CHAPTER V

DISCUSSION AND CONCLUSIONS

The activation of microglia under various pathological conditions such as Alzheimer's disease, Parkinson's disease, AIDS-related dementia, multiple sclerosis (MS) and stroke is progressive and viewed as a double-edged sword. Consistently, it has been reported that the activation of microglia is accompanied by intracellular accumulation of iron (Craelius et al., 1982; Levine et al., 1997; Kaneko et al; 1989). Little is known about functions of iron loaded activated microglia. Previously, cellular iron status has been shown to modulate the production of NO and TNF-α and a set of functional genes identified by microarray analysis in LPS-activated microglia (Cheepsunthorn et al., 2001a, b). Recently, the effect of iron on microglial functions as brain phagocytes was further investigated using an *in vitro* model of iron loading in a rat microglial cell line, the HAPI cell (Cheepsunthorn et al., 2001a). The results demonstrated that iron loading in activated microglia significantly increases the secretion of matrix-degrading enzyme MMP-9, whereas it decreased the secretion of MMP-2. Iron loading also decreased phagocytic activity of activated microglia (Cheepsunthorn et al., in preparation). All together, these findings suggest that functions of iron-loaded activated microglia may be different from that of non-iron loaded activated microglia. Thus, understanding the role of iron in activated microglia should provide insight into mechanisms of microglia activation for targeting microglia-mediated neurodegenerative processes.

Estrogen has emerged as a potential ligand to deactivate microglia. In different experimental paradigms, 17β-estradiol (E_2) has been shown to attenuate superoxide production, phagocytosis and the expression of the iNOS RNA in LPS-activated activation (Bruce-Keller et al., 2001; Deshpande et al., 1997; Annadora et al.,

2000; Hayashi et al., 1998). Then asked in term of cell-mediated secretory products and cytokine gene expression whether iron loading could modify the responses of activated microglia to estrogen.

Therefore, the present studies were designed to further examine the effect of iron loading on gene expression of iNOS and two major pro-inflammatory cytokines, TNF-α and IL-1β in activated mciroglia.

This study found that 17β-estradiol (E_2) diminished LPS-induced increase in MMP-9 secretion from iron-loaded activated microglia. However, such amount of MMP-9 was still greater than that of E_2 treated activated microglia, suggesting that iron loading could lower the response of activated microglia to E_2. This notion was further supported by these results that the up-regulation of the IL-1β RNA in iron-loaded activated microglia was not affected by increasing doses of E_2. Elevated level of IL-1β RNA may increase its protein secretion, which has been to be secreted in form of precursor protein (Giulian and Baker, 1986). These precursors can further be processed by MMP-9 into biological active form (Schonbeck et al., 1998; Chauvet et al., 2001). Taken together, iron loading in activated microglia could potentiate IL-1β mediated microglial inflammatory reaction. However, this hypothesis need to be further examined whether iron loading in activated microglia actually increases the secretion of IL-1β protein.

In this results, MMP-9 activities in iron loaded activated microglia were diminished by estrogen in dose dependent manner. However, such amount of MMP-9 is still greater than that of E_2 treated activated microglia. Several mechanisms have been proposed to explain how E_2 may act on MMP-9 expression. These proposals include a genomic estrogen receptor-mediated mechanism and non genomic mechanism involving MAPK and/or PI3K signaling (Dhandapani and Brann, 2002). Previous study suggested effect of E_2 could be ascribed to the inhibitory transcriptional activity of ER-α. However, no sequence reminiscent of an ERE can be found in MMP-9 promoter (Vegeto et al., 2001). Since MMP-9 promoter contains an essential

proximal AP-1 element and an upstream NF-κB site (Bond et al., 1998).It has been proposed that inhibitory effect of E_2 on MMP-9 expression could involve the interaction between ER-E_2 ligand complex and these transcription factor. ER-mediated transcriptional activation or repression model is dependent on the ligand and the nature of the response element in the target gene (Jakacka et al., 2001). Moreover, the antiinflammatory activity of estrogen has been attributed to interference of NF-κB activity occur through multiple mechanisms, including direct protein-protein interactions (Stein and Yang, 1995; Ray et al., 1994), inhibition of NF-κB DNA binding (Ray et al., 1997, Deshpande et al., 1997), induction of IκB expression (Sun et al., 1998), or by means of coactivator sharing (Harnish et al, .2000, Speir et al., 2000). Furthermore, research in our laboratory suggested that iron could be synergist the secretion of MMP-9 in LPS-activated microglia. Some studies demonstrated that iron acts as a dependent proinflammatory molecule via induction of the intracellular signaling for NF-κB activation. The results indicated that LPS induced NF-κB activation was a blocked by iron chelator in hepatic macrophage (Xiong et al., 2004). Thus, the effect of iron that synergist the secretion of MMP-9 in LPS-activated microglia might via NF-κB activation. However, these results suggesting that iron loading could lower the response of activated microglia to E_2.

Nitric oxide has pleiotropic effects in the CNS (Verity, 1994), excessive NO production in the CNS can be toxic to astrocytes and neurons (Hewett et al., 1994). Since NO released by activated microglia are considered as markers of active inflammatory responses (Fiebich et al., 2002). This study shown that estrogen decreased LPS-induced production of NO. These findings are in agreement with previous reports by other laboratories (Vetgeto et al., 2001).

In the present study shown that treatment of iron-load activated microglial cells with estrogen markedly reduced NO pattern of production in dose dependent manner, which is consistant with the effect of iron on iNOS expression. In case of E_2, the exact cellular mechanisms by which estrogen down-regulates NO

production in microglia have not yet been identified, but it is though to involve NF-κB. In addition, iNOS transcription levels were also decreased by treatment with estrogen in dose dependent manner. However, the levels of iNOS mRNA in microglia were not diminished by treatment with iron like seen in NO production. This data is not in agreement with previous report showing that iron decrese iNOS mRNA expression (Cheepsunthorn et al., 2001a). The result discrepancy could due to the differences in time course study and concentration of iron used, or cell type examined (murine macrophage vs microglia) (Weiss et al., 1994). The present studies demonstrated that estrogen significantly inhibited the expression of iNOS and NO production in the presence of iron in activated microglia, suggesting that estrogen exerts its activities might be through the estrogen receptors to affect the cellular responses and through a post-transcriptional mechanism.

It is reported that MAPKs regulate the production of cytokines IL-1β, TNF-α and reactive oxygen species (ROS) in LPS -activated microglia (Koistinaho and Koistinaho et al., 2002; Combs et al,.2001; Bhat et al,.1998; Pyo et al,.1998). p38 MAPK has been implicated in the signal transduction pathways responsible for increased iNOS and TNF-α gene expression in glial cells (Won et al, .2001; Xu and Malave, 2000). Since the above results have shown the beneficial effects of estrogen on protecting microglial cells from activation by LPS, the capacity of estrogen maybe interfere with MAPKs phosphorylation. In the other hand disrupt the signal transduction pathways elicited by LPS in microglial cells, and subsequently preventing the NO and TNF-α in microglia and production of neurotoxic mediators (Feinstein et al., 1994). However, this result shown that estrogen inhibited levels of TNF-α expression in iron-loaded activated microglia.

In conclusion, these results suggest that an intracellular iron loading activated microglia seem to increase resistance of activated microglia to E_2 treatment. These findings indicate that effector functions of activated microglia are different from that of iron-loaded activated microglia, suggesting the activation of microglia is linked

to cellular iron metabolism. Intracellular iron loading modifies the responses of activated microglia to E_2, suggesting iron could interfere with E_2 actions. Therefore, these findings raise the precaution whether estrogen therapy would be an effective strategy for reversing the pathology of neurological diseases (e.g. AD) that involved brain iron accumulation and microglial activation,praticulary through IL-1β dependent microglial mediated neuroinflammation.