular iron status, oxidative stress, mitochondrial function, GSK-3β activity, neuronal apoptosis, and Aβ production which hypothesized that cells with the H63D HFE variant had a phenotype that promotes oxidative stress, mitochondrial damage GSK-3β activity, Aβ production and neuronal apoptosis. Because it has been reported that Aβ peptide increased GSK-3β activity leading to increase neuronal apoptosis in AD. Therefore, the effect of Aβ peptide on neuronal GSK-3β activity and neuronal apoptosis in H63D HFE variant were also investigated.

2. Objectives of Research

2.1 To examine the effect of iron and LiCl on GSK-3β activity in cultures of activated microglia.

2.2 To examine the effect of iron and LiCl on NF-κB nuclear translocation in cultures of activated microglia.

2.3 To examine the effect of iron and LiCl on the expression of pro-inflammatory cytokines (IL-1β, TNF-α), inflammatory mediators (NO, MMP-9) and on neuronal toxicity of activated microglia.

2.5 To examine the effect of H63D HFE variant on the production of ROS, mitochondrial membrane potential and cytochrome c oxidase activity, a markers of mitochondrial function.

2.6 To examine the effect of H63D HFE variant on GSK-3β activity, Aβ production and neuronal apoptosis.

3. Hypothesis

It was hypothesized that elevated iron concentrations might be one factor that involved in the pathological processes which contributed to neurodegeneration. For example involved in neuroinflammation by enhancing GSK-3β activity, nuclear levels of NF-κB, expression of pro-inflammatory cytokines (IL-1β, TNF-α), inflammatory mediators (NO, MMP-9) and neuronal toxicity mediated by activated microglia. Moreover, elevated cellular iron concentrations in the H63D HFE variant might increase the production of ROS, mitochondrial membrane potential and cytochrome c oxidase activity, GSK-3β activity, Aβ production and neuronal apoptosis which might trigger the onset of neurodegeneration.

4. Key words

Alzheimer's disease

Apoptosis

Glycogen synthase kinase 3 beta

Iron

Microglial toxicity

Oxidative stress

5. Expected Benefits and Applications

Elevated iron concentrations in specific brain regions have been associated with many neurodegenerative disorders including AD, but its contribution to neurodegeneration has been controversial. Therefore, identification of novel molecular mechanisms or anomalies in proteins linked with iron-mediated neurodegeneration in AD could encourage the development of a drug which may help prevent or slow diseases progression.