CHAPTER I

INTRODUCTION

1. Background and Rationale

In response to various form of brain insults, "resting" microglia become activated, proliferate, and migrate to the site of injury, where they transform into brain macrophages removing tissue debris (Streit, 2000). The activation of microglia is rapid and often precedes reactions of any other cell types in the brain suggesting their apparent quiescence represent a state of vigilance to changes in their extracellular milieu (Gehrmann et al., 1993). During its activation, microglia produces several potentially cytotoxic substances such as nitric oxide (NO), reactive oxygen species (ROS), pro-inflammatory cytokines (e.g. tumour necrosis factor (TNF)-α, Interleukin (IL)-1β), excitatory amino acids, and proteases (Aloisi et al., 2000; Hesselgesser, 1999). Activated microglia also release chemokines (e.g. macrophage inflammatory protein (MIP)-1α and MIP-2) and increase cell surface expression of cytokine and chemokine receptors (Dopp et al.1997; Morris and Esiri, 1998; Simpson et al., 2000). The presence of these receptors makes them responsive to most inflammatory molecules that have been released into the damaged areas. It is currently viewed that chronic stimulation of microglia may play an important role in the progressive neuronal death that occurs in neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease (McGeer et al., 1994; McGeer and McGeer, 1995). Although, activated microglia are generally regarded as cytotoxic effectors cells, they may play a protective role by releasing trophic factors that act on neurons and other glial cells. Such growth factors include nerve growth factor (NGF), basic fibroblast growth factor (bFGF), neurotrophin (NT)-3, and transforming growth factor (TGF) -β (Mallat et al., 1989; Shimojo , 1991; Elkabes , 1996). Thus,

modification of microglial activation could form a rational basis for targeted intervention on microglial-mediated neurotoxicity.

At cellular level, it is important to note that activated microglia associated with neuropathological hallmarks in many brain diseases accumulate iron (Zecca et al., 2004). It is not known whether activated microglia function differently from iron-loaded activated cells. In case of AD, observations have suggested that the activity of amyloid plaque-associated microglia might be tightly connected to their cellular iron levels (Rogers et al., 2004; LeVine, 1997; Grundke-Iqbal et al., 1990; Kaneko et al., 1989; McGeer et al., 1987; Ohgami et al., 1991). Using an in vitro model of iron loading in activated microglia, it was shown that modulation of intracellular iron levels affected the production of NO and TNF-α from activated microglia (Cheepsunthorn et al., 2001). More recently, the preliminary results demonstrated that cellular iron loading in activated microglia enhances the secretion of MMP-9, which is a key player in the maturation of pro-IL-1β. These findings are in agreement with the reports showing an increase in plasma level of MMP-9 in patients with AD (Leake et al., 2000; Lorenzl et al., 2003) and inflammatory aspect of this disease (Yong et al., 2001; Leppert et al., 2001). Thus, iron-loaded activated microglia may contribute to the neuroinflammatory component of many brain diseases.

Several lines of studies have implicated the neuroprotective effects of estrogens in the CNS. Retrospective and prospective epidemiological studies have suggested a possible protective role for postmenopausal estrogen therapy toward neuro-degeneration in Alzheimer's disease (Henderson, 1994; Tang et al., 1996; Kawas et al., 1997; Yaffe, 1998). Estrogen has been reported to protect cultured neurons from β-amyloid-induced neuronal death. (Sortino et al., 2004; Zhang et al., 2004) as well as inducing neuronal maturation, differentiation (Murashov, et al., 2004; McEwen, et al., 1999; Singer, et al., 1996; Chang, 1997; Green, 1997; Behl, 1998), synaptic connections and neurotransmission release (Horvath, 1997; Yankova, 2001; McEwen, 2001; Saleh, 2003; Fink, 1996). Furthermore, it has been reported that

estrogen intervenes at the level of apoptotic signaling cascades to prevent onset of death in hippocampal CA1 neurons (Jover et al., 2002). However, estrogen actions on glial cells are little known. The presence of estrogen receptors (ER) -α and (ER) -β in microglia (Santagati, 1994; Mor, 1999) suggests that microglial activation could be modified by estrogen. In support to this view, exposure of microglia to17β-estradiol (E_2) diminished the production of NO induced by inflammatory stimuli (Vegeto et al., 2000). Therefore, in the present study we aim to examine the effect of estrogen on secretory products and inflammatory gene expression of iron-loaded activated microglia with expectation that estrogen could decrease inflammatory aspect of iron-loaded activated microglia.

2. Research Questions

Primary Question

Does estrogen influence secretory products of iron-loaded activated microglia?

Secondary Question

Does estrogen influence gene expression of pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS) in iron-loaded activated microglia?

3. Objectives of This Research

1. To examine the effect of estrogen on the secretion of MMP-9 from iron-loaded activated microglia using gel zymography assay.

2. To examine the effect of estrogen on the production of nitric oxide from iron-loaded activated microglia using Griess reagent assay.

3. To examine the effect of estrogen on the mRNA expression of pro-inflammatory cytokines (TNF-α and IL-1β) and iNOS in iron-loaded activated microglia by RT-PCR assay.

4. Hypothesis

Estrogen could exert its anti-inflammatory effects on iron-loaded activated microglia at the gene expression and secretory levels.

5. Keywords

17β-estradiol, microglia, NO, iNOS, TNF-α, MMP-9

6. Expected Benefits & Applications

Activated microglia appears to play a significant role in pathological and regenerative states of the brain, expressing both or either of two potentially opposing functions: cytotoxic and neurotrophic actions. The molecular mechanisms leading to neurotrophic and cytotoxic states of activated microglia are poorly understood. Thus, the results from these studies could provide an insightful look into the regulatory roles of brain endogenous ligands on the activation of microglia and could be essential for therapeutic intervention of inflammation mediated by glial cells.