CHAPTER II

Review Literatures

Alzheimer's disease

AD is the most common cause of dementia among people age 65 (Katzman, 1986), and risk goes up with age. During the course of AD, nerve cells die in particular regions of the brain. The brain shrinks as gaps develop in the temporal lobe and hippocampus, which are reponsible for storing and retrieving new information. This in turn affects people is ability to remember, speak, think and make decisions. It has not yet known what specifically causes nerve cells to die leaving certain characteristic appearances of the brain after death. In particular, neurofibrillary tangle (and plaques made from protein fragments are observed under the microscope in damaged areas of the brain confirming the diagnosis of AD (Selkoe *et al.*, 1991; LeBlanc *et al.*, 1996; Kosik *et al.*, 1994).

In AD, the affected nerve cell cytoskeleton is often altered and presented as neurofibrillary tangles (Selkoe *et al.*, 1991; LeBlanc *et al.*, 1996; Kosik *et al.*, 1994). NFTs are found in the cell bodies, dendrites, and axons. They are made up of poorly soluble hyperphosphorylated isoforms of tau, a microtubule-binding protein that normally is soluble (Figure 1). NFTs often first become evident in neurons of the entorhinal cortex, which receives inputs from isocortex and projects to the hippocampus. Later the neurofibrillary pathology extends to the neocortex. As these neurons die, the synaptic inputs in regions of the brain critical for normal cognitive and memory function are lost. Fundamentally, the cytoskeleton is essential for maintaining cell structure and intracellular trafficking of proteins and organelles, including transport along axons. Thus, disturbances of the cytoskeleton are likely to impair axonal transport and thereby compromise the functions of synaptic inputs and, eventually, the viability of neurons. The affected nerve cells eventually die and the

extracellular neurofibrillary tangles are left behind as tombstones of the cells destroyed by this disease.

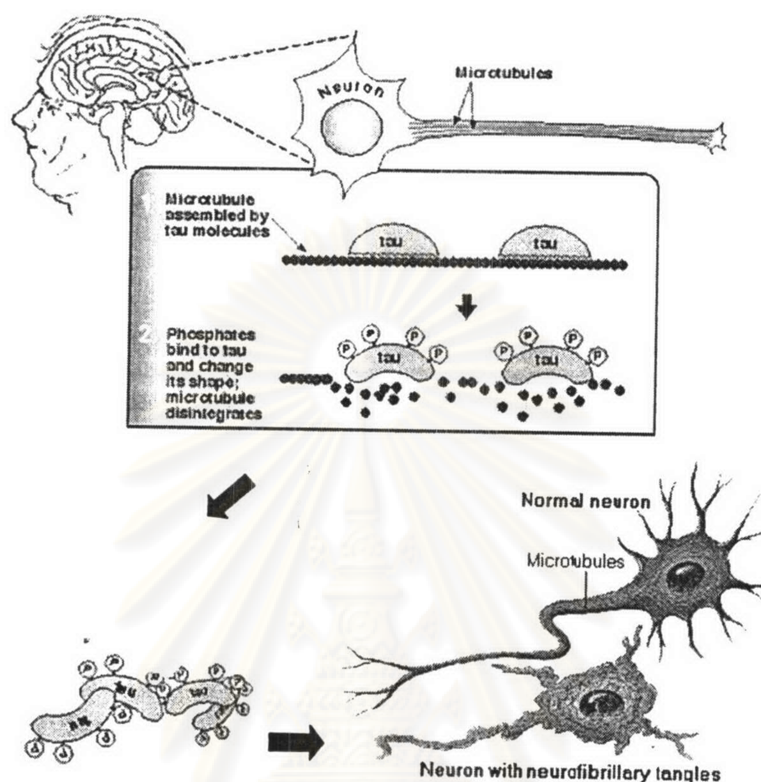

Figure 1 Neurofibrillary tangles. Biochemical analysis has shown that neurofibrillary tangles are composed mainly of abnormally-phosphorylated tau protein (a neuron-specific phosphoprotein that is the major constituent of neuronal microtubules)

The brain regions affected by AD also contain "senile plaques" in which extracellular deposits of beta amyloid (Aβ) (Selkoe *et al.*, 1991; LeBlanc *et al.*, 1996; Kosik *et al.*, 1994). Senile plaques are classified into two major types : the classical (neuritic) and the diffuse (preamyloid) plaques. The classical plaque is a complex lesion of the cortical neuropil containing several abnormal elements with a central deposit "the core" composed of extracellular amyloid fibrils or Aβ (Masters *et al.*, 1985; Glenner *et al.*, 1984) surrounded by dystrophic neurites (both dendrites and

axonal terminals), activated microglia, and reactive astrocytes (Terry *et al.*, 1994; Selkoe,1991). The diffuse plaques contain nonfibrillar (amorphous) Aβ and are devoid of amyloid core with very few surrounding dystrophic neurites or activated glia. The principal constituent of amyloid is a 4 kDa peptide called Aβ amyloid.

Aβ is cleaved from a larger precursor protein, amyloid precursor protein (APP). APP is present in the dendrites, cell bodies, and axons of neurons. Neuronal APP is likely the source of most of the Aβ deposited in the CNS in patients with AD. However, the functions of neuronal APP are not yet identified. APP is synthesized in the endoplasmic recticulum, glycosylated in the golgi apparatus, and delivered to the cell surface as an integral membrane protein. Some of these molecules are cleaved within the Aβ sequence, thus preventing the formation of the Aβ peptide. A fraction of the APP within the plasmalemma is internalized into the cell to generate various forms of amyloid Aβ (Aβ1-40, Aβ1-42, and Aβ1-43) as well as truncated forms of the Aβ peptide (β17-40), all of which are normally present in cerebrospinal fluid. According to one model, a β-secretase cleaves APP at the N terminus of the Aβ peptide sequence in endosomal compartments and a γ-secretase enzyme cleaves at the C terminus of the Aβ peptide at or near the cell surface (Annaert *et al.*, 1999; Wolfe *et al.*, 1999) (Figure 2). The Aβ peptide is predominantly 40 amino acids in length, that is, Aβ1-40. However, Aβ1-42 and Aβ1-43 nucleate more rapidly into amyloid fibrils than Aβ1-40 does. In the cerebral cortices of individuals with AD or Down syndrome, Aβ amyloid deposition begins with Aβ1-42 and Aβ1-43, but not with Aβ1-40. The Aβ1-42 peptide appears to be neurotoxic by mechanisms not yet fully understood.

Despite the widespread presence of neurofibrillary tangle and senile plaques in the brain of AD patients, their specific and individual roles in the pathogenesis of this disease are not clearly defined and the mechanisms by which neurons are injured and eventually lost in AD brains remain poorly understood. Many studies have supported the hypothesis that Aβ deposition may play an early and, in some cases, causative role

in the disease. One pathway of Aβ-induced neuron damage involve cellular activation of inflammatory cells such as reactive microglia/brain macrophages

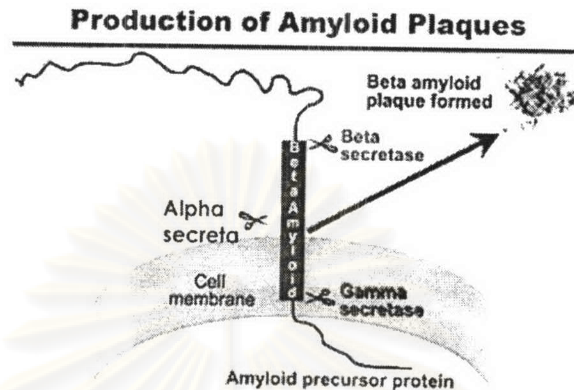

Figure 2 The detail of the peptide shows the sites of α-, β-, and γ-secretase cleavages. The endopeptidase α-secretase cleaves within the Aβ region, resulting in the secretion of the extracellular domain of APP; hence, the cleavage does not produce the Aβ peptide. In contrast, the β-secretase and γ-secretase cleavages do result in production of the peptide.

Microglia

The microglial cells are commonly thought to represent the CNS's macrophage precursors, functioning as part of the reticuloendothelial system like Kupfer's cells in the liver and Langerhans cells in the skin. Generally, they are said to have a mesodermal origin and to invade the developing CNS at the time when the vascular supply of the brain is being elaborated. The major sources of these cells are usually considered to be the pia mater, the walls of the vascular elements, and the tela choroidea. They are found in all regions of the brain, and there are more in gray than in white matter. The phylogenetically newer regions of the CNS (cerebral cortex, hippocampus) have more microglia than do older regions (brainstem, cerebellum).

Mature brain, microglia constitute from 5-20% of the neuroglial population (Lawson *et al.*, 1990). They seem to be inactive (resting, ramified) and adapt the morphology of their cell bodies and processes, and the expression of cell surface markers, to their microenvironment, as do the resident monocyte-derived macrophages of other tissues (Lawson *et al.*, 1990). Resting microglia in adult animals may become reactive and convert into macrophages after brain injury or degenerative process in the CNS (Streit *et al.*, 1988). Under such conditions the microglial cells undergo rapid proliferation, as may histiocytes derived from the meninges the vessel walls, and the blood. Activated microglial cells retract their processes and migrate toward the site of the injury, where they turn into macrophages and remove the debris (Figure 3). At this time, the cell may assume either a rod like form or rounded and globular shape as they become laden with lipids and cell remnants. They transport the debris to the vicinity of blood vessels, but whether they discharge their contents into the blood vessels, or themselves pass through is uncertain.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 3 In the normal brain, microglia constitue approximately 20% of all glial cells (brain cells that are not nerve cells). (A) In the healthy brain, microglia are in a resting state. With their large cell surface pleated into humerous delicate cell branches, they survey the surrounding tissue. (B) Activated microglia, they change in cell shape

(they start retracting many of the dilicate cell branches.) At this stage microglia begin to produce numerous important molecules, e.g. for cell-cell signalling.

In the brains of patients with AD, activated microglia are associated with virtually every amyloid deposit and are concentrated in regions of compact amyloid deposits (Dikson *et al.*, 1993) where they surround and infiltrate into the β-amyloid plaques (Streit *et al.*, 1988). Quantitative histopathology has determined that more than 80% of core plaques are associated with clusters of reactive microglia, whereas less than 2% of diffuse Aβ deposits show such an association (Lawson *et al.*, 1992). Reactive microglia are also found associated with plaques that develop in a trangenic expressing mutant APP (Gelman, 1993). Microglia are highly reactive to environmental changes and respond to several types of CNS injury (Barron, 1995). They sense threats to the integrity of the CNS and may respond by releasing cytotoxic and inflammatory modulators (Giulian, 1987; Dickson *et al.*, 1993), phagocytosis and cell killing (Banati *et al.*, 1993). Microglial cells are interesting for understanding AD because they are highly reactive to environmental changes. They can generate many molecules associated with inflammatory and immune functions such as complement proteins and their regulators, inflammatory cytokines, acute phase reactants and many proteases and protease inhibitors. Thus activated microglia seem to facilitate and amplify immune mechanisms locally within the CNS. Many of those proteins appear in association to neurodegenerative disease lesions. A strong inflammatory response may be autotoxic to neurons, exacerbating the fundamental failure underlying the neurological disorder. Reactive oxygen intermediates produced by microglia are particularly relevant. Production of oxygen free radicals can be initiated by several stimuli, including Aβ. Activated microglia can generate enormous quantities of superoxide anions. While oxygen radicals are very effective against abnormal cells, normal host cells are unable to avoid being damaged. Because of that, microglia acting

through these mechanisms can contribute substantially to tissue damage as a pathogenic factor of Alzheimer's disease.

Very little is known, however, about the function of microglia surrounding the plaques. Because microglia are phagocytic cells, it has been suggested that microglia may function as plaque-attacking scavenger cells. In vitro studies have clearly demonstrated that microglia can actively degrade Aβ isolated from AD brain (Shaffer *et al* 1995; Ard *et al.*, 1996) Therefore, understanding why microglia appear unable to remove plaque deposition in AD brain. In fact that microglia activation is progressive and generally accompanied by intracellular accumulation of iron as seen in AD (Grundke-Iqbal *et al.*, 1990; Connor *et al.*, 1992a; Connor *et al.*, 1992b) Furthermore iron enriched microglia are commonly observed in Parkinson's disease, AIDS-related dementia, Multiple sclerosis and Stroke (Craelius *et al.*, 1982; Levine, 1997; Kaneko *et al.*, 1989).

Brain iron

Iron is essential for normal CNS growth and development because it forms an important component of hemoproteins and enzymes involved in neuronal oxidative metabolism, neurotransmitter synthesis, and myelin synthesis (Dallman, 1986). Although iron is importance to normal neurological activity is clear, too much iron homeostasis may have even more devastating effects. The ability of iron to catalyze the generation of free radicals in biological systems is well known (Yagi *et al.*, 1992). In general, iron is tightly complexed with proteins, but can also be present in a soluble "pool" of low molecular weight complexes, such as ferric citrate and Fe (III) ATP (Weaver *et al.*, 1989). This latter soluble pool of iron reacts with hydrogen peroxide and superoxide, both normally produced in biological systems, to form hydroxyl radicals (Floyd, 1990). Hydroxyl radicals are extremely reactive causing lipid peroxidation, DNA strand breaks and degradation of biomolecules (Halliwell *et al.*,

1984) and are suspected to play a major factor in AD (Sayre *et al.*, 1997; Smith *et al.*, 1997; Smit *et al.*, 1995) and other CNS disorders (Floyd *et al.*, 1990; Hall, 1993; Halliwell *et al.*, 1992; Sayre, 1999). In AD iron riched microglia are associated with amyloid cores of the senile plaques and have been proposed to contribute to conversion of diffuse plaques to dense core plaques. These data lead us to hypothesize that iron loading in activated microglia could impair microglial phagocytic activity, therefore promoting extracellular deposits of tissue debris and protein fragment. However, it is not known what role of iron played in activated microglia on pathogenesis of AD, particularly in the formation of the senile plaques. Previous studies indicated that accumulation of iron in microglia could effect the production of nitric oxide (NO), tumor necrosis factor (TNF) as well as the expression of a family of zinc-dependent endopeptidase known as matrix metalloproteinases (Cheepsunthorn *et al.*, 2001b).

Matrix metalloproteinases (MMPs)

The matrix metalloproteinases (MMPs) are a family of highly conserved zinc dependent endopeptidases, which, collectively, are capable of degradation of most, if not all, components of the basement membrane and extracellular matrix. In particular, the MMPs include the only enzyme known to be capable of degrading fibrillar collagen. They are important roles in many biological processes such as morphogenesis, ovulation, embryo implantation, cell migration, tissue involution, angiogenesis, and wound healing. (Woessner, 1991; Kleiner *et al.*, 1993; Birkedal-Hansen *et al.*, 1991; Oh *et al.*, 2001). In excess, they participate in the destruction of the tissue associated with many connective tissue diseases such as arthritis, (Goldbach-Mansky *et al.*, 2000) nephritis (Urushihara *et al.*, 2002) cardiovascular disease (Spinale, 2002) and tumor cell invasion and metastasis (Curran, 1999; Forget *et al.*, 1999; Sternlicht *et al.*, 2000). However, very little is known about MMPs in neurodegeneration, particularly in AD

Structure, nomenclature and substrates of MMPs

Analysis of the MMPs reveals several distinct structural domains (Figure 4). There are an N-terminal propeptide domain, a catalytic domain, and a c-terminal haemopexin-like domain. The membrane type MMPs (MT-MMPs) are distinguished from the soluble secreted MMPs by the presence of hydrophobic transmembrane domain at the c-terminus. The MMPs are synthesized in a latent form, which requires extracellular activation; this is accompanied by cleavage of N-terminal propeptide, which includes a conserved sequence of amino acids (PRCGVPDV). The unpaired cysteine residue in this sequence is believed to operate a cysteine switch mechanism, forming a co-ordinate bond with the zinc ion in the active site and thereby rendering the enzyme inactive.

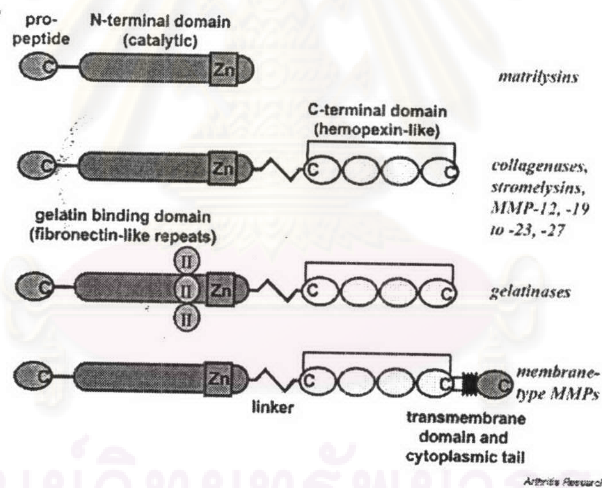

Figure 4 Modular domain structures of the matrix metalloproteinase (MMPs). The N–terminus propeptide region has about 80 amino acids and is common to all MMPs. An invariant cysteine residue in the propeptide domain ligates to the zinc ion (Zn) of the catalytic domain and blocks its activity. The C-terminus domain, present in all MMPs except matrilysin, has a high level of homology with members of the hemopexin family. C- and N-terminus domains are connected by a linker region or hinge that is short in collagenases and long in other MMPs (Murphy *et al.*, 1997).

At present, there are at least 19 know human MMPs, wich can be divided into at least four groups according to their in vitro substrate specificity: the collagenases, the stromelysins, the gelatinases, and the membrane-type MMPs (MT-MMPs). Four members of latter group have been identified to date : MT1-MMP to MT4-MMP. It is now reccognized that MMPs usually degrade multiple substrates, with considerable substrate overlap between individual MMPs. For example, interstitial collagenase (MMP-1) is capable of degrading casein, gelatin, pro-TNF and IL-1β, pro MMP-2, and -9. Gelatinase A (MMP-2) can degrade fibrillar collagen, elastin, and can activate MMP-1, -9 and -13. MMP-12 is highly active against type IV collagen, gelatin, fibronectin vitronectin and plasminogen, but it is not very effective at degrading elastin. The substrate and nomenclature of the MMPs is complex and is summarized in table 1.

Table 1 Nomenclature, chromosomal location, and substrate specificity of individual MMPs

MMP group	MMP	Alternative name	Substrates
Collagenases	1	Collagenase-1/ Interstitial Collagenase	Fibrillary collagens I, II, III, VI, IX
	8	Collagenase-2	Collagens I, II, III
	13	Collagenase-3	Collagens I, II, III
Gelatinases	2	Gelatinase A/72 kDa Gelatinase	Gelatin, Collagen type IV, V
	9	Gelatinase B/92 kDa Gelatinase	Gelatin , Collagen type IV, V
Stromelysin	3	Stromelysin-1	Fibronectin, lainin, proteoglycans, non-fibrilla collagen, casein
	10	Stromelysin-2	Fibronectin, lainin, proteoglycans, non-fibrilla collagen, casein
	11	Stromelysin-3	Laminin, fibronectin
Membrane-type MMPs	14	MT1-MMP	Pro-MMP-2, collagens, gelatin
	15	MT2-MMP	Pro-MMP-2, collagens, gelatin
	16	MT3-MMP	Pro-MMP-2, collagens, gelatin
	17	MT4-MMP	Pro-MMP-2, collagens, gelatin
Other	12	Metallelastase	Elastin
	19	Not koown	Aggrecan

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Regulation of MMP activity

Because MMPs can catalyze the degradation of all the protein constituents of the ECM, it is important that their activities are kept under tight control to prevent tissue destruction. The activity of MMPs is regulated in at least three ways : gene transcription, proenzyme activation and by the action of tissue inhibitores of metalloproteinases (TIMPs). Most MMPs are express at low levels or not at all in resting-state adult tissues. However, gene transcription can be induced by stimuli including phorbol esters, growth factors, inflammatory cytokines, oncogene products or cell-cell interactions. The MMPs are initially expressed as inactive pro MMP zymogens where a zinc atom present in the catalytic domain is bound to a cysteine residue in the pro-peptide region. Activating factors disrupt the cysteine-zinc interaction (cysteine switch) and thus expose the catalytic site; the result is a partially active intermediate form of the enzyme that can cleave the propeptide region by autocatalysis and render the enzyme fully active (Van-Wart *et al.*, 1990). An important physiological activator of pro-MMPs is plasmin, a serine proteinase that is generated from plasminogen by the action of tissue or urokinase-plasminogen activator (uPA). Activation of the MT-MMPs also requires removal of a propeptide, but this is catalyzed by a serine proteinase, furin. Activation pathways of MT-MMPs can co-operate, leading to the activation of additional downstream MMPs such as MMP-9, as shown in figure 5. The activation apparatus is localized on the surface of the cell, and an important consequence of this is that proteolysis is greatest in the immediate pericellular environment, where it can influence cell-cell and cell-ECM interactions.

Following activation, MMPs can be regulated by the formation of tight, 1:1 non-covalent complexes with TIMPs (Yong *et al.*, 1998). The four known TIMPs share many properties but also have distinct activities (Table 2), suggesting that they might have specific physiological roles. The functional relationship and interaction between TIMPs and MMPs is complicated. For examples TIMPs form complexes with

the inactive pro-enzyme of gelatinases via interactions that are different from those between TIMP and the active form of these enzymes. These complexes, such as the pro-MMP-9/TIMP-1, pro-MMP-2/TIMP-2 and pro-MMP-2/TIMP-3 complexes, control the rate at which physiological factors activate MMPs. For example, TIMP-2, when complexed with pro MMP-2, has been shown to be an adaptor molecule that allows pro-MMP-2 to become associated with MT-MMPs. Thus, an important aspect of TIMP-2 (and TIMP-3) function is that cell behavior critically depends on the stoichiometry between TIPM-2, MT-MMP and MMP-2. Low concentrations of TIMP-2, favor pro-MMP-2 activation, and high concentrations block activation by neutralization of all available MT-MMP binding sites.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 5 Cascade of matrix metalloproteinase (MMP) activation at the cell surface (Murphy *et al.*, 1997). The co-ordinate activation of several MMPs is initiated by the formation of plasmin. Plasmin is produced from plasminogen by the action of urokinase plasminogen activator (uPA) that is anchored by its receptor, uPA. Plasmin

can activate MMP-9 and stromelysin-1, and the later can in turn activate other MMPs, including MMP-9 and collagenase-1, thus amplifying and broadening the activation cascade. MMP-2 is activated by membrane-type MMPs (MT-MMPs) that are activated by furin proteinases.

Table 2 Properties of tissue inhibitors of metalloproteinases (TIMPs) (Murphy *et al.*, 1997; Edwards *et al.*, 1996)

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Chromosome gene location (human)	Xp11.23-11.4	17q2.3-2.5	22q12.1-13.2	unknown
Protein (kDa)	28	21	24	22
Predominant form of expressed molecule	Secreted	Secreted	ECM-associated	Secreted
Pro-MMP complex	MMP-9	MMP-2	MMP-2	unknown
Inhibition of MT-MMP	No	Yes	Yes	unknown
Inhibition of gelatinases	Yes	Yes	Yes	Yes

Biological role of MMPs

Understanding of the biological role played by the MMPs outside the brain may greatly aid in the elucidation of their function in this organ as well. Hence, it is noteworthy that the role of MMPs has been very well documented in embryonic development and tissue morphogenesis (Vu and Werb, 2000; Sternlicht and Werb, 2001). The evidence for developmental significance of the MMPs and TIMPs comes from studies on their expression patterns as well as from functional experiments with inhibitors and null mutations. Paramount evidence for MMP role in physiology has been collected in the case of implantation, mammary and bone development as well as wound healing. Vu and Werb (2000) proposed that MMP activity during development might be required for : degradation of the ECM in order to allow cell migration,

alteration of the ECM microenvironment to modify cell behavior; and, modulation of the activity of biologically important molecules by direct cleavage, release from bound stores or modifying their inhibitors. In addition, MMPs are upregulated following other types of insult to the nervous system, such as penetrating injury or peripheral nerve transections (La Fleur *et al.*, 1996). The up-regulation of MMPs following almost all CNS injury raises the possibility that some MMPs could function to enhance the recovery of the CNS. What might some of these functions be? It is likely that following an injury that leads to cell death, some degradation of the ECM must occur in order for the environment to be remodeled, and MMPs could fulfil this role. Although the adult CNS does not contain a well-defined parenchymal ECM, an ECM barrier does exist in the basement membrane that surrounds cerebral blood vessels, and a diffuse and amorphous mixture of ECM molecules can be found throughout the CNS (Bertolotto *et al.*, 1990). New blood vessels are likely to be required to facilitate recovery from various CNS insults, and MMPs might play a role in angiogenesis. When mononuclear phagocytes are recruited to the lesion, these might utilize MMPs to facilitate their migration or to engulf debris, or both. With respect to cell migration, neural-cell precursors might require MMPs in order to migrate to the lesioned area and replenish lost cells; a precursor cell for oligodendrocytes has been demonstrated to require MMPs for motility (Amberger *et al.*, 1997). New axonal growth and synaptic reconnections need to be established and their extension through the brain matrix might require MMPs; indeed, stromelysin-1 has been found to mediate the motility of growth cones through basal laminae (Nordstrom *et al.*, 1995). It has been found that oligodendrocytes utilize MMP-9 to extend their processes, which is a prerequisite for developmental myelin formation and remyelination. The upregulation of MMPs in the MS brain or in other CNS pathologies, might not always be harmful, and thus it is important to be able to discriminate between the beneficial and deleterious effects of MMPs. Lastly, the remodeling of the ECM might release several neurotrophic factors, such as basic fibroblast growth factor, that are anchored on the ECM. Thus, there are

several mechanisms by which the production of MMPs, following insults to the nervous system, might be beneficial.

Pathological role of MMPs

Under physiological conditions, MMP activity is precisely controlled; However, when too many MMPs are produced or are present at the wrong time, they can break down the material, known as extracellular matrix, that holds cells together inside tissues. This activity occurs in diseases in which healthy tissue is broken down or unhealthy tissue grows, as in cancer, rheumatoid arthritis, nephritis (Curran *et al.*, 1999; Forget *et al.*, 1999; Goldbach-Mansky *et al.*, 2000).

MMPs in cancer and rheumatoid arthritis

Cancer tumors grow and spread, interfering with the functions of healthy tissue. Some MMPs (MMP-2 and MMP-9) (Iijima *et al.*, 2004) have been shown to play a significant role in tumor development by contributing to three processes that lead to the progression of cancer: invasion, metastasis, and angiogenesis. In patients with osteoarthritis and rheumatoid arthritis, elevated levels of MMP-1, MMP-3 and MMP-13 are observed in the synovium and cartilage (Cawston, 1998; Andreakos *et al.*, 2003), where they are thought to play a pivotal role in the degradative changes in joint tissues associated with long term patient disability.

MMPs in diseases of the nervous system

A great deal of data has been collected regarding the MMP/TIMP system in a variety of brain pathologies. In particular, the MMPs have been implicated in gliomas (tumors of glial origin), viral infections, inflammation, MS, AD, brain trauma and ischemia (Rosenberg, 1995; Rooprai *et al.*, 1997; Yong *et al.*, 1998; Yong *et al.*, 2001;

Lukeset *et al.*, 1999; Leppert *et al.*, 2001). In the context of MS MMP-1, -2, -3 and -9 were immunolocalized to brain macrophages/microglia and astrocytes (Yong *et al.*, 1998; Yong *et al.*, 2001; Leppert *et al.*, 2001). The levels of those enzymes could be increased by various inflammatory cytokines in vitro as well as in animals with experimental allergic encephalomyelitis (EAE) serving as a model for MS. Furthermore, chemical inhibitors of MMP activity were shown to ameliorate the severity of the EAE (Yong *et al.*, 2001). In case of brain ischemia in rats and mice, expression and activation of the MMP-2 was observed in neurons, glia and the endothelium, whereas MMP-9 was found elevated in neurons and glia as well as myelinated fiber tracts, and MMP-3 was found in neurons (Rosenberg, 1996; Clark *et al.*, 1997; Mun-Bryce *et al.*, 1998; Gasche *et al.*, 1999; Heo *et al.*, 1999; Rivera *et al.*, 2002). Inhibition of MMPs with substances displaying especially high activity against gelatinases (MMP-2 and -9), as well as injection of a neutralizing antibody against MMP-9 and a targeted disruption of the MMP-9 gene, all diminished the severity of the damaging consequences of ischemia to the brain (Romanic *et al.*, 1998; Asahi *et al.*, 2000; Asahi *et al.*, 2001; Jiang *et al.*, 2001). In conclusion, the disease studies underscore the importance of the MMPs in brain dysfunctions, as well as clearly showing how important it is to investigate the cellular origins of the MMP activities within this organ, where non-neuronal cells, including the infiltrating inflammatory ones, provide the major component of pathology-related MMP overexpression. As Yong *et al.* (2001) have already pointed out, an intriguing question remains: to what extent the activation of MMPs in the response to brain-damaging treatments is detrimental to the tissue or, in contrast, represents a repair reaction.

Table 3 Potential consequences of matrix metalloproteinase (MMP) expression in the mature nervous system

Undesirable effects	Beneficial action
- Breakdown of blood-brain barrier	-Clearance of debris following injury
-Demyelination	-Remodeling of ECM for cell migration and axonal elongation
-Cytokine production and propagation of an inflammatory response	-Release of growth factors anchored on the ECM
-Deposition of amyloid proteins	-Breakdown of amyloid proteins
-Tumor invasion, metastasis and angiogenesis	-Angiogenesis
-Inappropriate degradation of extracellular matrix leading to alteration of structural integrity	-Process formation by oligodendrocytes

MMPs and Alzheimer's disease

One of the most outstanding histopathological features of AD is the accumulation of Aβ peptide in the gray matter of patients affected with this dementia (Selkoe, 1994). The Aβ is associated with extensive dystrophic neuritic pathology and glia cells, astrocyte and microglia. Histological evidence has implicated microglia in AD brain as plaque-attacking scavenger cells, as sources of cytokines (Griffin *et al.*, 1989; Giulian *et al.*, 1996), as producers of Aβ (Frackowiak *et al.*, 1992), and as secretors of complement proteins (Rogers *et al.*, 1992; Meda *et al.*, 1995). Activated microglia proliferate, exhibit phagocytic activity removing infectious agents or remnants of dying brain tissue, and produce a number of secreted factors such as secrete a set of extracellular matrix (ECM) degrading enzymes that include the MMPs. This enzyme is involved in the degradation of ECM proteins like collagen, fibronectin, laminin and elastin (Woessner, 1991). It has been reports that increase plasma level of MMP-9 in patients with AD as well as MMP-1 (Leake *et al.*, 2000; Lorenzl *et al.*, 2003). Although no protease for the cleavage of Aβ has identified in vivo, it has been recently reported that MMP-2 has the ability of degrading Aβ of AD in vitro (Roher *et*

al., 1994). Furthermore, it has been reported by Miyazaki *et al* (1993) that MMP-2 cleave Aβ at the α-secretase site. If this hydrolysis also occurs in the brain' extracellular matrix, the enzymatic action of MMP-2 could prevent the generation of Aβ. A previous study had reported that amyloid cores isolated from the brains of AD patients are ingested by cultured microglia (Ard *et al*., 1996; Shaffer *et al*., 1995). Therefore it is likely that the microglia surrounding senile plaques in AD brains are the main scavengers and remove of Aβ peptides from the brain (Giulian *et al*., 1995). But they would not appear to do so in the AD brain. Therefore, understanding how Aβ and other plaque components affect microglial phagocytosis would contribute greatly to understanding why microglia appear unable to remove plaque deposition in AD brain. In fact, the activation of microglia in AD brain is progressive and generally accompanied by intracellular accumulation of iron. More recent data has further suggested that accumulation of iron in microglia affect the expression of these MMP-1 and MMP-10 (Cheepsunthorn *et al*., 2001b).

Taken together, these data lead to the hypothesis that iron loading in activated microglia could impair microglial phagocytic capability, therefore promoting extracellular deposits of tissue debris and other protein fragments. In the present study we investigate whether iron loading in microglia could affect the expression of MMP-1, MMP-9, and MMP-10 in activated microglia. Furthermore, we also study whether an increase in intracellular iron could influence of phagocytosis of activated microglia.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย