CHAPTER I

INTRODUCTION

1. Background and Rationale

Alzheimer's disease (AD) is the neurodegenerative disorder characterized by progressive death of neurons in the brain areas associated with learning and memory, particularly in the hippocampus and cerebral cortex (Selkoe, 1991; Kosik, 1994; LeBlanc *et al.*, 1996). The specific mechanisms by which neurons are injured and eventually lost in AD brains have not yet been determined. At the microscopic examinations, the pathological hallmarks in the brains of patients with AD are the presence of neurofibrillary tangles and numerous senile plaques. The latter are extracellular deposits of the amyloid cores surrounded by activated glial cells (Selkoe, 1991; Itagaki *et al.*, 1989). The cores are composed mainly of insoluble fragments of β amyloid peptides (Aβ) with the predominant 1-40 and 1-42 isoforms. These Aβ fragments are derived from the proteolytic cleavages of a larger membrane spanning glycoprotein called amyloid precursor protein (APP) catalyzed by enzymes β and γ secretases (Annaert *et al.*, 1999; Wolfe *et al.*, 1999). When produced in a higher concentration, Aβ can aggregate together with other extracellular proteins including proteoglycans to form amyloid cores of the senile plaques (McGeer *et al.*, 1994). There is substantial evidence supporting the hypothesis that progressive cerebral accumulation of Aβ is an early and perhaps necessary feature of the disease (Selkoe, 1999).

One pathway of Aβ-induced brain damage may involve the activation of glial cells, particularly microglia which are considered to be key inflammatory mediators of the CNS (Selkoe, 1991; Kosik, 1994; LeBlanc *et al.*, 1996; McGeer *et al.*, 1994; Itagaki *et al.*, 1989) Quantitative histopathology has clearly demonstrated that nearly all amyloid cores are associated with clusters of activated microglia implicating

microglia as plaque-attacking scavenger cells to clear deposited Aβ (Giulian *et al.*,1995). In vitro studies have demonstrated that microglia are actually capable of uptake and degrading Aβ isolated from AD brains (Ard *et al.*, 1996; Shaffer *et al.*, 1995). But, they would not appear to do so in the brains of patients with AD. Therefore, understanding why microglia appear unable to remove senile plaques in AD brain is crucial for molecular targeting of this disease.

In deed, the activation of microglia under various pathological conditions such as AD, Parkinson's disease, AIDS-related dementia, Multiple sclerosis (MS) and Stroke is progressive and generally accompanied by intracellular accumulation of iron (Craelius *et al.*, 1982; Levine *et al.*, 1989; Kaneko *et al*; 1997). Iron-riched activated microglia are specifically associated with amyloid cores of the senile plaques. Histological evidences have implicated iron-riched activated microglia as the converter of diffuse plaques to dense core plaques (Grundke-Iqbal *et al.*, 1990; Styren *et al.*, 1990; Robinson *et al.*, 1995). Taken together, these observations have led us to hypothesize that iron loading in activated microglia could impair microglial phagocytic activity, therefore promoting extracellular deposits of cellular debris and protein fragments such as Aβ peptides and proteoglycans. The effect of iron on microglial function has just emerged. Recent study has demonstrated that at least microglial function as an inflammatory mediator can be modified by iron (Cheepsunthorn *et al.*, 2001a). More recent data has further suggested that iron could potentially affect microglial phagocytic activity by influencing the expression of matrix metalloproteinases (MMPs) (Cheepsunthorn *et al.*, 2001b), which is a large family of proteiolytic enzymes involved in the degradation of different components of ECM including serum amyloid and proteoglycans (Woessner, 1991; Stetler-Stevenson *et al.*, 1993)

Therefore, in the present study we have employed the HAPI cell (Cheepsunthorn *et al.*, 2001a) which is an established in vitro model for the study on

microglia to examine whether intracellular iron loading could modulate the expression of MMPs during the activation of microglia. Furthermore, we aim to investigate whether intracellular iron loading could also affect phagocytic activity of activated microglia.

2. Research Questions

Primary Question

Does iron influence the expression of MMP-9, MMP-1 and MMP-10 in activated microglial cells induced by lipopolysaccharide ?

Secondary Question

Does iron affect phagocytic capability of activated microglia during cellular activation induced by lipopolysaccharide ?

3. Objectives of This Research

1. To examine the effect of iron on the expression of MMP-9 in cultures of LPS- activated microglial cells using zymogram assay

2. To examine the effect of iron on the expression of MMP-1 and MMP-10 in cultures of LPS-activated microglial cells by RT-PCR and western blotting

3. To examine the effect of iron on phagocytic activity of cultured activated microglia induced by LPS using phagocytic assay.

4. Hypothesis

Intracellular iron loading could affect the expression of MMP-9, MMP-1, MMP-10 and phagocytic activity of activated microglia induced by LPS.

5. Keywords

Alzheimer's disease

Iron

Microglia

Matrix metalloproteinases

Phagocytosis

7. Expected Benefits & Applications

Activated microglia associated with senile plaques contain high levels of intracellular iron suggesting that the cellular activity of these microglial cells may be tightly connected to their iron requirements. Thus, modulation of brain iron levels may influence the involvement of microglia in the progression of AD. However, it is not known what role of iron plays in the activation of microglia, particularly in association with formation of senile plaques. The results from these studies could be essential for theraputic intervention of AD. For example, if iron loading compromises microglial phagocytic capability, lowering the level of available iron, intervention with antioxidants, or the administration of free radical scavengers could provide a therapeutic inroads in the fight against AD.