An Overview of Huperzine and its role in the Management of Alzheimer's disease.

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ABSTRACT

The Chinese Academy of Sciences developed a possible nootropic drug called huperzine A, an alkaloid that was extracted from Huperzia serrata. Phase III trials including huperzine A are presently underway in China to treat Alzheimer's patients. A renewable plant source has been obtained using an in vitro approach, and Huperzine A's chemical production has been refined. Pharmacological investigations revealed that the medication selectively and reversibly inhibits the acetyl cholinesterase enzyme. New techniques for chemical synthesis and biological production have been developed in response to the need for a dependable source of huperzine A, given low yield naturally existing in plants and the increasing demand for the compound. There is contradicting information regarding huperzine A's use in the US, despite the drug being widely used in China. This is mostly because there aren't enough large-scale, multicenter, randomized control trials that are well-designed. There's little doubt that concurrent clinical trials will give us additional knowledge about how to use huperzine A to treat AD. HupA has been shown to have greater penetration strength, greater bioavailability, and the ability to inhibit AChE for a longer period of time when compared to other cholinesterase inhibitors including donepezil, rivastigmine, and galantamineThe pathogenesis of Alzheimer's disease is not well understood, which makes it difficult to identify a suitable treatment. A naturally occurring substance from Huperzia serrata moss called huperzine A has been suggested as a possible AD therapy. Humans with mild to moderate AD and animal models have both benefited from favourable cognitive augmentation in clinical trials.

KEYWORDS: Huperzine A, Huperzia serrate, Alzheimer’s disease, Acetylcholinesterase and Clinical trials.

INTRODUCTION

The Chinese Academy of Sciences developed a possible nootropic drug called huperzine A, an alkaloid that was extracted from Huperzia serrata. Phase III trials including huperzine A are presently underway in China to treat Alzheimer's patients. It is proposed that huperzine A's gradual, reversible inhibition of acetylcholinesterase[1] facilitates its mechanism of action. Alzheimer's disease is manifested by the pile up of neurofibrillary tangles and neuritic plaques. It is the fourth leading cause of death among adults. The sole goal of current therapies is to reduce symptoms. The chemistry, pharmacology, and clinical effectiveness of huperzine A are evaluated, as a modern ailment for Alzheimer's disease. A renewable plant source has been obtained using an in vitro approach, and Huperzine A's chemical production has been refined. Pharmacological investigations revealed that the medication selectively and reversibly inhibits the acetylcholinesterase enzyme.

Huperzine A has demonstrated favourable pharmacokinetic properties throughout the body. While there is currently limited human toxicity data, animal studies have indicated that at therapeutic doses, huperzine A can cause minimal cholinergic side effects. Improvements in cognitions using tests such as the MMSE, MQ, ADAS-COG, and ADL in already conducted clinical trials. The ADAS-COG and MMSE tests revealed improved memory at 0.4 mg in Phase II trials, but no upgrade was evident at 0.2 mg. Significant cognitive upliftment is evident via the MMSE scores enhancement at the 0.4 mg. Strong evidence suggests that huperzine A is well accepted for up to 24 weeks at doses of up to 0.4 mg. Consequently, huperzine A appears to be a possible AD therapeutic alternative[2].

HUPERZINE

()-Huperzine A belonging to Huperziaceae, Lycopodiaceae, and Selaginella plant families that contain trideca-2(7),3,10-trien-5-one. The chemical was extracted from the dried herb of Huperzia serrata; the yield of huperzine A varies from 0.0047 to 0.025% based on the method of plant collection and extraction[3].

New techniques for chemical synthesis and biological production have been developed in response to the need for a dependable source of huperzine A, given the low yield naturally existing in plants and increasing demand for the compound. Studies conducted in vivo and in vitro revealed that the activity of chemically produced ()-huperzine A was equivalent to that of naturally occurring ()-huperzine A[4]. This study evaluated many synthetic and biological techniques for processing ()-huperzine A. Huperzine A is thought to enhance overall cognitive function, address behavioural disturbances, improve global status, and boost functional performance[5].

The majority of clinical trials conducted in China provide evidence for its usage in improving cognitive function. As of right now, China has approved huperzine A at 0.2 mg twice day for treatment of AD[6]. There is contradicting information regarding huperzine A's use in the US, despite the drug being widely used in China. This is mostly because there aren't enough large-scale, multicenter, randomized control trials that are well-designed. There's little doubt that concurrent clinical trials will give us additional knowledge about how to use huperzine A to treat AD.

MANUFACTURING OF DRUG SUBSTANCE

In order to address the limited natural occurrence of huperzine A in plants and to fulfil the growing need for the chemical in research and medicine dosage manufacture, scientists have spent the last thirty years investigating several ways to increase the compound's prevalence[7]. This has involved the creation of procedures for both the enhanced production via various biological processes, notably the preparation of biosynthesis, and the domain of chemical synthesis[4]. Because of its compact and rigid framework, huperzine A is a really unusual molecule containing fused pyridone moiety[8].

After the stereoisomers of huperzine A were identified, ()-huperzine A is considerably effective than (ξ)-huperzine A[9, 10]. Research into the synthetic preparation of huperzine A began in late 1989 and recently Koshiba et al described improved protocol for the entire of ()-huperzine A preparation. Some crucial elements of their methodology were notably different from those of earlier research. First off, a commercially accessible anhydride served as the foundation for the whole synthesis procedure. They originally developed the bicyclic ring system with this chemical. The Wittig reaction phase was one of the biggest distinctions between this process and those that have been previously published. Earlier synthetic pathways involving this step typically result in the predominant production of the undesired (Z)-isomer. To address this difficulty, Koshiba et al[8] added vinyllithium and treated the allyl alcohol intermediate with SOCl2 to enhance the production of the particular isomer. Subsequent reactions then made it possible to create ()-huperzine A in 23 stages with a 1.8% total yield.

FORMULATION

The production, enhancement, and characteristics of loaded nanostructured lipid carriers (NLC) containing Huperzine A (Hup A), a successful treatment for Alzheimer's disease in ancient Chinese Medicine. NLC was effectively produced by modifying the processes of melt ultrasonication and high pressure homogenization. The solid lipids in the emulsifiers were soybean phosphatidylcholine (Spc), liquid lipids Miglyol (R) 812, solid lipid Cetyl Palmitate (CP), and solutol HS15. Box-Behnken design with 3 components and levels was used to find the best formulation. Formulation's ratio of lipid: drug, amount of mixed lipid, and amount of emulsifier combination were all looked at as independent factors. The dependent variables studied were particle size, drug loading and entrapment efficiency. The characteristics of the nanoparticles, including morphology, dependent variables and drug release behaviour was assessed. The findings revealed that “the proposed nanoparticles had nearly spherical particles with an average size of 120 nm and a zeta potential of -22.93 ± 0.91 mV”. It is possible for the EE (%) and DL (%) to achieve values of 1.46 +/- 0.05% and 89.18 +/- 0.28%, respectively. With Hup A loaded NLC, differential scanning calorimetry (DSC) revealed no recrystallization propensity. Hup A was released from NLC in vitro in stages, with a burst release observed at the beginning and a protracted release lasting up to 96 hours. According to the findings, the Hup A loaded NLC system that has been demonstrated may be a useful delivery method for increasing drug loading capacity and achieving regulated drug release.

NON-CLINICAL STUDIES

Research has also suggested that HupA therapy may be superior to traditional medication and psychotherapy for treating epilepsy and dementia. Meanwhile, HupA has a dose-and time-dependent impact that can greatly enhance the cognitive function in Alzheimer's disease

patients. In numerous animal models of neurological disorders, low dosages of HupA have been demonstrated to significantly improve cognitive impairment, lower neuroinflammation, improve neuroprotection by raising cortical inhibition, and influence these processes. Furthermore, HupA has been shown to have greater penetration strength, greater bioavailability, and the ability to inhibit AChE for a longer period of time when compared to other cholinesterase inhibitors including donepezil, rivastigmine, and galantamine[12].

NON-CLINICAL PHARMACOLOGY

A. Behavioural Test Analysis

Since the Morris water maze test accurately measures spatial memory ability, it is frequently used to evaluate the effectiveness of medications in AD models. A platform hidden beneath opaque water is used for the training, which takes place in a circular swimming pool. After being placed in the water, the animal is led to the platform. The probe test, which lasts for 60 seconds after the animal has been trained for days, involves letting it swim freely. The amount of time spent in the target quadrant and the escape delay are the markers for spatial memory.

Seven out of the sixteen articles were tested, and the forest plot looked at the two markers that were previously mentioned. Image J (Rawak Software Inc., Stuttgart, Germany) and RevMan 5.4 (Cochrane Community, London, UK) were used to study the data. The escape delay meta-analysis included 91 rats and 30 mice. The time spent in the target quadrant was assessed for 30 mice and 70 rats. 0.1 mg/kg and 0.2 mg/kg was designated as 1 and 2, respectively.

Both rats and mice given huterzine A exhibited noticeably shorter escape latencies. The studies' heterogeneity, however, is significant (I2 (mice) = 56%, p = 0.13; I2 (rats) = 74%, p = 0.004). In the rat subgroup, there is considerable heterogeneity in the amount of time spent in the target quadrant (I2 (rats) = 92%), whereas there is a significant (p = 0.004) increase in time in the mice group with low heterogeneity (12 = 0). The research employed various Alzheimer's disease (AD) models, including APPswe/PS1dE9 mice, quinolinic acid-induced AD, AMPA-induced AD, Aβ-induced AD, and D-galactose-induced AD. This notable variety in models might account for differing results. Surprisingly, amount of time spent in the target quadrant was not elevated by huperzine A[13].

B. Neuroprotective Mechanism Analysis

Inhibition of Aβ pathway

Numerous studies have discovered neuroprotective strategies, including as modulating the cholinergic system, attenuating mitochondrial dysfunction, inhibiting Aβ aggregation or associated pathways, and controlling apoptotic factors. Huperzine A has been demonstrated to exhibit inhibitory action on Aβ aggregation or associated pathways; Aβ levels were quantified through investigations employing immunohistochemistry and ELISA. Huperzine A therapy enhanced the non-amyloidogenic pathway in the brains of APP/PS1 mice and considerably declines the levels of soluble Aβ40 and Aβ42 in both cortex and hippocampus. A diet high in iron could, however, undo these modifications. Huperzine A also decreased ABAD levels, oligomeric Aβ42, Aβ plaques, and mitochondrial dysfunction. To establish the Aβ burden, other investigations employed immunohistochemistry, thioflavin S, and Congo red staining. The findings demonstrated that Huperzine A therapy significantly decreased the quantity of Aβ

plaques in the hippocampus and cortex, with the hippocampus group exhibiting low heterogeneity and the cortex group exhibiting high heterogeneity. Overall, studies on a variety of brain areas have demonstrated the potential neuroprotective effects of huperzine A.

Enhancement of Cholinergic Activity

It has been noted that the brains of AD patients have lower levels of choline acetyltransferase (CAT/ChAT), but the process of developing AD can be stopped by upregulating the levels of ChAT in the cortex and hippocampus. AChEIs (donepezil, rivastigmine, galantamine) and memantine are currently approved medications for the treatment of AD; huperzine A is well-known for its effectiveness in treating cholinesterase inhibitors (ChEIs). Research has indicated that huperzine A is more effective than AF64A in attenuating memory deficits caused by AChE in the cerebral cortex and hippocampus. AChE activity has been demonstrated to be inhibited by huperzine A; notable enhancement was noted in the rat subgroup and inhibition in the mouse subgroup.

Pharmacokinetic Studies

Based on in vitro findings, liver liposomes (HLMs) do not significantly inhibit or induce CYP enzyme activities, nor do they metabolize HupA at clinically relevant quantities. This goes against earlier research in renal liposomes (RLMs), where it was discovered that CYP1A2 metabolized primarily at a concentration in line with its plasma levels of therapeutic HupA. The metabolic stability experiment carried out in RLMs indicates that the HupA can be digested at a concentration of 10 ng/mL with a t1/2 of 61.9 minutes. The difference between HLMs and RLMs indicates that more clinical trials are required to guarantee pharmaceutical safety and that findings made from rat studies should not be applied to humans. In rats and mice, HupA is primarily distributed in the kidney and liver, and it is primarily excreted from kidney.

According to the in vivo investigation, older patients expelled 35% of their total HupA through urine 48 hours after receiving a single dose. Additionally, the study discovered that HupA was mostly removed from the kidney in an unaltered state. *In vitro* metabolic stability research and the *in vivo* pharmacokinetic investigation showed HupA was primarily removed from the kidney intact. According to the study, modifications in CYP isoenzyme activities can affect a drug's plasma concentration, pharmacokinetic mechanism, and therapeutic characteristics. To predict possible drug interactions, a dosage of 20 ng/mL HupA—250 times higher than the current dosage—is probably not acceptable. Given HupA's low CYP isoenzyme interaction propensity, there may not be any medication interactions in clinical settings. HupA is primarily intended for older patients; hence, it could be a good substitute for elderly AD patients who require combination medication therapy with hypoglycemia or hypotensive medications. To reduce adverse effects, HupA should be used in conjunction with medications that are excreted by the kidneys, however care should be taken regarding the possibility of DDI. To completely assess the potential for medication interactions brought on by the co-administration of HupA, more extensive clinical research is required[14].

Toxicology

There were no morphological or behavioral changes in animals exposed to huperzine. Thirty minutes after being treated to 625 µg/kg of huperzine, the last group experienced tonic-clonic seizures. Among the markers under investigation are TBARS, FRAP, GR, caspase 3, and a few

other biochemical markers. The temporal and parietal lobes were more susceptible to huperzine's highest dose, which also enhanced caspase 3 activity in the cerebellum, frontal lobe, and temporal lobe. Temporal and parietal lobes showed the most noticeable lipid peroxidation, while the frontal lobe, cerebellum, spleen, and kidney all showed increased expression of GR.

The biochemical markers remained constant and did not indicate any signs of disease. According to the study's findings, exposure to huperzine significantly increased brain lipid peroxidation, and as dosage grew, the ratio of AST to ALT decreased. The ability of huperzine to lower lipid peroxidation and oxidative stress, as shown by elevated GR and TBARS levels. The rise in antioxidants is greater than the amount of direct oxidative stress, indicating that even a small amount of oxidative stress can cause antioxidants to respond appropriately. It has been observed that huperzine A lowers oxidative stress and nerve apoptosis. On the other hand, it has been discovered to cause mild liver damage, resulting in a dose-dependent reduction in the AST/ALT ratio. Huperzine A also has the capability to inhibit caspase 3 activity induced by amyloid beta and reduce amyloid beta levels generated by oxidative stress. Huperzine can have negative effects in addition to being associated with the strengthening of antioxidant barriers. Through the cholinergic anti-inflammatory route, huperzine's effect on the immune system can boost cognitive abilities. Unfounded expectations are inappropriate since the role of huperzine in antioxidant homeostasis is poorly known[15].

Administration in Animals

In an in vivo trial with dogs, transdermal Huperzine A (HupA) patches demonstrated effective controlled release compared to oral treatment. The patches had a longer t_{max} (24 hours vs. 3 hours), lower C_{max} (3.4 ng/ml vs. 9.8 ng/ml), and generally stable serum levels over 84 hours. They also resulted in localized and mild cutaneous adverse reactions, making them suitable for administration twice a week.

Research has also shown that administering HupA intranasally disperses it more evenly throughout the rat brain than administering it intravenously. In addition, when delivered intranasal as demonstrated in rats, HupA has a C_{max} of 190 µg/l at a t_{max} of 41 min. Rat liver microsomal HupA metabolism mostly proceeds through CYP1A2, a monooxygenase belonging to the cytochrome P450 superfamily of proteins that catalyzes processes including drug metabolism. Mass spectroscopy and nuclear magnetic resonance have identified 13,14-Epoxy HupA, a metabolite of HupA, in rat blood; however, it is not obvious whether this is the chemical that is expelled and whether it has biological activity.

Furthermore, through pregnane X receptor-related pathways, in LS174T (colorectal adenocarcinoma) cell lines, HupA induces CYP3A4 mRNA and protein expression. Similarly, although total CYP levels did not rise, oral administration of HupA at doses of 0.1, 1, and 2 mg/kg over 14 days showed changes in CYP3A4 activity which was observed to increase CYP1A2 transcription and protein production in rat liver microsomes. If these findings are applied to humans, they may indicate that CYP1A2-metabolized medications and HupA interact; however, more research is needed to confirm this. Studies on animals have shown that radiolabeled HupA is primarily (73%) eliminated in urine; nevertheless, more research in humans is required.

CLINICAL STUDIES

Pharmacology

Acetyl cholinesterase is strongly, competitively, and reversibly inhibited by huperzine A, blocking endogenous acetylcholine breakdown. Compared to existing inhibitors such as tacrine, donepezil, rivastigmine, and physostigmine, it is more potent, or equally potent. Little is known regarding its impact on AD, despite the existence of suggested mechanisms of action. Inhibiting acetyl cholinesterase may improve cognitive improvement in AD patients. Five sites—the active-site gorge, the parent chain ketone group of His440, ethylidene Me group, the H-bonding with Tyr130, and hydrophobic interactions—are where huperzine A interacts with acetyl cholinesterase. The enhanced potency of huperzine A over other inhibitors can be explained by these interactions. Huperzine A holds promise for treating Alzheimer's disease (AD) due to its protective effects against cytotoxicity and apoptosis induced by H2O2, β-amyloid protein, glutamate, ischemia, and staurosporine. It is better than other acetyl cholinesterase inhibitors because it binds to both acetyl cholinesterase and peripheral butyrylcholinesterase. Huperzine A is a desirable alternative for treating AD because it likewise inhibits the NMDA receptor ion channel without causing psychotic effects and has few negative cholinergic effects[2].

Pharmacokinetics

Drug development relies heavily on pharmacokinetic studies, and huperzine A's pharmacokinetic characteristics have been investigated. Twelve human individuals in good health were given a single oral dose of 0.4 mg in the form of a tablet during an in vivo research. The study discovered a biphasic pharmacokinetic profile, which is characterized by rapid absorption and distribution and a delayed rate of elimination. Huperzine A has an area under the plasma vs. time curve of 2450.34233.32 mg/l min and was dispersed broadly in the body, even managing to breach the blood-brain barrier. Six Chinese participants received a single dosage of 0.99 mg in a tablet study, and reversed-phase HPLC was used to measure the drug's plasma concentrations. Additionally, the study showed that huperzine A was removed at a more modest rate and that it was rapidly absorbed and distributed widely throughout the body[2].

Toxicology

Although huperzine A is not hazardous to humans, research on animals have reported subtle to modest cholinergic side effects. The LD50 is 2-4 mg/kg and 0.6 mg/kg/d in rats and rabbits respectively. Heart and brain problems are caused by high dosages of huperzine A. Acute cholinergic toxicity has been reported in dogs, with 0.1 mg/kg/d being the “no-observed adverse effect threshold” (NOAEL) for dogs[2].

Reduced toxicity of β-amyloid: Extracellular senile plaques are made up of β-amyloid (Aβ) fragments, which result from the proteolysis of the membrane-bound amyloid precursor protein (APP). Aβ fragments are produced by the β/γ-secretase amyloidogenic pathway, which cleaves APP into insoluble form, and soluble α-APP fragments are produced by the α-secretase non-amyloidogenic pathway. It has been demonstrated that Aβ triggers apoptotic responses, which could result in AD therapy choices. Multiple studies showing a link between Ach-E activity and Aβ generation have shown that AChE co-localizes with Aβ deposits in AD patients and forms a stable complex with Aβ, promoting the cluster of Aβ deposits. This bond increases the neurotoxicity of the Aβ deposit and changes the enzymatic activity of AChE[17].

Treatment Regimen in Humans

Every tablet containing the active medicine contains 50 μg of HupA. For eight to twenty-four weeks in a row, patients in the HupA group were given oral HupA pills. The daily mean dosage varied between 300 and 500 μg and patients in the control group were given blank pills. In a study conducted by Zhang et al, in addition to this vitamin E (100 mg/day) was administered to both treatment and control groups[18].

Efficacy and Safety

Four RCTs with 474 participants—two single-blind and two double-blind trials showed that MMSE and ADL scores were improved when huperzine A was used instead of a placebo. Regression analysis revealed a similar trend for ADL and improved efficacy with time for MMSE. The huperzine A group experienced minor peripheral cholinergic side effects more frequently than the placebo group[19].

Adverse Effects

With frequent adverse medication reactions that impact neuropsychiatric, gastrointestinal, general, and cardiovascular diseases, the effectiveness of ChEIs is still inadequate, especially in patients with advanced AD. In order to address the rising incidence of AD, new, focused, safe, and effective treatments are required. Anorexia, indigestion, bradycardia, insomnia, nasal obstruction, ankle oedema, diarrhoea, dizziness, festinating gait, headache, hyperactivity, hypopraxia, moderate abdominal pain, nausea and vomiting are the adverse effects that are anticipated[20].

CONCLUSION

The pathogenesis of Alzheimer's disease is not well understood, which makes it difficult to identify a suitable treatment. A naturally occurring substance from Huperzia serrata moss called huperzine A has been suggested as a possible AD therapy. Humans with mild to moderate AD and animal models have both benefited from favourable cognitive augmentation in clinical trials. However, doctors in USA are unable to prescribe huperzine A as a routine treatment for memory problems like dementia due to the death of research-backed literature regarding the drug's safety and effectiveness. Approval of huperzine A as conventional treatment for AD and other dementias may be aided by a more extensive, carefully planned phase III clinical trial.

CONFLICT OF INTEREST:
The authors have no conflicts of interest regarding this investigation**.**

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