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Review article

The psychopharmacology of huperzine A: an alkaloid with cognitive enhancing and neuroprotective properties of interest in the treatment of Alzheimer's disease

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Abstract

Huperzine A (HupA), extracted from a club moss (*Huperzia serrata*), is a sesquiterpene alkaloid and a powerful and reversible inhibitor of acetylcholinesterase (AChE). It has been used in China for centuries for the treatment of swelling, fever and blood disorders. It has demonstrated both memory enhancement in animal and clinical trials and neuroprotective effects. Recently it has undergone double-blind, placebo-controlled clinical trials in patients with Alzheimer's disease (AD), with significant improvements both to cognitive function and the quality of life. Most of the clinical trials are from China, but HupA and derivatives are attracting considerable interest in the West, where AD is a major and growing concern. Furthermore, both animal and human safety evaluations have demonstrated that HupA is devoid of unexpected toxicity. Other interesting aspects of HupA pharmacological profile relate to its neuroprotective properties: it has been shown in animal studies that HupA can be used as a protective agent against organophosphate (OP) intoxication and that it reduces glutamate-induced cell death.

Keywords: Cholinesterase; Cognitive enhancer; Neuroprotective; Alzheimer's disease; Natural product; Huperzine A

1. Introduction

1.1. From traditional Chinese medicine to huperzine A

Traditional Chinese medicine tends to raise the natural defences of the organism instead of trying to restore its natural functions, and it offers a vast repertory for pharmaceutical research. The experience accumulated during centuries inspires the search for new drugs in modern times. Huperzine A (HupA) is a good example of this continuum.

HupA is a plant-based alkaloid. In China the folk medicine *Qian Ceng Ta* (*Huperzia serrata*), a source of HupA, has been used for centuries to treat fever, inflammation, blood disorders and schizophrenia (Liu et al., 1986). HupA acts as a potent, highly specific and reversible inhibitor of acethylcholinesterase that crosses the blood–brain barrier. Its potency of acetylcholinesterase (AChE) inhibition is similar or superior to that of physostigmine, galanthamine, donepezil and tacrine (Table 1) (Wang and Tang, 1998a; Wang et al., 1986). The latter three are acethylcholinesterase inhibitors (AChEIs) approved for Alzheimer's disease (AD) in the United States and some European countries. Cholinesterase inhibitors increase the amount of ACh at the neuronal synaptic cleft by inhibiting the enzyme responsible for the hydrolysis of ACh and consequently improve neuronal transmission.

1.2. Alzheimer's disease

HupA has been found to reverse or attenuate cognitive deficits in several animal models, such as passive footshock avoidance (Gao et al., 2000; Zhu and Tang, 1987, 1988; Lu et al., 1988), water maze (Liu et al., 1998; Ye et al., 2000), spatial radial arm maze discrimination (Xiong and Tang, 1995), and delayed response performance (Tang et al., 1986). Similarly, cognition enhancement was obtained in aged monkeys in a delayed recognition task (Ye et al., 1999). Clinical trials in China, where it has been approved and clinically used as a symptomatic agent for AD, demonstrated a significant improvement in memory of aged subjects and patients with AD, with minimal peripheral

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Table 1
Cholinesterase power of HupA, donepezil, tacrine and galanthamine

IC_{50} (μM)			
	AChEIs		K_i (nM)[a]
	AChE (rat cortex)	BuChE (rat serum)	
HupA	0.082	74.43	24.9
Donepezil	0.010	5.01	12.5
Tacrine	0.093	0.074	105.0
Galanthamine	1.995	12.59	210.0

From Bai et al. (2000).
[a] Rat erythrocyte membrane AChE.

cholinergic side effects typical of other AChEIs in use, particularly without the dose-limiting hepatotoxicity induced by tacrine (Xu et al., 1999; Zhang et al., 1991, 2002b; Zhang and Wang, 1990). Adverse effects have been reported at a very low rate in all the clinical trials, and are mainly cholinergic, such as dizziness, nausea, gastroenteric symptoms, headaches and depressed heart rate.

In the United States, where HupA is not yet approved by the FDA, it is sold as a dietary supplement for memory loss and mental impairment. It has rapidly become a cult supplement in the smart-drugs market and a best-selling item, being available without prescription in "natural product" shops and over the Internet.

The *Journal of the American Medical Association* (*JAMA*) in March of 1997 stated, "Huperzine A appears to be strongly specific for AChE, which suggests that it can be effective without the adverse effects that have been caused by drugs used to treat memory loss and dementia" (Skolnick, 1997).

1.3. Neuroprotection

HupA may also reduce neuronal cell death caused by an excess of glutamate (Ved et al., 1997), an action that further enhances the potential value of HupA as a therapeutic agent for AD. Recently, additional pharmacological properties have been demonstrated. It is a prophylactic drug against the irreversible AChEI soman and other nerve gases (Lallement et al., 1997, 2002a; Grunwald et al., 1994). It is also a powerful neuroprotective and antioxidant agent (Xiao et al., 2000; Wang et al., 2002a; Zhou et al., 2001; Shang et al., 1999) and a protective against amyloid beta peptide-induced neuronal cell death (apoptosis) (Xiao et al., 2000).

This review expands upon previous reviews (i.e., Tang and Han, 1999; Pilotaz and Masson, 1999), providing a comprehensive survey of preclinical and clinical studies with HupA, covering the literature up to the end of 2002.

2. Phytobiology

HupA is found in the Chinese club moss known as *H. serrata* (from a new botanical classification), or *Lycopodium serrata*. In the Devonian period, the Lycopods, some of the oldest vascular plants (according to fossil records), were large trees, but nowadays they are found in forests as common club mosses, and one of them is the *H. serrata*. Natural AChEIs have been identified from other plants as well, e.g., both alkaloids physostigmine from *Physostigma venenosum* and galanthamine from *Galanthus nivalis*.

Fig. 1. *H. serrata*.

The plant source, traditionally called *Qian Ceng Ta*, meaning thousand-laid pagodas (due to the tall, multileafed structure of the plant), has a second name in China: *Jin Bu Huan*, meaning "more valuable than gold," a definition applied to plants with analgesic properties. The powerful analgesic and antipyretic properties of the plant alkaloids were the reason for their traditional use in the treatment of fever and swelling (Pilotaz and Masson, 1999).

The whole herb (Fig. 1) contains triterpenoids (they may have a role in the traditional uses of the plant) and various alkaloids, including lycodoline, lycoclavine, serratinine and huperzines. Alkaloids represent 0.2% of the total content. Huperzine occurs in different chemical species, with similar properties but different strengths (HupA is about 10 times as strong as the B form). The average content of HupA in plants is 0.011% (Liu et al., 1986). The (−)-A species was first isolated by Chinese scientists from the herb *L. serrata* in 1986 (Liu et al., 1986). Lycopodium alkaloids comprise four rings, one of which may be open. The huperzines contain a nitrogen within one of the rings and an NH_2 group attached to the ring structure (Fig. 2).

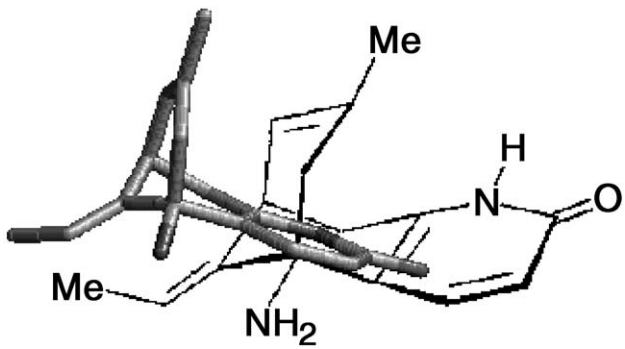

Fig. 2. Chemical structure of HupA and a 3-D view superimposed.

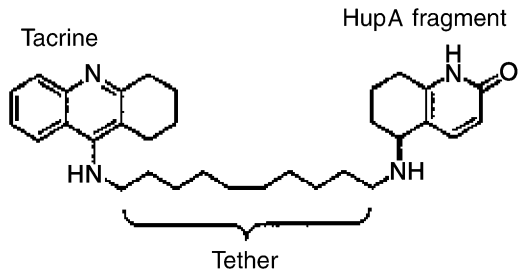

Fig. 3. The tether.

The original process for making the herbal extract was released in 1986 without patent protection. This is one of the reasons why HupA is sold in the United States as a dietary supplement and pharmaceutical companies have not yet invested in the clinical studies required by the FDA to approve a new drug. The low content of this alkaloid in nature has prompted successful attempts to synthesise this molecule or analogues (Qian and Ji, 1989; Kozikowski and Xia, 1989; Kozikowski et al., 1998; Mulzer et al., 2001). Paul Carlier, Associate Professor of Chemistry at Virginia Tech, in Blacksburg, Virginia, extended previous investigations (Pang and Kozikowski, 1994) and synthesised a drug similar to the natural compound, exploring the fusion products of (−)-HupA and tacrine (Fig. 3) joined by a tether (Carlier et al., 1999). These initial patented tacrine–HupA hybrids had slightly higher affinity for AChE than tacrine itself. Recently, huprine X, a hybrid that combines the carbobicyclic substructure of HupA with the 4-aminoquinoline substructure of tacrine, has been synthesised with one of the highest affinities yet reported (K_i of 26 nM) for human AChE (Camps et al., 2000). Under equivalent assay conditions, this affinity was 180 times that of HupA, 1200 times that of tacrine and 40 times that of donepezil. Compared with other classes of drugs available for the treatment of AD, such optimisation of an AChE inhibitor may provide much more effective symptomatic treatment. Another interesting analogue is ZT-1, a HupA derivative. It represents a prodrug that is progressively hydrolysed into HupA, the active principle.

3. Biochemistry

HupA is an unsaturated sesquiterpene alkaloid with a pyridone moiety and primary amino group. Its empirical formula is $C_{15}H_{18}N_2O$, and molecular weight 242. Chemically, HupA is 9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3.8)]trideca-3(8),6,11-trien-5-one (structure in Fig. 2). The compound is optically active and in the plant is present only in its (−)-enantiomer. It is a very stable molecule, with a white-crystal appearance, soluble in aqueous acid and $CHCL_3$ (Geib et al., 1991).

HupA is a potent reversible inhibitor of AChE over butyrylcholinesterase (BuChE) (Ashani et al., 1992), which is inhibited 1000-fold less than AChE, and works at nanomolar concentrations (see next section), with a stereoselective mechanism: (−) HupA, the naturally occurring form, is the more potent enantiomer, whereas (+) HupA inhibits the enzyme 38-fold less potently (Hanin et al., 1993).

4. Pharmacology

4.1. Anticholinesterase action

HupA, a potent reversible inhibitor of AChE (K_i = 20–40 nM), binds with aromatic residues in the active site gorge of AChE, localising between Trp86 and Tyr337 in the enzyme (Ved et al., 1997). The formation of the AChE–HupA complex is rapid, and the dissociation is slow (Ashani et al., 1992). This complex has been studied utilising kinetic, computer-aided docking, and X-ray crystallography approaches. In particular, the X-ray structures of AChE from the *Torpedo californica* fish (one of the richest sources of this enzyme) complexed with HupA demonstrated a high affinity for the AChE (Fig. 4). HupA appears to bind more tightly and specifically to the deep chasm known as the active-site gorge of AChE (which guides ACh molecules to the enzyme's cleaving machinery) than do other AChE inhibitors, such as tacrine and edrophonium. The structural analysis surprisingly revealed that HupA bears no resemblance to ACh and that the HupA–AChE complex binds to the active-site gorge of AChE with few direct contacts with the protein. Only one strong hydrogen bond is seen, as well as some hydrophobic interactions within the crystalline complex (Raves et al., 1997). The 3-D computer image of AChE–HupA binding generated in the Raves study revealed how the HupA blocks the enzyme by sliding smoothly into the active site of AChE where acetylcholine (ACh) is broken down, and latches onto this site via a large number of subtle chemical links. Joel Sussman, one of the authors of the study, commented: "It is as if this natural substance were ingeniously designed to fit into the exact spot on AChE where it will do the most good." It was also

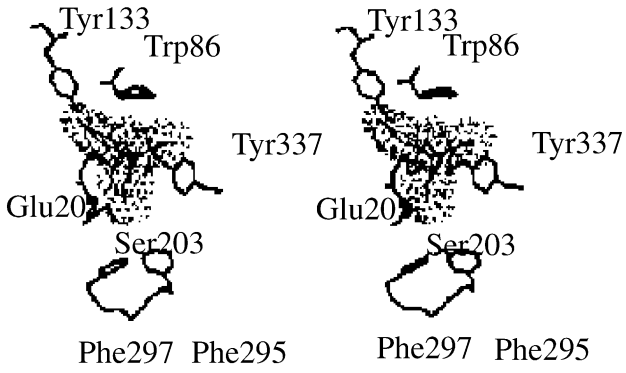

Fig. 4. Stereoscopic view of a model human AChE complex with HupA according to the X-ray structure of the *Torpedo* AChe complex (see text).

demonstrated that HupA can form an extra hydrogen bond with Tyr 337 within the choline site that exists only in mammalian AChE, but not in *Torpedo* enzyme and BuChE (Saxena et al., 1994; Sussman et al., 1991). The stronger inhibitory property of HupA for mammalian AChE than for the other two enzymes may rely on this particular interaction.

The cholinesterase inhibition activity of HupA has been evaluated in vitro and in vivo using spectrophotometric methods (Ellman et al., 1961).

4.1.1. In vitro

The concentration of inhibitors producing 50% inhibition of enzyme activity (IC_{50}) are listed in Table 1 for HupA and other AChEIs.

HupA initiated AChE (rat cortex) inhibition at 10 nM. The inhibition of AChE activity of HupA was more effective than that of tacrine and galanthamine, but less than that of donepezil. The pattern of inhibition is of the mixed competitive type. The inhibition on BuChE demonstrated a different profile: HupA inhibited BuChE at a higher concentration than needed for AChE compared with donepezil. The K_i values (inhibition constants, in nM) revealed that HupA was more potent than tacrine and galanthamine, but about twofold less potent than donepezil (Table 1). Compared with AChE in animals such as horse and rat, HupA is a weaker inhibitor of human serum BuChE. This selectivity for AChE as opposed to BuChE (similar to that of galanthamine) may suggest a better side-effects profile (Scott and Gao, 2000). However, a stronger inhibition of BuChE could be important in the later stage of AD (Ballard, 2002), and offer more protection over A-beta amyloid plaque deposition (Guillozet et al., 1997). In contrast to isoflurophate (DFP), the AChE activity did not decrease with the prolongation of incubation with HupA in vitro, and the AChE activity returned to 94% of the control after being washed five times, demonstrating a reversible inhibitory action (Wang et al., 1986).

4.1.2. In vivo

Following administrations of oral HupA at doses of 0.12–0.5 mg/kg, a clear, dose-dependent inhibition of AChE was demonstrated in brains of rats (Cheng and Tang, 1998; Tang et al., 1989). In contrast to the AChE inhibition in vitro, the relative inhibitory effect of oral HupA over AChE was found to be about 24- and 180-fold, on an equimolar basis, more potent than donepezil and tacrine, respectively. In rats, HupA injected intraperitoneally (ip) exhibited similar efficacy of AChE inhibition as demonstrated following oral administration, whereas ip administration of tacrine and donepezil showed greater inhibition on both AChE activity and serum BuChE (Wang and Tang, 1998a). The inhibitory action of HupA on brain AChE was less than that of donepezil after the intraventricular injection, but more effective than that of tacrine (Cheng and Tang, 1998). Maximal AChE inhibition in rat cortex and whole brain was reached at 30–60 min and maintained for 360 min following oral administration of 0.36 μg/kg HupA (Tang et al., 1989, 1994a; Wang and Tang, 1998a). The oral administration of HupA produced greater AChE inhibition compared with donepezil and tacrine, which indicated that it has greater bioavailability, and more easily penetrates the blood brain barrier (Table 2). Repeated doses of HupA showed no significant decline in AChE inhibition as compared to that of single dose, demonstrating that no tolerance to HupA occurred (Laganiere et al., 1991).

4.2. Effects on neurotransmitter levels

HupA caused a significant increase in ACh levels in rat brain. Rats treated with HupA at doses of 0.3, 0.5 or 2 mg/kg demonstrated increased brain ACh 6 h after administration (Tang et al., 1989, 1994b; Zhu and Giacobini, 1995). HupA produced a more prolonged increase of ACh levels in whole brain than did tacrine, heptylphysostigmine, physostigmine and metrifonate (De Sarno et al., 1989). The degree of ACh elevation was regionally selective: the maximal increase was seen at 60 min in the frontal and parietal cortex, intermediate at 30 min in the hippocampus and at 5 min in the medulla oblongata, and only slight increases at 30 min in the striatum (Tang et al., 1989, 1994a; Wang and Tang, 1998a). Considering that the level of ACh is particularly low in the cerebral cortex of patients with AD (Perry et al., 1978a; Bowen et al., 1983), this regional specificity produced by HupA may represent a therapeutic advantage. The choline levels or the levels of choline acetyltransferase were not altered in any region of the rat brain, suggesting that the biosynthesis of ACh was not altered (Laganiere et al., 1991; Tang et al., 1994a).

There was a significant dose-dependent increase over baseline of brain norepinephrine (NE) and dopamine (DA) as a result of systemic (ip) or local administration via microdialysis probe into the rat cortex (Zhu and Giacobini, 1995). This increase lasted for 6 h. Systemic HupA significantly increased ACh levels above baseline at doses of

Table 2
AChE inhibition of oral HupA, donepezil, and tacrine in rats

AChE inhibition (%) $n=6$				
ChEI	mg/kg	cortex	hippocampus	striatum
HupA	0.36	20±6*	17±3*	18±4*
	0.24	16±6*	15±3*	16±8*
	0.12	10±6*	8±7	13±10†
Donepezil	6.66	18±6*	12±5*	12±8
	5.00	11±6*	10±4*	10±6
	3.33	9±11	6±8	8±6
Tacrine	28.2	20±6*	11±10†	11±10†
	21.1	8±6*	9±6	8±41*
	14.1	7±7	2±2	2±5

From Tang and Han (1999).
Values expressed as percent inhibition ± standard deviation.
* $P<.01$.
† $P<.05$ vs. saline group.

0.1, 0.3 and 0.5 mg/kg by 54%, 129% and 220%, respectively. NE and DA levels were increased more than 100% after the 0.3 and 0.5 mg/kg doses. There were no changes at the 5-HT levels. These effects might contribute to the cognitive enhancing effects of HupA, because there is evidence of interaction between cholinergic and monoaminergic systems in the control of cognitive function (Decker and McGaugh, 1991), and the clinical effect of ChEIs has been related to the stimulation of both cholinergic and monoaminergic systems (Alhainen et al., 1993).

4.3. Cholinergic receptors

Studies on displacement of [^{3}H]QNB and [^{3}H]-(−) nicotine binding have shown little direct effect of HupA on cholinergic receptors, compared to other ChEIs, such as galanthamine and tacrine (De Sarno et al., 1989; Tang et al., 1989). HupA also lacks an effect on muscarinic receptors, whereas huprine X, a hybrid between tacrine and HupA, exhibited micromolar activity at M(1) and M(2) receptors, probably agonistic (Roman et al., 2002). This additional muscarinic activity of huprine X could be relevant and provide therapeutical advantages in dementia therapy (Fisher et al., 2002).

4.4. Protective properties

4.4.1. Nerve gas poisoning

Other interesting properties of HupA pharmacology relate to a broad range of protective actions. HupA has been tested as a prophylactic drug against soman and other nerve gas poisoning with excellent outcome (Grunwald et al., 1994). It works by protecting cortical AChE from soman inhibition and preventing subsequent seizures. This prophylactic use makes HupA a potential protective agent against chemical weapons. It has been demonstrated that rats can be protected against low doses of soman with pretreatment with only HupA, and without typical cholinergic side effects (Grunwald et al., 1994). This was confirmed in a study with primates, where HupA was compared with pyridostigmine (Lallement et al., 2002a): the cumulative dose of soman needed to produce convulsions and epileptic activity was 1.55-fold higher in the animals who received HupA compared to the group of primates pretreated with pyridostigmine. The same study demonstrated that huperzine selectively inhibited red cell AChE activity, whereas pyridostigmine also inhibited plasma BuChE. Thus, the superior protection offered by HupA appears related both to the selectivity of HupA for red cell AChE, preserving the scavenger capacity of plasma BuChEs for organophosphate (OP) agents, and to protection conferred on cerebral AChE (Lallement et al., 2002b).

4.4.2. Glutamate toxicity

HupA also protects primary neuronal cell culture and animals from glutamate toxicity. Glutamate activates *N*-methyl-D-aspartate receptors and increases the flux of calcium ions into the neurons (Gordon et al., 2001), and calcium at toxic levels can kill the cells (Sattler and Tymianski, 2000).

Pretreatment of primary neuronal cells with HupA reduced glutamate- and OP-induced toxicity and decreased neuronal death (Ved et al., 1997). The consequence of excitatory amino-acid-induced overstimulation has been implicated in a variety of acute and chronic neurodegenerative disorders, including Parkinson's disease, dementia, neuroleptic drug-induced side effects, spasticity, ischemic brain damage, epilepsy, anxiogenesis, traumatic brain injury, AD, OP-induced seizures and neuronal cell death (Choi, 1994). Other cholinesterase inhibitors available, such as donepezil, physostigmine and tacrine, also exhibit an antagonist effect on NMDA receptor in addition to their inhibitory effect on AChE (Wang et al., 1999). A comparative study (Ved et al., 1997) demonstrated that HupA is the most powerful in protecting mature neurons, followed by donepezil, physostigmine, and tacrine. In this research, HupA was particularly effective in protecting more mature neurons against neurotoxicity due to the presence of more functional NMDA receptors in mature neurons.

In addition to the loss of cholinergic function in patients with AD, glutamatergic and GABAergic neurotransmitter systems may also be compromised (Vajda, 2002). Thus, HupA, with its ability to attenuate glutamate-mediated toxicity, may be used to treat dementia and as a preventive agent by slowing down or blocking the pathogenesis of AD at an early stage (Gordon et al., 2001). Memantine, a partial NMDA antagonist, is a new drug that protects the brain against the excess of glutamate observed in AD. Its action differs from AChEIs such as HupA, which also temporarily boost levels of ACh. Memantidine could be used in combination with AChEIs or as stand-alone treatment in moderately severe to severe AD (Doraiswamy, 2002).

4.4.3. Oxidative stress

Increased oxidative stress, resulting from free radical damage to cellular function, can be involved in the events leading to AD, and is also connected with lesions called tangles and plaques. Plaques are caused by the deposition of amyloid beta-peptide (Abeta) and observed in brains of AD patients (Perry et al., 1978b; Selkoe et al., 1986). It is not clear how much these lesions and the oxidative stress are associated with other neurodegenerative diseases. HupA and tacrine were compared for their ability to protect against Abeta-induced cell lesion, level of lipid peroxidation, and antioxidant enzyme activities in rat PC12 and primary cultured cortical neurons (Xiao et al., 2000). Following pretreatment of both cells with HupA or tacrine (0.1–10 mM) before Abeta exposure, the survival of the cells was significantly elevated. Xiao et al. found that both drugs are similarly protective against Abeta toxicity, which results in a reduction of cell survival and glutathione peroxidase (GSH-Px) and catalase (CAT) activity, and both increase the

production of malondialdehyde (MDA) and superoxide dismutase (SOD). Administration of HupA reduced the apoptosis (programmed cell death) that normally followed beta-amyloid injection (Wang et al., 2001). Prevention in the expression of apoptosis-related proteins and limitation in the extent of apoptosis in widespread regions of the brain were also seen. Wang et al. suggested that these actions may reflect a regulation of expression of apoptosis-related genes.

It has been recently demonstrated that the neuroprotective properties of HupA enantiomers have no relation to anticholinesterase activity: preincubation with (+)-HupA or (−)-HupA (0.1–10 mM) protected cells with similar potency against Abeta toxicity and significantly enhanced survival (Zhang et al., 2002a). This result contrasted with the stereoselectivity of cholinesterase inhibition in vitro and in vivo, in which (−)-HupA is more potent than (+)-HupA.

4.4.4. Hypoxic–ischemic brain injury

It has been suggested that by having effects in the cholinergic system and also on the oxygen free radical system and energy metabolism, HupA may be useful for the treatment of vascular dementia (Wang et al., 2002a).

The protective effect of HupA on hypoxic–ischemic (HI) brain injury was investigated in neonatal rats in which a combination of common carotid artery ligation and exposure to a hypoxic environment caused great brain damage (Wang et al., 2002b). HupA administrated daily to neonatal rats, at the dose of 0.1 mg/kg ip for 5 weeks after HI injury produced significant protection from damage after HI injury, and on behaviour (decreased escape latency in water maze) and neuropathology (less extensive brain injury). Consequently, Wang et al. concluded that HupA might be effective in the treatment of HI encephalopathy in neonates. Similar protection was obtained by administering subchronical oral doses of HupA (0.1 mg/kg, twice daily for 14 days) following 5 min of global ischemia in gerbils (Zhou et al., 2001). An herbal extract from the plant *Ginkgo biloba* (ginkgolide A, B), which showed nootropic effects (Kennedy et al., 2000; Wesnes et al., 2000), was tested together with HupA for neuroprotective actions. The nitric oxide (NO) production from human BT325 astrocytoma cells was concentration-dependently inhibited by Hup A, and ginkgolide A or B, 0.01–10 micromol l^{-1} for 24 h (Zhao and Li, 1999).

4.4.5. Free radical level

A reduction in the level of abnormal free radicals was demonstrated in a study on the effects of HupA on lipid peroxidation and superoxide dismutase in the hippocampus, cerebral cortex and serum of aged rats (Shang et al., 1999). A reduction in the plasma and erythrocyte oxygen free radicals was also demonstrated in a clinical study (Xu et al., 1999).

Huperzine B showed neuroprotective properties similar to HupA, and to other AChEIs (donepezil, galanthamine, tacrine), attenuating the hydrogen-peroxide-induced injury (Zhang and Tang, 2000).

4.5. Pharmacokinetics

The pharmacokinetics of HupA has been studied in rats and humans. An autoradiographic study in mice after intravenous (iv) injection of a dose of 183 μg/kg. (Tang et al., 1989) showed the presence of HupA in all regions of the brain, with particularly high concentrations in the frontoparietal cortex, striatal cortex, hippocampus and nucleus accumbens. In mice, radiolabelled HupA was found highest in kidney and liver 15 min after iv administration. After 12 h, no radioactivity was found in any part of the body. In pregnant mice (Wang et al., 1988), a small amount of radioactivity was detected in the foetus. HupA was mainly (73%) excreted in the urine 24 h after iv injections, and only 2.4% of radioactivity was found in the faeces. The HupA eliminated from the kidney was part as metabolites and part as prototype.

In six young healthy volunteers, oral HupA was absorbed rapidly, distributed widely in the body and eliminated at a moderate rate. Drug concentrations in plasma were monitored at various time points by reverse-phase high-pressure liquid chromatography. HupA conformed to a one-compartment open model with first absorption and first elimination. Following a supratherapeutic oral dose of 0.99 mg, peak serum concentrations were reached in 79 min and half-life was 288 min, suggesting a daily dose schedule of two or three administrations. No notable side effects were observed with doses between 0.18 and 0.54 mg (Qian et al., 1995).

4.6. Toxicology

Toxicological studies conducted in different animal species indicated less severe undesirable side effects associated with cholinergic activation for HupA than for other ChEIs such as physostigmine and tacrine (Yan et al., 1987; Wang and Tang, 1998a). In mice, the LD_{50} doses were 4.6 mg po, 3.0 mg sc, 1.8 mg ip, and 0.63 mg iv. Histopathological examinations showed no changes in liver, kidney, heart, lung and brain after administration of HupA for 180 days, in dogs (0.6 mg/kg im) and in rats (1.5 mg/kg po). No mutagenicity was found in rats (Zenghong and Meiying, 1990), and no teratogenic effect in mice or rabbits.

5. Preclinical studies

HupA has been shown to have memory-enhancing activities in mice, with a superior safety/efficacy ratio when compared with other AChEIs (Table 3).

Beneficial effects on learning and memory performance were seen in rodents following the administration of 0.001–0.5 mg/kg on various tasks including spatial dis-

Table 3
Comparison of efficacy/toxicity of cholinesterase inhibitors in mice

ChEIs	Memory enhancement (μM/kg po)	Acute LD_5 (μM/kg po)
HupA	0.83	17.31
Physostigmine	1.09	6.14
Galanthamine	5.43	71.96
Tacrine	68.17	199.83

From Tang and Han (1999).
Memory enhancement (retention memory) assessed by step-down passive avoidance performance.

crimination of radial arm maze (Xiong and Tang, 1995;), water maze (Liu et al., 1998) and passive footshock avoidance (Tang et al., 1986; Zhu and Tang, 1987, 1988; Lu et al., 1988). Memory enhancement was shown in rats and monkeys by reversing effects of both aging (Lu et al., 1988; Ye et al., 1999) and experimental cognitive impairment produced by cholinergic lesions (Xiong et al., 1995, 1998), scopolamine (Ye et al., 1999; Gao et al., 2000; Wang and Tang, 1998b), electroshock, cycloheximide, $NaNO_2$, CO_2 (Lu et al., 1988), reserpine and yohimbine (Ou et al., 2001). As with other cognitive enhancers, the dose–response curve for HupA is of the inverted U-shape type. The effects on learning and memory retention lasted longer than those obtained with the other AChEIs, physostigmine, galanthamine and tacrine (Tang et al., 1994a). Further, after repeated daily administrations of HupA, no significant tolerance in cognitive improvement was seen (Xiong and Tang, 1995).

Scopolamine (0.15 mg/kg ip) causes significant impairment in the ability of rats to complete the radial maze task. HupA (0.2–0.4 mg/kg po; 0.1–0.4 mg/kg ip) Donepezil (0.6–0.9 mg/kg po; 0.3–0.6 mg/kg ip) and tacrine (1.5–2.5 mg/kg po; 0.3–0.6 mg/kg ip) have been compared for effects on scopolamine-induced working and reference memory errors (Wang and Tang, 1998b). The AChE inhibitors were administered 30 min before the behavioural testing. All three compounds tested had an inverse bell-shaped dose-dependent effect. HupA was the most potent and orally active in reversing the scopolamine-induced errors. More improvement was seen on working memory than on reference memory, an effect that, if transposed to AD, may be relevant to improving impairment in memory for recent information.

The dose-related deficit induced by scopolamine (0.01, 0.02 and 0.03 mg/kg) in young adult monkeys was reversed significantly up to 24 h with various doses of HupA (0.001, 0.01, 0.1 and 0.2 mg/kg) (Ye et al., 1999). The improvement was marked and highly significant on a delayed-response task (choice accuracy with food reward). The range of delays (five different delay lengths distributed over 30 daily test trials) were adjusted during training sessions to produce performance levels of 90% correct trials. The performance changed from an average of 27/30 trials correct on control to 20 of 30 correct trials after scopolamine. After administration of 0.1 mg/kg HupA, the correct trials changed to an average of 25 of 30. The dose–response curve was bell-shaped with maximum improvement at 0.1 mg/kg. Neither the lowest nor the highest doses had significant effects, and the cognitive-enhancing effect of HupA was more evident at the longest delay. In the same experiment, a second group of monkeys, older and not pretreated with scopolamine, performed significantly better in a similar choice accuracy task (25/30 trials correct) following a lower dose of HupA (0.001–0.01 mg/kg), compared with placebo (20/30 trials correct). The dose–response was again bell-shaped, similar to that observed in scopolamine-pretreated monkeys. The beneficial effect lasted for 24 h in both groups, and no adverse signs were observed, even at the highest doses.

On the Morris water maze task, rats pretreated with scopolamine performed better after receiving either natural or synthetic HupA (Liu et al., 1998), showing a similar cognitive-enhancing effect of the enantiomers. Subchronic administration of HupA in guinea pigs was recently studied again using the Morris water maze test (Filliat et al., 2002). HupA did not induce deleterious effects on spatial memory, but the effect of HupA on learning appeared not significant.

6. Clinical studies

HupA satisfies criteria for a potential new drug for the symptomatic treatment of AD. It is specific for AChE over other enzymes, selective for brain AChE over peripheral AChE (reducing peripheral cholinergic side effects), has a long duration of actions, high bioavailability after oral administration, and has stronger or equivalent inhibition over AChE when compared with current prescribed inhibitors. Further, it is effective against glutamate-induced neuronal death. Its clinical evaluation is now in phase IV clinical trials of AD.

One of the first clinical studies of HupA, and the only known study of HupA for myasthenia gravis, demonstrated an improvement in muscle weakness in 128 patients (Cheng et al., 1986). All the patients before the start of the study received prostigmine intramuscularly to stabilise their conditions. Treatment and control groups were matched for age and gravity of their conditions. HupA 0.4 mg im daily was administered to 59 patients for 10 days, whereas the control group received neostigmine 0.5 mg per day every other day and HupA 0.4 mg daily on intervening days. The duration of effect was 7 h, 3 h more than that of neostigmine. Secondary effects were mainly cholinergic and, with the exception of nausea, were significantly milder than with neostigmine.

In a group of 100 aged subjects, 17 with AD and the others reporting memory problems, acute treatment of HupA (30 μg im), was compared to a treatment of 6 mg Hydergine (dihydroergotoxine). HupA had a positive effect, in comparison with dihydroergotoxine, on the memory of the subjects between 1 and 4 h after administration, with no remarkable side effects. (Zhang, 1986).

A multicenter, randomised, double-blind, placebo-controlled study (Zhang et al., 1991), evaluated HupA for the treatment of memory disorders in the elderly. Fifty-six patients with multi-infarct dementia or AD were given 0.05 mg im of HupA im or placebo twice daily for 1 month. A further 104 patients with presenile or senile memory disorders were given 0.03 mg im of HupA or placebo twice daily for 2 weeks. All patients had a diagnosis of disease for at least 2 years and stopped all other medication 7 days before the start of the study. Therapeutic effects were measured with the Wechsler Memory Scale (WMS). Patients treated with HupA showed significant improvement in their memory quotient (MQ), and only few side effects were reported (mainly nausea and dizziness).

In another randomised, double-blind placebo-controlled study, 103 patients, enrolled after a diagnosis of AD according to the *Diagnostic and Statistical Manual of Mental Disorders—Third Revision* (DSM III-R) criteria, were given 200 μg of HupA or placebo orally and twice daily for 8 weeks (Xu et al., 1995). Their conditions were evaluated with the WMS, Hasegawa Dementia Scale (HDS), Mini-Mental State Examination (MMSE), and Activities of Daily Living scale (ADL). Baseline measurements of blood pressure, heart rate, electrocardiogram, electroencephalogram and samples of blood and urine were obtained. The patients stopped all the medications 1 week before the study. Twenty-nine (58%) of the patients treated with HupA showed a significant improvement in memory over baseline in all the tests, compared with 19 (36%) of the placebo group. The improvement over placebo was still significant when measured after 8 weeks (Table 4) in the WMS scale, in MMSE and HDS. The average improvement over placebo was evident when comparing results in the MMSE between HupA-treated patients (3.0 points) and the placebo group (0.4 points). Side effects were principally cholinergic and mild. Incidence of diarrhea (10%), anorexia (10%) and hyperactivity (10%), nausea or vomiting (8%) was comparable with the rate in the placebo group. There were no changes from baseline in the laboratory tests, but the vital signs revealed a clinical relevant bradycardia (mean heat rate decreased from 72 to 47 beats/min).

Table 4
Efficacy of HupA in patients with AD

	Placebo ($n=53$)	HupA (0.2 mg, $n=50$)
Memory quotient		
Before trial	47.9 ± 21.5	55.8 ± 21.1
8 weeks trial	51.6 ± 25.6*	64.4 ± 26.2*
Mini Mental State Scale		
Before trial	14.4 ± 4.7	16.0 ± 5.0
8 Weeks trial	14.9 ± 6.4	18.9 ± 6.2*,†
Hachinski ischemic scale		
Before trial	15.6 ± 5.3	16.1 ± 5.6
8 Weeks trial	15.4 ± 6.7	19.7 ± 6.5*,†
Activity of daily living scale		
Before trial	30.7 ± 9.3	32.6 ± 9.6
8 weeks trial	31.9 ± 0.7	29.1 ± 9.3*

See text for details. From Xu et al. (1995).
* $P<.01$ vs. before trial.
† $P<.01$ vs. placebo group.

The efficacy and safety of HupA for treating AD were tested by comparing both capsules and tablets on 60 patients who satisfied the AD criteria of DSM III-R and National Institute of Neurological and Communicative Disorders and Stroke (NINCDS-ADRDA), using multicenter, double-blind, placebo-controlled, parallel, positive controlled and randomised methods (Xu et al., 1999). Patients were divided into two equal groups, one receiving four capsules of HupA (each containing 50 μg) and four tablets of placebo, while the other received four tablets of HupA (each containing 50 μg) and four capsules with placebo. Administration was twice daily and for 60 days. Vital signs and laboratory tests were taken monthly. Evaluation of cognitive and behavioural functions was based on monthly administration of MMSE, HDS-revised (HDS-R), instrumental activity of daily living (IADL), Gottfries–Bräne–Steen Scale for dementia syndromes (GBS-SDS), and Treatment Emergent Symptoms Scale (TESS). Measures of ECG, EEG, and WMS were taken at the start and the end of the trial. There were significant differences on all the psychological evaluations between "before" and "after" the 60 days of HupA treatment, but there was no significant difference between the two groups. The changes of oxygen free radicals showed marked improvement in the plasma and erythrocytes, but the correction was partial, and the distance from the reference value of healthy aged was still significant, leading the authors to suggest that symptomatic treatment with HupA in patients with pathological changes, must be undertaken for long periods. The incidence of peripheral cholinergic side effects (mild to moderate nausea and mild to moderate insomnia) of HupA revealed with TESS score was up to 33%, and they diminished or disappeared by the end of the trial. The efficacy and safety of HupA in tablets and capsules appears to be equal; in other words, Xu concluded that HupA is a safe and promising drug for symptomatic treatment of AD, but further, long-term observations and direct clinical comparison with other AChEIs are needed.

In the United States the safety and efficacy of HupA were evaluated in 26 patients meeting the DSM IV-R and the NINCDS-ADRDA criteria for uncomplicated AD and possible or probable AD (Mazurek, 1999). This study (office-based) lasted 3 months and was open label. Other therapies, including tacrine, donepezil and *G. biloba* were continued. An oral dose of 50 μg HupA was given twice a day to 22 patients, and the 4 other patients received a dose of 100 μg twice daily. A mean dementia baseline score of 22.6 was measured with the MMSE. The changes in this score, for the 50-μg group and for the 100-μg group, respectively, were 0.5 and 1.5 points at 1 month; 1.2 and 1.8 points at 2 months; and 1.1 and 1.0 points at 3 months. Despite the

Table 5
Memory quotient in the two groups before and after 4 weeks

Memory quotient	HupA	Placebo
Baseline	92 ± 7*	94 ± 8
4 Weeks trial	115 ± 6	104 ± 9
Odds	23 ± 7*	11 ± 10

Data from Sun et al. (1999).
Data are expressed as value ± S.D. ($n = 34$).
* $P < .01$.

small number of patients, the authors observed dose-related improvements with higher MMSE scores at higher dosage, and no serious side effects.

Zhang et al. (2002b) have recently evaluated the use of HupA in patients meeting the DSM IV criteria for possible or probable AD. Two-hundred and two patients aged between 50 and 80 years were enrolled from 15 centers in five cities in China. The study was multicenter, double-blind, randomised and placebo-controlled, and the testing period was 12 weeks. Patients were randomly divided into a HupA treatment group ($n = 100$) and a placebo group ($n = 102$). The initial dose of HupA was 100 μg twice daily, taken orally with breakfast and dinner. The dose was increased to 150 μg twice daily from Week 2 to Week 3, and up to 200 μg twice daily from Week 4 to Week 12. The dosage was adjusted according to the reaction of the patients. The placebo group followed the same scheme. Both groups were given an oral dose of vitamin E 100 mg twice daily as basic treatment. The results were assessed using ability of daily life (ADL), ADAS-Cog, ADAS-non-Cog, MMSE, and CIBIC-plus scales. Safety checks including vital signs, physical, neurology and laboratory tests were carried out every 6 weeks. In comparison with the baseline data, in the HupA-treated patients, the cognitive functions (MMSE, ADAS-Cog), noncognitive function (mood and behavior-ADAS-non-Cog) and ADL were all improved significantly at Week 6, and particularly at Week 12. Though the study did not have overall evaluation for the symptom of psychological behavior, the results from ADAS-non-Cog showed that huperzine had positive effect for symptoms of depression, delusions and repetitive activities. At the end of Week 12, there was a significant improvement over the placebo group in the CIBIC score. This indicated that HupA improved cognitive function and indices of daily life and behavior. This results were confirmed by the care givers who reported a significant improvement of the patients in daily life. There was a significant difference between the two groups at 6 weeks, indicating that HupA improved the condition of the patients from Week 6. The average age in the study was 70 years, so there was a high percentage of patients using other drugs for the treatment of other long-term disease. The percentage of mild and transient adverse events was 3% (insomnia and bilateral ankle oedema), similar to the placebo group. The study duration was not long enough, and did not test the maximum dosage possible. The authors concluded that while HupA appears to be a safe and effective treatment for AD, this needs to be confirmed in larger and longer clinical trials, using different doses (400 μg is not the maximum dose).

6.1. Cognitive effects in nonpatient populations

The effect of HupA on the performance of young adults was studied using a double-blind, matched-pair design (Sun et al., 1999). Junior middle school students ($n = 68$) complaining of learning and memory problems were divided into two paired groups according to normal psychological health inventory, similar memory quotient, same gender and class. For 4 weeks, the HupA group was administered orally two capsules (HupA 50 μg each) twice daily, and the placebo group two capsules of placebo, twice daily. At the beginning and end of the trial, the students were evaluated with the WMS and the TESS, and they performed tests of English, Chinese and mathematics. The findings of this study were that HupA improved memory. There were significant differences on MQ in both groups between "before" and "end" of the trial ($P < .01$), but the MQ of the HupA group was also significantly higher ($P < .01$) at the end of the 4 weeks, than that of the placebo group (Table 5).

Analysis of the WMS factors revealed that HupA increased the scores of "accumulation," "recognition," "association," "factual memory" and "number of recitations," but not "understanding," a result the authors found consistent with the findings from learning performance, where HupA enhanced the results of Chinese and English language lessons, but not mathematics (Table 6).

Table 6
Learning performances in the two groups after 4 weeks

	HupA	Placebo
English language		
Baseline	59 ± 20	68 ± 18
4 weeks trial	66 ± 18*	70 ± 17
Odds	6 ± 8	3 ± 12
Chinese language		
Baseline	59 ± 16	68 ± 9
4 weeks trial	70 ± 12*	70 ± 10*
Odds	10 ± 9	2 ± 7
Mathematics		
Baseline	58 ± 21	58 ± 22
4 weeks trial	68 ± 21*	69 ± 18*
Odds	9 ± 11	10 ± 14
Average of the three courses		
Baseline	59 ± 14	64 ± 12
4 weeks trial	68 ± 14*	70 ± 12*
Odds	9 ± 6	6 ± 7

Data from Sun et al. (1999).
Values are expressed as score ± S.D. ($n = 34$).
* $P < .01$.

Both scores of TESS in two groups were zero, indicating no occurrence of any side effects in the 4-week trial. Sun et al. (1999) concluded that HupA was a promising candidate drug for improving memory functions and learning aptitude in adolescent students, and that it should be studied in more controlled studies in the future.

A study conducted using the Cognitive Drug Research computerised test battery in 10 healthy elderly volunteers suggested that ZT-1, a HupA derivative, had the ability to antagonise the cognitive impairment caused by scopolamine (CDR, unpublished results). Scopolamine administered to healthy volunteers reproduces the attentional and secondary memory deficits seen in AD (Wesnes et al., 1991).

7. Conclusions

HupA has an appropriate pharmacological and cognitive-enhancing profile for AD and age-related memory impairment. It has been proven to have a powerful and lasting effect on the brain while keeping side effects to a minimum. In addition, HupA can lower neuronal cell death attributed to glutamate.

More research is needed to further explore the actions of this alkaloid and its analogues. However, the multiple benefits (and minimal side effects) of HupA already assessed in animal studies and clinical trials (clinical evaluation of HupA is now in phase IV in China) make it a promising treatment for AD and a very effective and safe pretreatment against chemical weapons as nerve gases.

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