CHAPTER II

LITERATURE REVIEW

Dementia, Degenerative Disease, and Alzheimer's disease

Dementia is the product of a progressive failure of cognition, language, memory, and other intellectual functions. The most common type of dementing disease is attributed to cerebral atrophy (2009b) and Alzheimer's disease is

the most common and important degenerative disease of the brain (2009a), having an immense societal impact especially at the present when the world population has longevity than the past. The incidence rate of clinically diagnosed Alzheimer's disease is similar throughout the world, and it increases comparably with age, approximately 3 new cases yearly per 100,000 persons younger than age 60 years and staggering 125 new cases per 100,000 of those older than 60 years. The prevalence of the disease per 100,000 population is nearly 300 in the group aged 60 to 69 years; it is 3,200 in the 70- to 79-year-old group and 10,800 in those older than age 80 (2009a). In the year 2008, it is estimated to be more than 2 million persons with Alzheimer's disease in the United States (2009a).

Table 1 Relative frequency of dementing disease

Dementing Disease	Relative Frequency %
Cerebral atrophy, mainly Alzheimer but including Lewy-body, Parkinson, frontotemporal, and Pick disease	50
Multiinfarct dementia	10
Alcoholic dementia	7
Intracranial tumors	5
Normal-pressure hydrocephalus	5
Huntington chorea	2
Chronic drug intoxications	3
Miscellaneous diseases (hepatic failure; perni cious anemia; hypo- or hyperthyroidism; dementias with amyotrophic lateral sclerosis, cerebellar atrophy; neurosyphilis; Cushing syndrome, Creutzfeldt-Jakob disease; multi ple sclerosis; epilepsy)	6
Cerebellar trauma	2
AIDS dementia	2
Pseudodementias (depression, hypomania, schizophrenia, hysteria, undiagnosed)	8

Source: Adapted from Allan H. Ropper and Martin A Samuels, 2009

In Thailand, aging persons are also at higher risk to be dementia. Among the affected persons about 50% are in the eighties or more. There were 229,000 dementia persons in the year 2005 and estimated to be 450,000 and more than million persons in the next 20 and 50 years.

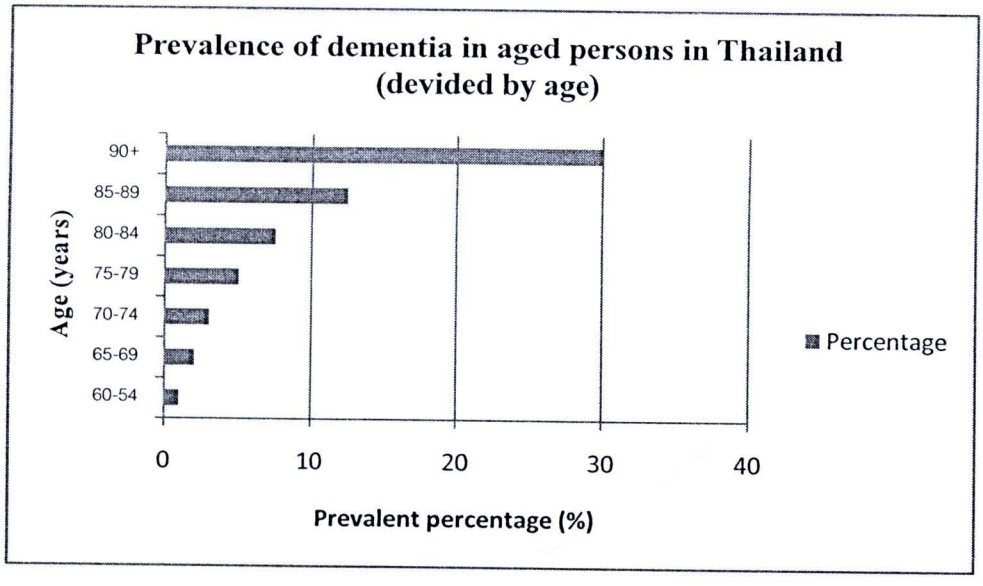

Figure 1 Prevalence of dementia divided by age in Thailand

Source: Adapted from Ageing and Long-term Care: National Policies in the Asia-Pacific, 2002

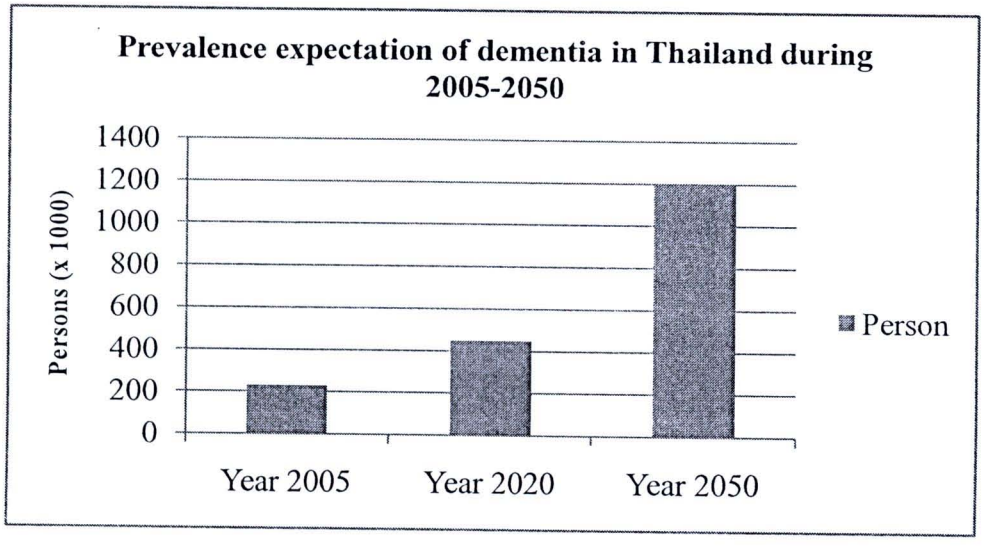

Figure 2 Prevalence expectation of dementia in Thailand during 2005 - 2050

Source: Adapted from Global prevalence of dementia: a Delphi consensus study, 2005

Pathology of Alzheimer's disease

When the Alzheimer's disease progresses, the brain displays a diffusely atrophy and weight loss by 20% or more. The atrophy process usually occurs at frontal, temporal, and parietal lobes even through their degree may be varied among the patients. In macroscopic scale, nerve cells are loss throughout the brain. Additionally, three microscopic changes provide the outstanding pathologically characteristic to the disease: (1) the intracellular presence of thick, fiber-like strands of silver-staining material in the form of loop, coil, or tangle masses called Alzheimer neurofibrillary changes or "tangles". The small part of these strands is a hyperphosphorylated form of microtubule associated protein "tau" paired together to form helical filament at the ultrastructure. (2) Spherical deposition of amorphous materials, of which at core of aggregate is the protein "amyloid" seen by periodic acid-shiff (PAS) and silver staining. Surrounded by degenerating nerve terminal, amyloid proteins aggregate to form "neuritic plaques". (3) Granulovacuolar degeneration of neurons, most evident in the pyramidal layer of hippocampus. This last change is the least important in diagnosis (2009a). Comparatively, neurofibrillary tangles (NFTs) and quantitative neuronal losses, not the amyloid plaques, correlate best with the severity of dementia although both neuritic plaques and neurofibrillary tangles are found in all association areas of the cortical cortex (Arriagada, et al., 1992a).

Pathogenesis of Alzheimer's disease

1. Protein aggregation

Neurofibrillary tangles and amyloid plaques are the two microscopic changes and outstanding pathological characters of Alzheimer's disease. Amyloid plaques are composed of aggregation of Aβ proteins. Aβ proteins occur when amyloid-precursor protein (APP) is cleaved by the action of protease α, β, and γ secretase. On the current hypothesis developed by Selkoe et al., APP is cleaved by either α or β secretases during normal cellular metabolism. The products of this reaction are then cleaved by γ secretase isoform of the enzyme. The consequence of the cleavage by α and then γ secretase produces tiny fragments that are not toxic to neurons. However, cleavage by β and then γ secretase produces a 40-amino acid product, Aβ40, and a longer 42 amino-acid form. The latter Aβ42 is toxic in several models of Alzheimer's disease. Several findings support the view that elevation Aβ42 levels contributes to the aggregation of amyloid and neuronal toxicity.

Because of its more association with severity of dementia, tau protein aggregation or neurofibrillary tangle is the focus in this study thus pathogenesis of tau aggregation will be mentioned specificially in the following part.

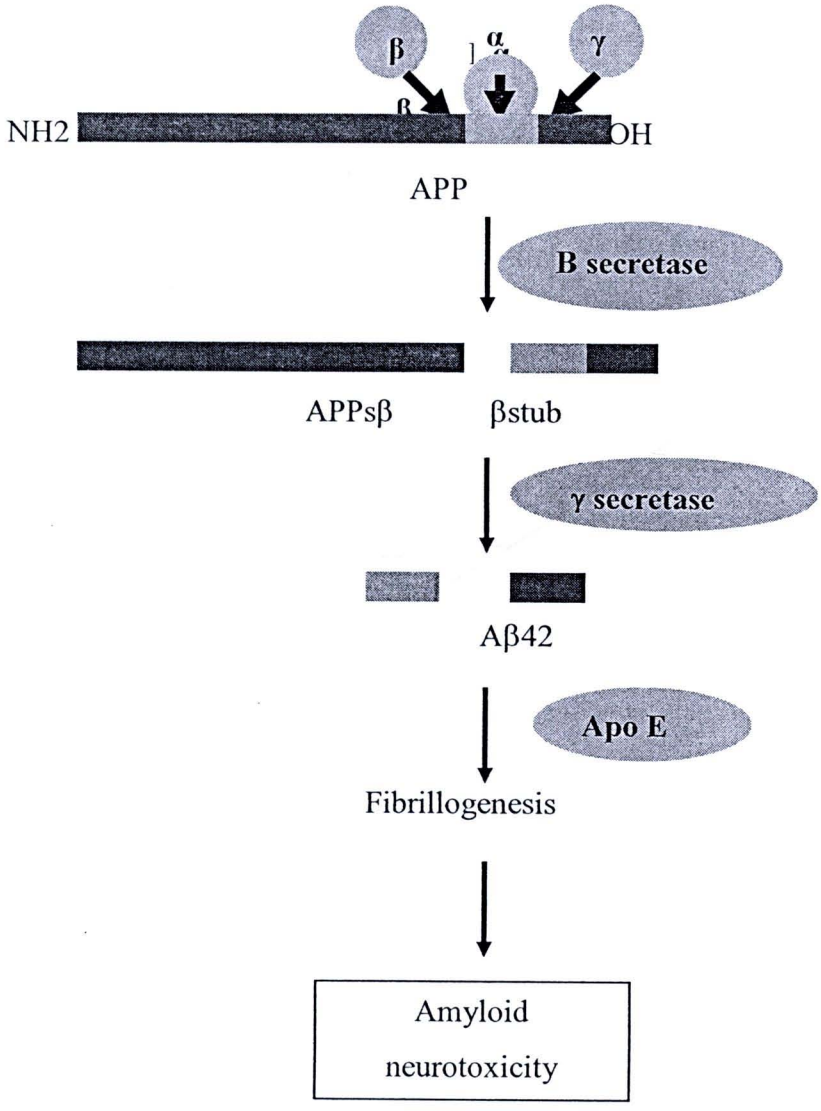

Figure 3 Diagram of proteolysis of amyloid precursor protein (APP)

Source: Adapt from Allan H. Ropper and Martin A Samuels, 2009, p.1019

2. Neurotransmitter Abnormality

In Alzheimer's disease patients, there was the finding that choline acetyltransferase (ChAT) and acetyl choline in hippocampus and neocortex were reduced. This loss of cholinergic synthetic capability was caused by a reduction in the number of cells in the basal forebrain nuclei (mainly the nucleus basalis of Meynert), from which the major portion of neocortical cholinergic terminalis originate

(Whitehouse, et al., 1981). However, many regions such as the caudate nucleus which present neither amyloid plaques and neurofibrillary tangles had the reduction of ChAT activity by 50% (Selkoe, 1999). Alzheimer's brain also exhibits a loss of monoaminergic neurons and diminution of noradrenergic, GABAnergic, and serotonergic functions in affected neocortex. The amount of amino neurotransmitters especially glutamate also decreased in cortical and subcortical areas (Sasaki, et al., 1986). Though several neuropeptides, notably substance P, somatostatin, and cholycystokinin were also found to be diminished in Alzheimer's cortex, it remains elusive whether any of these biochemical abnormalities, including cholinergic ones, are primary or secondary to heterogeneous neuronal loss. Nevertheless, the administration of cholinomimetics either acetylcholine precursor (e.g., choline or lecithin), degradation inhibitors (e.g., physostigmine), or muscarinic agonists that acts directly on postsynaptic receptors have had a mild and unsustainable therapeutic effect (2009a).

3. Genetic Aspect

For genetic aspect, some series of discoveries revealed the defective gene coded for errant APPs localized to chromosome 21 in patients with inherited forms of Alzheimer's disease (St George-Hyslop, et al., 1987). This similar notion can be explained for the Alzheimer changes that characterize the brains of practically all patients with trisomy 21 defect (Down syndrome) who are beyond twenties overproducing amyloid because of triplication of the gene. However, only a small portion of familial cases and miniscule percentage of disease overall is caused by the gene defect of chromosome 21. For up to 50% of familial cases, Alzheimer's disease has been linked to rare mutation of the presenilin gene on chromosome 14 (presenilin 1; (Sherrington, et al., 1995)) and on chromosome 1 (presenilin 2) for many of the remaining one (Levy-Lahad, et al., 1995). In these familial forms, the patient age of onset is earlier than that of sporadic forms.

Table 2 Genetic defect of Alzheimer's

Notation	Chromosome	Gene	Genetic	Clinical Features
APP	21	Amyloid precursor protein	AD	Rare but clinically simulates sporadic AD
PS1	14	Presinilin 1	AD	As above
PS2	1	Presinilin 2	AD	As above
ApoE	19	Apolipoprotein E	Haplotype	These variants modify susceptibility to typical AD
BQLN 1	9	Ubiquitin 1	SNP	Familial cases only
Trisomy 21	21	Amyloid precursor	Triploidy	AD is almost universal in Down syndrom

Source: Adapted from Allan H. Ropper and Martin A Samuels, 2009, p.1020

For some evidence, only an excess or aberrant amyloid can't be pathological for the disease. Another genetic marker, ApoE, a regulator of lipid metabolism, has had an affinity for Aβ in Alzheimer plaques and only modifies the risk of acquiring Alzheimer's disease. Among several ApoE isoforms, the expression of E4 (and its corresponding allele 4 on chromosome 19) is associated with a tripling of the risk of developing Alzheimer's disease (Polvikoski, et al., 1995; Roses, 1995; Strittmatter, et al., 1993). In fact, elevated amount of Aβ deposition correlates with the possession of the e4 allele (McNamara, et al., 1998). Another modifying gene at the UBQLN (ubiquilin 1) coding for a protein interacting with PS1 and PS2 and participating in proteasome degradation has also been found in familial cases of Alzheimer's disease. The summary of these genetic defects of the disease is following viewed on the table 2.

Tau protein

Tau is one of microtubule-associated proteins (MAP) (Weingarten, et al., 1975) found in many animal species including Caenorhabiditis elegans (Goedert, et al., 1996; McDermott, et al., 1996), bovine (Himmler, 1989; Himmler, et al., 1989), goat (Nelson, et al., 1996), monkeys (Nelson, et al., 1996), and human (Goedert, et al., 1989a; Goedert, et al., 1989b). In human, tau is mainly expressed in neurons (Schoenfeld and Obar, 1994; Tucker, 1990) in spite of trace amounts in non-neuronal cells.

There is the single human tau gene with 16 exons locating over 100 kb on the long arm of chromosome 17 at the band position 17q21 (Neve, 1986). The tau nascent transcript contains all 16 exons. Splicing to peripheral tau proteins, three of them (exon 4A, 6, and 8) are never present in any mRNA in human brain. Exon 4A is found in human, bovine, and rodent peripheral tissues with high degree of homology. Tau mRNAs containing either exons 6 or 8 have not been described in human.

As a part of promoter, exon -1 is transcribed but not translated onto any RNA. Exons 1, 4, 5, 7, 9, 11, 12, and 13 are constitutive exons expressed in all mRNAs. Exon 14 is found in mRNA but not translated into protein (Andreadis, et al., 1992; Goedert, et al., 1989a; Goedert, et al., 1989b; Sawa ,et al., 1994). Exon 2, 3, and 10 are alternatively spliced onto adult brain-specific mRNAs. Exon 3 always expresses dependently on exon 2 (Andreadis, et al., 1995). Thus, there are six combinations of

alternative splicing of these three exons, i.e. 2-3-10-; 2+3-10-; 2+3+10-; 2-3-10+; 2+3-10+; 2+3+10+, providing six mRNAs.

In the brain, there are six major isoforms of tau proteins carrying 352 to 441 amino acids with their weight ranging from 45 to 65 kDa. For a structural stand-point, tau protein is characterized by the presence of a microtubule-binding domain repeats of highly conserved tubulin-binding motif (Lee, Gloria, et al., 1989) at the carboxy terminal (C-terminal) half of the protein, followed by a basic protein-rich region and an acidic amino-terminal (N-terminal) region referring a the "projection domain". The six tau variants differ from each other by the absence or presence of exon 10 expression providing either three or four microtubule-binding domain repeats (3R or 4R tau isoforms) and by whether the expression of either one or two 29 amino-acid-long insert (0N, 1N, or 2N tau isoforms) at the N-terminal portion of the protein, which is not instrumental for microtubule-binding.

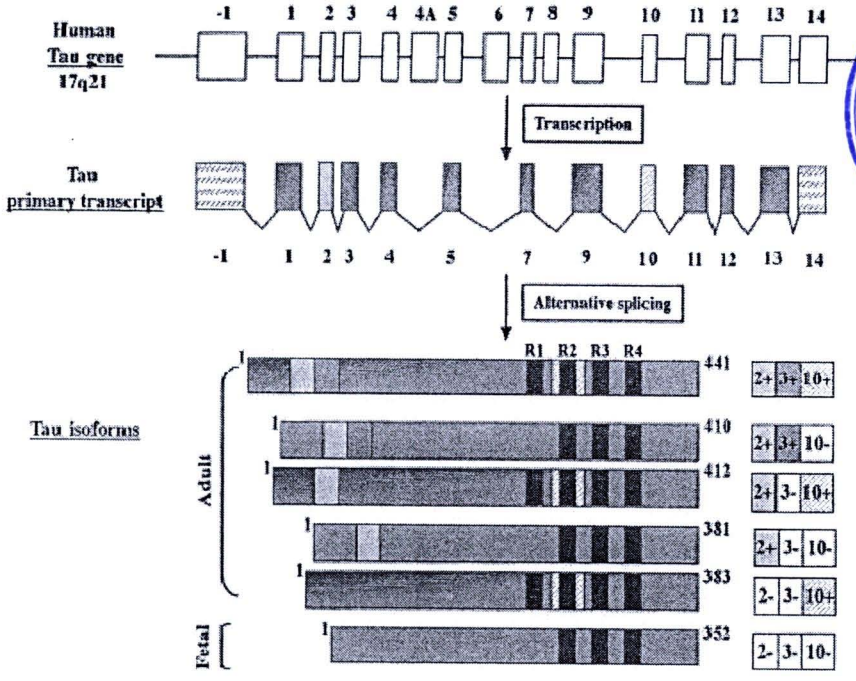

Figure 4 Tau gene and Tau protein structure

Source: Buée L., et al., 2000, pp. 95-130

Etiopathogenesis

Two major functions of the microtubule-associated protein tau particularly abundant in axons of the neurons are its ability to promote assembly and to maintain structure of microtubule (MT) (Weingarten, et al., 1975). MT-bound tau is essential to MT integrity. Additionally, the normal dynamic equilibrium of tau, on and off the MTs, respectively regulates the physical obstacle and movement for vesicles and cargoes transporting along the axons as shown in figure 5. These two functions of tau are regulated by its degree of phosphorylation (Alonso, et al., 1994; Iqbal, et al., 1994; Khatoon, et al., 1995; Lindwall, 1984).

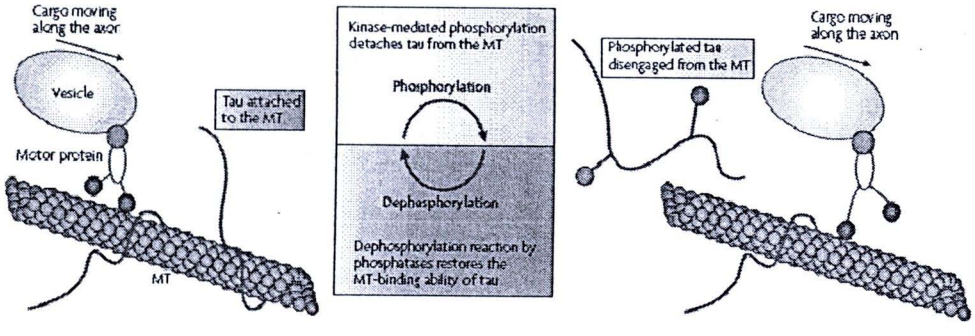

Figure 5 Dynamic equilibrium of tau protein and microtubule association

Source: Ballatore C, M. Y. Lee V. and Trojanowski JQ., 2007, pp.663-672

There are seventy-nine putative Ser or Thr phosphorylation sites on the longest brain tau isoform (441 amino acids). Using phosphorylation-dependent monoclonal antibodies against tau, mass spectrometry and sequencing, at least thirty phosphorylation site have been described, including Thr39, Ser46Pro, Thr50Pro, Thr69Pro, Thr153Pro, Thr175Pro, Thr181Pro, Ser198, Ser199Pro, Ser202Pro, Thr205Pro, Ser208, Ser210, Thr212Pro, Ser214, Thr217Pro, Thr231Pro, Ser235Pro, Ser237, Ser241, Ser262, Ser285, Ser305, Ser324, Ser352, Ser356, Ser396Pro, Ser400, Thr403, Ser404Pro, Ser409, Ser412, Ser413, Ser416 and Ser422Pro (Hasegawa, et al., 1992; Lovestone and Reynolds, 1997; Morishima-Kawashima, et al., 1995; Paudel and Li, 1999; Roder, et al., 1997; Takahashi, et al., 1995). All of these site are localized

outside the microtubule-binding domain with the exception of Ser262 (R1), Ser285 (R1-R2 inter repeat), Ser305 (R2-R3 inter repeat), Ser324 (R3), Ser352 (R4) and Ser356 (R4) (Goedert, et al., 1989a; Roder, et al., 1997; Seubert, et al., 1995). Most of these phosphorylation sites are on Ser-Pro and Thr-Pro motives. A number of Ser/Thr-Pro sites have also been identified (Morishima-Kawashima, et al., 1995). Phosphorylation of Ser262 dramatically reduces the affinity of tau to MT in vitro (Biernat,et al., 1993). Nevertheless, this site alone, which is present in fetal tau, adult tau as well as in hyperphosphorylated tau proteins found in NFTs, is insufficient to eliminate tau binding to MT (Seubert, et al., 1995). There is a finding that the heptapeptide 224KKVAVVR230 located in the proline-rich region has a high MT binding activity in combination with the repeat regions (Goode, et al., 1997), suggesting intra molecular interaction between the both regions. Thus, phosphorylation outside the MT-binding domains can strongly influence tubulin assembly by modifying the affinity between tau and MT (Buee, et al., 2000). The more phosphorylation of tau protein, the less tau capability to bind to MT. Tau, a phosphoprotein, which normally contain 2-3 molecule of phosphate/ 1 mololecule of the protein (Kopke, et al., 1993), but AD brain contains 4-8 fold of abnormal hyperphosphorylated tau (Khatoon, et al., 1992, 1994). Indeed, the abnormal hyperphosphorylation of tau provides a basis of tau pathology in all known human tauopathies including AD. The loss of tau's normal MT-stabilizing functions would inevitably lead to a pathological disturbance in the normal structure and regulartory functions of the cytoskeleton, which would compromise axonal transport and these contribute to synaptic dysfunction and neurodegeneration (Ballatore, et al., 2007). Abnormal disengagement of tau from MTs increases cytosolic unbound tau concentration, leading to a key event for the toxic gains-of-function of tau-mediated neurodegeneration. Imbalance of tau kinases and/or phosphatases, mutations of the tau gene, covalent modification of tau can contribute to abnormal disengagement of tau from MTs and promotes misfolding and other causes such as other post-translational modification. Once tau is unbound from the MT, it becomes more likely to misfold and then early deposits in the form of "pretangles". Later, these intermediate forms transform themselves to be the β-sheet containing structures and pair together in the form of paired-helical filaments (PHFs). A structural transition

leads to more organized aggregate and eventual development of neurofibrillary tangles (NFTs). The studies by immunohistochemical techniques for determining the level of both NFTs and SPs in different brain regions of AD patients and non-demented elderly individual reveal that the number of NFTs, but not the number of SPs, correlates with the degree of cognitive impairment (Arriagada, et al., 1992a; Arriagada, et al., 1992b; Ballatore, et al., 2007). These provide the initial circumstantial evidence to suggest that the toxic gains-of-function by NFTs might be an important part in the progression of the disease (Ballatore, et al., 2007).

It is possible that the toxic effect of NFTs may partly arise from the relatively large size of the fibrillary material accumulating inside the neuron which may directly disrupt physical functions of the cells such as axonal transportation (Ballatore, et al., 2007). Moreover, NFTs may aggravate the disease progression by effective sequestering more tau and other proteins, reinforcing and amplifying the loss of normal function.

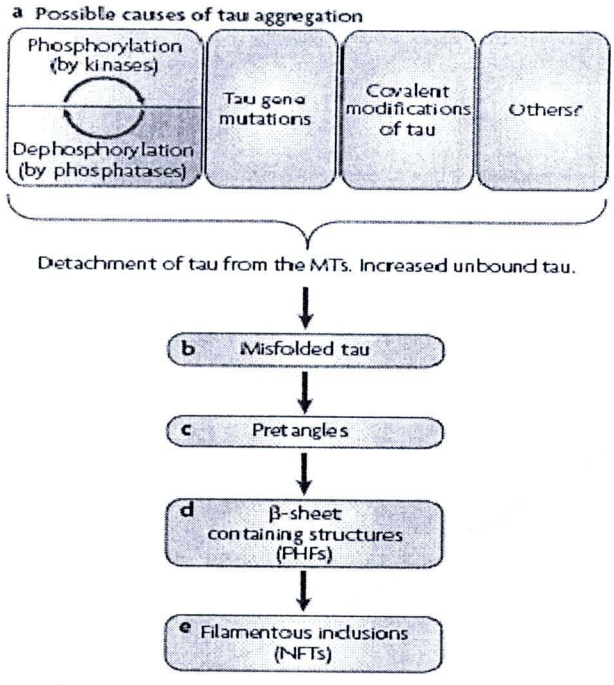

Figure 6 The progression of tau protein aggregation.

Source: Nature Review/Neuroscience, 2007, pp. 663-672

Tau protein and dementia

Tau protein aggregation also exhibited in other dementia including frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (Hutton, et al., 1998; Spillantini, et al., 1998), corticobasal degeneration, Pick disease, and progressive supranuclear palsy (Iqbal and Grundke-Iqbal, 2008), which are collectively named as "tauopathy". Tau protein levels present correlation with memory performance in many studies both in human and animals. Concentration of total tau (t-tau) was elevated in AD (Lewczuk, et al., 2004). Increasing in both CSF total tau (t-tau) and CSF phosphorylated-tau (p-tau) levels were consistently found in patients with mild cognitive impairment (MCI) compared to healthy cognitive controls (Blennow, et al., 2006; Hansson, et al., 2006; Herukka, et al., 2007; Schonknecht, et al., 2007; Winblad, et al., 2004). Additionally, Anderson et al., report that CSF P-tau levels were significantly increased in initially non-demented patients with impair episodic memory (SIM) at baseline, who decline cognitively over time and progress to

dementia at a high rate, during 3 year follow-up, while they were unchanged in most moderate impaired memory (MIM) and non-impaired memory (NIM) patients who remained cognitively stable over time (Andersson, et al., 2008). Tau pathology was assumed as a contributor of age-dependent learning impairment when 12-month-old tau-infected mice presented cognitive deficit in an object recognition memory task and exhibited spatial memory deficit as indicated by the impaired performance in the Morris water maze (Polydoro, et al., 2009). The appearance of NFTs in memory-related temporal lobe, strongly related with score on test of memory function in non-demented individual, suggested the responsible of accumulation of NFTs for memory loss associated with aging as well as the memory deficit seen in some case of MCI (Guillozet, et al., 2003). Moreover, numerous neurofibrillary tangles (NFTs) in the hippocampal area are the characteristic of limbic neurofibrillary tangle dementia (LNTD), a subset of senile dementia, which has an absence or scarcity of amyloid deposit throughout the brain (Yamada, et al., 2001). These all inevitably accentuated the putative pathological substrate of tau protein and its aggregation for progression of dementia.

Tau protein and apoptosis

As previously mentioned, neuronal atrophy is the most common type of neurodegenerative dementia. Correlatively, recent findings reported the elevation of tau hyperphosphorylation in apoptotic neuronal cells by both DNA damage (Mookherjee and Johnson, 2001) and by removal of trophic factor (Davis and Johnson, 1999a, b; Nuydens, et al., 1997; Shelton and Johnson, 2001; Zhang and Johnson, 2000). In the former paradigm, tau phosphorylation is increased significantly in early stages of apoptosis in human SH-SY5Y neuroblastoma cells induced by camptothecin, however at the late stage tau levels and tau phosphorylation decrease significantly. For the later paradigm, apoptosis was induced in differentiated neuronal PC12 cells by removal of both serum and neurotrophic factor, NGF. In this case tau and high molecular weight (HMW) tau phosphorylation increased significantly at tau-1 (Davis and Johnson, 1999a,b; Shelton and Johnson, 2001) and its subset AT-8 (Nuydens, et al., 1997) epitopes. Tau-1 phosphorylation significantly enhances throughout the cell body, especially in the neuritis, with the exception of neucleus and

perinuclear region (Davis and Johnson, 1999b; Shelton and Johnson, 2001). Alteration in tau and HMW tau phosphorylation state during neuronal apoptosis reduced the microtubule-binding capacity of tau and HMW tau significantly (Davis and Johnson, 1999b). Phosphate incorporation into a phosphopeptide of tau and HMW tau is increased in apoptotic neuronal PC12 cells (Davis and Johnson, 1999a) and numerous specific sites mainly serine and threonine residues, are hyperphosphorylated during apoptosis (Zhang and Johnson, 2000). This elevation may be partially due to dysregulation of certain kinases (Nuydens, et al., 1997) such as cyclin-dependent kinase 5 (cdk5) and cell division control protein 2 (cdc2) because there is an increase in the association between these protein kinases and tau during apoptosis (Zhang and Johnson, 2000).

***Bacopa monnieri* (L.) Wettst.**

Bacopa monnieri or water hyssop is a revered ancient Indian Ayurvedic herb specific for cognition enhancing benefit. (Kidd, P. M., 1999) Its popular name Brahmi, the literature meaning is which expands consciousness, is derived from Brahma, one of the gods of trinity in Hindu mythology who created and controlled the world. The synonyms of Brahmi in the ancient texts are Sureshta-liked by god, Divy-divine, Sarasvati, Sharad, Bharati-goddesses of learning, Vayastha-arrests old age, Sharma-charming and Medhya-good for mental work. (Singh, H. , 2003) Belonged to the family of Scrophulariaceae, this creeping annual herb grows in wet, damp, and marshy areas in tropical regions. The stem of this plant is prostrate, having ascending branches and entire, oblong, succulent, 2.5 cm long and 0.6 cm wide leaves with solitary flowers and ovoid capsule fruits in summer. (Russo and Borrelli, 2005; Singh, H., 2003)

Chemical constituents

In 1931, Bose and Bose reported the isolation of the alkaloid "bramine" from BM (Bose K.C., 1931). Later, Chopra et al. discovered other alkaloids like nicotine and herpestine from the plant (Chopra, 1956). In 1959, Sastri et al. extracted D-manitol, and a saponin, hersaponin and potassium salts provided further details of the chemical components of BM (Sastri, 1959).

Among its all components, Bacoside A or 3-(α-L-arabinopyranosyl)-O-β-D-glucopyranoside-10, 20-dihydroxy-16-keto-dammar-24-ene is responsible for the memory-facilitatingaction (Chatterji, 1965). Bacoside A usually present concomitantly with Bacoside B, its optical isomer different from each other only in optical rotation (Rastogi, R.P., 1990). Bacoside A was found revo-rotatory and Bacoside B detro-rotatory (Deepak and Amit, 2004). The chemical composition of bacosides contains in the polar fraction of BM extract. On acid hydrolysis, bacosides yield a mixture of aglycones, bacogenin A1, A2, A3 (Chandel, 1977; Kulshreshtha, 1973), which are the artifacts, and two genuine saponins, jujubogenin and pseudojujubogenin (Rastogi, S., et al., 1994).

Many other compounds isolated from BM have been consecutively revealed such as bacogenin A4 (Rastogi, S., et al., 1994), a minor saponin bacoside A1 (Rastogi, S., et al., 1994), a triperpenoid saponin bacoside A3 (Rastogi, S., et al., 1994), three dammarane-type triterpenoid saponin bacopasaponin A, B, and C (Garai, et al., 1996), pseudojujubogenin glycosides bacopaside I and II (Chakravarty, et al., 2001), etc.

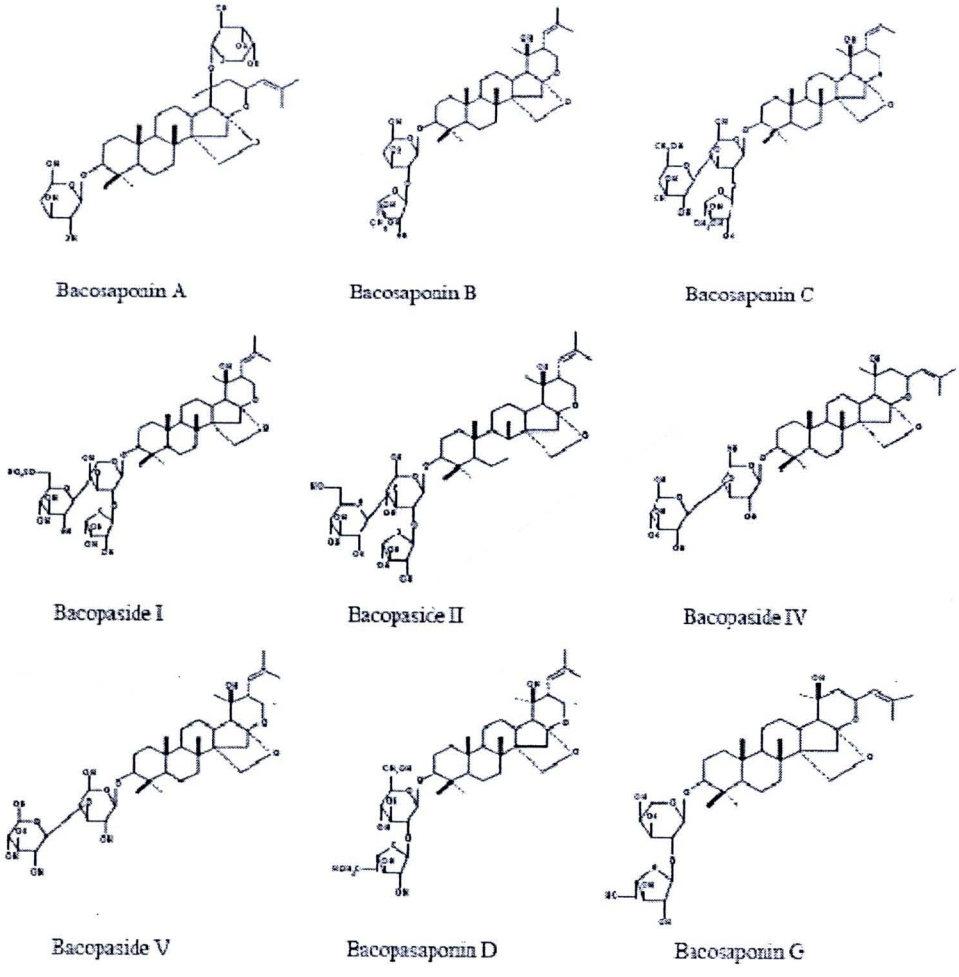

Figure 7 Structure of some components in Bacopa monniera

Source: Phytomedicine, 2005, pp. 305-317.

Biological activity

The biological effects of BM have been documented in traditional and in scientific literatures. The earliest mention of BM is in several ancient Ayurvedic Indian treatises including the Caraka Samhita (6th century A.D.), in which it is recommended in formulations for management of a range of mental conditions including anxiety, poor cognition, and lack of concentration, and the Brahprakash Var-Prakarana (16th century A.D.) (Russo and Borrelli, 2005). The traditional Ayurvedic medicine has used BM for centuries as an anti-inflammatory, analgesic, antipyretic,

antiepileptic, and sedative agent. The most important of these is especially the use of the plant, plant extract and isolated bacosides as an enhancing agent for cognition and memory function.

Beneficial effects on learning and memory

Preliminary studies showed that, the treatment with the plant (Malhotra C.L., 1959) and with the alcoholic extract of BM plant (Singh, H. K. and Dhawan, 1982) enhanced learning ability in rats. The cognition-facilitating effect of BM was reported that it is attributed to the two active saponins, bacoside A and B present in ethanolic extract (Singh, H. K., Dhawan, B.N., 1992). In 1990, Singh et al. suggested that bacosides induce membrane dephosphorylation concomitantly with increasing the turnover of protein and RNA in specific brain areas (Singh, N.K., Srimal, R.C., Srivastava, A.K., Garg, N.K., Dhan, B.N., 1990). Further, it was reported that BM enhanced protein kinases activity in the hippocampus which could also contribute to its nootropic action (Singh, H. K., Dhawan, B.N., 1997). Later, Bhattacharya et al. reported the cognitive deficit could be reversed by colchicines and ibitenic by administration of standardized bacoside-rich of BM for 2 week (Bhattacharya, S.K., Kumar, A., Ghosal, S., 1999). Additionally, BM is a potential cognitive enhancer and neuroprotectant against Alzheimer's disease rats induced by AF64A (Uabundit, et al., 2010)

Antioxidant activities

Several findings suggest that the cognition-promoting functions of BM may be partially attributed to the antioxidant effect of the bacosides. Antioxidant action mechanism of BM could be due to metal chelation at the initiation level of the free radical-induced chain reaction or to the scavenging of free radical at the propagation level (Tripathi, et al., 1996). BM induced a dose-related increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities in frontal cortical, striatal, and hippocampal rat brain after treatment for 14 or 21 days (Bhattacharya, S. K., et al., 2000). Additionally, the hydroalcoholic extract of the whole BM plant revealed an inhibitory effect on superoxide released from polymorphonuclear (PMN) cells in nitroblue tetrazolium (NBT) assay (Pawar, et al., 2001). BM is able to directly inhibit the production of free radical superoxide anion in dose-dependent manner by the use of Paoletti assay (Russo, et al., 2003b). Further,

methanolic extract of BM added to the culture of rat astrocytes during the treatment with NO donor, S-nitroso-N-acetyl-penicillamine (SNAP), reduced the intracellular NO oxidant, which consequently prevented DNA damage (Russo, et al., 2003a). Additionally, Uabundit et al., demonstrates that BM extract can relief the memory impairment and the degeneration of neurons in hippocampus in Wistar rat model of Alzheimer's disease induced by AF64A (Uabundit, et al., 2010).

Clinical studies

Learning and memory, improvement of early information processing, retention of new information and cognitive performance could be enhanced by chronic treatment of BM extract in children and healthy or anxiety adult subjects (Calabrese, et al., 2008; Roodenrys, et al., 2002; Sharma, 1987; Singh, R.H., Singh, L, 1980; Stough, et al., 2001) but could not be significantly affected by that acute treatment even when concomitantly treated with other potent herb, *Ginko biloba*.

Table 3 Clinical study of BM extract in human

Substances/Treatments	Subjects/patients	Results/comments	References
BM crude extract; Chronically 4 weeks	Patients with anxiety neurosis	Enhancing of memory	(Singh, R.H., Singh, L, 1980)
BM extract; chronically for 12 week	Children	Enhancing memory and learning	(Sharma, 1987)
BM standardized extract	Mentally adult subjects	Effective in enhancing learning and in controlling abnormal behavior	(Dave, et al., 1993)
BM standardized extract (300 mg); chronically for 12 weeks	Healthy adult subjects	Improving early information processing, verbal learning	(Stough et al., 2001)
BM standardized extract (300 mg); acute treatment for 2 h	Healthy adult subjects	No significant changes were found	(Nathan, et al., 2001)
BM standardized extract (300 mg) combined with Ginko biloba extract (120 mg); acute treatment for 90 and 180 min	Healthy adult subjects	No acute effect on cognitive was found on any of the tests	(Maher, et al., 2002)

Table 3 (Cont).

Substances/Treatments	Subjects/patients	Results/comments	References
BM standardized extract (300 mg); one trial after chronic treatment for three months and another trial and for 6 weeks after the completion of the trial	Healthy adult subjects	Significant effect on a test for retention of new information. Task assessing attention, verbal and visual short term memory and the retrieval of pre-experimental knowledge were unaffected	(Roodenrys, et al., 2002)
Dried BM extract (300 mg) for 12 weeks	Healthy ≥65 volunteers	Enhanced AVLT delayed word recall memory scores, Stroop results were similarly significant	(Calabrese, et al., 2008)

Source: Applied from Phytomedicine, 2005, pp. 305-317.

Toxicological study

BM has been found to be well tolerated and without any untoward reaction or side effects in regulatory pharmacological and toxicological studies. The LD_{50} of aqueous and the LD_{50} of alcoholic crude extracts of BM in rat were 1000 and 15 g/kg by intraperitoneal route, respectively (Martis G., 1992). The aqueous crude extract given orally at a dose of 5 g/kg did not show any toxicity. The LD50 of the alcoholic crude extract was 17 g/kg given orally. Single doses of bacoside A and B (100 and 200 mg) administrated for 4 weeks have been tolerated well and devoid of any untoward reaction or side effects in 31 healthy male human volunteers (Singh, H. K., Dhawan, B.N., 1997).