



DEPARTMENT OF HEALTH & HUMAN SERVICES  
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

. OCT - 1 1999

Date

From

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

Subject

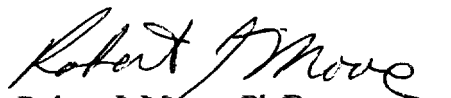
75-day Premarket Notification for New Dietary Ingredient

TO

Dockets Management Branch, HFA-305

New Dietary Ingredients:	Huperzine A
Firm:	Solgar Vitamin and Herb
Date Received by FDA:	September 30, 1999
90-day Date:	December 28, 1999

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-03 16** after December 28, 1999.

  
Robert J. Moore, Ph.D.

95S-03/6

RPT 55



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Washington, DC 20204

OCT - 1 1999

Ms. Karla LaSasso  
International Registration Coordinator  
Solgar Vitamin and Herb  
500 Willow Tree Road  
Leonia, New Jersey 07605

Dear Ms. LaSasso:

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 September 28, 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 September 30, 1999. Your submission will be kept confidential for 90 days from the date of receipt, and after December 28, 1999, your submission will be placed on public display at Dockets Management Branch (Docket No. 953-03 16). 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,

A handwritten signature in black ink, reading "Robert J. Moore", is written over the typed name.

Robert J. Moore, Ph.D.

Senior Regulatory Scientist

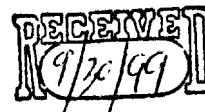
Division of Programs and Enforcement Policy

Office of Special Nutritionals



**SOLGAR VITAMIN AND HERB**  
WORLD HEADQUARTERS

500 WILLOW TREE ROAD, LEONIA, NJ 07605 USA  
PHONE 201-944.2311 FAX 201-944.7351



September 28, 1999

Office of Special Nutritionals (HFS-450)  
Center for Food Safety and Applied Nutrition  
FOOD AND DRUG ADMINISTRATION  
200 C Street, S.W.  
Washington, D.C. 20204

**RE: Premarket Notification For A New Dietary Ingredient**

Dear Sir/Madam:

In compliance with Dietary Supplement Health and Education Act of 1994, Solgar Vitamin and Herb hereby makes its official Premarket Notification for a new Dietary Ingredient, Huperzine A. Accordingly, enclosed please find two (2) copies of this Notification.

Please be advised as follows:

- I. The name and address of the manufacturer is:

**Solgar Vitamin and Herb**  
**500 Willow Tree Road**  
**Leonia, New Jersey 07605 USA**

2. The name of the new Dietary Ingredient is:

**Hupenine A**

3. A description of the dietary supplement:

**Dietary supplement Huperzine A is the alkaloid compound extracted from the herb *Huperzia serrata* present in tablet form.**

- (a) the level of the new dietary ingredient is:

**50 mcg per tablet**

- (b) the conditions of use suggested on the label are:

**Suggested Use: As a dietary supplement for adults, one (1) to four (4) tablets daily, preferably at mealtimes, or as directed by a healthcare provider.**



**SOLGAR VITAMIN AND HERB**

WORLD HEADQUARTERS

500 WILLOW TREE ROAD, LEONIA, NJ 07605 USA

PHONE 201-944-2311 FAX 201-944-7351

September 28, 1999

Page Two

Enclosed please find documentation that establishes this dietary ingredient, Huperzine A, when used under the conditions suggested on the label, will reasonably be expected to be safe. This documentation includes a Certificate of Analysis, toxicity information, review articles and efficacy studies. Additionally, in support of this Notification, Solgar includes by reference correspondence dated August 25, 1997 from John P. Troup, Ph.D., Vice President Scientific Affairs of GENERAL NUTRITION CENTER (GNC) and the supporting documentation annexed thereto. In addition, reference is made to correspondence from James Tanner, Ph.D. of the Office of Special Nutritionals responding to said letter.

Thank you for your time and attention to this matter. If you have any questions or comments, please do not hesitate to contact the undersigned.

Very truly yours,  
SOLGAR VITAMIN AND HERB

Karla LaSasso  
International Registration Coordinator

Enclosure

Certified Mail - Return Receipt Requested (P035906605)



## CERTIFICATE OF ANALYSIS

**Marco Hi-Tech JV Ltd.**369 Bayview Avenue  
Amityville, New York 11701Phone 516-789 8228  
Fax 516-789 1240**Certificate of Analysis**

Sample Name	Hyperzine A	Quantity	10 grams
Packing	Plastic bottle	Batch Size	350 g
Deliverer	Plant-extra workshop	Manufacturer	
Batch No.	980601	Receiving Date	98-06-01
Criteria	WS-127(X107)94	Reporting Date	98-06-10

**Result**

1. **Characteristics:** A white needle-like crystalline powder; odorless; hygroscopic.
2. **Melting Point:** 228.5 - 229.5 °C (should be 227 - 231 °C)
3. **Identification:** (1)  $\mu_{max}$ : 231 nm, 313 nm  
(2), (3) positive
4. **Loss on Drying:** 2.8% (not more than 5.0%)
5. **Related Substances:** alkaloid impurities I in accord  
alkaloid impurities II in accord  
other impurities I in accord
6. **Assay:** 99.7% (should be 98.0 - 102.0%)

**Conclusion:**

This batch of product is in conformity with the above criterion.

**See Addendum A attached**

# ADDENDUM A

## 1. Product Specifications:

Chemical Classification

Physical Classification

Identification:

IR

UV

Color

Odor

Solubility

pH (1% Solution)

Moisture (%)

Melting Point (°C)

Identification:

UV max

Active Ingredients:

Huperzine A (%)

Heavy Metals:

Pb (ppm)

As (ppm)

Hg (ppm)

Cd (ppm)

Infestation

Foreign Material

Microbiological Assays:

Total Plate Count (CFU/gm)

Yeast & Mold (CFU/gm)

*E. coli* (CFU/gm)

*Salmonella* (CFU/gm)

Hygroscopic

Organic, Nutritive  
Powder

Conforms to standard supplied by Seller

Conforms to standard supplied by Seller

White needle-like crystalline powder

Odorless

To be determined

To be determined

No more than 5

227 - 231

231 nm, 313 nm

98 - 102

Less than 2

Less than 2

Less than 2

Less than 1

None

None

Less than 1000

Less than 100

None

None

Positive

## 2. Representations and Warranties:

a) Common or Usual Name - Huperzine A

b) Product Description - Product consists of a standardized extract of Huperzine A, a natural alkaloid from the club moss *Huperzia serrata*. Product may also consist of synthetically derived Huperzine A, but only upon prior written approval from Buyer and at mutually agreed upon prices.

c) Product Application - Product is a nutritional ingredient for use in food, beverage and dietary supplement products.

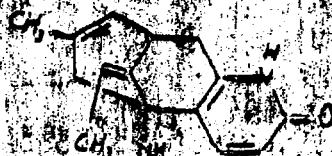
d) Product Dosage - Recommended dosage is 100 micrograms per day.



Hyperzine A (Fordine)

Reference Material  
Quality Specification Protocol

HYPERZINE A



(C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>)

C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>

Hyperzine A contains not less than 98.0 percent and not more than 102.0% of Biological Alkaloid C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub> calculated on the dried basis.

Description: White or off-white, odorless, hygroscopic, needle shape crystals, very slightly soluble in water, slightly soluble in ether, freely soluble in anhydrous ethanol and chloroform.

Melting point: (CP 1990 edition, Part 2 appendix page 15) between 227 and 231°C (but the melting range between beginning and end of melting does not exceed 2°C). It decomposes upon melting.

Identification:

(1) The absorption spectrum determination (CP 1990 edition, part 2, appendix Page 24) of a solution obtained under assay preparation exhibits maxima at wavelengths 231±1 nm and 313± nm.

(2) Spot a filter paper with a drop of sample preparation solution obtained under related substances and allow to dry. Repeat the procedure once more. Place 1 mg of p-dimethyl-amino-benzaldehyde and 1 drop of benzene on the spot and allow to dry. Place the filter paper over hot acetic acid vapor for 1-2 min. a yellow spot appears.

(3) Add 3 drops of water and one drop of Bromine TS to 0.1 ml of a test preparation solution obtained under related substances and shake. Yellow color disappears and a white precipitate is produced.

**Testing :**

**Loss on Drying:** (CP 1990 edition, part 2 appendix page 55)  
Dry about 0.5 gm sample at 80°C to constant weight: it loses not more than 5% of its weight.

**Related Substances:** (1) Biological Alkaloid Impurity I (simply named isomer)

A. Test preparation-- Dissolve a suitable quantity of the product in anhydrous ethanol to obtain a solution having a concentration of 20mg/ml.

B. Standard preparation-- Dissolve a suitable quantity of isomer reference standard in anhydrous ethanol to obtain a solution having a concentration 0.2 mg/ml.

**Procedure:** Apply separate 4  $\mu$ l portions of standard preparation, and test preparation on a 5 X 10 cm Chromatographic Plate coated with GF254 silica gel and CMC (method of making plate see below). Dry with hair dryer. Develop the chromatogram in a solvent system consisting of a mixture of dichloromethane, acetone, methanol, 95% ethanol, and water (3.5 : 1.5 : 1.0 : 0.20 : 0.25). Develop the chromatogram vertically twice. (After the first development when the solvent front has reached the pre-determined end of the plate, allow it to dry for 10 minutes. Discard the used solvent and replace with fresh solvent for subsequent development of the chromatogram the second time.) Allow the solvent to evaporate. Examine the plate under UV lamp (254 nm). Mark the major spots. Spray the plate with 0.1% KMnO<sub>4</sub> solution. Examine the plate after 2 minutes. Compare the intensities and sizes of spots observed in the chromatograms of the Test Preparation to those spots in the chromatograms of the Standard Preparation. No isomer spots observed from the chromatograms of the Test Preparation is larger or more intense than those spots obtained from Standard Preparation. (<1%)

## (2) Biological Alkaloid Impurity II.

A. Test Preparation: same as (1)A above.

B. Standard Preparation: Dissolve a suitable quantity of Huperzine Reference Standard in anhydrous ethanol to obtain a solution having a concentration of 20 mg/ml.

Procedure: Apply separate 5  $\mu$ l portions of standard preparation and test preparation on a 5 X 20 cm Chromatographic Plate coated with GF254 silica gel and CMC (method of making plate see below). Continue the procedure as under Biological Alkaloid Impurity I, starting from "Dry with hair dryer". Examine the plate after 2 minutes. Major spots obtained from test preparation correspond in R<sub>f</sub> value to that of standard preparation and no secondary spots are observed (<4.5%).

## (3) Other Impurities.

Procedure: Apply separate 4  $\mu$ l of standard and test preparation prepared under Biological Alkaloid Impurity II on a 5 X 10 cm Chromatographic Plate coated with GF254 silica gel and CMC (method of making plate see below). Dry with hair dryer. Develop the chromatogram vertically in a solvent system consisting of a mixture of water saturated n-butanol (shake a quantity of n-butanol and water in a separatory funnel. Allow the layers to separate for two days. Separate and discard water layer before use) and glacial acetic acid (4:1:0.5). Allow to dry. Examine under UV light (265 nm). The number of impurity spots produced by the test preparation should be the same as that of standard preparation and no other spots are observed.

Assay: Accurately weigh about 10 mg of the raw material previously dried to constant weight. Dissolve in ethanol to obtain a solution having a concentration of 10  $\mu$ g per ml. Determine the absorbance (CP 1990 part 2 appendix page 24) of the solution on 1 cm cell at a wavelength of maximum absorbance at 312 nm. Calculate the concentration based on the extinction coefficient of C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> ( $E_{1\%}^{1\text{cm}}$ ) = 331.

Indication and Uses: Truly reversible cholinesterase inhibitor, used for treatment of benign memory impairment, brain functional memory impairment, and dementia.

Dosage and administration: Omitted.

Precaution: Omitted

Storage: Tight sealed and protect from light.

Preparation of (5 X 10 cm) thin layer plate: Weigh 1.0 gm of silica gel GF 254 (10-40  $\mu$ ). Add 2.3 ml of 0.5% CMC solution. Add sufficient water and mix to form a paste of batter consistency. (Along the two 10 cm edges apply 0.5 mm thick tape so the width of adsorbant will be about 4.3 cm.) Transfer and apply the adsorbant onto the plate. Level it with a spreader. Place the plate horizontally and allow to dry naturally. Activate the dried adsorbant the next day at 110°C for 30 minutes. Store in silica gel desiccator before use.

Preparation of (5 X 20 cm) thin layer plate: Weigh 2.5 gm of silica gel GF 254 (10-40  $\mu$ ). Add 6 ml of 0.5% CMC solution. Add sufficient water and mix to form a paste of batter consistency. Follow the rest of the steps as in preparation of (5 X 10 cm) thin layer plate.

11/20/2019 11:20:00 AM

11/20/2019 11:20:00 AM



## TOXICITY INFORMATION



## **WILKE RESOURCES**

Phone: (800) 779-5545 15036 W. 106th Street, Lenexa, KS 66215 FAX: (913) 438-5544

**Date: Wednesday, July 29, 1998**

**Pages: 1**

**To: Carl Germano  
Solgar Vitamin & Herb Co. Inc.  
Fax: 201-944-7351**

**From: Jim France  
Wilke Resources, Inc.  
Fax: 438-5554**

**Subject: Huperzine A Toxicity Information**

Our Chinese pharmaceutical manufacturer of the natural extract, Hupenine A, has provided us with the following results of **inhouse** animal toxicity testing for this product:

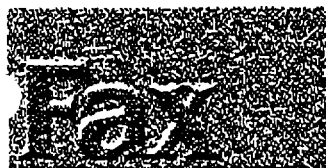
Acute tests showed that Hup A will cause **ChE** inhibition poisoning if it is taken at a very high dosage by mouse, rat, rabbit and dog. In mice, the **LD<sub>50</sub> (ig)** = 5.2 **mg/kg**, **LD<sub>50</sub> (iv)** = 0.63 **mg/kg**, and **LD<sub>50</sub> (ip)** = 1.8 **mg/kg** while in rats the **LD<sub>50</sub> (ig)** = 25.9 **mg/kg**, **LD<sub>50</sub> (iv)** = 2.55 **mg/kg**, and **LD<sub>50</sub> (ip)** = 5 **mg/kg**. The toxicity of **HupA**, however, is much lower than Physostigmine. In mice via **IP** testing, the treatment index (**LD<sub>50</sub>/ED<sub>50</sub>**) is 23.1 for Hup A, 8.6 for Neostigmine, and 3.8 for Physostigmine. In rats via IV testing, the treatment index is 72.9 for **HupA**, 34.0 for Neostigmine, and 7.2 for Physostigmine.

In a subacute toxicity test, two dosages were used to treat dogs - 0.3 **mg/kg** and 0.6 **mg/kg**. After a period of 180 consecutive days of Hup A treatments, no clear side effects were found in any of the treated animals. The dosage used in this test was 45 times higher than the recommended clinical dosage. Other toxicity tests were **also** conducted, including mutagenic and teratogenic tests, with no effects found. Therefore, Huperzine A is a safe and very effective new herbal extract.

While we **find** the above results to be indicative of Huperzine A's safety, we are asking the manufacturer to expand on the statement that "no clear side effects were found." Specifically, we are asking how this was determined and what tests if any were done on the liver, kidney, and heart to support this statement.

It is also important to note that the above refers to the natural herbal extract, **(-)Huperzine A** in its 98% plus purity form. **Lower** percent extracts will potentially contain additional plant chemicals that may or may not be toxic. In addition, this information does not relate to any synthetically prepared form of huperzine A which differs dramatically in reduced efficacy compared to the natural extract.

If you have any questions, please don't hesitate to call Jim France at (800) 779-5545.



# WILKE RESOURCES

15036 W. 106th Street, Lenexa, KS 66215

Date: Monday, July 20, 1998

Pages: /

To: Carl Germano  
Solgar Vitamin & Herb Co. Inc.  
Phone: 201-944-2311  
Fax: 201-944-7351

From: Jim France  
Wilke Resources, Inc.  
Phone: 913-438-5544  
Fax: 438-5554

Subject: Huperzine-A (98%)

Wilke Resources has developed sourcing for a high purity (98%) **Huperzine-A** which is an alkaloid extracted from herb **Huperzia serrata**. **Huperzine-A** has demonstrated an excellent potential for memory enhancement as related to Benign Senescent Amnesia and, more importantly, **Alzheimer's Disease**.

**Alzheimer's** disease is believed to be attributed to aging and, as the population lives longer, it is becoming a common ailment among the elderly. Ten percent of the US population between the ages of 80 and 85 reportedly suffer from **Alzheimer's** with the percentage increasing to 25% for those over 85 years of age. **Alzheimer's** is a chronic and progressively degenerative neurological disorder characterized by dementia and behavioral symptoms that severely reduces the quality of life of both the victim and the immediate family.

It has been shown that a severe deficiency of choline acetyl transferase and a decrease in the synthesis of acetylcholine represent the most prominent neurochemical changes that occur with **Alzheimer's**. The concentration of acetylcholine, however, can be increased by inhibiting the reduction of the enzyme acetylcholinesterase (**AChE**), thus relieving certain symptoms such as cognition. A number of such inhibitors have been developed and two of which, tacrine (**Cognex**) and donepezil (**E2020; Aricept**) are FDA approved for the symptomatic treatment of mild-to-moderate **Alzheimer's** disease in the US.

Research in China has revealed that **Huperzine-A** is a potent reversible inhibitor of **AChE**. Further studies at Weizmann Institute of Sciences in Rehovot, Israel and Gerogetown University in Washington suggest that **Huperzine-A** is even more potent than either tacrine or donepezil. As reported in the Journal of the American Medical Association on March 12, 1997, **Huperzine-A** appears to be more selective and possibly less toxic than either of the FDA approved drugs. Compared to tacrine and donepezil, **Huperzine-A** has a longer half-life and the **AChE-HupA** complex has a slower rate of dissociation, which may make it a more effective therapeutic agent.

Reports from China, where an estimated 100,000 people have been treated with **Huperzine-A**, further support the contention that the extract has low toxicity. In fact, since the herb is a traditional Chinese medication and used for generations, it may be reasonably believed to be safe without excessive application and marketed as a dietary supplement under DSHEA.

**Huperzine-A** is available via Wilke Resources from the original Chinese pharmaceutical manufacturer in powder form with a minimum purity of 98%. The literature reports that the effective daily dosage for the 98% pure product to be in the 100 to 200 microgram range. Based on a delivered selling price of US\$750.00 per gram, the ingredient cost for a single individual's one month's supply would range from \$2.25 to \$4.50. This could easily translate into a retail price of \$25 to \$30 for one month's supply. The current prescription pricing for one month's supply of donepezil and tacrine is around \$100 and \$117-\$234 respectively.

**Huperzine-A (98%)** is priced as follows: (1) 1-9 grams = \$750/gram; (2) 10 to 99 grams = \$700/gram; and 100 grams or more = \$650/gram (pricing includes air freight; all applicable duties and taxes are the responsibility of the buyer). Payment must be made in advance of shipment with the understanding that the customer can return a given order within 10 days of receipt for a full refund. All shipments are by air and are fully insured. Additional information is being sent via FEDEX. If you have any questions, please call Jim France at (800) 779-5545 for more information.



10/10/10



## **REVIEW ARTICLES**

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

XU Si-Sun, G.40 Zhi-Xu<sup>\*</sup>, WENG Zheng<sup>\*</sup>, DU Zun-Ming<sup>1</sup>, XU Wei-An<sup>4</sup>, YANG Jian-Shen<sup>5</sup>, ZHANG Ming-Lian<sup>6</sup>, TONG Zhen-Hua, FANG Yong-Sheng<sup>1</sup>, CHAI Xin-Sheng<sup>2</sup>, LI Shu-Lan<sup>\*</sup> (Zhejiang Supervision and Detection Station of Drug Abuse, Zhejiang Medical University, Hangzhou 310009; <sup>1</sup>Shanghai Mental Health Center, Shanghai 200030; <sup>2</sup>Shandong Mental Health Center, Ji-nan 250014; <sup>3</sup>Hangzhou Central Hospital of Railway, Hangzhou 310009; <sup>4</sup>Shanghai 3rd Mental Hospital, Shanghai 201905; <sup>5</sup>Suzhou Puji Hospital, Suzhou 215008; <sup>6</sup>Suzhou 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, Hasegawa 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 (36 %, 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<sup>(2)</sup>. 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<sup>(1)</sup>, is a potent, centrally active, and reversible cholinesterase inhibitor (ChEI)<sup>(4)</sup> with better therapeutic index than those of physostigmine and THA<sup>(5)</sup>. It was reported to ameliorate learning and memory retention in rodents and improve memory in aged<sup>(6,7)</sup>.

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

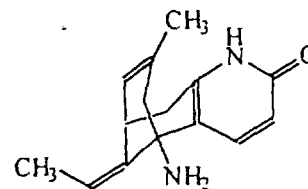


Fig 1. Molecular representation of Huperzine-A.

### MATERIALS AND METHODS

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

of DSM-III-R<sup>23</sup> 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); mini-mental 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 8 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  $\chi^2$  method.

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

## RESULTS

The blind was declassified on 1994-05-06 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 AD.**  
All data showed no statistical significance between Hup and Pla group.

	Pla (n=53)	Hup (n=50)
Sex: ♂	29	28
♀	24	22
Age: $67 \pm 11$		$66 \pm 11$
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	1
married	38	32
Course: (year)	$3.0 \pm 1.8$	$3.1 \pm 1.6$
<2	8	8
2-	34	24
4-	8	16
6-	1	0
$\geq 8$	2	2
Severity:		
mild	27	33
moderate	23	17
severe	3	0
MQ baseline	$48 \pm 21$	$56 \pm 21$
MMS baseline	$14 \pm 5$	$16 \pm 5$
HDS baseline	$16 \pm 5$	$16 \pm 6$
ADL baseline	$31 \pm 9$	$33 \pm 10$
TESS baseline	$1 \pm 4$	$1 \pm 3$
Identified cerebral atrophy by CT or MR.	25 (47%)	22 (44%)

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

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 <sup>d</sup>
8-wk trial	52 ± 26	64 ± 26 <sup>e</sup>
t (paired-t)	2.69	5.15 <sup>e</sup>
MMS baseline	14 ± 5	16 ± 5 <sup>d</sup>
8-wk trial	15 ± 6	19 ± 6 <sup>f</sup>
t (paired)	0.76 <sup>f</sup>	5.62
HDS baseline	16 ± 5	16 ± 6 <sup>d</sup>
8-wk trial	15 ± 7	20 ± 6 <sup>f</sup>
t (paired)	0.30 <sup>f</sup>	7.04 <sup>f</sup>
ADL baseline	31 ± 9	33 ± 10 <sup>d</sup>
8-wk trial	31.9 ± 0.7	29 ± 9 <sup>d</sup>
t (paired)	1.64 <sup>f</sup>	4.51 <sup>f</sup>

**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)	$\chi^2$
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 <sup>f</sup>
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. \* $P > 0.05$ .

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

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<sup>13</sup>, 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 similarity to the discovery of some au-

thors<sup>(9,10)</sup>.

Throughout 8-wk study, no patient ALT value exceeded the upper limit of normal or renal toxicity in both groups, only a slight increase in some mild peripheral cholinergic side 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<sup>(4,6,7,11,12)</sup>, ie Hup produced less peripheral side effects at optimal doses, it indicates that Hup is a safe drug and suitable for treating patients with Alzheimer's disease.

Both Hup and tetrahyd oaminoacrine (THA, tacrine) approved by FDA in 1993 belong to cholinergic agents, but the latter has a potential liver toxicity<sup>(13,14)</sup>, and only a mean improvement of 2.0 points on MMS over 30 wk was noted for patients receiving THA 160 mg d<sup>-1</sup> with 43 % of these patients improving by 3.0 points or more<sup>15</sup>, 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 the 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

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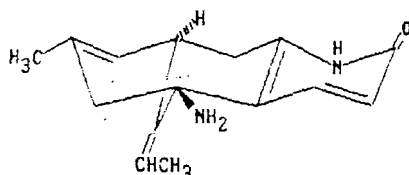
**目的:** 评估石杉碱甲片治疗阿耳茨海默病的疗效及其安全性。 **方法:** 采用多中心、前瞻性、双盲、平行、空白对照和随机方法, 给50例阿尔采末病一日两次口服石杉碱甲片4片(每片含50微克), 并给53例阿耳茨海默病一日两次口服安慰剂片4片, 共8 wk。所有病人都用韦氏智力量表、简易精神状态量表、长谷川痴呆量表、日常生活能力量表、副反应量表和其他实验室检查。 **结果:** 发现58 % (29/50) 的石杉碱甲片服用者改善了所有的记忆、认知和行为能力, 而安慰剂组仅为35.8 % (19/53), 两组疗效有显著差异 ( $\chi^2 = 5.07$ ,  $P < 0.05$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

## HUPERZINE A

9R,11E)-5-Amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocteno[b]pyridin-2(1H)-one  
= 122-853

Cholinesterase Inhibitor  
of Myasthenia Gravis  
Inhibition Acrivarol



$H_{18}N_2O$ ; Mol wt: 242.33  
74.35%; H 7.49%; N 11.56%; O 6.60%

### Isolation

Huperzine A is a new lycopodium alkaloid isolated from the Chinese folk medicine *Huperzia serrata* (Lamb.) Trev. The powdered herb is extracted with 70% ethanol. The phenolic alkaloids are separated by treatment with dilute sodium hydroxide. The mixture is taken up on a basic silica gel column, and eluted with chloroform-methanol. Huperzine A is crystallized from acetone after evaporation of the solvent solution (2).

### Description

Slightly yellow crystals, m.p. 229-30°;  $\alpha_D^{25} = -150.4^\circ$  ( $c = 0.498$  MeOH). UV absorption:  $\lambda_{max} = 231$  (log  $\epsilon$  4.01), 313 (log  $\epsilon$  3.893). IR  $\nu_{max}^{KBr}$ : 3180, 1650, 1615, 1550 (1).

### Pharmacological Actions

The anticholinesterase activity of huperzine A was studied *in vitro* (3). Rat erythrocyte membrane and kidney nuclei of pigs were used as sources of acetylcholinesterase, and rat serum was chosen as source of butyrylcholinesterase. The  $IC_{50}$  of huperzine A towards erythrocyte membrane and kidney nuclei was 7.2 and 7.9, respectively. The inhibiting effect was about 3 times as potent as that of physostigmine. Huperzine A was less potent than physostigmine towards rat serum. The alkaloid thus belongs to the class of mixed and reversible

cholinesterase inhibitors.

Rats were placed on an electrified grid in a Y-maze and learned to run into the light arm (safe area). The criterion of learning or retrieval was met after they had chosen the light arm 10 trials in succession. Huperzine A 100 and 167  $\mu$ g/kg i.p. administered 20 min before training caused a significant decrease in the number of trials to criterion. Facilitation of retrieval was also dose-dependent at doses of 36-167  $\mu$ g/kg i.p. Scopolamine 0.2 mg/kg s.c., atropine 5 mg/kg s.c. or hemicholinium 20  $\mu$ g/10  $\mu$ l i.c.v. antagonized the positive effects of huperzine A 0.1 mg/kg on retrieval process, but methylatropine 2 mg/kg s.c. did not do so. Under the same conditions, physostigmine 80-160  $\mu$ g/kg i.p. improved the learning and retrieval process, but neostigmine 30  $\mu$ g/kg i.p. did not. The facilitating actions of huperzine A were due to an effect on the central cholinergic system, especially on the muscarinic system (4).

### Clinical Studies

One hundred twenty eight cases of myasthenia gravis were treated with huperzine A 0.4 mg i.m. once daily. Clinical manifestations were controlled or improved. Huperzine A showed a duration of action of  $7 \pm 6$  h, whilst that of prostigmine was  $4 \pm 5$  h (5).

The therapeutic effects of huperzine A and hydergine were studied by a double-blind method in 100 aged patients with memory impairment. Memory was improved 1-4 h after injection of huperzine A, and the effect was sustained for about 8 h. The therapeutic value of huperzine A 30  $\mu$ g was superior to that of hydergine 600  $\mu$ g (6).

Except nausea, the side-effects of huperzine A, such as fasciculation, dizziness, sweating, blurred vision, etc., were less and milder than those of prostigmine (5).

### Manufacturer

Shanghai Inst. Materia Medica (China).

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# LORATADINE

Prop INN: USAN

## Loratadine (Sp)

11-[N-(Ethoxycarbonyl)-4-piperidylidene]-8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine

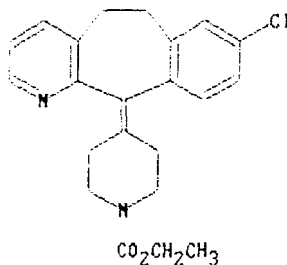
4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylic acid ethyl ester

Sch-2985 1

[CAS-79794-75-5]

EN = 90-791

Antihistamine


 $C_{22}H_{23}ClN_2O_2$ , Mol wt: 382.89

C 69.01%; H 6.05%; Cl 9.26%; N 7.32%;  
O 8.35%

## Synthesis

This compound can be obtained by two different ways (Scheme 1):

1) By carboxylation of 8-chloro-6,11-dihydro-11-(4-piperidylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (I) with ethyl chloroformate (II) in refluxing benzene (1, 2).

2) By reaction of 8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one (III) with the Grignard reagent (IV) to give the tertiary carbinol (V), which is dehydrated with 85%  $H_2SO_4$  affording 8-chloroazatadine (VI) (3). Finally compound (VI) is treated with ethyl chloroformate (II) in toluene (4).

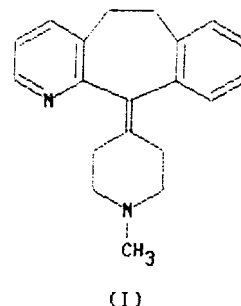
## Description

Crystals, m.p. 128-30°.

(CES)

## Introduction

Antihistamines became widely used in the mid to late 1940's. They quickly became established in the treatment of various allergic disorders, particularly rhinitis, conjunctivitis and urticaria. The problem of sedation limited the use of classical antihistamines and the search for  $H_1$ -antagonists without sedative potential has been a goal within the pharmaceutical industry. Several non-sedating  $H_1$ -antihistamines have been recently introduced in the clinic, others are under investigation and others are continuously emerging from patent literature (Table I). Recently, conversion of the basic tertiary amino function of the potent antihistamine azatadine (Optimine®) (1) to a neutral carbamate function led to the preparation of compounds which retained significant antihistamine activity, with little or no CNS effects. The most potent antihistamine, Sch-29851 (loratadine), in this series was selected for further evaluation in view of its lack of CNS side-effects (4).



## Pharmacological Actions

Loratadine has been shown to be a potent antihistamine in laboratory animals and to have no CNS effects in a variety of animal species and test paradigms (4-7). One explanation for these findings is the weak affinity of loratadine for CNS receptors involved in sedation, including histamine  $H_1$ -receptors,  $\alpha_1$ -adrenoceptors and acetylcholine receptors (5). In a recent study displacement of [ $^3H$ ]-mepyramine binding was studied in membranes from guinea-pig lung vs. cerebral cortex as a measure of affinity for peripheral vs. CNS histamine receptors. Loratadine was selective for lung vs. cortex, while other antihistamines, including terfenadine, astemizole, mequitazine and chlorpheniramine, were not selective. From these results it was concluded that the lack of significant sedative effects shown by loratadine was due to its poor

# HUPERZINE A - AN INTERESTING ANTICHOLINESTERASE COMPOUND FROM THE CHINESE HERBAL MEDICINE

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**Summary:** Huperzine A, alkaloid from the Chinese herbal medicine Qian Ceng Ta, which is prepared from the moss *Huperzia serrata*, has been used in China for centuries to treat fever and inflammation. Huperzine A is a strong inhibitor of cholinesterases with high selectivity to acetylcholinesterase and in China is developed as therapeutic against Alzheimer's disease. May be that huperzine A will be better than other centrally active anticholinesterases in treating this neurodegenerative disorder. Huperzine A appears to have additional pharmacological properties that make it an attractive candidate therapy for clinical trials.

**Key words:** Huperzine A; Alkaloid; Inhibitor of Acetylcholinesterase; Alzheimer's Disease; Treatment

## Introduction

The alkaloid compound, huperzine A, was discovered in the Chinese herbal medicine called Qian Ceng Ta (14). This traditional remedy, which is prepared from the moss *Huperzia serrata*, has been used in China for centuries to treat fever and inflammation.

## Chemistry

Huperzine A is an unsaturated sesquiterpenic compound with pyridone moiety and primary amino group (Fig. 1)  $C_{15}H_{18}N_2O$ , MW = 242.32. Chemically 9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3.8)]tridec-3(8),6,11-trien-5-one. Compound is optically active and in the moss is present only its (-)-enantiomer. The pyridone ring is planar and the stereochemistry of the C(11)-C(12) double bond is E. It is white solid soluble in aqueous acids and  $CHCl_3$  (3).

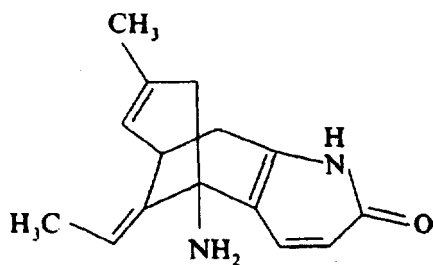


Fig. 1: Chemical structure of huperzine A

## Biochemistry

Huperzine A is a potent reversible inhibitor of cholinesterases, i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) with on- and off-rates that depend on both the type and the source of enzyme. A low dissociation constants  $K_i$  was obtained for mammalian-AChE-huperzine A (2040 nM) compared to mammalian BuChE-huperzine A (20-40  $\mu$ M) (1). This indicates that the thermodynamic stability of huperzine-cholinesterase complex may depend on the number and type of aromatic amino acid residues in the catalytic pocket region of the enzyme molecule. The mechanism of the inhibition of acetylcholinesterase (AChE) is stereoselective. (-)-Huperzine A, which is in drug, was the more potent enantiomer with a  $K_i$  value of 8 nM. (+)-Huperzine A inhibited the enzyme 38-fold less potently with a  $K_i$  value of 300 nM. Racemic huperzins A was about two-fold less potent than the more active enantiomer. The mechanism of inhibition of rat cortical AChE for all three compounds was of the mixed linear competitive type (9). Very similar results were obtained with enzymes from other sources (13). The crystal structure of the complex of AChE with optically pure huperzine A at 2.5 Å resolution shows an unexpected orientation for the inhibitor with surprisingly few strong direct interactions with protein residues to explain its high affinity. An analysis of the affinities of structural analogues of huperzine A, correlated with their interactions with the protein, shows the importance of individual hydrophobic interactions between huperzine A and aromatic residues in the active-site gorge of AChE (12, 13). Based on docking studies and the pharmacologi-

cal results reported for huperzine A and its analogues. it was predicted that huperzine A binds to the bottom of the binding cavity of **AChE** with its ammonium group interacting with **Trp84**, **Phe330** and **Asp72** and to the opening of the gorge with its ammonium group partially interacting with **Trp279**. At the catalytic site, three partially overlapping **subsites** of huperzine A were identified which might provide a dynamic view of binding of huperzine A to the catalytic site (7. IO).

### Neurochemistry

**AChE** was assessed in rats after acute and chronic administration of huperzine A. Forty-five min after a single injection of huperzine A (0.5 **mg/kg**, i.p.) the activity of **AChE** was significantly decreased by 15-30 per cent in hippocampus, striatum and septum. The activity of **cholineacetyl transferase (ChAT)** was not altered. In the hippocampus high affinity choline transport (**HACT**) was altered by 25 per cent, whereas no effect in the striatum was observed. After 90 min. both inhibition of **AChE** and attenuation of **HACT** had returned to control values. After 7 days chronic application of huperzine A (twice a day) at 0.5 **mg/kg**, the activity of **AChE** was significantly reduced by 20-30 per cent in every region of the brain studied. **HACT** in the hippocampus was reduced by 28 per cent, 45 min after the last injection, but in the striatum there was no effect. The activity of **ChAT** was not affected in any region of the brain studied (8).

Tang et al. (15) show that huperzine A can produce a long-term inhibition of **AChE** activity in the **acetylcholine** levels up to 40 per cent at 60 min. There is considerable regional variation in the degree of **acetylcholine** elevation after huperzine A with maximal values seen in frontal (125 per cent) and parietal (105 per cent) cortex and smaller increases (22-65 per cent) in other brain regions. A comparable effect was also observed in studies, in which, over a range of 0.1-2.0 **mg/kg** of huperzine A administered i.p., significantly inhibits of **AChE** activity in all brain region tested (hippocampus, hypothalamus, striatum and frontal cortex) and decreases level of brain **acetylcholine** (16).

### Pharmacology

Huperzine A at concentrations 1 to 100  $\mu\text{M}$  does not significantly alter the electrically evoked release of  $^3\text{H}$ -**acetylcholine** from cortical slices. With the exception of the highest concentrations (600 **M**) the displacement effect of huperzine A for cholinergic lipands is -stronger for  $^3\text{H}$ -**nicotine** than for  $^3\text{H}$ -**QNB**. Autoradiographic study in the mouse shows that 60 min after i.v. injection (183  $\mu\text{g/kg}$ ) huperzine A is particularly concentrated in certain areas such as frontoparietal cortex, nucleus accumbens, hippocampal, and striatal cortex. Radioactivity is practically absent in the whole body at 12 hr (15).

Huperzint A in doses from 0.4 o 0.5 **mg/kg**, i.p., significantly ameliorated the **AF64A-induced** memory deficit in rats in the radial maze. These results suggest that dissruping working memory induced by cholinotoxine **AF64A** can be effectively ameliorated by huperzint A (18). Very similar effects were obtained with huperzine A in doses from 0.1 to 0.4 **mg/kg**, p.o., on **memory** impairments induced by scopolamine. The comparison with other **AChE** inhibitors shows that huperzine A is the most selective **AChE** inhibitor, and improved the working memory deficit significantly better than did **tacrine** or **dontpezil** (2). The results with natural (-)-**huperzine A** and synthetic (+/-)-**huperzine A** indicate a similar biological effects, but the racemic mixture of (+/-)-**huperzine A** has a weaker biological activity than the natural product (6).

Huperzine A in dose 0.1 **mg/kg**, in conscious rabbits produced, already 30 sec after i.v. administration, an alert **EEG** pattern, which showed decreases of lower frequency components and the total EEG power in cortical area, and the dominant frequency transferred from delta rhythm to theta rhythm in hippocampus and the same effects were observed with physostigmine in the dose of 0.1 **mg/kg**. Intravenously administered huperzine A in dose 0.2 **mg/kg** as well as physostigmine in dose 0.3 **mg/kg** antagonized the EEG effects of scopolamine (0.3 **mg/kg** i.v.). That results indicate that the effects of huperzine A are closely related to the action on the central cholinergic system (5).

Huptrzint A appears to have additional pharmacological properties that make it an attractive candidate therapy for clinical trials. In studies using cell cultures from the hippocampus and cerebellum of rat embryos, have been shown that huperzine A decreases neuronal cell death caused by toxic level of glutamate (14). In addition to the loss of cholinergic function in patients with AD, glutamatergic and **GABAergic** neurotransmitter systems may also be compromised. -Glutamate activates N-methyl-Daspartate receptors and increases the flux of calcium ions into the neurons, which in sufficient concentration can kill the cells.

Huperzine A has been also testing as a prophylactic drug against **soman** and other **nerve** gas poisoning with very good effect (4).

### Pharmacokinetics

Pharmacokinetic of huperzine A was studied in six volunteers after a single oral dose of 0.99 **mg** and drug concentrations were assayed by reverse phase HPLC from 0.5 to 10 hrs. The time course of plasma concentrations conformed to a one-compartment open model with a first order absorption with  $T_{0.5}$ ,  $k_a = 12.6$  min,  $T_{0.5\text{ ke}} = 288.5$  min.  $T_{\text{max}} = 79.6$  min.  $C_{\text{max}} = 8.4$   $\mu\text{g/litre}$ ,  $\text{AUC} = 4.1$   $\text{mg/litre.min}$ . From this result is clear that huperzine A is absorbed rapidly, distributed widely in the body, and eliminated at a moderate rate (11).

## Medical use

Huperzine A has similar action to the drugs currently approved to treat Alzheimer's disease - tacrine (Cognex) and donepezil (Aricept). i.e. inhibits brain AChE and blocks the breakdown of acetylcholine, a chemical messenger in the brain that is important to memory function (14, 19). Reports from China, where perhaps 100.000 people have used huperzine A, suggest that it is at least as safe as the two approved Alzheimer's drugs. Not all informations from China are available and trustworthy. It is evident that huperzine A in China was not only clinically tested, but this compound is used as remedy in the form of tablets in Alzheimer's disease (17). Nevertheless, huperzine A is probably still a long way to medical use in Europe (14).

The ability of huperzine A to decrease neuronal cell death caused by toxic level of glutamate may make this compound a potential drug for reducing neuronal injury from strokes, epilepsy, and other disorders.

Huperzine A is a candidate drug against organophosphate nerve agent toxicity for its long-lasting antidotal efficacy and low toxicity (4). Prophylactic study make this drug promising as a protective agent against chemical weapons.

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## Pharmacokinetics of tablet huperzine A in six volunteers<sup>1</sup>

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**AIM:** To study pharmacokinetics of tablet huperzine A (Hup-A) in Chinese volunteers to help establishing its drug administration schedule. **METHODS:** For 6 volunteers after a single oral dose of 0.99 mg, drug concentrations in plasma were assayed by reverse phase high pressure liquid chromatography (HPLC) at 0.5, 0.75, 1.0, 1.25, 1.5, 2, 4, 6, 8, and 10 h. The pharmacokinetic parameters were calculated with a 3P87 program by computer. **RESULTS:** The time course of plasma concentrations conformed to a one-compartment open model with a first order absorption. The pharmacokinetic parameters were as follows:  $T_{1/2\alpha} = 12.6 \text{ min}$ ,  $T_{1/2\beta} = 288.5 \text{ min}$ ,  $T_{\text{max}} = 79.6 \text{ min}$ ,  $C_{\text{max}} = 8.4 \mu\text{g L}^{-1}$ ,  $\text{AUC} = 4.1 \text{ mg L}^{-1} \text{ min}$ . **CONCLUSION:** Hup-A was absorbed rapidly, distributed widely in the body, and eliminated at a moderate rate.

**KEY WORDS** huperzine A; cholinesterase inhibitors; high pressure liquid chromatography; pharmacokinetics; phase I clinical trials

Huperzine A (Hup-A), a new alkaloid first isolated from Chinese herb *Huperzia serrata* (Thunb) Trev<sup>[1]</sup>, exhibited a selective inhibition on acetylcholinesterase (AChE)<sup>[2]</sup>. It potentiated the skeletal muscle contraction and increased muscle tones<sup>[3]</sup>, and enhanced rodent learning and memory<sup>[4]</sup>. Clinically, Hup-A improved muscle weakness of myas-

thenia gravis<sup>[5]</sup> and memory in patients with impaired memory or Alzheimer's disease<sup>[6]</sup>. The plasma level of Hup-A following *iv* or *ig* [<sup>3</sup>H]Hup-A 13.9 MBq kg<sup>-1</sup> in rats declined in two phases, the distribution phase and the elimination phase, with half-lives of 6.6, 149 min (*iv*) and 10, 203 min (*ig*) respectively<sup>[7]</sup>. This paper was to study the pharmacokinetics of Hup-A in healthy volunteers to help establishing its drug administration schedule in clinic.

### MATERIALS AND METHODS

**Drug** According to Chinese National Standard tablet Hup-A (batch No 940112) was prepared by the Institute of Materia Medica, Zhejiang Academy of Medical Sciences. The purity of Hup-A was 99.5%. Each tablet contains Hup-A 0.09 mg. ( $\pm$  minor Hup-A as internal standard was synthesized and presented kindly by Dr HE Xu-Chang, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and 3 mg L<sup>-1</sup> was used for experiment.

**Subjects** Six Chinese volunteers (M 3, F 3), aged 27  $\pm$  6 a and weighing 58  $\pm$  7 kg were all healthy, not in pregnant or menstruation. Each volunteer was told about the aim and process of the study. Agreements were obtained from them before study. Each subject was given a single oral dose of 0.99 mg Hup-A tablet at 8 am after an overnight fasting. Breakfast was served at 10 am. Blood (5 mL) was collected from an indwelling catheter in antecubital vein before and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2, 4, 6, 8, and 10 h after *po*. Plasma (2 mL) was taken for HPLC. Pharmacokinetic parameters were obtained by first calculating the parameters from each person and then taking average of the 6 parameters, using a 3P87 program provided by Chinese Mathematic-Pharmacological Society on the computer.

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HPLC Shimadzu LC-GA liquid chromatography was connected to SPD-6A uv spectrophotometric detector (Shimadzu) and Rheodyn 7125 sampler, recorded on C-R3A integrator (Shimadzu). The column was a Spherisorb C18 (150 mm × 5 mm inner diameter; 5 μm particle size). The mobile phase was methanol: water (45:55, vol/vol), 1.0 mL min<sup>-1</sup> at 30 °C column oven. The column effluent was monitored at 313 nm.

**Plasma sample** Add (±)-dinor Hup-A 100 μL to plasma 2 mL, add Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer 1 mL (using NaOH 1 mol L<sup>-1</sup> to adjust pH to 11.9). Then add chloroform 7.5 mL, shake 2 min, and centrifuge at 1000 × g for 10 min. The organic phase was blown to dryness by N<sub>2</sub> at 40 °C. Dissolve the residue with HPLC mobile phase 50 μL, and 20 μL was applied to HPLC. Hup-A peak and (±)-dinor Hup-A peak were separated clearly. The retention times (Rt) of (±)-dinor Hup-A and Hup-A were 3.5 and 8.3 min, respectively (Fig 1).

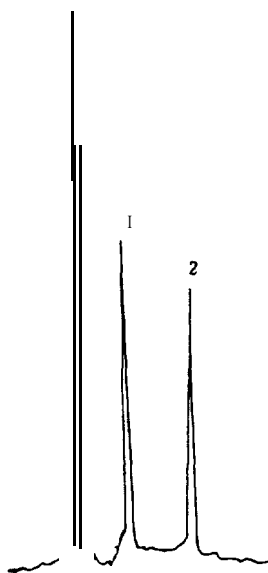


Fig 1. Chromatogram of blank plasma spiked with internal standard (peak 1, retention time 3.5 min) and Hup-A (peak 2, retention time 8.3 min).

**Standard curve** To the plasma containing (±)-dinor Hup-A add Hup-A 2.20, 4.43, 7.08, 8.85, and 17.70 μg L<sup>-1</sup>, according to the ratio of Hup-A peak area to (±)-dinor Hup-A peak area in HPLC, a linear equation  $\bar{Y} = 0.0188X - 0.0069$  was obtained ( $r = 0.9988$ ). The minimal detect limit of plasma Hup-A

was 1.60 μg L<sup>-1</sup>. The recovery of Hup-A was 95.7 ± 5.5 % ( $n = 9$ ) and coefficient of variation was 6.4 %. According to measurements of 3 standard plasma Hup-A concentrations, intraday and interday variances were 5.5 × 7.4 % ( $n = 9$ ) and 6.0 % - 9.9 % ( $n = 9$ ), respectively.

## RESULTS

The plasma concentrations of Hup-A after oral administration of 0.99 mg within 10 h were fitted well to a one-compartment open model with a first-order absorption (Fig 2).

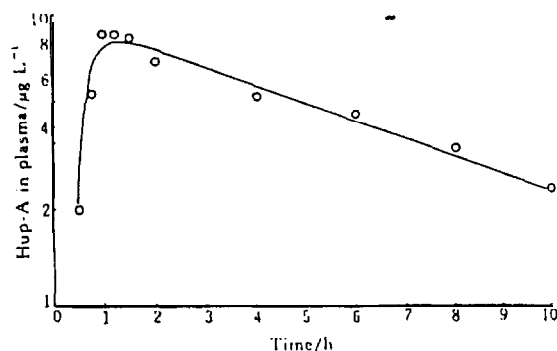


Fig 2. Mean plasma concentration-time curve after po tablet Hup-A 0.99 mg in 6 adults.

Hup-A was absorbed quickly after po with  $T_{1/2\alpha} = 12.6$  min and time peak for plasma averaged 79.6 min. It indicated that Hup-A was released and absorbed quite well *in vivo*. Plasma mean peak concentration after po was 8.4 μg L<sup>-1</sup>,  $V_d/F$  was 0.108 L kg<sup>-1</sup>, indicating that Hup-A was widely distributed *in vivo*. Mean elimination half life  $T_{1/2\beta}$  was 288.5 min, suggesting that Hup-A have a mild elimination rate (Tab 1).

## DISCUSSION

Hup-A showed some advantages, compared with the first generation of ChE inhibitors such as physostigmine (Phy) and tetrahydroaminoacridine (THA), LD<sub>50</sub> value in mice for Hup-A ip was 1.8 mg kg<sup>-1</sup> and

Tab 1. Pharmacokinetic parameters of Hup-A after po tablet 0.99 mg in 6 healthy volunteers.  $\bar{x} \pm s$ .

Parameter	$\bar{x} \pm s$
$K_e$ min <sup>-1</sup>	0.061 ± 0.017
$K_r$ min <sup>-1</sup>	0.0025 ± 0.0006
$T_{1/2\alpha}$ min	13 ± 5
$T_{1/2\beta}$ min	288 ± 63
$T_{max}$ min	80 ± 9
$C_{max}$ μg L <sup>-1</sup>	8.4 ± 0.9
$T_{lag}$ min	25.4 ± 1.8
$V_d/F$ L kg <sup>-1</sup>	0.108 ± 0.008
AUC mg L <sup>-1</sup> min	4.1 ± 1.2

that for Phy was 0.6 mg kg<sup>-1</sup>. Hup-A at optimal doses has a long term inhibition of AChE in rat brain (up to 360 min) and only 60 min for Phy<sup>(9)</sup>. The results of this paper showed that in human being  $T_{1/2\beta}$  of Hup-A was 288.5 min. However, for bhy the  $T_{1/2\beta}$  was 20 min<sup>(8)</sup>. Hup-A was absorbed rapidly, distributed widely in the body and eliminated at a middle rate<sup>(9)</sup>. Therefore it is better to take tablet Hup-A orally 2-3 times a day.

As a new ChE inhibitor, Hup-A shows some interesting cholinomimetic properties and its effects satisfy more closely established criteria for therapeutic use than effects of previously tested compounds. Hup-A is a new promising ChE inhibitor.

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## 石杉碱甲片在六名志愿者体内的药物动力学

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**目的:** 了解石杉碱甲片在人体内的药物动力学过程, 为设计临床用药方案提供依据。 **方法:** 用反相高效液相色谱法测定六名健康志愿者口服片剂0.99 mg后的血药浓度, 按3P87程序计算动力学参数。 **结果:** 石杉碱甲片在体内的药时过程符合一级吸收的一室开放模型。主要动力学参数:  $T_{1/2\alpha}$  12.6 min,  $T_{1/2\beta}$  288.5 min,  $T_{max}$  79.6 min,  $C_{max}$  8.4 μg L<sup>-1</sup>, AUC 4.1 mg L<sup>-1</sup> min。 **结论:** 石杉碱甲吸收迅速, 属于中等速率消除类药物。

**关键词** 石杉碱甲; 胆碱酯酶抑制剂; 高压液相色谱法; 药物动力学; I期临床试验

## ACUTE AND CHRONIC STUDIES WITH THE ANTICHOLINESTERASE HUPERZINE A: EFFECT ON CENTRAL NERVOUS SYSTEM CHOLINERGIC PARAMETERS

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**Summary**—High affinity choline transport, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) were assessed in rats after acute and chronic administration of the AChE inhibitor Huperzine A. Acute treatment: Forty-five min after a single injection of Huperzine A (0.5 mg/kg i.p.) the activity of AChE was significantly decreased by 15–30% in hippocampus, striatum and septum. The activity of ChAT was not altered. In the hippocampus high affinity choline transport was attenuated by 25%, whereas no effect in the striatum was observed. After 90 min, both inhibition of AChE and attenuation of high affinity choline transport had returned to control values. A dose of 0.1 mg/kg (i.p.) did not produce significant effects. Similar results were obtained with physostigmine (0.25 mg/kg), although the duration of inhibition of AChE was shorter than that with Huperzine A.

Chronic treatment: After 5 days (twice a day), at 0.5 mg/kg, the activity of AChE was significantly reduced by 30–35% in every region of the brain studied. High affinity choline transport in the hippocampus was reduced by 25%, 45 min after the last injection, but in the striatum there was no effect. The activity of ChAT was not affected in any region of the brain studied. Thus, acute or chronic treatment with Huperzine A: did not alter ChAT; reduced high affinity choline transport in the hippocampus in a transient manner; and had a longer duration of action as an AChE inhibitor than physostigmine. Moreover, tolerance to low-toxicity doses of Huperzine A was minimal, contrary to what has been observed with other inhibitors of AChE.

**Key words**—chronic, Huperzine A, anticholinesterase, HAChT, ChAT, Alzheimer's disease.

The new cholinesterase (AChE) inhibitor Huperzine A (Fig. 1) is an alkaloid extracted from a *Lycopodium* found in China. It was reported to ameliorate learning and memory retention in rodents (Lu, Shou and Tang, 1988; Tang, Han, Chen and Zhu, 1986; Zhu and Tang, 1988). Moreover, improvements in memory, lasting for several hours after a single intramuscular injection, were reported in patients affected by impairment of memory or Alzheimer's disease (AD) (Zhang, 1986).

Recently, the acute action of Huperzine A was investigated in the CNS of the rat by Tang, De Sarno, Sugaya and Giacobini (1989), who showed a sustained increase in levels of acetylcholine (ACh) in brain of several hours duration. At the doses used, the inhibition of cholinesterase lasted three times longer than with physostigmine as well as producing significantly fewer side effects than physostigmine or tetrahydroaminoacridine (THA) (Tang et al., 1989).

However, the effect of Huperzine A on other central cholinergic parameters, such as the high affinity transport of choline and activity of choline acetyltransferase (ChAT) was not assessed *in vivo*. Neither was it determined if Huperzine A would be an effective cholinergic modulator during chronic

treatment. Here, it is reported that the inhibitory action of Huperzine A on AChE *in vivo* was effective at smaller doses than previously reported and, moreover, it persisted after chronic treatment in all areas of the brain. Huperzine A also produced a transient inhibition of the high affinity transport of choline in the hippocampus.

### METHODS

#### Animals

Male Sprague-Dawley rats (Zivic Miller Laboratories, Allison Park, Pennsylvania) were used. At the time of the experiment, the rats weighed between 275 and 350 g. For the duration of the experiment, the rats were housed in groups of 2 on a 12-hr light-dark cycle. Food and water were available *ad libitum*.

#### Administration of Huperzine A

Huperzine A and the reference inhibitor of AChE physostigmine salicylate were solubilized in saline and injected intraperitoneally (i.p.). The chronic treatment consisted of 9 injections, over a period of 4 days (twice a day, hence). The 9th and last injection was administered 45 min prior to sacrifice.



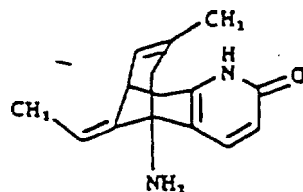


fig. 1. Molecular representation of Huperzine A.

### Dissection of tissue

After decapitation, the brain was quickly removed and the various regions of the brain studied were dissected from each hemisphere, on a chilled metallic plate, according to Glowinski and Iversen (1966).

### Activity of ChAT

Dissected areas of the brain were homogenized in 19 volumes of sodium phosphate buffer (75 mM, pH 7.4, 4°C) and the homogenate was frozen at -70°C, until subsequent analysis of enzyme. After thawing, homogenate (10 µl, 6 mg protein per ml) was added in duplicate to 10 µl of buffer-substrate mixture (McCaman and Hunt, 1965; Spyku, Goldberg and Sparber, 1972) comprising: sodium phosphate, 75 mM (pH 7.4); NaCl, 600 mM; MgCl<sub>2</sub>, 40 mM; physostigmine, 2.0 mM; bovine serum albumin, 0.05%; choline (Ch) iodide, 10 mM and [<sup>3</sup>H]acetyl-coenzyme A, 0.57 mM. After 30 min of incubation at 37°C, the tubes were placed on ice and 150 µl of 3-heptanone, containing 75 mg/ml sodium tetraphenylboron, were added to each tube to extract the ACh (Fonnum, 1969). After vortexing, the samples were centrifuged and a 100 µl aliquot of the top (organic) layer was assayed for radioactivity, using liquid scintillation spectrometry.

### Activity of acetylcholinesterase (AChE)

Dissected areas of the brain were homogenized in 19 volumes of sodium phosphate buffer (75 mM, pH 7.4, 4°C) and the homogenate was frozen at -70°C until subsequent analysis of enzyme. After thawing, the homogenate (10 µl, 6 mg protein per ml) was added in duplicate to 40 µl of buffer-substrate mixture, which contained: sodium phosphate (75 mM, pH 7.0, 4°C) and [<sup>3</sup>H]ACh iodide (10 mM). After 20 min of incubation at 37°C, the tubes were placed on ice and 150 µl of sodium tetraphenylboron/3-heptanone were added to each tube to separate ACh from the acetate (Fonnum, 1969). The samples were vortexed, centrifuged and placed at -70°C, until the bottom (aqueous) layer was frozen; the top (organic) layer was then removed by aspiration. Subsequently, the aqueous layer was thawed and a 25 µl aliquot was assayed for radioactivity, using liquid scintillation spectrometry.

### High affinity transport of choline

Dissected areas of the brain were homogenized in 19 volumes of sucrose (0.32 M, 4°C) and centrifuged

(1000 g, 10 min, 4°C). The supernatant was then recentrifuged (20,000 g, 20 min, 4°C) and the resultant pellet was resuspended in 19 volumes of sucrose (0.32 M, 4°C). Duplicate aliquots (50 µl) of the suspension were then added to 500 µl of buffer (pH 7.4) comprising: Ch, 1.0 µM; [<sup>3</sup>H]Ch, 0.28 µCi; NaCl, 125 mM; KCl, 9.6 mM; MgSO<sub>4</sub>, 4.2 mM; CaCl<sub>2</sub>, 2.4 mM; dextrose, 10.0 mM and Tris base, 40.0 mM. In Na<sup>+</sup>-free buffer, 252 mM sucrose was substituted for sodium. After 8 min of incubation at 30°C, 1 ml of buffer (4°C) were added to each sample and tissue was collected onto GF/F filters (Whatman), by vacuum filtration. After washing with 10 ml of cold buffer, the filters were placed in scintillation vials and were assayed for radioactivity by liquid scintillation spectrometry. The Na<sup>+</sup>-dependent high affinity transport of choline was defined as the amount of choline transported into tissue, in the presence of Na<sup>+</sup>, minus that accumulated in the absence of Na<sup>+</sup> (Yamamura and Snyder, 1973). Protein was assayed according to Lowry, Rosebrough, Farr and Randall (1951).

### Statistical analysis

Differences were compared by multiple analysis of variance and *post-hoc* analysis, using the SYSTAT Statistical System (Evanston, Illinois, U.S.A.).

## RESULTS

Figure 2 illustrates the effects of a single injection of small doses of Huperzine A on the activity of AChE in various regions of the brain. The data indicate that the inhibition of esterase was dose- and time-dependent in hippocampus, striatum and septum. At 45 min after the injection, the dose of 0.1 mg/kg (i.p.) induced a slight but non-significant reduction in specific activity of AChE. At 0.3 mg/kg (i.p.), the activity of AChE was more strongly reduced ( $P < 0.01$ ,  $x0.001$ ,  $< 0.005$  in hippocampus, striatum and septum, respectively). At these small doses,

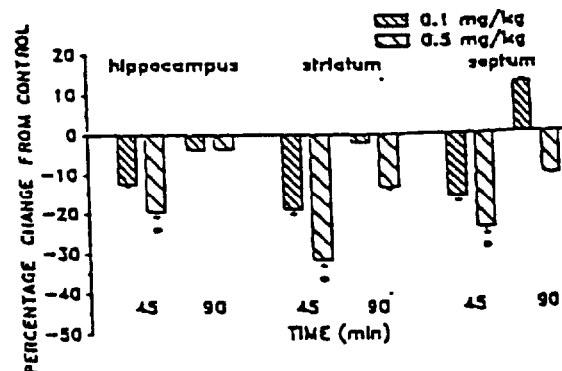


Fig. 2. Effect of acute injection of Huperzine A on activity of AChE in various regions of the brain. Values represent mean  $\pm$  SEM. Two-way ANOVA (repeated measures),  $P < 0.001$ . Multiple contrasts analysis for dose-effect at 45 min: 0.1 mg/kg,  $P = 0.52$ ; 0.3 mg/kg,  $*P < 0.01$ . At 90 min: non-significant (N.S.).  $N = 4-8$  rats/group.

the inhibition of AChE was mostly reversed by 90 min after the injection in all regions of the brain studied.

By comparison, a single injection of physostigmine (as the salicylate, 0.25 mg/kg i.p.) resulted in a more profound reduction in activity of AChE than has been seen with Huperzine A (Fig. 2), ranging from 30 to 50% in parietal cortex, septum, hippocampus and striatum at 15 min after the injection (results not shown). However, the activity of AChE had reverted to control levels by 30 min after injection of physostigmine.

The specificity of Huperzine A on the metabolism of ACh was assessed by determining, in parallel, the activity of ChAT in each sample. The ACh-forming enzymatic activity was not influenced *in vivo* in the hippocampus or in the striatum by Huperzine A (results not shown). The specificity of Huperzine A on this cholinergic parameter, ChAT, was further compared to that of physostigmine (0.3 mg/kg i.p., 15 min after the injection) in cortex, septum, striatum and hippocampus. Physostigmine had essentially no effect on the activity of ChAT *in vivo* (results not shown).

As shown in Fig. 5, a single injection of Huperzine A produced a transient inhibition of the high affinity transport of choline in hippocampal synaptosomes. The transport activity was significantly ( $P < 0.01$ ) reduced at 45 min. at the dose of 0.5 mg/kg (i.p.), whereas there was essentially no effect at 0.1 mg/kg (data not shown). By 90 min, the transport had returned to control values. High affinity transport of choline in the striatum was measured in parallel in the same animals at 45 and 90 min after the injection. The data in Fig. 3 show clearly that no inhibition of the uptake of choline took place at 0.5 mg/kg (i.p.), or at 0.1 mg/kg (i.p.) (data not shown).

The high affinity transport of choline was also assessed in various regions of the brain of rats injected with physostigmine (0.25 mg/kg i.p.). At

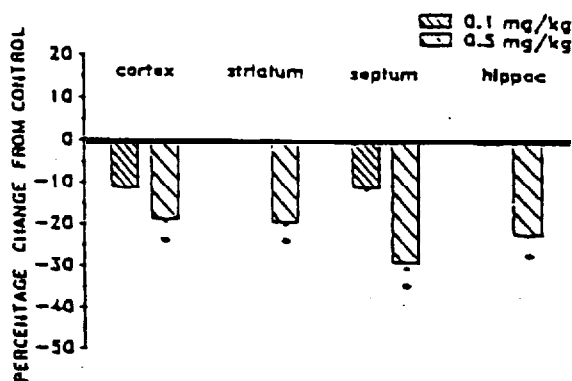


Fig. 4. Effect of 9 chronic injections of Huperzine A (4.3 days) on activity of AChE in various regions of the brain. Values represent mean  $\pm$  SEM. Two-way ANOVA (repeated measures),  $P < 0.001$ . Multiple contrasts analysis for dose-effect: at 0.1 mg/kg, N.S.; 0.5 mg/kg,  $*P < 0.01$ .  $N = 6$  rats/group.

15 min, the transport was reduced significantly in hippocampus and parietal cortex by 34% and 37%, respectively, but not in the striatum (results not shown). By 30 min after the injection, the inhibition persisted significantly in the cortex and hippocampus.

The data in Figs 4 and 5 relate to the chronic treatment (twice a day for 4.5 days) with Huperzine A on the same parameters which were studied acutely. As shown in Fig. 4, the reduction in activity of AChE in the various regions of the brain, at the dose of 0.1 mg/kg (i.p.), did not reach significance. However, at 0.5 mg/kg, the results showed that activity of AChE was significantly reduced by 20–30% in every region of the brain studied.

The high affinity transport of choline was similarly influenced by chronic treatment with Huperzine A, as is shown in Fig. 5. The slight reduction in transport of choline in the hippocampus was not significant at

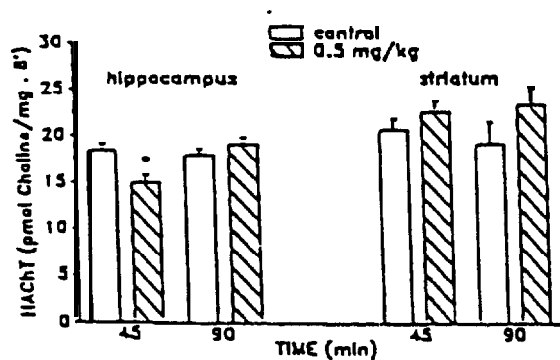


Fig. 3. Effect of acute injection of Huperzine A (0.5 mg/kg) on high affinity choline transport (HACHT) in hippocampus and striatum. Values represent mean  $\pm$  SEM. Two-way ANOVA (hippocampus),  $P < 0.001$ . Single contrast analysis for dose-effect: at 45 min,  $*P < 0.01$ ; at 90 min, non-significant. Striatum: no significant differences.  $N = 7$ –10 rats/group.

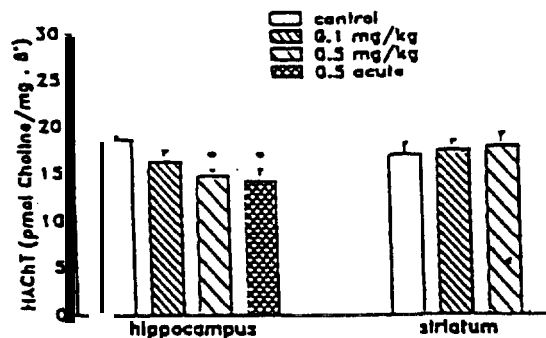


Fig. 5. Effect of 9 chronic injections of Huperzine A (4.5 days) on high affinity choline transport (HACHT) in hippocampus and striatum. Values represent mean  $\pm$  SEM. One-way ANOVA (hippocampus),  $P < 0.005$ . Single contrast analysis for dose-effect: at 0.1 mg/kg, N.S.; at 0.5 mg/kg,  $*P < 0.01$ . Striatum: no significant differences. The 0.3 mg/kg group (cross-hatch) was injected acutely and used as control.  $N = 5$ –7 rats/group.

0.1 mg/kg. However, at 0.5 mg/kg, high affinity transport of choline was reduced by 28% ( $P < 0.01$ ), 45 min after the last injection of Huperzine A approximately to the same extent as in the acute controls included in this experiment. The high affinity transport of choline in striatal synaptosomes (Fig. 5), was not influenced by chronic treatment with Huperzine A, at either dose. The activity of ChAT was not affected *in vivo* in the hippocampus, striatum, cortex and septum after chronic treatment with Huperzine A (0.1 or 0.5 mg/kg i.p.) (results not shown).

Huperzine A-induced inhibition of the high affinity transport of choline was further investigated *in vitro*. Hippocampal synaptosomes were incubated with Huperzine A, at concentrations ranging from  $10^{-7}$  M to  $10^{-4}$  M, for periods of 5, 15 and 45 min. No consistent effect of the inhibitor of cholinesterase on high affinity transport of choline could be detected *in vitro*.

#### DISCUSSION

From the present data it is clear that Huperzine A-induced inhibition of AChE activity was as potent after chronic as it was after acute treatment. These results indicate that minimal tolerance to the drug occurred. This is important, since it is well established that tolerance develops to many of the effects of physostigmine (Costa, Schwab and Murphy, 1982; Genovese, Elsmore and King, 1988). It has also been shown that the response to various inhibitors of AChE varies considerably after a second injection (360 min), especially in the case of THA (Hallak and Giacobini, 1989).

In their recent study with Huperzine A, Tang *et al.* (1989) used doses of 2 mg/kg (i.m.), with maximum inhibition of AChE occurring at 60 min and reported side effects, such as fasciculations. Inhibition of AChE was also studied at 30 min using smaller doses (ranging from 0.1 to 2 mg/kg i.p.) and maximum inhibition of AChE with minimal side effects occurred between 0.50 and 1 mg/kg (i.p.) (Tang *et al.*, 1989). In the present study, using two small doses of Huperzine A, administered intraperitoneally, at 45 min, it was observed that inhibition of AChE was not very effective at 0.1 mg/kg (i.p.). However, although inhibition of AChE attained 30–50% with physostigmine (0.25 mg/kg i.p.), as compared to 15–25% with Huperzine A (0.5 mg/kg i.p.) in various regions of the brain, it was observed that the duration of inhibition of AChE was longer than that with physostigmine. These results agree with previous findings (Tang *et al.*, 1989). Furthermore, at the small dose of 0.5 mg/kg (i.p.), no mortality or any side effects were observed, even after chronic treatment.

The action of Huperzine A on the activity of ChAT was also investigated *in vivo*. Acute or chronic treat-

ment with Huperzine A did not alter the activity of ChAT in any region of the brain studied. This finding complements the study of Hallak and Giacobini (1989), who reported no effect of various inhibitors of AChE *in vitro* (other than Huperzine A) on purified ChAT. Therefore, the reported *in vivo* increase in levels of ACh by Huperzine A (Tang *et al.*, 1989) was likely not to be mediated through an increase in the rate of synthesis of ACh.

In the same study, Tang and his coinvestigators showed that electrically-evoked release of ACh was not influenced by Huperzine A in slices of hippocampus. Neither was the release of ACh influenced by physostigmine, unless large concentrations were used (Hallak and Giacobini, 1989). Thus, it appears that release of ACh *in vivo* also may not be influenced by Huperzine A.

Another important effector of metabolism of ACh is the high affinity transport of choline (Tudek, 1985). According to the present studies, acute or chronic administration of Huperzine A was a potent inhibitor of high affinity transport of choline in the hippocampus *in vivo*. Physostigmine (Atweh, Simon and Kuhar, 1975; Sherman and Messamore, 1988) and THA (Sherman and Messamore, 1988) were also found to have a similar effect on transport of choline *in vivo*. However, in those studies, large doses of inhibitors of AChE, often accompanied by toxic effects, were used. Atweh *et al.* (1975) clearly showed that drugs affecting the turnover of ACh *in vivo* influenced the high affinity transport of choline, accordingly. For instance, physostigmine was shown to reduce turnover of ACh (Saelens, Simke, Schuman and Allen, 1974; Trabucchi, Cheney, Hanin and Costa, 1975) and muscarinic agonists, which increase turnover of ACh, increased high affinity transport of choline (Atweh *et al.*, 1975). The effect of inhibition of AChE on uptake of choline is believed to be mediated through a regulatory pre-synaptic control of high affinity transport of choline in response to the increase in content of ACh following inhibition of AChE (Yamamura and Snyder, 1973; Jope, 1979; Tamaru and Roberts, 1988; Breer and Knipper, 1990). The present results support this contention, since the effect of Huperzine A was completely reversible with time (Fig. 2) and not mediated through a direct interaction with the transporter (results not shown). Physostigmine also did not show any direct effect *in vitro* on synaptosomes in brain (Yamamura and Snyder, 1973), contrary to neostigmine (Yamamura and Snyder, 1973; Simon, Mittag, and Kuhar, 1975). These results indicate that inhibition of AChE may influence the high affinity transport of choline through a feedback-type regulation, rather than by operating directly on the transporter.

Hallak and Giacobini (1987) have hypothesized that *in vivo* treatment with an inhibitor of AChE "which would not decrease turnover of ACh, would maintain long-lasting levels of the neurotransmitter

in the brain". Such may indeed be the case with Huperzine A. Although the present results could be interpreted as an indication that Huperzine A operates in the CNS according to the same mechanisms as those postulated for physostigmine, only the specific determination of the turnover of ACh will resolve the issue.

Another finding of this study that remains to be addressed is why the high affinity transport of choline was not decreased in the striatum, despite a potent reduction in the activity of AChE by both Huperzine A and physostigmine in this region of the brain. The striatum contains the greatest concentration of ACh in the brain (Sethy, Roth, Kuhar and Van Woert, 1973). Nevertheless, inhibition of AChE may not be accompanied by a significant elevation of ACh in striatum (Tang *et al.*, 1989). De Sarno, Pomponi, Giacobini, Tang and Williams (1989) have also shown that, after injection of a long-lasting derivative of physostigmine, increases in levels of ACh showed marked regional differences. Moreover, it has been appreciated for some time that regional variations exist among the effects of drugs on the high affinity transport of choline (Jope, 1979) and that the striatum often differs from other areas of the brain in its cholinergic responses to pharmacological challenges (Wecker and Dettbarn, 1979; Sherman, Zigmond and Hanin, 1979).

In conclusion, it has been demonstrated that low-toxicity doses of Huperzine A could be used for several consecutive days and still exhibit full patency; hence, tolerance to Huperzine A, if it occurred, was minimal. Furthermore, the differences that have been shown in inhibition of AChE induced by Huperzine A and physostigmine are further indications that Huperzine A may be more effective and less toxic than physostigmine when a long term inhibition of AChE is required, e.g. in clinical treatment of diseases manifesting a cholinergic hypofunction.

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