



DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . NOV 19 1999

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient: Huperzine A
Firm: Pharmavite Corporation
Date Received by FDA: November 17, 1999
90-day Date: February 14, 2000

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after February 14, 2000.


Robert J. Moore, Ph.D.

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955-0316

RPT 58



NOV 19 1999
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Mr. David Kropp
Acting Director
Regulatory and Consumer Affairs
Pharmavite Corporation
15451 San Fernando Mission Boulevard
P.O. Box 9696
Mission Hills, California 91346-9606

Dear Mr. Kropp:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) dated November 15, 1999, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., Huperzine A) was received by the Food and Drug Administration (FDA) on November 17, 1999. Your submission will be kept confidential for 90 days from the date of receipt, and after February 14, 2000, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

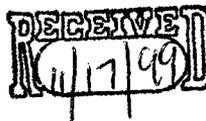
Sincerely,

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Programs and Enforcement Policy
Office of Special Nutritionals



November 15, 1999

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.
Washington, DC 20204



Dear Sir or Madam:

In accordance with 21 CFR 190.6, Pharmavite Corporation is hereby notifying the Food and Drug Administration that we intend to market a dietary supplement containing a new dietary ingredient.

1. The name and complete address of the manufacturer or distributor of the dietary supplement that contains a new dietary ingredient:
Pharmavite Corporation
15451 San Fernando Mission Blvd.
Mission Hills, CA 91345
2. The name of the new dietary ingredient that is the subject of the premarket notification:
huperzine A
3. A description of the dietary supplement or dietary supplements that contain the new dietary ingredient including:
 - (i) The level of the new dietary ingredient in the dietary supplement:
Maximum daily intake of 5 mcg huperzine A
 - (ii) The conditions of use recommended or suggested in the labeling of the dietary supplement:
Maximum daily intake of 5 mcg huperzine A
4. The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe:
See attached articles:
Double-blind trial of Huperzine-A (HUP) on Cognitive Deterioration in 314 Cases of Benign Senescent Forgetfulness, Vascular Dementia, and Alzheimer's Disease

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Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
November 15, 1999
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Efficacy of tablet huperzine-A on memory, cognition, and behavior in
Alzheimer's disease
Huperzine A: Boost Your Brain Power
Cognition Improvement by Oral Huperzine A: A Novel Acetylcholinesterase
Inhibitor

An original and two copies of this notice are being filed. Pursuant to 21 CFR 190.6(c), please confirm your receipt of this notice. We also request that this information be kept confidential for 90 days under the provisions of 21 CFR 190.6(e).

Sincerely,

David Kropp
Acting Director, Regulatory and Consumer Affairs

DK/FDA/huperzine notice 1

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ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 854

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TOWARDS PROLONGATION OF THE HEALTHY LIFE SPAN

Practical Approaches to Intervention

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Imp
#2

Edited by Denham Harman, Robin Holliday, and Mohsen Meydani

*New York Academy of Sciences
New York, New York
1998*

Double-blind Trial of Huperzine-A (HUP) on Cognitive Deterioration in 314 Cases of Benign Senescent Forgetfulness, Vascular Dementia, and Alzheimer's Disease

MA YONG-XING, ZHU YUE, GU YUE-DI, YU ZHEN-YAN, YU SAI-MEI,
AND YE YONG-ZHEN

*Research Division of Aging and Antiaging, Shanghai Geriatric Institute,
Huadong Hospital, 221 West Yan An Road, Shanghai 200040, China*

HUP, a new alkaloid extracted from *Huperzia serrata* (Thumb) Trev, by Liu, is a potent anticholinesterase with minimal toxicity. Tong has found that HUP may improve the learning and retrieval function of rats, and its facilitation actions were due to an effect on the central cholinergic system.

BENIGN SENESCENT FORGETFULNESS (BSF) TREATED WITH 0.03-0.05 mg HUP im b.i.d.

The first clinical trials used the double blind method on 120 patients with cognitive deterioration, with memory quotient (MQ) (WMS) < 100. The mean values of MQ of 60 treated and 60 controls were 76.27 ± 14.08 and 77.97 ± 12.55 ($p > 0.05$), respectively. The dosage was 0.03 mg im B.i.d. for 14-15 days. The mean values of MQ after the treatment (the interval between pre- and posttests of WMS with A and B form, respectively, is 1-2 months) of treated and controls were 91.85 ± 13.73 ($p < 0.01$) and 82.24 ± 15.10 ($p > 0.05$), with the MQ increase of 15.82 ± 10.02 and 4.40 ± 8.72 , respectively, ($p < 0.01$). The effective rates were 68.33 and 26.37% in the two groups. No significant side effects were observed.

The second trial included 16 patients of the HUP treatment group (0.03-0.05 mg im B.i.d for 4 wks) with IQ (WAIS) < 105 (95.0 ± 7.6). The IQ increased to 100.7 ± 12 ($p < 0.01$) after the treatment. The value of IQ increase is 5.7 ± 0.68 as compared with 3.0 ± 0.36 in the hyperbolic oxygenation treatment group (2.5 ATA, 80', 4 wks in 10 patients ($p < 0.01$)).

* BSF TREATED BY 0.1 MG HUP(po)q.i.d.

The clinical trials used the double blind method on 88 patients with cognitive deterioration, with MQ (WMS) < 100. The mean values of MQ of 44 treated and 44 controls were 82.8 ± 14.3 and 81.5 ± 14.4 ($p > 0.05$), respectively. The dosage was 0.1 mg po Q.i.d. The mean values of MQ after the treatment (the interval between pre- and posttests of WMS with A and B form, respectively, is 2 months) of treated and controls were 93.5 ± 14.5 ($p < 0.01$) and 85.5 ± 16.5 ($p < 0.01$). The effective rates were 68.18 and 34.09% in the two

MA *et al.*: HUP

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groups. No significant side effects were observed except gastric discomfort (2), dizziness (1), insomnia (1), and mild excitement (1) in the treated group.

VASCULAR DEMENTIA AND ALZHEIMER'S DISEASE

A clinical trial of vascular dementia (25) and Alzheimer's disease (55) was conducted on 40 treated and 40 control patients with the same dosage as for BSF. The MQ of the treated group increased from 50.40 ± 18.49 to 59.74 ± 18.73 ($p < 0.05$); that of the control group increased from 53.95 ± 14.74 to 55.85 ± 16.28 ($p > 0.05$). The MQ increase of 9.37 ± 10.38 is significantly higher than that of 1.90 ± 10.36 ($p < 0.01$). The effective rate of the treated group was 60%, significantly higher than that of 35% in the control group ($p < 0.05$). No significant side effects were observed except gastric discomfort or nausea (3) and dizziness (3) in the treated group.

It is concluded that huperzine is an effective and safe drug to improve cognitive and memory function in the aged and preaged.

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1995 Sep; 16 (5): 391-395

Efficacy of tablet huperzine-A on memory, cognition, and behavior in Alzheimer's disease

XU Si-Sun, GAO Zhi-Xu¹, WENG Zheng², DU Zun-Ming², XU Wei-An⁴, YANG Jian-Shen⁵, ZHANG Ming-Lian⁶, TONG Zhen-Hua, FANG Yong-Sheng¹, CHAI Xin-Sheng², LI Shu-Lan³ (Zhejiang Supervision and Detection Station of Drug Abuse, Zhejiang Medical University, Hangzhou 310009; ¹Shanghai Mental Health Center, Shanghai 200030; ²Shandong Mental Health Center, Ji-nan 250014; ³Hangzhou Central Hospital of Railway, Hangzhou 310009; ⁴Shanghai 3rd Mental Hospital, Shanghai 201905; ⁵Suzhou Puji Hospital, Suzhou 215008; ⁶Suzhou Guangji Hospital, Suzhou 215008, China)

AIM: To evaluate the efficacy and safety of tablet huperzine-A (Hup) in patients with Alzheimer's disease. **METHODS.** Using multicenter, prospective, double-blind, parallel, placebo controlled and randomized method. 50 patients were administrated orally 0.2 mg (4 tablets) Hup and 53 patients were given *po* 4 tablets of placebo *bid* for 8 wk. All patients were evaluated with Wechsler memory scale, Hascgawa dementia scale, mini-mental state examination scale, activity of daily living scale, treatment emergency symptom scale, and measured with BP, HR, ECG, EEG, ALT, AKP, BUN, Cr, Hb, WBC, and urine routine. **RESULTS.** About 58 % (29/50) of patients treated with Hup showed improvements in their memory ($P < 0.01$), cognitive ($P < 0.01$), and behavioral ($P < 0.01$) functions. The efficacy of Hup was better than placebo (38 %, 19/53) ($P < 0.05$). No severe side effect was found. **CONCLUSION:** Hup is a promising drug for symptomatic treatment of Alzheimer's disease.

KEY WORDS huperzine-A; cholinesterase inhibitors; Alzheimer's disease; multicenter studies; double-blind method; randomized controlled trials; Wechsler scales; memory; cognition disorders; activity of daily living

The loss of cholinergic neurons of the brain observed in Alzheimer's disease is considered an important pathogenetic element of dementia^[1]. These finding provoked a series of pharmaceutical studies to look for a drug which might supplement the cholinergic function for its symptomatic treatment. Huperzine-A (Hup), a new Lycopodium alkaloid (Fig 1) first isolated from Chinese herb *Huperzia serrata* (Thunb) Trev by Chinese^[2], is a potent, centrally active, and reversible cholinesterase inhibitor (ChEI)^[3] with better therapeutic index than those of physostigmine and THA^[4]. It was reported to ameliorate learning and memory retention in rodents and improve memory in aged^[5,7].

The present study was to confirm the clinical efficacy and safety of Hup in treatment of Alzheimer's disease.

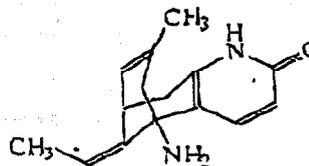


Fig 1. Molecular representation of Huperzine-A.

MATERIALS AND METHODS

Patients Patients ($n=103$) who met AD criteria

Received 1994-12-26

Accepted 1995-06-23

Hup treatment ($P < 0.01$), but not in the placebo group except the MQ score ($P < 0.01$): resulting in a significant difference on MQ, MMS, and HDS between 2 groups ($P < 0.01$) (Tab 2). Rank sum test of WMS and MMS between 2 groups showed a significant improvement in 'number of recitation' item of WMS and 'time orientation' item of MMS.

Tab 2. comparison of MQ, MMS, HDS and ADL between 2 groups of AD.

* $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs before treatment; # $P > 0.05$, ## $P < 0.05$, ### $P < 0.01$ vs placebo.

	Pla (n=53)	Hup (n=50)
MQ baseline	48 ± 21	56 ± 21 ^d
8-wk trial	52 ± 26	64 ± 26 ^a
t (paired)	2.69 [*]	5.15 ^{**}
MMS baseline	14 ± 5	16 ± 5 ^c
8-wk trial	15 ± 6	19 ± 6 ^f
t (paired)	0.76 [*]	5.02 ^{**}
HDS baseline	16 ± 5	16 ± 6 ^d
8-wk trial	15 ± 7	20 ± 6 ^f
t (paired)	0.30 [*]	7.04 ^{**}
ADL baseline	31 ± 9	33 ± 10 ^d
8-wk trial	31.9 ± 0.7	29 ± 9 ^d
t (paired)	1.64 [*]	4.51 ^{**}

Subjective evaluation According to the reports of patients' intimate relatives, the 3 positive results ('clear headed', 'memory improving', and 'language improving') of Hup group were increased, whereas the negative result (complaint of 'unchanged') of the placebo group was increased. A significant difference was found between two groups ($P < 0.01$) (Tab 3).

Tab 3. Complaints of the patient's legal representatives between 2 groups of AD. * $P < 0.01$.

Complain	Pla (n=53)	Hup (n=50)	χ^2
Clear headed	13 (17.37)	26 (21.63)	
Memory improving	8 (10.69)	16 (13.31)	
Language improving	2 (4.45)	8 (5.55)	12.29 [*]
Unchanged	34 (24.49)	21 (30.51)	
Total	57	71	

Neither the TESS score nor the laboratory changes showed any significant difference between 2 groups with paired or group t test (Tab 4, 5).

Tab 4. Comparison of all measured data between 2 groups of AD. * $P > 0.05$.

no 3 test significantly difference

Item	Pla (n=53)		Hup (n=50)	
	baseline	8-week trial	baseline	8-week trial
BP/kPa				
systolic	17.4 ± 2.1	17.5 ± 2.4 [*]	17.6 ± 2.9	17.1 ± 2.5 [*]
diastolic	11.0 ± 1.1	11.0 ± 1.2 [*]	11.0 ± 1.6	10.9 ± 1.3 [*]
HR/min	74 ± 9	74 ± 8 [*]	72 ± 9	47 ± 9 [*]
Hb/g L ⁻¹	128 ± 18	129 ± 15 [*]	128 ± 18	129 ± 13 [*]
WBC/1 × 10 ⁹ L ⁻¹	6.1 ± 1.2	6.1 ± 1.4 [*]	6.0 ± 1.4	6.2 ± 1.5 [*]
BUN/mmol L ⁻¹	5.1 ± 0.8	5.1 ± 0.9 [*]	5.1 ± 1.0	5.1 ± 1.1 [*]
Cr/μmol L ⁻¹	103 ± 21	102 ± 21 [*]	94 ± 19	94 ± 19 [*]
AKP/U L ⁻¹	19.6 ± 2.8	20 ± 3 [*]	19 ± 4	19 ± 4 [*]
ALT/U L ⁻¹	29 ± 6	28 ± 6 [*]	29 ± 7	29 ± 6 [*]

of DSM-III-R⁽¹⁾ were selected for this study. Their entrance criteria were age over 50 a; memory quotient (MQ) < 90; Hasegawa dementia scale (HDS) < 15 (illiteracy), < 20 (primary), < 24 (middle); minimal state examination scale (MMS) score 13-23; activity of daily living scale (ADL) > 16; Hachinski ischemic scale (HIS) score < 4. Depression, severe physical or psychotic disorders, and non-AD dementia were ruled out. Their procurators agreed with the patients to participate in this study.

Methods Patients were abstained from any CNS stimulants, steroids, and nootropics for 1 wk. They were randomly divided into 2 groups given 4 tablets (0.2 mg of Hup or 70 mg of placebo) orally twice a day for 3 wk. The tablets, same in shape, color, weight, taste and the packaging, were provided by Hong-Qi pharmaceutical Factory of Shanghai Medical University. The clinicians and the patients were double-blind.

Assessment BP and HR were measured weekly. ECG and TESS were repeated half a month. ALT, AKP, BUN, Cr, Hb, WBC, and urine routine were repeated monthly. EEG, WMS, HDS, MMS and ADL were repeated at the end of trial.

Data analysis The statistical analysis of the results were performed by POMS software. Pair *t* test was used for MQ, MMS, HDS, and ADL before and after trial. We analysed 4 additional items ('clear headed', 'memory improving', 'language improving' and 'unchanged') with χ^2 method.

Duration of trial From 1993-09-01 to 1994-04-30

RESULTS

The blind was declassified on 1994-05-05 in Shanghai: 50 patients were in Hup group and 53 patients were in placebo group. The pretreatment data between the 2 groups showed no significant difference (Tab 1).

The intraclass correlation (ICC) ICC on MMS, HDS, and ADL from 4 districts (Zhejiang, Shanghai, and Shandong, Suzhou) were 0.98, 0.87, and 0.96, respectively ($P > 0.05$).

Psychological assessment There were

Tab 1. Background data between the 2 groups of All data showed no statistical significance bet Hup and Pla group.

	Pla (n=53)	Hup (n=50)
Sex: ♂	29	23
♀	24	22
Age:	67±11	66±11
range	55-89	53-90
Occupation:		
worker	30	21
peasant	3	2
administrator	13	16
technician	5	6
home-maker	2	5
Culture:		
college	5	6
senior high	9	9
junior high	9	10
elementary	26	18
illiteracy	4	7
Marriage:		
single, divorced	15	17
unmarried	0	2
married	38	32
Course: (year)	3.0±1.8	3.1±1.6
<2	8	8
2-	34	24
4-	8	16
6-	1	0
≥8	2	2
Severity:		
mild	27	33
moderate	23	17
severe	3	0
MQ baseline	48±21	56±21
MMS baseline	14±5	16±5
HDS baseline	16±5	16±6
ADL baseline	31±9	33±10
TESS baseline	1±4	1±3
Identified cerebral atrophy by CT or MR.	25 (47 %)	22 (44 %)

significant differences on MMS, HDS, and ADL between 'before' and 'after' the 8-

Tab 5. Comparison of cholinergic side effects between 2 groups of AD. * $P > 0.05$.

Cholinergic side effects	Pla ($n=53$)	Hup ($n=50$)
Exciting	3 (5.7 %)	3 (6 %)*
Hyperactivity	3 (5.7 %)	5 (10 %)*
Nasal obstruction	4 (7.5 %)	4 (8 %)*
Nausea or vomiting	1 (1.9 %)	4 (8 %)*
Diarrhea	2 (3.8 %)	5 (10 %)*
Insomnia	4 (7.5 %)	5 (10 %)*
Anorexia	3 (5.7 %)	5 (10 %)*
Dizziness	6 (11.3 %)	4 (8 %)*

DISCUSSION

In order to avoid many interfering factors of treatment study in dementia⁽⁹⁾, such as influences of intercurrent disease, age-related changes in pharmacokinetics, poor compliance with drug regimes, cognitive impairment, and loss of insight etc, we designed this strict study, in addition, there was a high ICC in evaluators, and comparable background data between 2 groups, we considered that the results of this study are reliable.

The results of this study exhibited that about 58 % (29/50) of patients treated with Hup showed clinical improvements in their memory ($P < 0.01$), cognitive ($P < 0.01$) and behavioral ($P < 0.01$) functions. The efficacy of Hup was better than placebo (36 %, 19/53) ($P < 0.05$). According to the MMS evaluation, an average improvement of 2.98 points was noted for patients treated with Hup, and with 54 % of these patients improving by 3.0 points or more. But the placebo group increased an average of 0.43 points, only with 30.2 % of them improving by 3.0 points or more ($P < 0.05$).

As to the findings of significant improvement in 'number of recitation' item of WMS and 'time orientation' item of MMS, it was similar to the discovery of some au-

thors^(9,10).

Throughout 8-wk study, no patient value exceeded the upper limit of normal toxicity in both groups, only a slight increase in some mild peripheral cholinergic effects such as nausea or vomiting and diarrhea were occurred in Hup group. But there was no statistical significance in comparison with placebo group. This clinical finding is similar to the results of several experimental studies^(4,6,7,11,12), ie Hup produced less peripheral side effects at optimal doses, it indicates Hup is a safe drug and suitable for treatment of patients with Alzheimer's disease.

Both Hup and tetrahydroaminoacridine (THA, tacrine) approved by FDA in 1991 are long to cholinergic agents, but the latter has potential liver toxicity^(13,14), and only a slight improvement of 2.0 points on MMS over 8-wk was noted for patients receiving THA 16 mg d⁻¹, with 43 % of these patients improving by 3.0 points or more⁽¹⁴⁾, therefore THA is not an ideal drug in the treatment of patients with Alzheimer's disease. Whereas, according to the results of present study, both Hup efficacy and the safety of Hup are significantly better than THA, we consider that Hup is a promising candidate drug for symptomatic treatment in patients with Alzheimer's disease.

Although the results of this study are encouraging, there is no extensive, long-term and high-dose observation, especially there is no direct clinical comparison with THA. At the same time, we think the present study is not sufficient, so we hope that further studies will be undertaken to develop methods for identifying the efficacy and safety of Hup.

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Fu for technical help in manuscript.

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石杉碱甲片对阿耳茨海默病记忆、认知和行为的疗效

徐莉芬¹, 高之超¹, 翁正², 杜孝铭³, 徐维安⁴, 杨健身⁵, 张明康⁶, 董振华⁷, 方雍生¹, 柴新生², 李淑兰³ (浙江医科大学浙江省药物滥用监测站, 杭州310009; ¹上海市精神卫生中心, 上海200030; ²山东省精神卫生中心, 济南250014; ³杭州铁路中心医院, 杭州310009; ⁴上海市第三精神病院, 上海201905; ⁵苏州普济医院, 苏州215008; ⁶苏州广济医院, 苏州215008, 中国)

目的: 评估石杉碱甲片治疗阿耳茨海默病的疗效及其安全性。 **方法:** 采用多中心、前瞻性、双盲、平行、空白对照和随机方法, 给50例阿尔采末病一日两次口服石杉碱甲片4片(每片含50微克), 并给53例阿耳茨海默病一日两次口服安慰剂片4片, 共8 wk。所有病人都用韦氏智力量表、简易精神状态量表、长谷川痴呆量表、日常生活能力量表、副反应量表和其他实验室检查。 **结果:** 发现58% (29/50)的石杉碱甲片服用者改善了所有的记忆、认知和行为能力, 而安慰剂组仅为35.8% (19/53), 两组疗效有显著差异($\chi^2=5.07, P<0.05$), 而两组均无严重不良反应发生。 **结论:** 石杉碱甲片显著增高记忆、认知和行为功能, 是治疗阿耳茨海默病的一个有前景的药物。

关键词 石杉碱甲; 胆碱脂酶抑制剂; 阿耳茨海默病; 多中心研究; 双盲法; 随机对照试验; 韦氏量表; 记忆; 认知障碍; 日常生活活动

#15 Dup

MEMORY MOSS

Huperzine A is a natural compound isolated from the club moss, *Huperzia serrata*. It is a Chinese folk medicine, called "qian ceng ta" and has been used for centuries to improve memory, focus and concentration and to help alleviate memory problems among the elderly. Reports from China, where an estimated 100,000 people have been treated, indicate that Huperzine A is safe and effective. It has other properties such as protecting nerve cells from toxic substances including nerve gas poisons, and from damage produced by strokes and epilepsy. It is also used to treat persons afflicted with Alzheimer's disease and myasthenia gravis. Based on laboratory studies some researchers believe that Huperzine A may be more effective than drugs presently available for the treatment of Alzheimer's disease.

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HUPERZINE A: BOOST YOUR BRAIN POWER

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INTRODUCTION

The aging process, physical and mental stress, and environmental toxins induce deleterious effects in the human brain. Initial symptoms can include short-term memory loss, lack of focus and reduced concentration. More seriously, they can lead to degenerative diseases of the brain including long-term memory loss and Alzheimer's dementia, the fourth leading cause of death in the United States.

The harmful effects of environmental toxins alone on the nervous system or brain are a major threat to the human race. Many people will recall the case of Minamata disease in Japan in the 1950s and 1960s and in Iraq in 1971-72. Those who ate fish and shellfish contaminated by methylmercury developed damage of the brain, spinal cord and peripheral nerves (those coming from the brain and spinal cord). Symptoms of the disease included disruption of visual and sensory nerve function, muscle uncoordination (ataxia) and impairment of hearing, speech and gait. Chronic poisoning from environmental toxins can also cause general weakness of the extremities, memory loss, multi-infarct dementia (resulting from small strokes) and various forms of memory impairment including Alzheimer's dementia. Dementia is a loss of intellectual function (thinking, remembering, reasoning) so severe that it interferes with an individual's daily functioning and eventually results in death. While the causes of Alzheimer's disease are not known and are currently undergoing intense scientific investigation, suspected causes include defective genes or a genetic predisposition, abnormal protein build-up in the brain (this will be explained later) and environmental toxins.

While the liver and other organs can regenerate and form

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new cells after a toxic insult, brain or nerve cells which die as a result of irreversible damage are not replaced. Alzheimer's disease is a degenerative disease. There is a progressive loss of nerve cells (neurons), and the loss is selective, meaning that it affects one or more groups of neurons while leaving others intact. Also, the disease arises without any clear "inciting event" in a patient having no known (previous) neurologic deficits. Although most cases are sporadic, at least 5 to 10 percent of cases have a genetic (hereditary) component. For Alzheimer's, the destruction occurs in the cerebral cortex, which is the outer tissue of the brain—the "gray matter." This tissue is made up of nerve fibers involved in movement, sensation, vision, hearing and other higher brain functions. The main symptom of degeneration of these nerves is dementia, i.e., progressive loss of cognitive function independent of the state of attention. Dementia is not part of normal aging and always represents a disease process. The best that can be done for Alzheimer's patients is to help them use their remaining healthy brain tissue fully, and protect their brains from the ravages of the disease. One characteristic that all degenerative brain diseases, including Alzheimer's, share is that oxidative stress increases the rate at which the disease progresses.¹

Oxidative stress results from free radical damage. Free radicals are destructive molecules that are produced in the body as a result of exposure to environmental toxins or occur as a result of normal body metabolism. Free radicals damage cells in every part of the body. Brain cells, however, are particularly susceptible to oxidative stress for two reasons. First, nerve cells do not divide, so no new cells are made. The brain thus has no means of escaping damage through cellular replenishment. Second, neurons possess low levels of antioxidant defenses such as vitamins C and E and beta carotene; it is thus difficult for these cells to protect themselves from free radical attack.¹

An ideal therapy for incurable degenerative brain diseases would protect the brain from free radical damage, maintain

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or enhance key neurotransmitter action and help the brain to function optimally. Huperzine A, a natural product isolated from the club moss *Huperzia serrata*, fulfills these requirements. Huperzine A (HupA) has been shown to be a promising agent for a wide range of memory and brain disorders, including Alzheimer's disease. It works by the following mechanisms.

Acetylcholine is a neurotransmitter that is essential for normal learning and memory function *in vivo* (in living organisms). Neurotransmitters carry chemical messages from one nerve cell (the presynaptic nerve) to the next, causing the receiving neuron (the postsynaptic nerve) to "fire" its neurotransmitters (see figure on page 16). Because nerve cells are close together (separated by a space called a "synaptic cleft") the released neurotransmitter (in this case, acetylcholine) will activate the next cell in the sequence. It will bind to a "cholinergic" receptor (one that binds acetylcholine) on the postsynaptic nerve. In the human and animal brain the enzyme "acetylcholinesterase" (AChE), normally breaks down acetylcholine, keeping it from repeatedly firing the nerve and thus regulating the availability of this neurotransmitter in the brain. Acetylcholine function is deficient in patients with memory impairments or Alzheimer's dementia, probably due to selective degeneration of acetylcholine-producing neurons in the brain. It has been demonstrated in Alzheimer's disease patients that inhibitors of AChE (also called acetylcholinesterase inhibitors) reduce the breakdown of acetylcholine, and thus increase its availability. This can conceivably lead to an improvement in the patient's condition. HupA is a potent inhibitor of AChE, and thus helps to increase levels of acetylcholine in the brain.

Short- or long-term administration of Huperzine A has been demonstrated to induce a potent inhibitory effect of high affinity transport of choline in the brain.² Choline is the starting material for the synthesis of acetylcholine. Inhibiting choline's transport allows more to be available for synthesis of acetylcholine (see figure on page 16).

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An important property of HupA is that there is no significant development of tolerance to it; it can be used continuously.² Tolerance develops in response to many of the effects of physostigmine, and in the case of tacrine, a second dose results in a varied response to the AChE inhibitor.

Scientific research has demonstrated the diverse therapeutic potential of HupA: it is a potent, selective and reversible inhibitor of AChE, with a longer duration of action than other AChE inhibitors and minimal side effects. HupA has been identified to have profound beneficial effects in the following areas:

- Learning and memory retention
- Improve focus and concentration
- Treatment of cognitive and memory impairment
- Improved nerve transmission to muscles
- Powerful and reversible long-term inhibitor of AChE activity in the brain
- Dementia resulting from strokes and senile or pre-senile dementia
- Improved clinical picture for patients with myasthenia gravis
- Improved short-term and long-term memory in patients with cerebral arteriosclerosis (hardening of arteries in the brain)
- Alleviation of symptoms related to glaucoma
- Prevention of organophosphate pesticide toxicity
- Prevents nerve gas toxicity
- A novel psychotherapeutic agent for improving cognitive function in Alzheimer's patients
- A superior safety and efficacy profile compared to other cholinesterase inhibitors

HupA has neuroprotective ability also: it can stop damage to nerve cells and can block the effects of glutamate toxicity. Glutamate is an excitatory (stimulatory) neurotransmitter. During a stroke or other brain injury, excess glutamate is released in the brain, causing inappropriate release of enzymes inside cells that leads to cell death. This protective

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ability of HupA may be helpful in treating strokes, epilepsy and other neurological disorders.

WHAT IS HUPERZINE A?

Huperzine A (HupA) is found in an extract from the club moss *Huperzia serrata*, which grows at high elevations and in cold climates. It has been used for centuries in Chinese folk medicine,³ and is also known as *Qian Ceng Ta*. In China it was used to treat fever and inflammation, and for the past several years has been a prescription medication for treatment of dementia. Researchers claim that it helps alleviate memory problems in the elderly, as well as in those individuals with Alzheimer's disease (AD). HupA, a *Lycopodium* alkaloid, was first isolated from *Huperzia serrata* (Thumb) Trev. (also known as *Lycopodium serratum* Thumb) at the Zhejiang Academy of Medicine and Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.⁴ Dr. Alan Kozikowski, Professor of Chemistry in the Neurology Department in Georgetown University's Drug Discovery Program, in Washington, DC, has studied it extensively, and is the researcher who first synthesized HupA. As he reported in JAMA (The Journal of the American Medical Association), HupA has been used to treat an estimated 100,000 people in China, suggesting that it is safe to use.⁵ The use of AChE inhibitors to alleviate symptoms of Alzheimer's disease has been the most promising approach thus far in dealing with this unresponsive condition. HupA, a natural, potent, and selective cholinesterase inhibitor has proven superior to other acetylcholinesterase inhibitors now recommended for the management of Alzheimer's disease; its neuroprotective action is an added benefit. Scientists have thus demonstrated that HupA holds tremendous promise for improving the quality of life for people with a wide range of memory impairments, including Alzheimer's dementia.

ALZHEIMER'S DISEASE DEFINED

Alzheimer's disease (pronounced Alz'-hi-merz) is a progressive, degenerative disease that attacks the brain and results

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in impaired memory, thinking and behavior. Damage is irreversible. The disease was first described by Dr. Alois Alzheimer in 1906. This mental decline is related to a loss of nerve cells and the links (synapses) between them. Alzheimer's disease (AD) is the most common form of dementia. It is not part of the normal aging process and always represents a disease process. AD is the fourth leading cause of death in adults after heart disease, cancer and stroke. Men and women are affected almost equally.

Researchers have developed a deeper understanding of the changes that occur in the brain (plaques and tangles) and behavioral changes that characterize the disease. Identified risk factors are age and family history. Most people diagnosed with AD are older than age 65; however, AD can occur in people in their forties and fifties. The course of the disease varies from person to person, as does the rate of decline. On average, AD lasts from four to eight years after diagnosis; however, it can continue for up to twenty years.

The first symptoms of AD include loss of recent memory, faulty judgement and personality changes. In the disease's early stages, people with AD may forget how to do simple things such as washing their hands. Often, they can no longer think clearly or remember the words for familiar objects or people's names.

The causes of AD are not known and are currently undergoing rigorous scientific investigation. Suspected causes include multiple mini strokes, defective genes or a genetic predisposition, abnormal protein build-up in the brain (amyloid—see below) and environmental toxins.

Amyloid is a protein-like substance deposited between cells and found in many organs of the body in a wide variety of diseases. In Alzheimer's disease, one of the main abnormalities seen in the brains of patients with the disease are plaques made of tangled nerve cells surrounding a central amyloid core. Amyloid consists of fibrils (string-like chains of protein) which represent 95 percent of the substance. The other 5 percent is glycoprotein (a sugar joined to protein).

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There are several types of amyloid. Beta-amyloid protein (A β) is the type comprising the core of the plaques found in the brains of people afflicted with AD. It is derived from a much larger protein called amyloid precursor protein (APP). Normally, APP is cleaved (broken), releasing a fragment called APPsec. This fragment does not form amyloid. During abnormal processing of APP, as is found in AD, the A β (also called amyloid beta-protein) fragment is cleaved from APP. This results in the accumulation of A β which then forms amyloid. In cases where AD runs in families, the genetic defect can be found in the processing of APP. The fibrillar amyloid beta-protein has been implicated in the development of Alzheimer's disease due to its neurotoxicity and its ability to activate an inflammatory response in the brain.

The importance of decreasing the brain's inflammatory response is demonstrated by the fact that people with rheumatoid arthritis seem to be protected from developing Alzheimer's disease.⁶⁷ The theory behind this finding is that patients with arthritis are usually taking large amounts of anti-inflammatory medication. Although aspirin and non-steroidal anti-inflammatory drugs are not the best way to control inflammation, the fact that the inflammation has been reduced does make a difference in the incidence of AD. Any substance that reduces inflammation, such as HupA, will decrease the level of oxidative stress in the brain tissue.

WHY HUPERZINE A?

It has been demonstrated repeatedly that memory loss or impairments and cognitive dysfunctions are accompanied by a dramatic reduction in acetylcholine synthesis and/or release in the nerve cells.⁶⁷ Interest in the functions of the central cholinergic system (the nerves in the brain that use acetylcholine as a neurotransmitter) has been stimulated by the finding that a decline in function in this area may underlie part of the cognitive deterioration seen in normal and pathologic (disease-related) aging. Postmortem (at autopsy) analysis of the brains of patients with senile dementia of

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the Alzheimer type has shown a decline in central (brain) cholinergic activity that correlates with their mental test scores. As in the case of dopamine replacement (a treatment approach used in Parkinson's disease) therapeutic trials of cholinomimetics (compounds similar to acetylcholine) and cholinesterase inhibitors have been used with the goal of enhancing cerebral cholinergic nerve transmission. The finding of a severely damaged and underactive cholinergic system in the brain of patients with memory impairments and Alzheimer's dementia has encouraged such research.

Acetylcholinesterase inhibitors have been reported to improve memory in a number of patients suffering from Alzheimer's dementia in its early stages. It has also been demonstrated that these inhibitors increase secretion of the beneficial amyloid precursor protein (APP) in the brains of rats. APP, when processed normally, has been demonstrated to enhance the working and reference memory function as well as increasing the synthesis and release of acetylcholine in the brain. The two types of memory are discussed on page TK, in the Animal Studies section. Thus, the long-term use of cholinesterase inhibitors such as HupA not only improves cholinergic function, but an increase in normal APP metabolism will further help patients suffering from memory impairments including Alzheimer's dementia.

The main reason for studying cholinesterase inhibitors such as HupA, therefore, is that these compounds will increase extracellular acetylcholine levels. Based on the available experimental and clinical information,^{8,9} an ideal cholinesterase inhibitor suitable for symptomatic treatment of memory and cognitive impairment including Alzheimer's dementia should satisfy the following requirements:

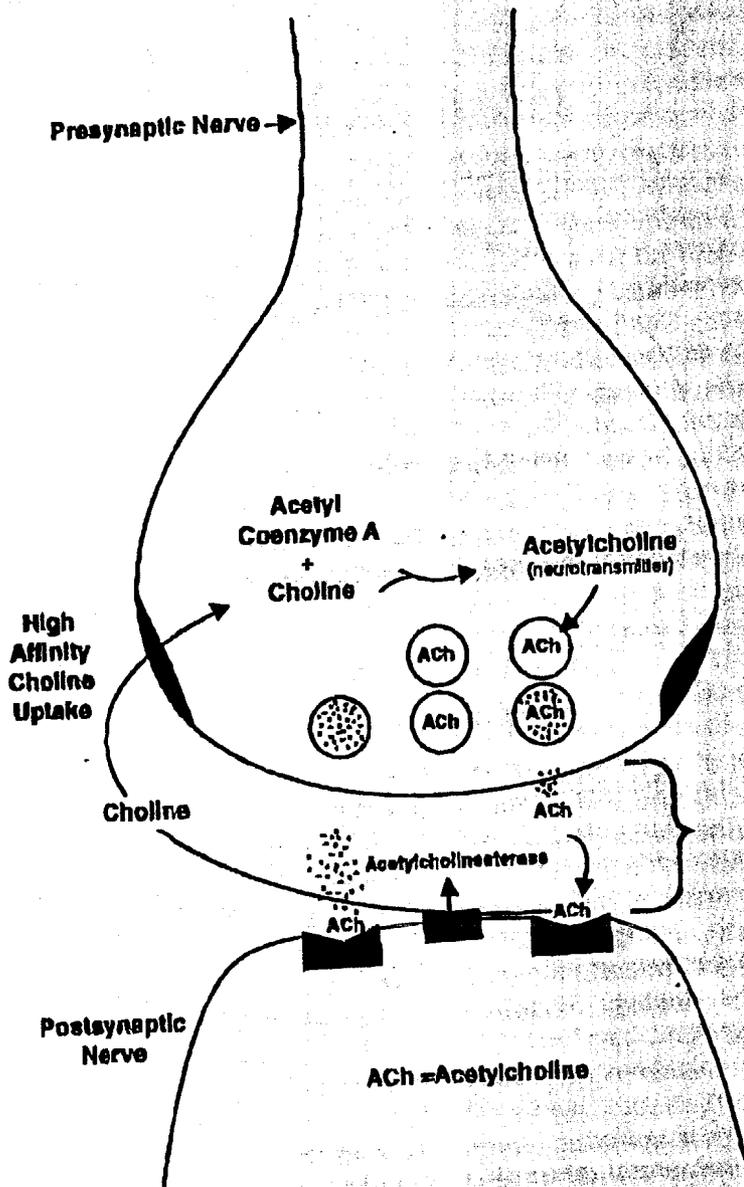
- a) Produce a long-term reversible acetylcholinesterase inhibition in the brain with increased synthesis or release of acetylcholine in the brain;
- b) Not inhibit acetylcholine synthesis or release in nerve endings; and
- c) Produce only mild side effects at therapeutic doses.

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Huperzine A fulfills all of these requirements. It is an excellent psychotherapeutic agent, a potent, selective and long-term reversible inhibitor of acetylcholinesterase^{10,12} and it induces the synthesis and release of acetylcholine. It also has a long resident time of interaction with acetylcholinesterase which may make it more effective. Its long half-life means that it can be given at lower dosage and fewer times per day than inhibitors with shorter half-lives. Half-lives are discussed further on page TK. With the compound physostigmine, AChE is strongly (80 percent) inhibited in the brain within a few minutes, but the effect lasts only 60 minutes.¹³ With HupA the inhibition is maintained for six hours after one dose. HupA also possesses unique blood-brain barrier penetration (it reaches its site of action) with minimal side effects at the therapeutic dose.

Other AChE inhibitors range from some of the most toxic agents to ever be synthesized (VX, Sarin, Soman) to therapeutic agents that are useful in the treatment of glaucoma (physostigmine) and myasthenia gravis (neostigmine). Only a few cholinomimetic compounds, physostigmine (also called eserine), tacrine and donepezil, have been evaluated extensively in dementia on a large scale, although there are more therapeutic candidates in various states of study. So far there have been problems with some of the compounds. The therapeutic effect of physostigmine, for example, is limited by its short duration of action, narrow therapeutic window and peripheral cholinergic effects. The term "narrow therapeutic window" means that the drug becomes toxic at doses above that needed to be effective. Drugs that have peripheral cholinergic effects will act on nerves outside the brain, causing side effects. For example, there may be fasciculations (involuntary contractions, or twitchings, of groups of muscle fibers in the muscles in the limbs). The most frequent and important side effect of tacrine is hepatotoxicity (liver toxicity), which limits its clinical value. Tacrine does not appear to alter the neurodegenerative disease; unlike HupA it is not neuroprotective. It also does not provide any benefit in non-Alzheimer's dementia.

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Huperzine A Figure

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Compared to physostigmine and tacrine, HupA is more potent at inhibiting acetylcholinesterase both *in vitro* (in the laboratory) and *in vivo*; it is about three times more potent than physostigmine.¹⁴ HupA also persists in the body for a longer period of time than physostigmine. At therapeutic doses, the side effects of HupA were minimal when compared with those caused by physostigmine and tacrine.

MECHANISM OF ACTION

Huperzine A works by a unique mechanism that has been scientifically discovered and reported in many research journals. It is a potent acetylcholinesterase inhibitor. Acetylcholine is the neurotransmitter in the brain that is responsible for carrying electrical impulses from one nerve to another (see appendix). It is made in the end section of nerve fibers and packaged into small vesicles where it is stored until released (see figure). Once acetylcholine has been secreted by the nerve ending, it persists for a few seconds. In a normal brain, the enzyme acetylcholinesterase serves a housekeeping function by breaking down the acetylcholine. It is split into an acetate molecule (from the "acetyl" part) and choline. The choline (a member of the B-vitamin family) is then transmitted back into the nerve ending to be used again to make acetylcholine. The brains of people with Alzheimer's demonstrate a deficiency of acetylcholine because of damage to the nerve cells that secrete it. Even with this deficiency, the acetylcholinesterase enzyme keeps working to get rid of whatever acetylcholine is released from the damaged nerve cell. This creates a deficiency. Huperzine A stops this enzyme from breaking down acetylcholine, thus preventing deficiency and improving mental function.

The finding of a severely damaged and underactive cholinergic system in the brains of patients with memory impairments and Alzheimer's dementia has led to clinical trials of new cholinomimetics including cholinesterase inhibitors. It has been demonstrated repeatedly that memory loss or impairments and cognitive dysfunctions are accompanied by dramatic reduction

in acetylcholine synthesis or release in the nerve cells. Investigating acetylcholine release is one way of testing the function of cholinergic synapses (the transmission of acetylcholine across the gap to the next nerve cell). It can be done with different drugs or compounds in animal or human autopsy tissue slices⁶ using techniques that measure extracellular acetylcholine (amounts outside the cell)⁷—an important way to assess drug effects on the body. Acetylcholine release is governed by complex factors such as membrane integrity and cholinergic receptor biochemistry. Receptors, such as those for acetylcholine, will function to different degrees depending on factors such as heredity (i.e., fewer insulin receptors increase the probability of getting diabetes) and adverse conditions during early childhood when the brain is still developing (some types of schizophrenia).

All cells, including nerve cells, are surrounded by a cell membrane. This membrane allows materials such as nutrients, hormones and neurotransmitters into the cell and allows cell products or waste material to leave the cell. If the integrity of this membrane is not maintained, normal function will be impaired. Cell membranes can be degraded by free radicals, or can stiffen because of a deficiency in the essential fats. Cell membranes contain good fats such as omega-3 fatty acid and oleic acid; without them cell walls harden and transport in and out is decreased or stopped.¹⁵ Cells die as a result. Huperzine A protects cell membranes from the effects of free radicals, with the result that nerve cells do not die as quickly, or in as large numbers as with the absence of such protection.

THE CHEMISTRY OF HUPERZINE A

The chemical name for Huperzine A is (5R, 9R, 11E)-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methano-18 / HUPERZINE A: BOOST YOUR BRAIN POWER

cyclooctenol[b]pyridin-2 (1H)-one. It is easy to understand why its name was shortened. The longer name describes the chemical structure of the molecule (see appendix). Although a lesson in chemistry is beyond the scope of this Guide, some of the facts about its structure are worth mentioning. For HupA to inhibit AChE, it must interact with it and prevent the enzyme from working. To accomplish this successfully, HupA contains various chemical groups and interactive abilities that give it a high affinity for AChE.¹⁶ Thus, the chemical structure of HupA allows it to maintain its unique ability to reversibly inhibit acetylcholinesterases.¹⁷

The chemical stability of Huperzine A is excellent. It is resistant to structural changes when placed in acidic or alkaline solutions.⁶ Furthermore, long-term incubation of HupA with acetylcholinesterases at 24°C (75°F) resulted in no detectable changes in the chemical structure of HupA.⁶ The stability in various solutions and the persistence of its effectiveness against AChE at different temperatures indicates that HupA will persist longer in the body and that tablets or capsules containing HupA will have a longer shelf life. Sophisticated laboratory techniques allow the determination of the exact quantity of HupA in an extract of *Huperzia serrata* (see appendix).

Regarding the structure and function of HupA, Joel Sussman, Ph.D., Professor of Structural Biology at the Weizmann Institute of Science in Rehovot, Israel, says that HupA can bind to AChE better than other AChE inhibitors.⁵ Dr. Sussman stated in an article published in the *Journal of the American Medical Association (JAMA)*,⁵ "It is as if this natural substance were ingeniously designed to fit into the exact spot in acetylcholinesterase where it will do most good."

The three-dimensional structure of the complex between HupA and AChE was worked out by a Weizmann Institute graduate student, Mia Raves, along with her professors at Weizmann and with Dr. Alan Kozikowski of Georgetown University (formerly at the Mayo Clinic, Jacksonville, FL).¹⁶

HupA has a longer half-life compared to the AChE inhibi-

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tors physostigmine, and tacrine (Cognex®). The half-life is the time during which half of the drug or compound has been metabolized (by breakdown and excretion from the body). The longer half-life means that the drug stays in the body longer and that less of it has to be given (and less often) than a similar compound with a shorter half-life. This characteristic is especially beneficial if the compound has few side effects as is the case for HupA. The half-life for HupA is 288.5 minutes (4.8 hours), while for physostigmine it is twenty minutes.¹⁸ Tacrine has a half-life of 2.7 hours.

According to Dr. Kozikowski the longer half-life and the fact that the complex of HupA and AChE dissociates (breaks apart) slowly may indeed make it a more effective therapeutic agent. Also, the strong selectivity of HupA for AChE suggests that HupA will have fewer side effects than tacrine or donepezil. A compound that acts very specifically, as does HupA, will do only the job it is intended to do. Compounds that are not as specific will bind with other proteins in the cell (non-specifically) as well—interfering with various cellular reactions. This results in adverse side effects from the unwanted interaction, i.e., nausea, vomiting, salivation and sweating. HupA can produce some of these side effects when given in high doses, but at therapeutic doses it produces very few. Donepezil (Aricept® or E2020) with a 72-hour half-life may cause bradycardia (slowing of the heart beat), which could be a problem for patients with heart disease. Tacrine causes elevation in liver enzymes (an indication of liver damage) in about half of treated patients, and weekly blood monitoring is needed; donepezil does not appear to affect liver enzymes.

Synthesis of Huperzine A

Because Huperzine A is difficult to procure in large quantities from natural sources, attempts were made to synthesize HupA in the laboratory. A racemic mixture of HupA and a variety of its chemical structural analogs (molecules with similar structure and function) have been synthesized by Kozikowski and colleagues.^{19,20} A racemic mixture means that there

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are two forms of the molecule—one active and one not. When scientists try to copy natural molecules in the laboratory, the racemic mixture is easiest to synthesize. Separating the two molecules in the racemic mixture is difficult and expensive, so the inactive form is usually just left in as part of the finished product. Such a product will not be as active as the original substance because of the presence of the inactive molecule. In fact, the synthetic racemic mixture of HupA was found to be three times less potent than natural HupA.²¹ The Food and Drug Administration (FDA), however, required that the inactive form be removed from the product. Dr. Kozikowski accordingly synthesized an "optically pure" form of HupA—one that contains only the active molecule. This optically pure form can be found in tablet form.

Whenever a therapeutic compound is developed, researchers always try to improve it by adding different chemical groups at various places on the molecule. Sometimes this works and a more effective compound (analog) is produced. In the case of HupA, however, none of the analogs proved as potent as the original, natural parent compound.

Both natural and synthetic HupA were shown to be more potent than physostigmine (a drug used to enhance memory in patients with Alzheimer's disease) as inhibitors of acetylcholinesterase *in vitro*. Moreover, this inhibitory effect on acetylcholinesterase *in vivo* was of a longer duration (peak activity of 20 minutes for physostigmine versus 60 minutes for the Huperzine A variants). These results indicate a similar biological mechanism of action between the two, but that synthetic racemic HupA exhibits a weaker biological activity than the natural product.²²

PHARMACOKINETICS OF HUPERZINE A

The process by which a drug or other compound is absorbed, distributed, metabolized and eliminated by the body is called pharmacokinetics. Understanding the pharmacokinetics of a drug, nutritional supplement or food in the human body is very important to understanding the characteristics of that

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agent as it acts in a biological system. An ideal therapeutic agent will be absorbed rapidly distributed widely, produce biological or therapeutic efficacy, and be eliminated at a moderate rate. It will also cause minimal or no toxicity in the body. The pharmacokinetics of HupA have been investigated in rodents and human volunteers. In these studies, HupA was absorbed rapidly, distributed widely in the body and eliminated at a moderate rate. In mice, 15 minutes after intravenous (IV) administration of HupA labeled radioactive for ease of detection, the HupA concentration was highest in the kidney and liver, moderate in the spleen, lung and heart, and lower in the brain. In pregnant mice, a small amount of detectable (radioactive) HupA was found in the fetus after administration. The majority (73 percent) of the radioactivity was excreted in the urine 24 hours after IV administration, while only 2.4 percent was recovered from feces.¹⁹ In a similar study, Tang and colleagues²³ have demonstrated that 60 minutes after IV injection, the drug is present in all brain regions but is particularly concentrated in certain areas such as frontoparietal and striatal regions of the cerebral cortex. It is these cortical areas that are low in acetylcholine in the brains of patients with Alzheimer's disease, so this specifically may indicate a therapeutic advantage of HupA use. It was also noted that lower doses of HupA were needed in rats to produce inhibition of AChE than with other compounds. If this proves true in humans, it may not be necessary to give high doses to produce a therapeutic effect. The result would be fewer side effects.

PRECLINICAL AND CLINICAL STUDIES WITH HUPERZINE A

Animal Studies

Extensive laboratory testing of HupA has been done. The Ames mutation test (done on bacteria) was conducted using increased dosages of HupA. When a compound is toxic it will cause abnormalities or mutations in bacteria which either affect the rate of growth of the bacteria or kill them. Such a substance is termed a "mutagen." HupA was compared with

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cyclophosphamide (a known mutagen). The test results indicated no noticeable differences between Huperzine A and a group of untreated bacteria that spontaneously mutate.

Teratological tests that determine the effect of a compound on offspring of animals given the substance were done in mice and rabbits. No external, internal organ or skeleton deformities were observed for any of the dosages.

The therapeutic index of HupA was also determined. This property is defined as the ratio of dose required to produce a toxic effect versus the dose needed to give the desired therapeutic response. The therapeutic index of a drug is also an approximate statement about its relative safety. The larger the ratio, the greater its relative safety.

Dr. Yan and colleagues²¹ determined the comparative therapeutic indices of five leading acetylcholinesterase inhibitors including Huperzine A, Huperzine B (also isolated from *Huperzia serrata*), neostigmine, physostigmine and galanthamine in mice and rats. HupA was compared for its biological efficacy and potency in various tests using isolated animal muscle tissues. The relative order of magnitude of the therapeutic indices (and safety order) of these different compounds was, in mice, Huperzine B (26.5) > Huperzine A (23.1) > neostigmine (8.6) > physostigmine (3.8), and in rats, the relative magnitudes were Huperzine B (294.8) > Huperzine A (72.9) > galanthamine (36.0) > neostigmine (34.0) > physostigmine (7.2). HupA was given in a lower dose than HupB. Based on these findings the researchers recommended that Huperzine A and Huperzine B should be of therapeutic value in the treatment of various peripheral or central nervous system diseases manifested by a cholinergic hypofunction (low acetylcholine levels). HupA would be the choice over HupB because less is used and side effects are thus fewer.

A similar toxicity comparison has also been reported by Tang²⁵ in mice using different cholinesterase inhibitors including Huperzine A, tacrine, physostigmine and galanthamine (table 1). In the table the therapeutic dose is compared with the LD₅₀. The LD₅₀ is the dose at which 50 percent of the experi-

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mental animals would die. The products that are the safest will have a therapeutic dose that is much lower than the LD₅₀.

Table 1. Biological Efficacy and Safety of Acetylcholinesterase Inhibitors in Mice

Compounds	Therapeutic Dose (mg/kg, oral)	LD ₅₀ (mg/kg, oral)
* Huperzine A	0.2	4.6
Tacrine	16.0	53.1
Physostigmine	0.3	1.96
Gаланthamine	2.0	27.1

mg = milligrams

kg = kilograms of body weight

LD₅₀ = the dose at which 50 percent of recipients are dead

HupA is safe because it would take more than 20 times the therapeutic dose to reach the LD₅₀.²⁵ This is determined as follows.

$$\frac{LD_{50}}{\text{Therapeutic Dose}} = \frac{4.6}{0.2} = 23$$

Example: If the therapeutic dose was one milligram, it would take 23 milligrams to reach toxicity.

As an example, for tacrine, that number would be 3.3. In other words, taking just 3 milligrams instead of one milligram would result in the death of 50 percent of the test population.

Huperzine A failed to cause liver toxicity in dogs and rabbits or any other side effects such as nausea, vomiting, gastrointestinal upset, depression, etc., which are common following physostigmine treatment. These data demonstrate the relative safety of HupA as compared to other cholinesterase inhibitors. In most human clinical trials, HupA was used at low therapeutic doses and no adverse side effects have been reported.

The effect of Huperzine A was shown to be more potent in improving memory impairments than with the AChE inhibitor
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tors E2020 (aricept or donepezil) and tacrine.²⁶ The memory impairments induced by scopolamine, a drug that produces amnesia similar to that in Alzheimer's disease, were evaluated using a maze task. Both working and reference memory were affected by scopolamine. Working memory refers to tasks that were first learned. Reference memory is the ability to recall a task learned in the past. For example, if a rat was being tested for its ability to run through a maze and find the bait, there would be a deficit in working memory if the rat went back to the same maze corridor where the bait had just been found. If a rat ran first into an unbaited maze, this would be an error in reference memory. Re-entry into an unbaited maze would represent a deficit in both types of memory.

In this experiment, to compare the inhibition of AChE by HupA with the effects of E2020 and tacrine, the scopolamine dose (0.2 mg/kg) significantly impaired spatial memory in rats. Huperzine A (0.1–0.4 mg/kg, orally), E2020 (0.5–1.0 mg/kg, orally) and tacrine (1.0–2.0 mg/kg, orally) reversed these scopolamine-induced memory deficits. Huperzine A was found to be the most selective acetylcholinesterase inhibitor. It improved the working memory deficit and amnesia induced by scopolamine significantly better than E2020 or tacrine. The action of the three AChE inhibitors was also tested in isolated mouse muscle.²⁷ It is easier to measure the effects of these inhibitors at the neuromuscular junction (the place where nerve and muscle meet) than in the synapses between nerve cells in the brain. It was found that HupA was more effective in inhibiting AChE than tacrine as shown by a decrease in muscle movement. Although E2020 (aricept, donepezil) had a stronger effect on the muscle than HupA, the E2020 was less selective. Such a decrease in specific inhibition of AChE may result in increased side effects. The researchers in both studies stated that the results confirm that HupA is a promising agent to evaluate for clinical therapy of cognitive impairment in patients with Alzheimer's dementia.

The effect of Huperzine A on maze performance in rats was assessed by Drs. Xiong and Tang²⁸ in a comparison with phy-

sostigmine. The maze is a valuable apparatus in the study of spatial memory. It offers an alternative that closely resembles the natural food-seeking behavior of species such as rats. The rats were trained to run in a spatial, radial arm maze using a procedure to determine the two memory functions (working and reference memory). The rats were trained so that baseline error rates were low (they practiced beforehand), which permitted the deficit in reference memory to be observed. Both scopolamine and mecamylamine have been reported to disrupt both the working and reference memory of rats.^{19,20} Low doses of scopolamine (0.125, 0.15 and 0.2 mg/kg, intraperitoneally or into the body cavity, 30 minutes before a session) impaired both working and reference memory in the radial maze. In contrast to scopolamine, mecamylamine (5, 10 and 15 mg/kg) did not cause any deficit in working and reference memory. Huperzine A (0.1, 0.2 and 0.3 mg/kg, intraperitoneally, 20 minutes before testing) and physostigmine (0.3 mg/kg, intraperitoneally, 20 minutes before testing) could reverse scopolamine-induced deficits in the task. Huperzine A completely reversed the scopolamine-induced deficit of maze performance. The memory improvement achieved with Huperzine A was comparable to that produced by physostigmine. Huperzine A exhibited a wide dose range (it worked at many different doses) for decreasing the scopolamine-induced memory impairment. Long-term treatment with Huperzine A (0.25 mg/kg, orally, once a day) for eight consecutive days was as potent as immediate high dose treatment in reducing scopolamine-induced amnesia.

The study further indicated that there was no significant tolerance to the memory-improving effect of Huperzine A. This is important because tolerance develops when physostigmine is used. This finding was consistent with the study conducted by Laganier and colleagues, who reported that Huperzine A-induced inhibition of acetylcholinesterase activity was as potent long-term as it was after immediate treatment.² These researchers have further demonstrated that following *in vivo* treatment with Huperzine A there was no decrease in turnover

of acetylcholine in the brain (it remained available for use). HupA also maintained long-lasting levels of acetylcholine for neurotransmission in the brain. Laganier and colleagues concluded that HupA may be more effective and less toxic than physostigmine. This would be especially true in diseases where long-term inhibition of AChE is required. This is the case with Alzheimer's disease.

The effects of Huperzine A were assessed on the performance of rats treated with a specific cholinergic neurotoxin, AF64A.²¹ This neurotoxin was used to treat rats before the animals entered the radial maze. AF64A is known to disrupt the working memory processes by altering cholinergic brain function. AF64A caused significant impairment in a rat's ability to perform the necessary spatially-oriented tasks needed to succeed in the maze. The behavioral impairment was associated with a significant decrease in the activity of mechanisms requiring acetylcholine in the hippocampus, which is a portion of the brain highly involved in neurotransmission animals and humans. Huperzine A significantly decreased the AF64A-induced memory deficit and improved cholinergic function. There were no side effects.

These animal experiments demonstrate that Huperzine A is a highly promising therapeutic agent in alleviating neurological disorders including short- and long-term memory dysfunctions and Alzheimer's dementia.

Human Studies

Administration of acetylcholinesterase inhibitors is a major pharmacological approach currently employed in the treatment of Alzheimer's disease. This strategy is used with the purpose of inhibiting acetylcholine degradation *in vivo*, and hence alleviating the cholinergic deficiency, which has been shown to occur in patients with Alzheimer's disease. Human studies (clinical) have been conducted to examine the effects of HupA.

The pharmacokinetics of Huperzine A tablets were monitored in both human male and female subjects aged 27 ± 6 years and weighing 58 ± 7 kg.¹⁸ All volunteers were healthy.

and not pregnant or menstruating. The volunteers were given a tablet containing 0.09 milligrams of HupA two hours before breakfast. Huperzine A levels in the blood plasma of these volunteers were then determined by sophisticated laboratory techniques. It was found that AChE was inhibited for 288 minutes with HupA, while inhibition lasted 20 minutes for the AChE inhibitor, physostigmine. These data demonstrate that Huperzine A was released and rapidly absorbed *in vivo*, widely distributed in the body and eliminated at a moderate rate. Based on these findings the researchers indicated that it would be best to take HupA in tablet form two to three times a day.

A recent clinical study was conducted by Dr. Xu and colleagues³² at Zhejiang Medical University, Shanghai, China, in patients with Alzheimer's disease. Using a multicenter, prospective, double-blind, parallel, placebo-controlled and randomized method, 50 patients were administered Huperzine A (200 micrograms in 4 tablets, orally) twice a day, and 53 patients were given a placebo (a "sugar pill") for eight weeks. All subjects were evaluated using the Wechsler memory scale, the Hasegawa dementia scale, the mini-mental state examination scale, the activity of daily living scale and the treatment emergency symptom scale. These are the "gold standard" measurement tests for cognitive function. About 58 percent of patients treated with Huperzine A evidenced improvements in their memory, cognitive and behavioral functions. The efficacy of HupA outperformed the placebo by 36 percent. Improvements noted by the patient's family members are shown in the following table. A significant difference was found between the two groups:

Table. Number of reports of Patient's Behavior from Family Members.

Observation	Placebo (53 patients)	HupA (50 patients)
Clear headed	13	26
Memory improving	8	16
Language improving	2	8
Unchanged	34	21
Total changes	57	71

No adverse side effects were observed and the overall efficacy of HupA was higher than the placebo.

These studies are very significant because they demonstrate a dramatic improvement in memory following administration of Huperzine A to patients with age-associated memory impairments and disorders. Compared to other cholinesterase inhibitors, Huperzine A possesses a clearly superior safety and efficacy profile. Furthermore, the comparatively long duration of action of Huperzine A and minimal side effects make it a potentially useful therapeutic agent.

HUPERZINE A AND HEALTH BENEFITS

Neuropsychiatric Illness and Alzheimer's Dementia

The recognition of Alzheimer's disease as the fourth leading cause of death in the United States in adults (after heart disease, cancer and stroke), combined with its profound morbidity (diseased state), has led to the development of therapeutic strategies aimed at reducing the effects of, if not preventing, this disorder. The cost of caring for Alzheimer's patients—including the cost of diagnosis, treatment, nursing home care and formal or paid care—is estimated to be more than \$100 billion per year. The federal government covers \$4.4 billion and the states \$4.1 billion. Much of the remaining costs are borne by patients and their families. A therapy that could prevent, or even slow the progression of this disease, would surely be welcomed by health professionals and families.

Cholinergic mechanisms play a major role in controlling cerebral blood circulation and cerebral blood flow. These

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Alzheimer's disease (AD) is a slowly worsening brain disease. AD is marked by changes in behavior and personality and by a decline in thinking abilities that cannot be reversed. This mental decline is related to a loss of nerve cells and the links between them. The course of the disease varies from person to person, as does the rate of decline. On average, AD lasts from 4 to 8 years after diagnosis, however, it can continue for up to 20 years. AD advances in stages, from mild forgetfulness to severe dementia, or mental decline. Signs (or symptoms) of AD appear most often between the ages of 60 and 70. The first symptoms include loss of recent memory, faulty judgment and personality changes. Early in the disease, people with AD may forget how to do simple things like washing their hands. Often, they can no longer think clearly or remember the words for familiar objects or people's names. Later on, people with AD lose all reasoning abilities and become completely dependent on other people for their care. Patients often live for years. The disease eventually becomes so debilitating, however, that patients are likely to develop other diseases. Most commonly, AD patients die from pneumonia.

mechanisms depend on adequate amount of acetylcholine. Acetylcholine can act to dilate or constrict blood vessels, depending upon where in the brain it is released. A deficiency in acetylcholine will prevent proper regulation of blood vessel diameter and result in impairment of brain function. The destructive process occurring in the brain of an Alzheimer's patient will worsen when an adequate blood supply to the area is not maintained. As the loss of cholinergic function progresses, memory becomes increasingly impaired. This impairment results in major neuropsychiatric dysfunction in the elderly.

The degree and extent of cholinergic deficit in this type of senile dementia correlates with the severity of cognitive impairment: the more pronounced the acetylcholine deficiency, the worse the cognitive function. Since a deficiency in the cholinergic system is believed to constitute one of the hallmarks of Alzheimer's dementia, reversible inhibitors of acetylcholinesterase (the enzyme that breaks down acetylcholine) that make their way into the central nervous system

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may serve as palliative agents in the treatment of the disease.

Studies using cholinesterase inhibitors with the goal of alleviating cholinergic deficiency have shown the most encouraging results in Alzheimer's dementia to date. Huperzine A gained considerable interest because of its unique anti-acetylcholinesterase activity and biochemical properties. As discussed earlier, its long half-life, unique blood-brain barrier penetration, minimal side effects, and high therapeutic indices suggested that Huperzine A may work better than existing drugs, including physostigmine and tacrine, for the treatment of Alzheimer's disease.

There is a considerable pharmacological evidence that the central (brain) cholinergic function plays an important role in learning as well as for the working and reference memory functions. Postmortem (autopsy) analysis of the brains of patients with senile dementia of the Alzheimer type has identified a decline in central cholinergic activity that correlates with their previous mental test scores. Interest in the functions of the central cholinergic system has been stimulated by the hypothesis that decline in this system may underlie the part of the cognitive deterioration seen in the normal as well as pathologic (disease-induced) aging brain. Huperzine A, considered one of the new generation of acetylcholinesterase inhibitors, has gained considerable notice because of its unique anti-acetylcholinesterase activity as well as memory-enhancing effects in a broad range of behavioral models. Huperzine A exhibits significant inhibition of acetylcholinesterase activity in all brain regions tested including hippocampus, striatum, hypothalamus and frontal cortex. Structures in the hippocampus regulate behaviors such as rage, passivity, learning and motivation. The striatum is closely connected to the amygdala, a part of the brain associated with fear, confusional states, disturbances of awareness and amnesia. The involvement of the amygdala in emotional states has important bearing on several forms of dementia. In Alzheimer's, the amygdala suffers from se-

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were neuronal loss; this part of the brain shrinks. This is accompanied by marked memory impairment and changes in emotional behaviors, including a loss of spontaneity. The hypothalamus regulates sleep, food and drink intake, and endocrine (hormonal) functions throughout the body. The frontal cortex is the area of higher cognitive functions: learning and memory.

Learning Ability, Memory Enhancement and Cognitive Performance

In a series of behavioral studies, Huperzine A was found to improve cognitive performance in a broad range of animal models involving mice, rats and monkeys with induced amnesia.³³⁻³⁶ HupA also markedly improved the retention of a learned task when tested 24 hours later in aged mice. Enhancement of learning and memory performance, increased retention and faster retrieval processes were observed. The loss of cholinergic neurons in the brain has been demonstrated to be part of the aging process itself. This loss is considered an important element in the process of memory loss or memory impairment including dementia. Huperzine A improved cholinergic function by inhibiting acetylcholine degradation in the brain.

In other behavioral experiments, Huperzine A has been shown to improve mice and rats' performance in running through mazes,³⁷⁻³⁹ and to protect young and aged animals against sodium nitrite, scopolamine, cycloheximide, carbon dioxide-treated and electroconvulsive shock-induced disruption of a passive avoidance response.^{13,35} It also improved accuracy of memory in squirrel monkeys.³⁵ These studies imply that Huperzine A is effective in a variety of classical behavioral tests designed to test an animal's learning ability and memory function. The duration of improved effects on learning and memory retention processes with oral Huperzine A were longer than that obtained with physostigmine, tacrine and galanthamine, the existing acetylcholinesterase inhibitors.

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Huperzine A and Muscle Contraction

Huperzine A, as discussed earlier, potentiates the skeletal muscle contraction and increased muscle tone in rats.²⁴ Clinically, in another study, Huperzine A was shown to improve muscle weakness significantly as well as memory in patients with impaired memory.⁴⁰ Because Huperzine A prevents the selective degeneration of acetylcholine-producing neurons in the brain and enhances the availability of acetylcholine in the brain of patients suffering from neurological disorders including Alzheimer's dementia, the connections between nerves and muscles function better. The improvement in neuromuscular cholinergic transmission leads to an improvement of the patient's condition.

More Memory Improvement

The study conducted in human subjects to assess improvement in muscle function using Huperzine A, also showed favorable effects in the treatment of age-related memory impairment.⁴⁰ In another comparative study with Hydergine[®] (a vasodilator, 600 micrograms), 30 micrograms of Huperzine A (given intramuscularly) appeared to improve memory for 1-4 hours after it was given to 100 aged individuals (ranging in age from 46-82; sex: 54 males, 46 females) suffering from memory impairment.⁴⁶ In this population of 100 subjects, 83 had no demonstrable brain disease but were suffering from age-related amnesia or memory dysfunction, and only 17 had probable Alzheimer's disease. Note that Alzheimer's disease cannot be definitely diagnosed before death. It is only at autopsy that the determination can be made with precision. The results of this experiment were very encouraging. Minimal or no side effects were observed.

A more comprehensive study was later conducted by Zhang and colleagues.⁴¹ The therapeutic effects of Huperzine A were studied by the random, matched and double-blind method (a scientifically valid and accepted method) on 56 patients with multi-infarct dementia (resulting from repeated small strokes). The patients were age 64 ± 7 years;

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there were 52 males, 4 females. Also studied were patients with senile dementia and 104 patients of senile and presenile simple memory disorders (age: 63 ± 7 ; sex: 58 males, 46 females). Each group was divided into two smaller groups—one given HupA with the other serving as a control group. The control groups were treated intramuscularly with only saline (salt) solution. The intramuscular dose of Huperzine A for multi-infarct dementia was 50 micrograms twice a day for four weeks, whereas that for senile and presenile simple memory disorders was 30 micrograms, twice a day for two weeks. HupA is also available and effective in oral (tablet) form. The Weschler memory scale was used to determine whether there was improvement of memory function in these patients. Huperzine A treatment significantly improved the memory of patients in both treatment groups (those that received the HupA) and did so significantly and with minimal observed side effects. Only a few patients felt slight dizziness and this did not affect the therapeutic effects.

Alleviation of Symptoms Related to Glaucoma

Glaucoma is a disease in which there is an increase in pressure within the eye. If the increase is high enough and persistent enough, there will be damage to the optic nerve and irreversible blindness can result. AChE inhibitors are used to alleviate this condition. Since Huperzine A can inhibit the transport of choline in the brain, this allows more to be available for synthesis of acetylcholine, the neurotransmitter important in maintaining normal eye function. It has been suggested that HupA may be a better therapeutic drug than other AChE inhibitors such as physostigmine, neostigmine or lacrine for the treatment of glaucoma.⁴¹

Improvement of Symptoms in Myasthenia Gravis Patients

In a clinical trial, Huperzine A has been demonstrated to significantly improve muscle weakness associated with myasthenia gravis.⁴² This is a neuromuscular disease character-

ized by weakness and marked fatigability of skeletal muscle. The defect caused by the disease involves nerve transmission at the neuromuscular junction, the area where nerve meets muscle. When a nerve "fires" and activates a muscle at the neuromuscular junction, the muscle should contract or move. Although patients with myasthenia gravis can initially move the muscle, repeated activation causes a diminished response and muscle movement cannot be maintained. AChE inhibitors, by keeping acetylcholine levels high, can increase muscle response. The study, conducted by Cheng and colleagues was done in 1986. The clinical effects of Huperzine A were compared with prostigmine, another AChE inhibitor. In 128 patients with myasthenia gravis, 99 percent demonstrated controlled or improved clinical symptoms of the disease. The duration of action of Huperzine A was 7 ± 6 hours. Side effects, except nausea, were minimal when compared with those induced by prostigmine.

Protection Against Nerve Agents and Pesticide Toxicity

Organophosphates, chemicals used as pesticides and nerve gases during wartime, are well known to enter the nervous system, react with cholinesterase, irreversibly inhibit it, and induce potential brain injury leading to coma and death. When AChE is irreversibly inhibited, there is no control over levels of acetylcholine. All the effects of acetylcholine will become exaggerated and continuous. In the eyes, the pupil will markedly constrict with pain and spasm of muscles; effects on the lungs include "lightness" in the chest and wheezing due to constriction of bronchial tubes, as well as increased mucus. In the gastrointestinal tract, nausea, vomiting, cramps, diarrhea and extreme salivation are produced. Death usually occurs by respiratory (lung) failure, often accompanied by a heart attack.

The remarkable selectivity for acetylcholinesterase and superior blood-brain barrier penetration ability of Huperzine A, along with its chemical stability and reversibility, suggests that it should provide a safe and long-lasting prophylaxis.

lactic treatment against nerve agent toxicity in humans. A comparative study against organophosphate toxicity using the nerve agent soman, was conducted in animals who were pretreated with Huperzine A or physostigmine (which is currently being used to prevent nerve agent toxicity). The study was designed to determine prophylactic (preventative) efficacy of Huperzine A in mice. Huperzine A dramatically prevented irreversible phosphorylation of the enzyme (AChE) by soman. As mentioned earlier, continuous activity of the enzyme results in dangerous and eventually fatal consequences. The effect of HupA against soman toxicity lasted a long time; this was consistent with the finding that HupA levels persist in the brain for a longer period than physostigmine. According to the researchers, the data indicated that HupA might provide significantly longer therapeutic activity than other AChE inhibitors used to manage diseases where there is a deficiency in cholinergic neurons. In this *in vivo* model Huperzine A demonstrated significant antidotal efficacy and much better protection compared to physostigmine.⁴⁴

CONCLUSION

The preceding sections describe how Huperzine A may serve as a highly promising therapeutic agent. Huperzine A has attracted considerable notice because of its unique, reversible anti-acetylcholinesterase potency and pharmacokinetic properties. The preliminary evidence indicates its potential clinical value in the treatment of dementia, myasthenia gravis and glaucoma. The potentially superior inhibition characteristics of Huperzine A, as compared to other cholinesterase inhibitors, have been attributed to the very slow rate of dissociation of the acetylcholinesterase-Huperzine A complex in solution.⁴⁵ Also, the interaction of Huperzine A with acetylcholinesterase appears reversible and does not result in any detectable chemical modification of the inhibitor. Reversibility is important because the enzyme (AChE) will not be turned on permanently (as is the

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case with nerve gases). Because HupA (the inhibitor) is not altered when it inactivates the enzyme, it can be released and act again. Huperzine A produces a long-term inhibition of acetylcholinesterase activity in the brain (up to 360 minutes) and increases acetylcholine levels up to 40 percent at 60 minutes.¹³

Physostigmine, another AChE inhibitor, produces a rapid effect (15 minute peak of 55 percent inhibition), but this effect is over within 120 minutes. After attaining peak plasma (blood) concentration in humans at approximately 30-60 minutes, physostigmine is cleared from plasma with a half-life of about thirty minutes.⁴⁷ The anticholinesterase action of Huperzine A in cholinergic synapses is stronger than that of tacrine (also known as tetrahydroaminoacridine).²⁷ The terminal half-life of Huperzine A is 4.8 hours. While the potency of Huperzine A is comparable to that of physostigmine and tacrine (a classical acetylcholinesterase inhibitor), its duration of action is 10- to 12-fold longer *in vivo* (up to 8 hours), and the incidence of side effects induced by Huperzine A is considerably less than that of physostigmine or tacrine. Tacrine has been approved by the FDA for the clinical treatment of patients with Alzheimer's dementia,²⁷ but the therapeutic usefulness of tacrine is limited by its liver toxicity. Huperzine A more significantly improved learning and memory in mice with higher efficacy than tacrine. In Phase II clinical trials, Huperzine A improved memory in Alzheimer's disease patients with minimal side effects.³² The Phase II clinical study with Huperzine A also showed that 99 percent of patients with myasthenia gravis were controlled and/or improved following administration of Huperzine A.²⁵ Furthermore, its long half-life, high oral bioavailability, unique blood-brain barrier penetration ability and safe therapeutic indices support the fact that Huperzine A is a potential candidate for treatment of memory deficits in patients with Alzheimer's disease. Huperzine A also has potential for treating other nervous system-related dementias as well

as usefulness in protection against organophosphate pesticide or nerve agent toxicity.

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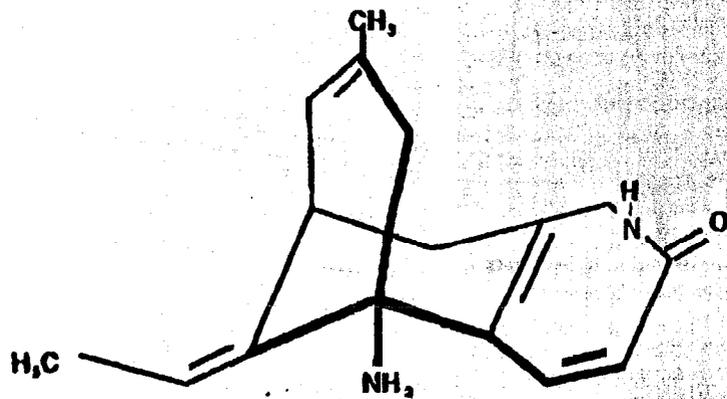
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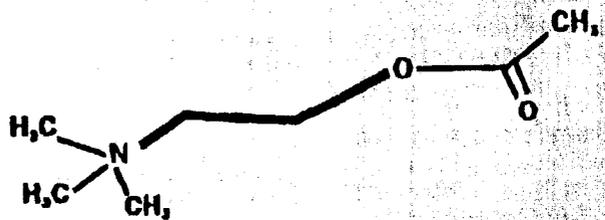
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Appendix



Huperzine-A



Acetylcholine

Chemical Structures of Huperzine A and Acetylcholine

C=carbon

H=hydrogen

N=nitrogen

HPLC Techniques for Quantitation of Huperzine A

Qian *et al.*¹⁸ has demonstrated a high performance liquid chromatography technique (HPLC) for the quantitation of Huperzine A. HPLC is a technique that uses a tube-like column filled with very small bead particles to separate the components of a liquid mixture of a substance, in this case the HupA extract. Depending on the molecular size of the component, it will either pass through the column or be trapped on the beads. By varying the size of the beads and the type of liquid flowing through the column, individual components in complex mixtures can be identified. The HPLC was equipped with an UV absorbance detector and a Spherisorb C₁₈ (150 mm x 5 mm I.D.; 5 μm particle size). The mobile phase was methanol: water (45:55, vol/vol) at a flow rate of 1 ml/min at 30°C column oven. The column effluent was monitored at 313 nm.

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IX. #16

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COGNITION IMPROVEMENT BY ORAL HUPERZINE A: A NOVEL ACETYLCHOLINESTERASE INHIBITOR

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INTRODUCTION

Alzheimer's disease (AD) is one of the most severe mental health problems commonly found in the aged population. The dementia disorders will continue to constitute a major burden upon social and medical care systems due to the mean age of the population as it continues to rise. Hence, a cure or prevention for the disease would be most desirable. Current efforts to develop an effective drug treatment for AD are in large part based upon the consistent finding that patients with this disease suffer from marked reduction of cholinergic neuronal function resulting in a deficiency in acetylcholine (ACh) concentration in the central nervous system (Whitehouse et al., 1982; Davies and Maloney, 1976; Coyle et al., 1983), and that these reductions have been associated with changes in memory (Giacobini, 1991). Of all the attempts at symptomatic therapy for AD based on the cholinergic hypotheses, studies using cholinesterase inhibitor (ChEI) has been the most encouraging up to now (Pomponi et al., 1990). Several ChEIs such as physostigmine (Phys) (Thal, 1991) and tacrine (THA) (Summers et al., 1986) have recently been the focus of extensive clinical investigation in patients who had AD (Becker and Giacobini, 1988). However, the liver toxicity with a higher dose of THA and short duration of action as well as a narrow dosing range with Phys were viewed as serious limitations to the development of these compounds as therapeutics. At present there is no therapeutic ChEI that has been shown to be both effective and safe in the treatment of AD. Thus the search for a potent, long-acting ChEI which exerts minimal side effects in the clinic for the treatment of AD is still most active.

Huperzine-A (Hup-A) was first isolated from clubmoss *Huperzia serrata* which is known as the Chinese folk medicine *Qian Chen Ta* (Liu et al., 1986). Huperzine-A, an alkaloid chemically unique from other agents under study for AD, is a reversible and mixed competitive ChEI, its potency rivals that of Phys, galanthamine (Gal) (Wang et al., 1986) and THA (DeSarno et al., 1989).

The duration of acetylcholinesterase (AChE) inhibition by a single dose of Hup-A in rats was over 6 hrs (Tang et al., 1989). Huperzine-A has been found to be an effective cognition enhancer in a number of different animal species (Tang et al., 1988; Vincent et al., 1987). Preliminary clinical trials conducted in China have indicated that intramuscular injection of Hup-A induced significant efficacy in the improvement of memory disruption in the aged and AD patients (Zhang, 1986; Zhang et al., 1991) being devoid of any remarkable side effects. In this paper, we report improving effects on cognition by oral Hup-A in rodents and in dementia patients.

RESULTS AND DISCUSSION

Effects in Rodent Behavioral Model

By using step-down passive avoidance task, effects of Hup-A, Phys, Gal and THA on learning and memory retention in mice were compared. Step-down latency was used as parameter of learning and memory retention performance (Kameyama et al., 1986). In learning trials, each dose of Hup-A (0.05-0.4 mg/kg), Phys (0.025-0.3 mg/kg), Gal (0.05-3 mg/kg) or THA (2-20 mg/kg) were orally administered 30 min before training task, respectively. The active dose on learning was 0.2-0.3 mg/kg (Hup-A), 0.05 mg/kg (Phys), 1 mg/kg (Gal) and 10-14 mg/kg (THA), respectively. Facilitation of learning performance exhibited the inverted U-shaped dose-response curve that is characteristic of known cognitive performance enhancing agents. Figure 1 shows the time course of each ChEI on learning tested with effective dosage. It is clear that Hup-A produced enhancing effect on learning lasts for up to 3 hrs. The longest enhancing effect of Hup-A was also observed in memory retention performance. Huperzine-A exhibited facilitating effect on memory retention up to 96 hrs (Table I). These results show that the effect of oral Hup-A on learning and memory retention processes are longer than those of Phys, Gal and THA.

Figure 2 shows that the amnesic action of NaNO_2 , indicated by the reduced step-down latency, was reversed significantly by Hup-A. The improving effect of Hup-A on NaNO_2 -induced amnesia was about 3-30 times stronger than those of Phys and Gal, respectively. The magnitude of improving effect produced by oral and intraperitoneal administration were nearly equivalent. Huperzine-A had a higher efficacy with oral route than that of Phys (Beller et al., 1985). The result coincides with previous data reported by Wang et al. (1988).

Cognition Improvement by Huperzine A

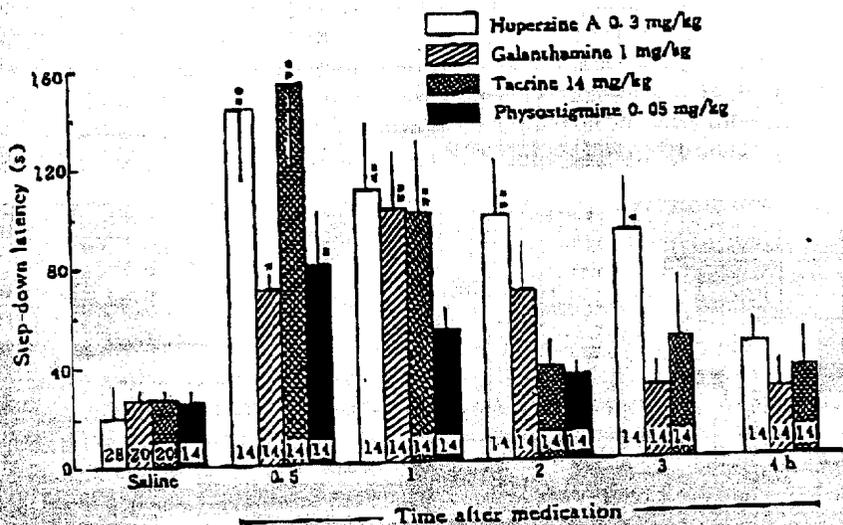


FIGURE 1. Facilitation of learning of a passive avoidance task in mice. A dose of ChEI or saline (10 ml/kg) was administered orally before training performance. Number of mice in bars. * $p < 0.05$; ** $p < 0.01$ vs saline.

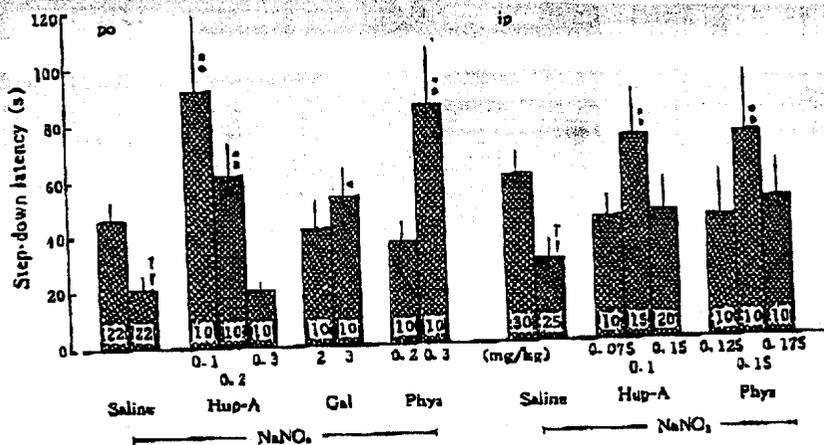


FIGURE 2. Reversal of NaNO₂-induced disruption of the retention of a passive avoidance task in mice treated with Hup-A ($X \pm SEM$). The retention test was performed 24 hr after training. NaNO₂ (120 mg/kg, s.c.) immediately after training. Huperzine-A or saline (10 ml/kg) was administered immediately after NaNO₂. Number of mice in bars. ++ $p < 0.01$ vs saline. * $p < 0.05$; ** $p < 0.01$ vs saline + NaNO₂.

ChEI	Dose (mg/kg)	Step-down latency (s \pm SEM)			
		24	48	72	96 (hr)
Saline	-	16.8 \pm 2.5	19.2 \pm 2.2	24.6 \pm 3.2	22.5 \pm 5.6
Hup-A	0.2	78.8 \pm 16.0**	34.5 \pm 5.9	67.1 \pm 19.9*	50.8 \pm 8.9*
Phys	0.3	66.0 \pm 13.1**	22.2 \pm 3.7	29.0 \pm 5.2	37.5 \pm 7.7
Saline	-	19.4 \pm 1.6	34.3 \pm 4.7	21.4 \pm 19.5	37.3 \pm 5.3
Gal	2.0	48.5 \pm 9.9**	67.8 \pm 11.8**	44.4 \pm 40.1	60.6 \pm 10.5
THA	16.0	44.6 \pm 6.1**	70.9 \pm 15.0*	31.5 \pm 33.7	59.1 \pm 14.0

TABLE 1. Facilitation effect of ChEI on memory retention of a passive avoidance task in mice ($n = 10-14$). * $p < 0.05$, ** $p < 0.01$ vs saline. An oral dose of ChEI or saline (10 ml/kg) was administered immediately after training. The retention test was performed 24, 48, 72, or 96 hr after training.

The effect of oral Hup-A on radial maze performance was evaluated using a 4-out-of-8 baiting procedure. Rats were trained to find and eat the dustless pellet (45 mg). At the start of each session, the four predetermined arms were baited at their distal end. Each rat was placed on the platform and left until all the four baited arms were collected or 10 min had elapsed, whichever came first. Rats choice accuracy stabilized over four days. An arranged criterion of 87% or better was used in the test. Scopolamine (Scop) pretreatment produced significant increase in error numbers of reference memory (RM), working memory (WM) as well as reference and working memory (RWM). However, when Scop-treated rats were pretreated orally with Hup-A, maze performance was improved (Fig. 3). The results also showed single dose and seven multiple doses of oral Hup-A did not exhibit difference on improving the amnesia induced by Scop ($p > 0.05$). These results indicated that Hup-A can significantly reverse effects of central cholinergic impairment of spatial memory. Huperzine-A induced improving effect was as potent after chronic, as it was after acute treatment indicating that no tolerance to the drug occurred.

* Effects in Human and Patients with Dementia

Phase I clinical studies of safety, tolerance and pharmacokinetics of oral Hup-A have been conducted in 22 young healthy volunteers. No significant side effects were observed at doses of 0.18-0.54 mg. Plasma levels of Hup-A determined by an HPLC method with electrochemical detector indicated that Hup-A is fairly rapidly absorbed following oral administration, with an average T_{max} of 1.27 hr. The terminal half-life is 349 min. Peak plasma levels after oral 0.99 mg dose was 8.1 ng/ml. Huperzine-A has a low plasma clearance of 14.4 L/hr and volume of distribution of 104.5 L in volunteers. Compared with THA and Phys (Hartvig et al., 1991), the terminal half-life of Hup-A was at least 4-17 times longer.

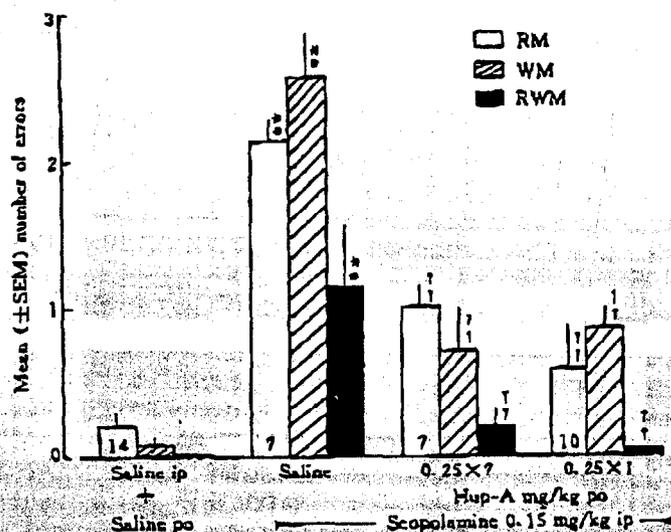


FIGURE 3. Improving effect of acute and 7 chronic administrations of Hup-A on Scop-induced amnesia on radial maze performance in rats ($X \pm SEM$). Scopolamine and Hup-A were administered 30 min before test. Number of rats in bars. ** $p < 0.01$ vs saline + saline; ++ $p < 0.01$ vs saline + Scop.

To examine the efficacy of oral Hup-A, cognitive function was studied in a sample of 20 patients with senile dementia impairment over 2-6 years. The subjects were ten males and 10 females in the range of 50-68 years. A double-blind design was used to compare efficacy of different dose levels. Tables of 50 μg of Hup-A were given t.i.d. in three consecutive days, then three days abrupt washouts were made between next dosage. The treatment response was evaluated by Buschke Selective Reminding (BSR) task. Comparison of all rating scores from oral Hup-A and placebo show significant difference (Table II). It can also be seen from Table II that an inverted U-shaped dose-response curve in memory as measured by objective verbal memory tests was observed. The results coincide with animal results (Tang et al., 1986) and oral Phys in human (Beller et al., 1985). The improving effect of oral Hup-A on memory has about 10 times more potency compared with physostigmine. Side effects that were recorded at doses of 0.2 mg were unremarkable. The most frequently occurring side effects with Hup-A were related to the cholinergic property of the compound. Some mild side effects, i.e. dizziness, nausea and sweating occurred in a few patients at doses over 0.2 mg. During the increase in Hup-A dose up to 0.4 mg fasciculation was recorded in two of 10 patients. There was no toxicity on liver and kidney at 0.5 mg dose tested.

Phase II clinical trials are in progress.

Dosage (mg, t.i.d.)	Group n=10	Rating Scores ($\bar{X} \pm SD$)			
		ER	LTR	LTS	CLTR
0	C	49 \pm 9	27 \pm 8	37 \pm 8	19 \pm 5
	M	50 \pm 9	28 \pm 8	37 \pm 8	19 \pm 5
0.1	C	50 \pm 9	28 \pm 8	37 \pm 8	18 \pm 5
	M	54 \pm 9	34 \pm 8	40 \pm 8	24 \pm 5
0.15	C	49 \pm 9	27 \pm 8	36 \pm 8	18 \pm 5
	M	63 \pm 9 ^{***}	44 \pm 8 ^{**}	46 \pm 9 ^{**}	39 \pm 6 ^{**}
0.25	C	50 \pm 9	27 \pm 8	37 \pm 8	18 \pm 5
	M	74 \pm 9 ^{***}	68 \pm 9 ^{***}	69 \pm 9 ^{***}	60 \pm 7 ^{***}
0.4	C	50 \pm 9	28 \pm 8	37 \pm 8	18 \pm 5
	M	55 \pm 9	37 \pm 8 [*]	47 \pm 9 [*]	41 \pm 6 ^{**}

TABLE II. Memory improvement by oral Hup-A in patients with senile dementia. * p < 0.05; ** p < 0.01 vs premedication. ER = ERrecall; LTR = Long-term retrieval; LTS = Long-term storage; CLTR = consistent LTR; C = control; M = medication, double-blind measurement.

On the basis of these findings, it is reasonable to expect that oral Hup-A is a promising candidate for clinical development as second generation of anticholinesterase in the treatment of cholinergic related neurodegenerative disorders such as AD.

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