

Perspective

New insights into huperzine A for the treatment of Alzheimer's disease

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Huperzine A, an active *Lycopodium* alkaloid extracted from traditional Chinese herb, is a potent, selective and reversible acetylcholinesterase (AChE) inhibitor and has been widely used in China for the treatment of Alzheimer's disease (AD). Accordingly, some new mechanisms of action for huperzine A have been discovered over the past decades. In addition to its AChE inhibitory effect, potent multifaceted neuroprotective effect through activating cholinergic system and directly acting on mitochondria have been explored. Moreover, in order to maximize the efficacy and safety of huperzine A therapy, great efforts have been made to optimize drug delivery system. In the present article, an attempt is made to discuss the current progress and future perspective for huperzine A therapy in AD.

Keywords: Alzheimer's disease; huperzine A; acetylcholinesterase; mitochondrion; controlled release

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by gradually loss of memory and other cognitive functions. Understanding the complexity of AD and its underlying pathophysiological mechanisms of AD provides a potential new paradigm that may help to develop new AD treatment^[1]. Among the limited therapeutic approaches, cholinergic replacement therapy especially acetylcholinesterase inhibitors (AChEIs), has been at the forefront of efforts to pharmacologically ameliorate the symptom of AD patients. So far, four AChEIs, tacrine, donepezil, rivastigmine, and galantamine, have been approved by the United States Food and Drug Administration for the treatment of mild or moderate AD.

Huperzine A is a novel *Lycopodium* alkaloid isolated from Chinese herb *Huperzia serrata* (Thunb) Trev (Qian Ceng Ta), which was traditionally used to relieve pain, antidote the poison, as well as alleviate swelling. Huperzine A is widely proved to be a potent, selective and well-tolerated inhibitor of acetylcholinesterase (AChE) (reviewed by^[2]). This effect was accidentally discovered in the 1970s while herbal extraction of *Huperzine serrata* was used to treat schizophrenia and found with significant cholinergic side effects. Huperzine A was

approved by State Food and Drug Administration of China for AD therapy in 1994. Since then, large amount of clinical studies have shown that huperzine A administration can significantly improve the memory, cognitive skills, and daily life abilities of AD patients with no severe side effects. Besides being indicated for AD, huperzine A is also used in treating memory impairment in vascular dementia (VaD) patients, schizophrenia patients and sleep disorder in insomniacs (reviewed by^[2]).

Suggestions on the multifaceted neuroprotective effects of huperzine A came from its superior preclinical and clinical benefits as compared with other clinically used AChEIs, and also from large numbers of laboratory studies evaluating the mechanisms of this compound (reviewed by^[3]). This perspective summarizes the evidences of novel insights of huperzine A to reduce AD risk, the molecular mechanisms involved in the anti-AD effects of huperzine A aside from its traditional AChE inhibition, and the novel delivery system which modified release patterns of this drug.

Multifaceted pharmacological effects of huperzine A: cholinergic-dependent and -independent mechanism

Although we are still at the stage of symptomatic treatments for AD since AChEIs remains the most widely used drugs, we are moving into an age in which disease-modifying agents, particularly, drugs with potent neuroprotective effect are considered to play a significant role in delaying AD development.

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Interestingly, recent studies reveal that huperzine A, might be of disease-modifying properties. The classical cholinergic effect and novel potential non-cholinergic actions of huperzine A are discussed as following paragraphs and summarized as Figure 1.

Multiple lines of evidences proved that huperzine A is a mixed-competitive and reversible AChE inhibitor, which shows higher potency and selectivity of AChE inhibition both *in vitro* and *in vivo* as compared with galanthamine, donepezil, tacrine, and rivastigmine (reviewed by^[2]). The potent inhibitory effect on AChE could in turn markedly enhance the synaptic ACh release and consequently cholinergic neurotransmission. Beside aforementioned classical effects, more and more beneficial characters of huperzine A are continuously discovered by employing various AD models, mainly from two aspects: expanded effects of cholinergic system in neuroprotection, and novel pharmacological target independent of its AChE inhibitory effect.

Cholinergic system is well established as an important part of the neuronal circuitry that modulates cognition, while, muscarinic and nicotinic ACh receptor antagonists are well known to produce or exacerbate cognitive impairments, respectively^[4-7]. Although ACh is generally considered to be a neurotransmitter, it can also function as a cytokine and might participate in various neuroprotective pathways: close association was found between ACh and the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor in the rat hippocampus^[8]; activating M1 muscarinic ACh receptor could activate the non-amyloidogenic APP pathway^[9, 10]; $\alpha 7$ nicotinic ACh receptor is increasing believed to be a critical link between inflammation and neurodegeneration in AD^[11].

In line with this observation, huperzine A administration was found to enhance the expression and secretion of NGF, as well as increase p75NTR mRNA in primary astrocytes^[12], enhance the non-amyloidogenic pathway by increasing the levels of sAPP α ^[13] possibly associated with M1 muscarinic ACh receptor mediated pathway^[14-16], and reduce the hypoxia ischemia-triggered inflammatory response through $\alpha 7$ nicotinic ACh receptor^[17-19]. Since previous study has proven that huperzine A had no direct effect on the amplitude or kinetics of nAChRs activation^[20], above cholinergic system-associated beneficial effects of huperzine A administration may mainly act through the enhancement of synaptic ACh level. These neuroprotective effects are probably potential common performance of cholinergic activation, since similar results are found from other AChEIs^[21-23].

As shown in Figure 1, effect of huperzine A on the cholinergic system may simultaneously contribute to symptomatic and disease modifying efficacy in AD. Meanwhile, huperzine A was recently found to exhibit additional benefits that appear to be independent of AChE inhibition, and differentiate the drug from other AChEIs. It is well known that mitochondria are the powerhouse of the cell which participates in a number of physiological functions^[24], and the mitochondrial dysfunction is considered as one of the key intracellular lesions associated with the pathogenesis of AD^[25]. We recently discovered that huperzine A was able to effectively ameliorate brain mitochondrial malfunction under A β ^[26-28] or ischemia insult^[29]. We further elucidated that the ameliorative effects of huperzine A on A β -induced mitochondrial dysfunction are associated with the reduced production in reactive oxygen species (ROS) and the increases in the activities of some key components of

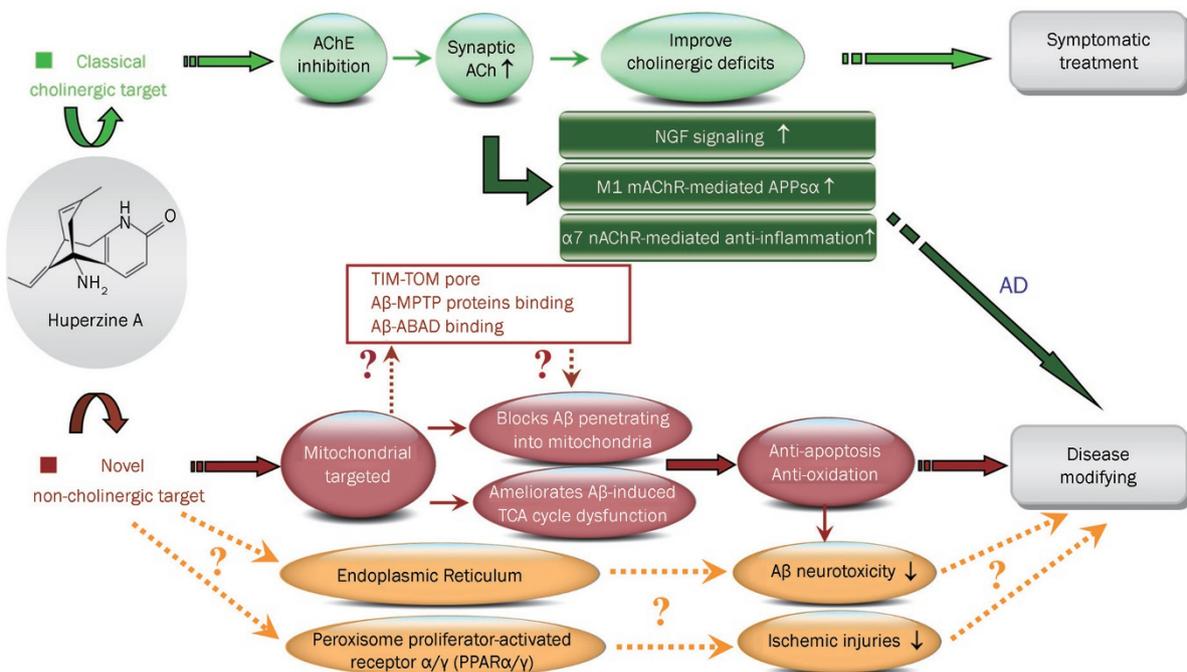


Figure 1. Summary of classical cholinergic and potential non-cholinergic pharmacological targets of huperzine A.

the respiratory chain and key enzymes in tricarboxylic acid (TCA) cycle^[26–28]. These findings clearly implicate that mitochondrion appears to be an important target of huperzine A, which is further supported by the findings that huperzine A inhibited the penetration of A β into mitochondria and ameliorated A β -induced TCA cycle dysfunction in isolated brain cortical mitochondria^[28]. The mitochondria-targeted effects of huperzine A are clearly independent of cholinergic system since there is no evidence indicated the existence of cholinergic system in isolated brain mitochondria. As mitochondria participate in a number of physiological functions that include calcium homeostasis, signal transduction, oxidative stress and apoptosis. This potential independent pharmacological target of huperzine A on mitochondria may further interpret the anti-oxidative stress^[30–36] and anti-apoptotic effects^[30, 31, 37–39] shown by huperzine A administration in various *in vivo* and *in vitro* models (Figure 1). Interestingly, several lines of evidence suggest that A β directly interacts with several proteins on and inside mitochondria^[40]: A β could import into mitochondria through a pore formed by the outer membrane (TOM40) and the inner membrane (TIM22)^[41]; A β could also interact with mitochondrial proteins from the membrane permeability transition pore (MPTP)^[42], which may in turn affect the mitochondrial membrane potential; A β was also reported to bind with β -amyloid binding alcohol dehydrogenase (ABAD) upon entering into mitochondria and leading to mitochondrial dysfunction^[43, 44]. Although the precise molecular target of huperzine A on A β -induced mitochondrial dysfunction remains to be clarified, whether and how huperzine A affects above mentioned A β -mitochondrion interactions could be a very promising future project.

Emerging evidence implies that a ligand-activated nuclear transcription factor, peroxisome proliferator-activated receptor (PPAR), could be a potential pharmacological target against inflammation and brain damage after ischemic injury^[45–47], and potent dual PPAR α/γ agonist exerted anti-inflammatory and neuroprotective effects^[48]. Moreover, similar as mitochondrion, endoplasmic reticulum (ER) is a multifunctional organelle that plays a central role in various malignant events in AD (reviewed by^[49]). Considerable studies have proved that huperzine A could effectively ameliorate ischemic injuries in various *in vivo* and *in vitro* models^[15, 17–19, 29], and our preliminary data also showed that huperzine A could affect A β -associated abnormal ER function (unpublished data). In order to further understand the above beneficial effects, it is also worthy to explore whether huperzine A could target on PPAR or ER.

Controlled delivery of huperzine A as a better drug administration strategy

Based on multifaceted beneficial profiles and well confirmed memory improvement effects, huperzine A has been proved to be one of the most promising agents for palliative therapy of cognitive deficits in patients with AD. The recommended dose of huperzine A for the clinical practice in China is 150–250 μg b.i.d., and the most-recently published phase II clinical trial

conducted in the United States showed that mild to moderate AD patients treated with huperzine A (400 μg b.i.d.) manifested significant improvement in cognitive function as compared to placebo^[50]. Meanwhile, it is also important to assess the side effects and tolerability of huperzine A therapy at the clinically used doses. As a specific AChE inhibitor, it will be easy to predict that adverse effects of huperzine A should relate to the well-known cholinergic activity. In fact, two US Phase I studies have shown that, although rated as mild scale, adverse symptoms included tachycardia, low energy levels, and hypertension at multiple dose ranges; bradycardia, headache, and intense dreams at a dose of 400 μg b.i.d.; muscle cramps at 400 μg b.i.d.; arthralgia at 300–400 μg b.i.d.

Taken together, the efficacy and safety data from clinical trails of huperzine A treatment indicated that larger doses of huperzine A were needed for better clinical effects in AD patients, however, the increasing of dosage may also increase the possibility of emergency of more severe side effects^[51]. It will be therefore very interesting to unravel the rational behind above phenomenon and search for proper solution. The pharmacokinetic data indicated that huperzine A had a rapid and nearly complete oral absorption and was extensively distributed into tissues after drug administration in dogs^[52]. The concentration of huperzine A in plasma quickly reached its peak at 1.25 h with a steep ratio after oral administration, the oral bioavailability is about 94%^[52], and approximately 20% of the huperzine A level in plasma reached the cerebrospinal fluid after both intranasal and intravenous administration^[53]. In combination of clinical, pharmacological and pharmacokinetic data, it is suggested that the increased side effect caused by high dosage of huperzine A may attribute to the promptly reached peak plasma drug level after oral administration, while the better efficacy shown by high dosage of huperzine A may due to higher level and longer duration of huperzine A in the plasma at this situation, besides, the high blood-brain-barrier penetration rate allows more huperzine A entering into the brain and maintain at a higher level, which could also enhance the efficacy of huperzine A.

At present, huperzine A is available in the market mainly as tablet or capsule, which has to be given orally 2–3 times per day. The pharmacokinetic parameters of huperzine A determined that it is difficult to optimize both efficacy and safety of the drug with the current two formulations, and it is not convenient for AD patient who suffers memory disorder not to miss scheduled self-medication. Therefore, developing new sustained released drug formulations with long-term efficacy may solve afore-mentioned problems. Currently, several new controlled released formulations are developed, most of them are performed on huperzine A loaded poly (lactic-co-glycolic acid) (PLGA) microspheres using an oil/water emulsion solvent evaporation technique^[54–56], and the period of the sustained release could range from one day to six weeks. While which delivery system exerts best efficacy still remain to be explored, it is reported that ACh has been implicated in cortical synaptic plasticity and memory processes, and suggested the existence of a circadian rhythm in central cholinergic trans-

mission, which modulates memory processes, with high ACh levels during wakefulness and reduced levels during slow-wave sleep^[57]. Therefore, huperzine A sustained release formulation with long lasting over than 12 h may not be a good choice, based on the evidence that low levels of ACh during slow-wave sleep were critical for the consolidation of declarative memory^[58]. Developing novel delivery system with stable and proper plasma concentrations during active cycle (light cycle for human) could be a promising strategy to create better clinical efficacy in AD therapy.

Conclusions and future prospects

AD is a multi-causal progressive neurodegenerative disease with complicated pathogenesis, the major current obstacle of this mysterious syndrome is the lack of effective therapeutic strategy. However, based on the fact that a big gap exists between bench research and clinical application, which is termed as “valley of death”^[59], large increases in spending on medical research have not produced corresponding increases in new effective therapeutic strategy. Recently, the concept of “translational medicine” is increasingly used to bridge benchside to bedside, in order to broaden and deepen the understanding of the real fact of AD pathogenesis, and also to shorten the period of drug development. One innegligible aspect is to further understand the beneficial profiles and weakness of an existed effective drug when used in clinical practice, trying to unravel the molecular mechanisms and optimize the medicine performance. This article reflects the research progress made in molecular pharmacology and pharmacological therapy for treating AD with huperzine A, showing that the classical cholinergic and potential non-cholinergic target of huperzine A may shed more light on the successes gained from huperzine A in the AD therapy. Accumulative evidence suggests that single target drug always exert limited clinical effects for AD therapy, while combined therapies or drugs with multi-pharmacological activities would be a promising future therapeutic approach to address the varied pathological aspects of the disease. The multiple neuroprotective effects of huperzine A together with the optimized drug delivery system will give this drug new opportunity to prove its merit, however, further studies still need to be performed to validate its efficacy, especially solid evidence from the bedside.

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