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AChE and BuChE vis-a-vis Dementia and Related Brain Disorders : A Therapeutic Strategies

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Memory and attention are cognitive functions that depend heavily on the cholinergic system. Local activity of acetylcholine esterase (AChE) and butyryl choline esterase (BuChE) are indicators of its integrity. Using very recently developed techniques patients with dementia of Alzheimer have been studied and compared with the findings on measurements of blood flow (CBF) and glucose metabolism. AChE activity was reduced significantly in all brain regions in demented subjects, reduction of glucose metabolism and CBF was more limited to temporo-parietal association areas. Present review indicates that, compared to non-demented controls, there is a global reduction of cortical AChE activity in dementia and increase of BuChE. It also discusses various plant-based brain boosters for the therapeutic goal through AchE. This article also reviews the pharmacological basis of some plants and their active constituents that have been used in traditional Ayurvedic medicine and TCM for their reputed cognitive-enhancing effects.

Keywords : Alzheimer's disease, Dementia, cholinergic system, acetylcholine esterase, cerebral blood flow, cerebral glucose metabolism.

Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disease that primarily affects the elderly population, and is estimated to account for 50–60% of dementia cases in persons over 65 years of age. The main symptoms associated with AD involve cognitive dysfunction, primarily memory loss (Förstl *et al.*, 1995; Grafman *et al.*, 1990 and Grosse *et al.*, 1991). Other features associated with the later stages of AD include language deficits, depression, behavioural problems including agitation, mood disturbances and psychosis (Kumar *et al.*, 1998; McGuffey, 1997 and Wragg and Jeste, 1989).

Alzheimer's disease : The dementia of Alzheimer's disease type, which occurs when nerve cells degenerate and cause pathological shrinking

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of the brain. The disease usually starts in late middle age or old age, and results in progressive worsening of cognitive functions (such as memory), abnormal behaviors, and speech disturbances. There is another type of dementia called vascular dementia that occurs when blood vessels in the brain are damaged by stroke, etc.

As shown in Figure 1, acetylcholine is released into the space between two nerve cells (the synapse), where it stimulates a "transfer" of the nerve impulse from one nerve cell to the next. After the nerve impulse has been transmitted, an enzyme called acetylcholine esterase breaks down acetylcholine and the nervous signal is ended. In some memory disorders, such as Alzheimer's disease and senile dementia, acetylcholine may be destroyed too quickly – so the nerve impulse is either too weak to be received or it id incompletely transmitted between nerve cells.

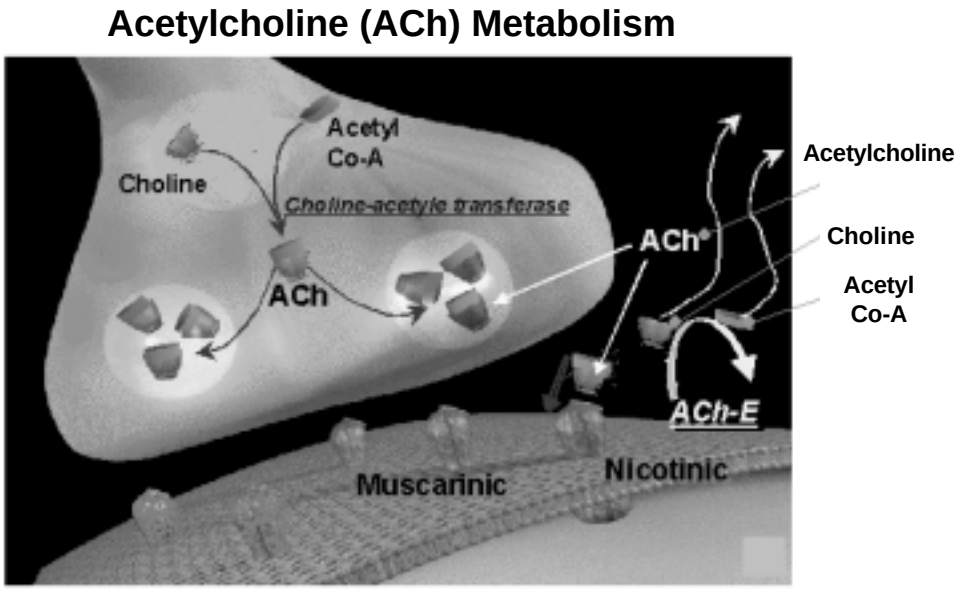

Fig. 1

The pathological features that have been identified in the central nervous system (CNS) in AD are senile plaques and neurofibrillary tangles, oxidative and inflammatory processes and neurotransmitter disturbances. A consistent neuropathological occurrence associated with memory loss is a cholinergic deficit, which has been correlated with the severity of AD (Bierer

et al., 1995; Collerton, 1986; Giacobini, 1990; Plotkin and Jarvik, 1986 and Read, 1987). Thus, attempts to restore cholinergic function have been a rational target for drugs used to treat the symptoms of AD. Approaches to enhance cholinergic function in AD have included stimulation of cholinergic receptors or prolonging the availability of acetylcholine (ACh) released into the neuronal synaptic cleft by inhibiting ACh hydrolysis by acetylcholinesterase (AChE); the latter may be achieved through the use of AChE inhibitors.

Acetylcholine Esterase Inhibitors : In patients with Alzheimer's disease (AD), the amount of a neurotransmitter (or chemical) called acetylcholine (ACh) – essential for normal brain function – is reduced. Current approved prescribed treatments for AD – called acetyl cholinesterase inhibitors – inhibit the production of the enzyme (acetylcholinesterase) that breaks down or degrades ACh, thus increasing the amount of ACh that is available to the brain. FDA approved drugs like REMINYL® (galantamine HBr) – treat some symptoms of the disease and have been reported in clinical trials to maintain or even improve cognitive function. However in medical studies the most frequent side effects experienced by patients taking such drugs were nausea, vomiting, diarrhea, anorexia, and weight loss. These side effects, if they occur, tend to happen with increasing the dosage. Acetylcholine is a neurotransmitter related to memory and learning. Acetylcholine esterase inhibitors are enzymes that limit the reduction of acetylcholine concentration in the brain by slowing its breakdown and increasing its availability to viable neurons.

Several cellular processes could be targeted if the complex nature of Alzheimer's disease (AD) was already understood. Most of the AD treatments have been focused on the inhibition of acetylcholinesterase (AChE) in order to raise the levels of its substrate, i.e., the neurotransmitter acetylcholine (ACh), to augment cognitive functions of affected patients. Effectiveness in AChE inhibition and side-effect issues of clin. (tacrine, Donepezil, galanthamine, and Rivastigmine) as well as of novel inhibitors are reviewed here. Novel design methods for the inhibition of AChE include the use of in silico tools to predict the interactions between AChE and the desired compound, both at the active site of the enzyme, responsible for hydrolyzing ACh and with the peripheral anionic site (PAS), which has been

described as a promoting agent of the amyloid .beta.-peptide (A.beta.) aggregation present in the senile plaques of the brain of AD individuals (Colombres *et al.*, 2004).

Fig. 2 : *BuChE staining is shown in the temporal cortex of a 71-year-old subject with AD (A) and an 89-year-old healthy control (B). Staining is limited to the glia (arrow) only in the 89-year-old healthy control. In contrast, BuChE staining is found in plaques (open arrows), tangles (curved arrow), dystrophic neurites (arrowheads), and glia (single arrow) in the AD patient. Scale bar = 100 µM [Guillozet A, et al. Butyrylcholinesterase in the life cycle of amyloid plaques. Ann Neurol 1997; 42: 909–918.*

Amyloid plaques

Deposits of amyloid material are seen many years before the occurrence of neuronal degeneration and dementia; this suggests that some plaques may be quite benign. Both AChE and BuChE are associated with amyloid and neurofibrillary tangles in the brains of elderly persons with and without relevant cognitive impairment. It's therefore important to know what factors may contribute to the transformation of 'benign amyloid' to one with pathogenic actions. BuChE is a candidate factor for this. Advanced amyloid plaques in Alzheimer brains have up to 87% BuChE reactivity, compared with 20% reactivity in early, benign deposits. The presence of BuChE distinguishes the neurotoxic plaques from those seen in normal aging. It is therefore possible that BuChE plays a role in transforming benign amyloid to the 'malignant' form associated with neuronal degeneration and clinical dementia.

- Amyloid deposits exist for many years in the brain as diffuse deposits or compact plaques before leading to neuritic degeneration and dementia. Therefore, it seems that some amyloid deposits are relatively benign while others lead to progressive neuronal damage. AChE and butyrylcholinesterase (BuChE) are both associated with amyloid plaques and neurofibrillary tangles in the brains of both individuals suffering from AD and elderly individuals without significant cognitive impairment.

- The factors that contribute to the transformation of a relatively inert plaque to a pathogenic one may involve interactions with additional plaque constituents. One such constituent is BuChE (Figure 1). Advanced plaques show as much as 87% BuChE reactivity compared with less than 20% reactivity in early, diffuse deposits. The presence of BuChE appears to distinguish the neurotoxic plaques seen in the AD brain from those observed in normal aging.

Insufficient cholinergic neurotransmission in AD is responsible for a progressive loss of cognition and motor capacities. The cholinergic hypothesis has provided the rationale for the current treatment approaches based on acetylcholinesterase inhibitors. However, recent data focus on the complex nature of AD and disclose the involvement of other neurotransmitters such as serotonin, noradrenalin, dopamine, histamine, excitatory amino acids, and neuropeptides too. Interestingly, recent research has revealed that in severe AD brains, the levels of AChE are considerably reduced whereas BuChE activity increases, thus aggravating the toxicity of .beta.A. In such instances, it is possible that BuChE may be a more suitable target than AChE. Oxidative stress has been implicated in CNS degenerative disorders such as AD and PD. Therefore, owing to their capacity to inhibit oxidative damage, MAOIs are potential candidates as anti-Alzheimer drugs. More recently, a novel drug - TV3326 - was designed, based upon 2 pharmacophores: the carbamate moiety from Rivastigmine, an AChE inhibitor; and the propargyl group from Rasagiline, a MAO inhibitor. This drug exhibits cholinesterase and selective brain MAO inhibitory activities, reduces apoptosis, and stimulates the processing of APP.alpha., hence reducing the possibility of generation of the toxic .beta.A. Thus, TV3326

may be expected to contribute posibility. to the cognitive benefits of Alzheimer's patients. Anyhow, the development of drugs with several targets and diverse pharmacological properties may conclusively demonstrate the most beneficial therapy (Carreiras and Marco, 2004).

A decline of cholinergic neurotransmission probably contributes to cognitive dysfunction occurring in Alzheimer's disease (AD) and vascular dementia (VaD). Acetylcholinesterase (AChE)/cholinesterase (ChE) inhibitors are the only drugs authorized for symptomatic treatment of AD and are also under investigation for VaD. Tayebati *et al.*, (2004) has investigated the influence of 2 doses of the AChE inhibitor rivastigmine (0.625 mg/Kg/day and 2.5 mg/Kg/day) on vesicular acetylcholine transporter (VAChT) and on choline acetyltransferase (ChAT) expression in frontal cortex, hippocampus, striatum, and cerebellum of normotensive and spontaneously hypertensive rats (SHR). Cholinergic markers were assessed by immunochem. (Western blotting) and immunohistochem. techniques. In frontal cortex and striatum of normotensive rats, treatment with the lower dose (0.625 mg/Kg/day) of rivastigmine had no effect on VAChT immunoreactivity and increased slightly ChAT protein immunoreactivity. The higher dose (2.5 mg/Kg/day) of the compound. increased significantly VAChT and ChAT protein immunoreactivity. In hippocampus rivastigmine induced a concentration dependent increase of VAChT protein expression and no significant changes of ChAT protein expression. A similar pattern of VAChT and ChAT protein expression was observed. in control SHR, whereas treatment of SHR with rivastigmine induced a more pronounced increase of VAChT protein immunoreactivity in frontal cortex, hippocampus, and striatum compared to normotensive rats. The authors' data showing an increase of VAChT after treatment with rivastigmine further support the notion of an enhancement of cholinergic neurotransmission by AChE/ChE inhibitors. The observation of a greater expression of this cholinergic marker in SHR suggest that AChE inhibition may provide beneficial effects on cholinergic neurotransmission in an animal model of VaD (Tayebati *et al.*, 2004)

Some AChE inhibitors have been licensed for clinical use to treat mild to moderate AD cases, but their effect is only to alleviate symptoms and they do not achieve any permanent improvement. The synthetic drug

tacrine (Cognex) was the first AChE inhibitor to be licensed, but its routine use has been restricted largely due to its hepatotoxicity (Watkins *et al.*, 1994). The use of tacrine has been eclipsed by the newer AChE inhibitors such as donepezil (Aricept, Eisai, Pfizer, UK), rivastigmine (Exelon, Novartis, UK) and galantamine (Reminyl, Shire, UK).

The use of anti-inflammatory agents has also been suggested to delay the progression of AD. Several studies have shown that **nonsteroidal anti-inflammatory drugs** (NSAIDs) may reduce the risk of developing AD, and that patients with rheumatoid arthritis, who often use NSAIDs, have a lower incidence of AD (Breitner, 1996; Breitner *et al.*, 1995; Jenkinson *et al.*, 1989; McGeer *et al.*, 1990 and McGeer *et al.*, 1996). Thus, the use of **anti-inflammatory drugs** has been proposed as a therapeutic target in AD. Also implicated in the pathology of many diseases, including neurodegenerative diseases such as AD, are free radical reactions, which are reported to initiate cell injury (Maxwell, 1995; Slater, 1984 and Spiteller, 1993). Consequently, the use of antioxidants has been explored in an attempt to slow AD progression and neuronal degeneration.

In traditional practices of medicine, plants have been used to enhance cognitive function and to alleviate other symptoms associated with AD. Plant constituents may not only act synergistically with other constituents from the same plant but may also enhance the activity of compounds, or counteract toxic effects of compounds from other plant species. This approach has been used in various practices of traditional medicine, including Ayurveda and **traditional Chinese medicine** (TCM) where a combination of plants is frequently prescribed. An ethnopharmacological approach may be useful in providing leads to identify plants and potential new drugs that are relevant for the treatment of cognitive disorders, including AD.

The pharmacological basis of some plants and their active constituents that have been used in traditional Ayurvedic medicine and TCM for their reputed cognitive-enhancing effects. The reputed effects for some traditional herbal drugs may not only be relevant in managing the cognitive decline that can be associated with general ageing but may also be relevant in the treatment of specific cognitive disorders such as AD. Thus, plants reputed to have

'antiageing' or 'memory-enhancing' effects could also be considered for potential efficacy in disorders now recognised to be associated with cognitive dysfunction, including conditions that feature dementia. Plants that have shown favourable effects in relation to cognitive disorders, including anticholinesterase (anti-ChE), anti-inflammatory and antioxidant activities, or other relevant pharmacological activities indicating the potential for clinical use, are discussed.

BuChE

Recent evidence suggests that both AChE and BuChE may have roles in the aetiology and progression of AD beyond regulation of synaptic ACh levels. The development of specific BuChE inhibitors and further experience with the dual enzyme inhibitor rivastigmine will improve understanding of the aetiology of AD and should lead to a wider variety of potent treatment options.

It has been shown quite convincingly that cholinesterase inhibitor therapy produces significant improvements in cognitive function in patients with Alzheimer's. Agents acting by inhibiting degradation of acetylcholine that are used include donepezil, galantamine, and rivastigmine. The first two drugs are selective acetylcholine esterase (AChE) inhibitors, while rivastigmine inhibits both AChE and butyrylcholine esterase (BuChE). Dr Ballard from Newcastle, England, has reviewed the potential role of BuChE in Alzheimer's disease.

BuChE has a similar molecular structure to that of AChE, but it lacks an anionic site and some aromatic residues. Contact with either enzyme causes acetylcholine to be cleaved and its neurotransmitter action terminated. While AChE is selective for acetylcholine breakdown, BuChE degrades several other substrates, including some interactive peptides.

Laboratory studies

Workers at the US National Institutes of Health (NIH) are developing specific BuChE inhibitors for possible therapy in Alzheimer's disease. They have been studied in learning models using elderly rats. Improvements in learning resulted with lower doses of BuChE-selective inhibitors than when

AChE-selective inhibitors were used. It was noted that high doses of the BuChE-selective agents did not cause typical cholinergic toxicity in animals.

Cholinergic therapy for Alzheimer's disease initially concentrated on AChE inhibition, as this is the main enzyme involved in the breakdown of acetylcholine in the normal brain. However, it now seems that the role of BuChE in hydrolysing acetylcholine may be relevant for brains with degenerative changes.

As Alzheimer disease progresses, AChE activity decreases in some brain regions, while BuChE activity increases. This is probably due to a relative increase in the numbers of BuChE-positive neurons. The increase in BuChE activity is greater in the hippocampus and in the temporal lobe in patients with Alzheimer's disease.

Acetylcholinesterase (AChE) predominates in the healthy brain, with butyrylcholinesterase (BuChE) considered to play a minor role in regulating brain acetylcholine (ACh) levels. However, BuChE activity progressively increases in patients with Alzheimer's disease (AD), while AChE activity remains unchanged or declines. Both enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral and global functioning characteristic of AD. The two enzymes differ in substrate specificity, kinetics and activity in different brain regions. Experimental evidence from the use of agents with enhanced selectivity for BuChE (cymserine analogues, MF-8622) and the dual inhibitor of both AChE and BuChE, rivastigmine, indicates potential therapeutic benefits of inhibiting both AChE and BuChE in AD and related dementias (Greig *et al.*, 2002).

Receptor Antagonist

The NMDA receptor is one of the glutamate receptors. Glutamate is a neurotransmitter in the brain that is related to memory and learning. Memantine hydrochloride is an NMDA antagonist that works to protect nerve cells in the brain from damage and degeneration caused by excessive stimulation of the receptors by glutamate.

Selective **muscarinic m1 acetylcholine receptor agonists** activate alpha-secretase processing of APP and decrease the generation of amyloid

beta-peptides (A-beta) in model systems.To determine whether **m1 agonists** decrease cerebrospinal fluid (CSF) levels of Abeta in patients with Alzheimer 's disease (AD), AF102B, talsaclidine or placebo were administered to AD. To control for specificity, two separate sets of AD patients were treated with the acetylcholine esterase inhibitor **physostigmine,** or the anti-inflammatory drug **hydroxychloroquine.** CSF was obtained before and during treatments by lumbar puncture, divided in aliquots, and frozen on dry ice immediately at the bed side. CSF levels of Abeta 1-42 and Abeta total were measured by specific and sensitive ELISAs. Paired samples obtained before and during treatments were compared. Analyses of covariates were used to test whether changes correlated with age, gender, dementia severity, or ApoE genotype. Treatment of AD patients with AF102B or talsaclidine significantly reduced CSF levels of Abeta total and Abeta 1-42, respectively. CSF levels of Abeta total neither changed significantly during treatments with the acetylcholine esterase inhibitor physostigmine nor with the anti-inflammatory drug hydroxychloroquine. Western blots with 6E10 confirmed the overall decreases in Abeta total , and changes in densitometric estimates of immunoreactive bands correlated significantly with relative changes measured by ELISA (r=.937). There were no significant correlations of changes in either Abeta 1-42 nor Abeta total levels with age, gender, ApoE genotype or dementia severity. The data show that selective m1 agonists can decrease CSF levels of Abeta peptides in AD patients within four weeks. Their data support the view that muscarinic m1 agonists have potential for the further development as amyloid-lowering drugs.

Memory and attention are cognitive functions that depend heavily on the cholinergic system. Local activity of acetylcholine esterase (AChE) is an indicator of its integrity. Using a recently developed tracer for positron emission tomography (PET), C-11-labeled N-methyl-4-piperidyl-acetate (C11-MP4A), Herholz *et al.* (2000) measured regional AChE activity in 4 non-demented subjects, 4 patients with dementia of Alzheimer type (DAT) and 1 patient with senile dementia of Lewy body type (SDLT), and compared the findings with measurements of blood flow (CBF) and glucose metabolism (CMRGlc). Initial tracer extraction was closely related to CBF. AChE activity was reduced significantly in all brain regions in demented

subjects, whereas reduction of CMRGlc and CBF was more limited to temporo-parietal association areas. AChE activity in SDLT was in the lower range of values in DAT. Their results indicate that, compared to non-demented controls, there is a global reduction of cortical AChE activity in dementia.

Herbal-based Medicines

"Why should anyone die who has sage in their garden", and "rosemary is for remembrance," as the old sayings go. Both of these herbs have been found to promote acetylcholine, the most versatile and common neurotransmitter. Rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) have both been shown to enhance the actions of acetylcholine through cholinergic activity and are listed as memory enhancers in old herbal compendiums. Lemon balm (*Melissa officinalis*), often called the gladdening herb, also has been found to improve cholinergic activity, and is recommended in old herbals for improving mood and cognition. Two other herbs more commonly associated with memory enhancement are *ginkgo* and *ginseng*.

Huperzine A (HUPA), a purified alkaloid compound isolated from Chinese club moss (*Huperzia serrata*), may benefit memory and cognition in several ways, one of which is influencing cholinergic activity. Huperzine's actions are very similar to that of cognitive drugs, because it is a highly purified single chemical.

Several studies indicate that HupA has a highly selective affinity for acetylcholinesterase (an enzyme that breaks down unused acetylcholine) and inhibits it primarily (Tang *et al.*, 1994). By doing so, HupA inhibits the degeneration of the neurotransmitter acetylcholine, which is rapidly broken down in the brains of Alzheimer's patients. A shortage of the neurotransmitter also appears to contribute to memory loss. In animal studies, daily oral administration of huperzine A has been shown to produce a significant improvement in learning ability (maze tasks) that is strongly correlated to promotion of blood flow and inhibition of acetylcholinesterase activity in various regions of the brain (cortex and hippocampus). HupA has also been observed to bind to nicotinic receptors in the central nervous system (Tang *et al.*, 1994), an activity believed to improve mental function.

Finally, HupA acts as a neuronal cell protector, which may be both therapeutic and preventive for Alzheimer's disease.

For these actions on the brain, HupA may be useful in cases of dementia and memory impairment. In animal tests, HupA is orally bioavailable and well absorbed (Raves *et al.*, 1997). HupA appears to act quickly and remain active for many hours (Wang *et al.*, 1988). Repeated doses do not appear to promote tolerance or unresponsiveness (Laganiere *et al.*, 1991). Animal studies show it easily crosses the blood-brain barrier and enters all areas of the brain (Tang *et al.*, 1989). HupA trials have not shown significant toxicity or side effects.

The theory of mechanism of action of Huperzine A may depend on acetylcholine. HupA seems to inhibit the activity of acetylcholine esterase – so the breakdown of acetylcholine is slowed and the strength and duration of the nerve impulse is improved. This inhibition of acetylcholine breakdown may be the reason for the effect of HupA on improving memory and overall cognitive processes. The effects of HupA have been investigated in laboratory and clinical settings – with the overall findings that HupA is known to be a potent, reversible and selective inhibitor of acetylcholine esterase, with a rapid absorption and penetration into the brain in animal tests. It exhibits memory-enhancing activities in animal and clinical trials. Compared to existing medications for the treatment of Alzheimer's disease, such as tacrine, physostigmine and donepezil, Huperzine A possesses a longer duration of action and higher therapeutic index, and the peripheral cholinergic side effects are minimal at therapeutic doses. HupA may also reduce neuronal cell death caused by glutamate – an action that further enhances the potential value of HupA as a therapeutic agent for Alzheimer's disease.

LYP/6-31G* method and IR spectrometry have been used to investigate the natural and binding structures of Huperzine B (HupB) in order to better understand the interaction nature between acetylcholinesterase (AChE) and its inhibitor, with the view of designing new AChE inhibitors. The predicted and exptl. results reveal that both the natural state and binding form of HupB adopt the chair conformation. Furthermore, the B3LYP/6-31G* results suggest that structure S1 should be the dominant form of the

two possible chair structures. The results also show that the condensed ring structure composing of rings A, B and C is very rigid. Therefore, its flexibility does not need to be considered when we try to dock this structure to its target. Indeed, this supposition is confirmed by the excellent alignment of the binding structure produced from our recent X-ray crystallographical structure of the HupB-AChE complex with the B3LYP/6-31G* predicted geometry. Among all the 111 predicted vibrational bands, the mode 110, which is resulted from the stretching of the bond N2-H and having the second highest frequency, is essential for the geometrical identification. The difference between the predicted strongest absorption band and experimental. IR spectrum suggests that a strong intermolecular interaction, which could be a hydrogen bond, exists in HupB crystal. The electrostatic potential surface of HupB derived from B3LYP/6-31G* CHelpG atomic charge suggests a mechanism of how HupB would interact with its target. In addition, the good agreement between predicted vibrational bands (scaled by a factor of 0.96) and experimental result shows that B3LYP/6-31G* is a good tool for studying such kind of natural compound (Luo et al, 2002).

In humans, the effects of Huperzine A are considered a promising therapeutic agent for Alzheimer's disease and memory deficit. In one study of 103 patients with Alzheimer's disease, (multi-center, prospective, double-blind, parallel, placebo controlled and randomized), 50 received 200 mcg of Huperzine A and 53 received a placebo for 8 weeks. Study results showed that about 58% (29/50) of patients treated with Huperzine A showed significant improvements in their memory, cognitive and behavioral functions versus only 36% of those receiving the placebo. No adverse side effects were reported. In another study of teenage Chinese students, the effect HupA on memory and learning performance was studied using a double-blind, matched pair, placebo controlled design in which 34 pairs of students complaining of memory inadequacy were given HupA (100 mcg HupA, taken twice per day) or a look-alike placebo for 4 weeks. At the end of trial, the students receiving HupA had significantly higher scores on tests of memory (memory quotient) compared to the placebo group.

Sage (*Salvia officinalis*) leaves are also traditionally used to support memory. Spanish sage is naturally very low in thujone, a potentially toxic compound. Many studies evaluate Spanish sage in patients with AD because

of the low thujone content. Since the herb has been used long-term for treating AD, a species with low thujone content is necessary. There are several small clinical trials in patients with mild-to-moderate AD, which show a positive effect on cognitive function. A typical dose is 3-5 mL, 3 times per day.

Garden Sage is a hardy perennial with leaves that are pebbly textured that may be green, golden-edged, tricolor, or purple. Plants range from 1 to 2 feet tall. Fresh sage has better flavor than dried. Use in stuffings, or with cheese, egg, or vegetable dishes. Snip off and dry flowering stems of older plants for decorative. It is harvested when plants are flourishing, but before flowering shoots develop, take stem tip cuttings with several sets of leaves.

Fig. 3 : ROSEMARY *Rosmarinus officinalis*

Perennial can withstand drought and poor, rocky soil. Grown for fragrant, resinous, grey-green foliage and beautiful blue flowers. Small, lance-shaped leaves and flowers grow in clusters from the leaf axils. ‘Arp’ is upright variety w/ grey-green foliage.

Rosemary is used historically as a memory enhancer, but research on it is lacking. A typical adult dose of fresh-herb tincture used alone is 3-5 mL, 3 times/day which is found apt for the study.

Fresh rosemary and sage were chopped and ensiled in 0.5-L anaerobic jars. Treatments comprised control (no additives), 0.5% glucose and lactic acid bacteria, and 1% cellulase plus 1% hemicellulase plus pectinase. Following storage at room temperature for 45 days (experiment 1) and 26 days (experiment 2), polyphenols were extracted from the silages in ethanol either by direct blending or by cold extraction. The enzyme treatment resulted in silages with the lowest pH values, lowest fiber content, highest water-soluble sugar content, and highest polyphenol recovery; this treatment resulted in increased polyphenol recovery from rosemary and sage, by 100 and 20%, respectively.

Fig. 4 : Sage Salvia Officinalis

***Curcuma longa* L.**

Regarded as a 'rasayana' herb in Ayurveda (to counteract ageing processes), *C. longa* (Zingiberaceae), known in English as 'turmeric,' has also been used for culinary purposes. Much research has focused on curcumin, a curcuminoid from *C. longa* rhizomes. In particular, studies have shown that some curcuminoids are associated with antioxidant and anti-inflammatory activities, but studies with particular attention to cognitive disorders and any clinical effects are lacking. In addition, further evaluation of potentially active compounds from *C. longa*, other than the curcuminoids, may contribute to the understanding of the traditional uses of this herb.

The antioxidant activity of curcumin is well documented (Das and Das, 2002; Miquel *et al.*, 2002; Priyadarsini, 1997; Scartezzini and Speroni,

2000). Curcumin was shown to be neuroprotective against ethanol-induced brain injury in vivo following oral administration; an effect that was related to a reduction in lipid peroxide levels and enhancement of glutathione in rat brain (Rajakrishnan *et al.*, 1999). Some compounds from *C. longa*, including curcumin, demethoxycurcumin, bisdemethoxycurcumin and calebin-A (and some of its synthetic analogues), were shown to protect PC12 cells from -amyloid insult in vitro (Kim and Kim, 2001 and Park and Kim, 2002); this activity was also suggested to be due to an antioxidant effect (Kim *et al.*, 2001).

Curcumin is also reported to be anti-inflammatory (Miquel *et al.*, 2002) and has been suggested to modulate eicosanoid biosynthesis and to inhibit cyclooxygenase (COX)-1, COX-2 and lipoxygenase (LOX) (Ramsewak *et al.*, 2000; Skrzypczak-Jankun *et al.*, 2000 and Srivastava *et al.*, 1995). Another activity that is perhaps relevant to the management of symptoms of cognitive-related disorders is antidepressant activity. An aqueous extract of *C. longa* demonstrated antidepressant activity in mice following oral administration, which was associated with inhibition of brain monoamine oxidase (MAO) A (Yu *et al.*, 2002).

A remarkable discovery in the retinula cells of *Mitopus morio* was the demonstration, by enzyme histochemistry, of acetylcholine esterase in large numbers of small vesicles. Dark specks in the vesicles (arrowed) close to the rhabdom are electron-opaque particles of reaction product produced by enzymic splitting of acetylthiocholine in a solution so that copper thiocholine is deposited - the dark specks.

AChE and PON1 Genes

Anxiety involves complex, incompletely understood interactions of genomic, environmental, and experience-derived factors, and is currently being measured by psychological criteria. Sklan *et al* (2004) reported nonperceived interrelationships between expression variations and nucleotide polymorphisms of the chromosome 7q21-22 acetylcholinesterase-paraoxonase 1 (ACHE-PON1) locus with the trait- and state-anxiety measures of 461 healthy subjects from the Health, Risk Factors, Exercise Training, and Genetics Family Study. The AChE protein controls the termination of

the stress-enhanced acetylcholine signaling, whereas the PON protein displays peroxidase-like activity, thus protecting blood proteins from oxidative stress damages. Serum AChE and PON enzyme activities were both found to be affected by demographic parameters, and showed inverse, reciprocal associations with anxiety measures. Moreover, the transient scores of state anxiety and the susceptibility score of trait anxiety both appeared to be linked to enzyme activities. Their finding supported the notion of corresponding gene expression relationships. Parallel polymorphisms in the ACHE and PON1 genes displayed apparent associations with both trait- and state-anxiety scores. It indicates that a significant source of anxiety feelings involves inherited and acquired parameters of acetylcholine regulation that can be readily quantified, which can help explaining part of the human variance for state and trait anxiety.

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