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Huperzine A: A promising anticonvulsant, disease modifying, and memory enhancing treatment option in Alzheimer's disease

Ugur Damar [a], Roman Gersner [a], Joshua T. Johnstone [b], Steven Schachter [c], Alexander Rotenberg [a,*]

[a] F.M. Kirby Neurobiology Center, Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA
[b] Biscayne Pharmaceuticals, Inc., Miami, FL, USA
[c] Department of Neurology, Beth Israel Deaconess Medical Center, and Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Alzheimer's disease (AD) is the most frequent cause of dementia. Besides cognitive deterioration, patients with AD are prone to seizures – more than 20% of patients diagnosed with AD experience at least one unprovoked seizure and up to 7% have recurrent seizures. Although available antiepileptic drugs (AEDs) may suppress seizures in patients with AD, they may also worsen cognitive dysfunction and increase the risk of falls. On the basis of preclinical studies, we hypothesize that Huperzine A (HupA), a safe and potent acetylcholinesterase (AChE) inhibitor with potentially disease-modifying qualities in AD, may have a realistic role as an anticonvulsant in AD.

Introduction

Alzheimer's disease (AD) is the most frequent cause of dementia worldwide, and remains incurable. FDA-approved drugs to relieve AD symptoms are acetylcholinesterase (AChE) inhibitors and memantine, a noncompetitive N-methyl-D-aspartate (NMDA) antagonist. AChE inhibitors address mnemonic symptoms referable to basal forebrain and cortical cholinergic deafferentation in AD [1]. Memantine limits neuronal glutamate excitotoxicity, interferes with pathological NMDA-mediated glutamate signaling, and increases normal signal-to-noise ratio [2–4].

Epilepsy, its treatment in AD and associated drawbacks

10–22% of AD patients have at least one unprovoked seizure [5] and 4–7% experience recurrent seizures [6–8]. Genetic factors and disease severity correlate significantly with AD epileptogenesis. Particularly patients with early-onset familial AD and patients with more severe AD symptoms are most likely to develop seizures [9]. Studies suggest that epileptiform activity in cortical and hippocampal network in mouse AD models decreases synaptic plasticity in dentate gyrus, which has been linked to cognitive decline in AD [10,11]. Hence, one could hypothesize that prevention of seizures in AD could stabilize and perhaps even reverse cognitive impairment.

Numerous antiepileptic drugs (AEDs) have been tested in AD animal models and AD patients, but none have ideal clinical profiles. Preclinical data are largely derived from studies in transgenic rodent models. For instance, levetiracetam reduces abnormal spike-wave activity, and in chronic use (12 days), reverses hippocampal remodeling and cognitive deficits in mice [12], benzodiazepines (BZ) increase inhibitory transmission and ameliorate enhanced synaptic potentiation in AD [13], and valproate may decrease beta amyloid (Aβ) production, and thereby provide neuroprotection and facilitate cognitive improvement [14,15].

In contrast to the encouraging animal work, human trials have not produced strong support for chronic use of conventional AEDs in AD. One study showed that levetiracetam, lamotrigine, and phenobarbital provided seizure freedom in only 30% of patients with AD, and reduced seizure frequency by >50% in 42, 35, and 36% of the patients, respectively [16]. Yet levetiracetam, lamotrigine, and phenobarbital caused frequent adverse effects, i.e. headache, dizziness, asthenia and somnolence, in 17, 28 and 43% of patients, respectively. While levetiracetam treatment corresponded to cognitive improvement in AD patients, in addition to seizure control, it caused somnolence and increased risk for infection [16,17]. Other AEDs such as phenytoin, topiramate, valproate and lacosamide have a considerable range of adverse effects with chronic

* Corresponding author at: 300 Longwood Ave, Department of Neurology, Boston Children's Hospital, Boston, MA 02115, USA.
E-mail address: Alexander.Rotenberg@childrens.harvard.edu (A. Rotenberg).

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use that renders them as suboptimal treatment options [16,18,19]. BZ use is also often impractical in the elderly due to the risk of sedation and falls [20] as well as BZ-related cognitive decline [21,22]. Overall, AED adverse effects are reported by about 34% of patients, and this figure almost doubles when written questionnaires are used to survey for symptoms [23]. Such adverse events lead to a withdrawal of AED treatment in about 25% of AD patients [24], which underscores a need for a novel therapeutic with a favorable side-effect profile in this syndrome.

Epileptogenic pathology in AD

Hippocampal sclerosis (HS) and Aβ deposition, the two major culprits in AD pathology, increase seizure propensity [25]. HS is accompanied by regional reduction of neuropeptide Y (NPY) which has anti-epileptic properties [26]. Also, NPY-neurogliaform interneurons which are GABAergic and have dense cholinergic input are reduced in number in rat brains after pilocarpine-induced status epilepticus which causes HS-like damage [27,28]. Aβ and neurofibrillary tangles (NFTs, excessively phosphorylated tau protein or paired helical filaments) are hallmarks of AD pathogenesis [29]. Aβ spontaneously assembles into dimers, oligomers, and fibrils. Intracellular Aβ (mainly Aβ42) and extracellular/secreted Aβ (mainly Aβ40) contribute significantly to Aβ toxicity [30]. Aβ42 forms amyloid fibrils faster than Aβ40, comprises the majority of amyloid plaques, and relates more intimately to AD pathology [31]. In vitro, cortical neurons from mice with mutant human amyloid precursor protein (hAPP) were exposed to extracellular Aβ, particularly fibrillar Aβ1-42 or Aβ25-35 show increased pyramidal cell membrane excitability and predispose mice to seizures [32]. Studies conducted in transgenic mice overexpressing hAPP also show that an increase in Aβ is sufficient to trigger spontaneous non-convulsive seizures recorded from cortex and hippocampus, even without overt neurodegeneration, suggesting that high Aβ levels directly cause disrupted network synchronization [11,25,33].

Aβ toxicity is dependent on the tau protein, which is phosphorylated and modulated by the Fyn tyrosine kinase [34]. While tau protein is necessary for tubulin to assemble into microtubules, hyperphosphorylated tau, as in AD, binds normal tau and microtubule-associated proteins and sequesters them into insoluble filament tangles, thereby inhibiting microtubule assembly [35]. Tau hyperphosphorylation has been associated with excitotoxicity, neurodegeneration, and various causes of epilepsy such as traumatic brain injury and focal cortical dysplasia [36,37]. In further support of the tau role in AD epileptogenesis, genetically modified tau-deficient animal models do not have Aβ-induced spontaneous epileptiform activity, and show reversed excitation: inhibition imbalance and reduced vulnerability to chemically-induced seizures [38].

Huperzine A as a therapeutic agent in AD

Huperzine A (HupA) is an AChE inhibitor extracted from Huperzia serrata, a firmoss. HupA is a more potent and specific central nervous system (CNS) AChE inhibitor than other inhibitors such as donepezil, tacrine, and pyridostigmine in preclinical studies [39]. HupA has been used clinically to improve cognition and memory in AD and other forms of dementia in China. It is currently marketed as a memory enhancing dietary supplement in the US. In clinical trials, HupA has demonstrable cognition-improving capacity in AD patients and is not associated with any serious side effects in either this vulnerable patient population or in healthy volunteers [40,41]. HupA reduces Aβ production and protects cells from Aβ1-42- or Aβ25-35-induced damage [42–45]. While HupA is also a weak N-methyl-D-aspartate glutamate receptor (NMDAR) antagonist [46], its NMDAR inhibitory activity is not likely achieved at tolerable doses. In addition, as recently shown by our laboratory, HupA has potent antiepileptic effects in the rat pentylenetetrazole (PTZ) model [47]. In light of these findings, HupA may have multiple benefits for mitigating the progression and AD symptoms.

AChRs and HupA effects

The 2 types of acetylcholinesterase receptors (AChRs) are nicotinic and muscarinic. They mediate fast and slow synaptic transmission, respectively. Muscarinic ACh receptors (mAChRs) are G-protein coupled and predominantly present in the parasympathetic nervous system. Nicotinic AChRs (nAChRs), neuronal- and muscle-type, are ion channels and have 5 subunits. Besides their roles in autonomic ganglia, nAChRs have a range of roles in neurotransmitter release, excitatory transmission, neurodevelopment, and plasticity throughout the CNS (primarily in cortex and hippocampus) [48]. In AD, the number of both mAChRs and nAChRs are decreased, in addition to loss of cholinergic neurons and the choline acetyltransferase enzyme in the basal forebrain [49]. This reduced cholinergic tone is most proximally related to cognitive impairment in AD [50].

HupA preferentially inhibits tetrameric AChE in the brain. Tetrameric AChE comprises the majority of membrane-bound AChE at the synapse, and hence HupA, unlike other AChE inhibitors, may have preferential synaptic effects [39,51]. Synaptic tetrameric AChE is the predominant AChE form in most brain regions where it co-localizes with other synaptic receptors, e.g. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, NMDARs, nAChRs and mAChRs [52]. The α7 subunit-containing nAChR (α7nAChR) has central anti-inflammatory effects in animal stroke and traumatic brain injury (TBI) models which suggest anti-seizure via a brain anti-inflammatory mechanism [53–55]. Moreover, GABAergic interneurons express abundant nAChR and thus enable AChE to mediate pyramidal cell activity [56]. Notably, the brain regions with both abundant tetrameric AChE and α7nAChR, such as the hippocampus and frontal cortex are those that are most affected by AD [39,57,58]. Hence, it is plausible that central HupA anti-seizure effects take place after indirect activation of α7nAChRs.

HupA may also modulate AD symptoms via mAChRs and α4β2-subunit containing-nAChRs (α4β2nAChRs). An in vitro study in rat hippocampal synaptosomes shows that GABA release is facilitated via α4β2nAChRs [59]. Another in vivo study demonstrates α4β2nAChRs increase GABAergic transmission in mouse corticolimbic neurons [60]. Another in vitro study also shows that mAChRs contribute to GABAergic inhibitory signaling in rat hippocampal neurons [61,62]. Taken together, these early preclinical data raise prospects for HupA to act indirectly via mAChRs and nAChRs to augment inhibitory tone and reduce cortical excitability in AD.

Hypothesis

Given that seizures in AD patients are difficult to control at tolerable doses of available AEDs, we hypothesize that HupA may be an effective and well-tolerated agent to both suppress seizures and improve cognition in AD by enhancing cholinergic and GABAergic signaling and mitigating AD-related neurotoxicity.

HupA as an antiepileptic drug in AD

HupA has acute anticonvulsant properties in the PTZ rat model [47] where it prophylaxes against seizures, enhances gamma oscillations on EEG cortical neurons from mice with mutant hAPP

which are associated with enhanced GABAergic cortical interneuron activity, and increases GABA-mediated cortical inhibition as measured by paired-pulse transcranial magnetic stimulation (ppTMS). Furthermore, inflammatory cytokines in brain probably mediate epileptogenic processes, and intervening with their actions are proposed to be potential targets for anticonvulsant drugs [55,63]. Several important mediators in this regard are tumor necrosis factor alpha (TNF-α), interleukin 1-beta (IL-1β), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). In addition, along with Aβ-mediated pathological changes, chronic neuroinflammation likely plays a role in AD pathogenesis [64]. Elevated levels of cytokines such as IL-1β, TNF-α and TGF-β are found in postmortem AD brains, especially in late-stage disease [65]. Moreover, increase in IL-1β, TNF-α and microglial activation have been correlated in aged rat hippocampus which in turn potentially causes loss of GABAergic cells [66]. In line with these, HupA has been shown to decrease IL-1β, NF-κB and TNF-α levels in animal models [67–69]. Hence, HupA may exert sub-acute anticonvulsant and antiepileptic effects indirectly via cholinergic anti-inflammatory mechanisms regulated by nAChRs. Additionally, HupA may facilitate GABAergic signaling by NF-κB and IL-1β pathway suppression [63,70]. In parallel, preclinical studies show that Aβ activates NF-κB pathway especially in hippocampus and prefrontal cortex, which subsequently causes induction of pro-inflammatory cytokines e.g. TNF-α and IL-1β [71]. By virtue of these plausible downstream effects, HupA may reverse the pro-inflammatory state in AD, which may also provide seizure protection.

Can HupA prevent seizures in AD-associated hippocampal sclerosis?

Preclinical HS models show an upregulation of microglial cell markers and genes related to NF-κB pathways in epileptogenic tissue [72]. In addition, decrease in expression of receptor subtype genes such as that of GABA-A were argued to play roles in epileptogenesis in HS. Reduction in expression of neuropeptide Y (NPY) was also noted. This reduction contributes to hyperexcitability, given that intrahippocampal NPY administration suppresses seizures in rats and NPY-neurogliaform interneurons are inhibitory via slow, GABA-A receptor mediated inhibitory post synaptic currents in hippocampus and cortex (Fig. 1) [27,73,74].

HupA has central anti-inflammatory effects plausibly via suppressing NF-κB pathway and interleukin release from neurons and microglial cells. In addition, HupA facilitates GABAergic signaling whose mechanism is still unclear [57]. Since NPY-neurogliaform interneurons have dense cholinergic input, HupA may enhance GABAergic transmission through NPY pathways in the hippocampus where tetrameric AChE is abundant and HupA displays a preferential targeting effect [27,39,75]. Taken together, HupA may be hypothesized to provide anti-seizure activity in AD-associated HS via its cholinergic and GABAergic effects.

Can HupA reverse Aβ- and hyperphosphorylated tau-related seizures?

Neuroligin-1 (NL1) is a transmembrane cell adhesion protein which helps organization and functioning of specifically excitatory synapses, ergo glutamatergic transmission and long-term potentiation [76]. NL1 has an extracellular domain homologous to AChE which interacts with Aβ oligomers and stimulates Aβ oligomerization [77,78]. An in vivo study suggests that NL1 overexpression causes an increased excitation:inhibition ratio in NL1-overexpressing transgenic mice, and another study reports that NL1 knockdown mice have less frequent and severe seizures [79,80]. We therefore hypothesize that interfering with NL1 related pathway by interacting with the AChE-like domain of NL1 with

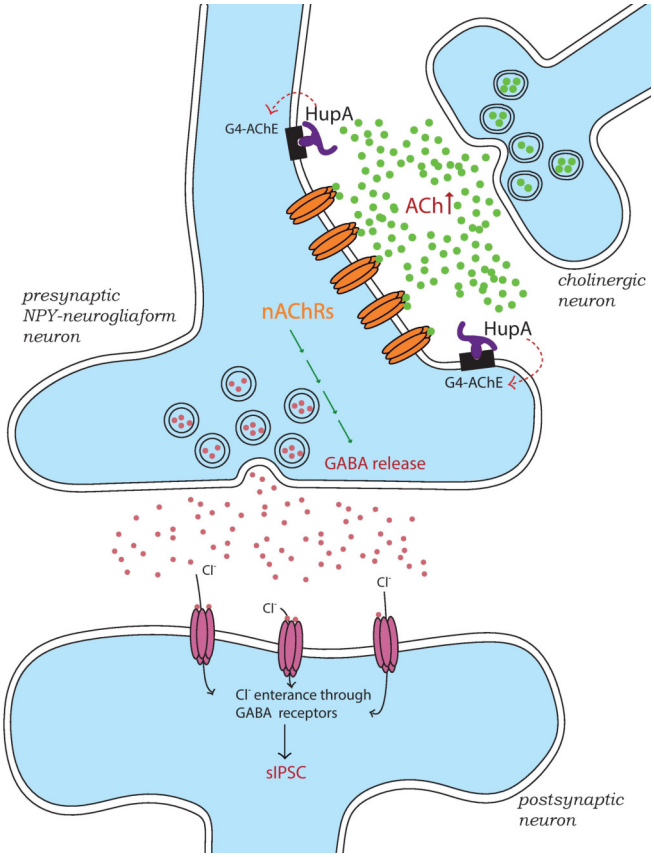

Fig. 1. Inhibiting AChE, HupA may indirectly activate nAChRs on NPY-neurogliaform neurons. Thereby, increased GABAergic signaling may augment inhibitory tone and decrease seizure propensity. (NP; neuropeptide Y, G4-ACh; tetrameric acetylcholinesterase, nAChR; nicotinic acetylcholine receptor, sIPSC; slow inhibitory postsynaptic currents).

HupA may disrupt Aβ-NL1 and prevent Aβ oligomerization-induced excitotoxicity.

Reducing tau hyperphosphorylation and interfering with tau functioning suppresses seizures in numerous animal models [81–83]. In addition, α7nAChR activation alleviates tau-mediated neurodegeneration [84,85]. Moreover, donepezil, an AChE inhibitor, enhances protein phosphatase 2A (PP2A) activity, therefore reducing tau-induced neuronal viability and neurodegeneration [86]. Given that HupA is a potent CNS AChE inhibitor and has plausible effects via α7nAChRs, it may also prevent tau-related seizure predisposition via PP2A enhancement.

Evaluation of hypothesis

An effective and tolerable AED for patients with patients with AD remains a significant unmet medical need. Given the mechanisms of epileptogenesis in AD, HupA is a promising antiepileptic agent that may normalize cortical excitation:inhibition ratio, and may also have disease-modifying properties.

Clinical trials show that HupA is a safe and well-tolerable drug at doses up to 0.4 mg twice daily in patients with AD and vascular dementia without serious adverse effects, except for nausea and mild dizziness [87,88], which could potentially be mitigated by slow-release formulations. Thus, clinical trials where outcome measures include seizure and interictal spike counts as recorded on EEG, as well as clinical screens for drug side-effects, are realistic. Furthermore, methods like florbetaben positron emission tomography (PET) and cerebrospinal fluid Aβ1-42 or hyperphosphorylated

tau levels may be used to detect Aβ burden, and track disease modification by HupA [89,90].

Another means of hypothesis testing may be in preclinical rodent experiments. Aged mice or rats, or transgenic animals expressing pathogenic APP gene variants are reasonable HupA study subjects [91,92]. In addition to measuring seizures by EEG, HupA-mediated modulation of cortical inhibitory tone can also be measured by ppTMS, a technique that is available in both humans and rodents which our group recently adapted to HupA research [47]. In this experiment, electromyography recordings from rat brachioradialis muscles were used to display motor evoked potentials (MEPs). It was found that the ratio of second-to-first MEP amplitudes were smaller after intraperitoneal HupA administration, suggesting increased cortical inhibition. That is, ppTMS can be used to detect CNS target engagement (GABA-A receptor modulation) by a range of pharmaceuticals, and we envision this technique to translate to human HupA trials. In parallel with in vivo animal studies, Aβ levels may be assessed over a range of HupA doses and treatment schedules ex vivo by immunohistochemistry, ELISA, size exclusion chromatography, or other common laboratory methods [93–95].

Conclusions

Unprovoked seizures affect approximately 20% of patients with AD, and a safe and well-tolerated antiepileptic treatment remains an unmet need in this population. While conventional AEDs may provide seizure control, their sustained use is associated with serious side effects such as lethargy, cognitive decline and susceptibility to falls. HupA, a potent cholinergic agent, has a favorable safety profile in the elderly, as supported by clinical trials in the AD population. In addition, HupA has acute anti-seizure effects in a rat preclinical model. Thus, HupA is a promising drug addressing both mnemonic and epileptic symptoms in patients with AD, without major, treatment-limiting, side-effects.

Overview box

1. While AD is a risk factor for the development of epilepsy, current approved treatment options for AD only help to improve cognition and are neither effective AEDs nor disease-modifying. Moreover, available AEDs may worsen cognition and have other serious side effects including sedation and falls. HupA is an AChE inhibitor that has been shown to be safe and that possesses newly discovered antiepileptic, cognitive-enhancing, and disease-modifying properties.
2. Given that there is no proven method to reduce seizures, improve cognition, and impact disease progression in AD, the proposed hypothesis may lead to the demonstration that HupA fills a substantial gap in AD treatment.
3. Initial animal studies should aim to confirm that HupA suppresses seizures and enhances cognition in relevant AD models. Further studies could then investigate the related effects on Aβ aggregation, preferably with transgenic animal models such as mutant chimeric human APP.

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