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Anticholinesterase effects of huperzine A, E2020, and tacrine in rats

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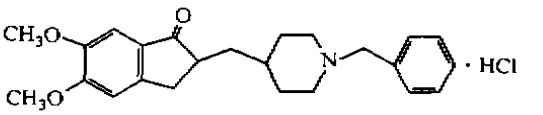
KEY WORDS cholinesterase inhibitors; cholinesterases; huperzine A; E2020; tacrine

AIM: To compare the anticholinesterase effects of huperzine A (Hup A), E2020, and tacrine in rats. **METHODS**: Spectrophotometry was used to determine AChE activity in brain and BuChE activity in serum. **RESULTS**: Following intragastric gavage, Hup A, E2020, and tacrine all produced dose-dependent inhibitions of brain AChE. Oral Hup A exhibited a higher inhibition than E2020 and tacrine. Tacrine was more effective in inhibiting serum BuChE correlated with severe peripheral adverse effects. The BuChE activity was less affected by Hup A and E2020. After a single oral dose of Hup A, a relatively steady state of AChE inhibition produced, which was longer than that after E2020 and tacrine. No change in the cholinesterase inhibition was seen for the 3 drugs following repeated ig medications. **CONCLUSION**: Hup A ig exhibited a higher efficacy, a longer duration of action, and a more selective inhibition on AChE than E2020 and tacrine.

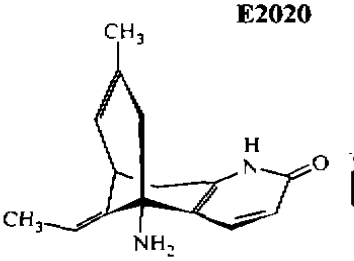
Since cholinergic hypofunction in hippocampus and cortex where age-related decline in acetylcholine (ACh) release and degenerative losses due to Alzheimer's disease (AD) has been found[1], the cholinesterase inhibitors (ChEI) have been the most promising approach[2]. An increase in the available ACh because of delayed hydrolysis can affect behavior through a cholinergic mechanism. Physostigmine, a classical ChEI, improved some memory in AD patients[3], and tacrine, approved by FDA in 1993, also produced an excellent improvement in several AD patients[4]. However, the short duration of action, the low bioavailability, and the frequent side effects of physostigmine limited its clinical value[5]; and tacrine caused dose-dependent hepatotoxicity[6]. Therefore, it is desirable to look for new ChEI that possess greater therapeutic window, longer duration of action, and significant fewer side effects.

Huperzine A (Hup A), an alkaloid extracted from Chinese herb *Huperzia serrata* (Thunb) Trev, is a potent and selective acetylcholinesterase (AChE) inhibitor. In controlled clinical trials, Hup A improved the memory of AD patients without significant side effects[7]. E2020, one of the piperidine derivatives, is a new ChEI under clinical investigation for AD in Japan and USA[8]. Both Hup A and E2020 show definite advantages as compared to the first generation of ChEI.

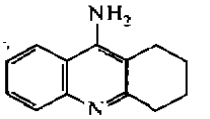
In the present study we compared Hup A, E2020, and tacrine to evaluate the clinical potentiality of the new generation of ChEI.



E2020



Huperzine A



Tacrine

MATERIALS AND METHODS

Materials Hup A (colorless powder, purity > 98 %) was prepared by Department of Phytochemistry in Shanghai Institute of Materia Medica. E2020 (colorless powder, purity > 98 %) was provided by Department of Synthetic Chemistry in this Institute. Tacrine was purchased from Sigma Chemical Co. Other chemicals were reagent grade.

Rats Sprague-Dawley rats ($n = 220$, weighing $250 \pm s$ 35 g) of either sex were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (clean, Certificate № 005).

Medication In acute experiment the rats were decapitated 30 min after ig or ip ChEI. In subacute experiment the rats were given ig, once daily (× 8 d), and killed 30 min after the 8th

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dose. The rats for assay of the duration of action were killed at various times following ig. Control rats were given saline.

Preparation of samples Rats were decapitated, the blood was collected, and the brains were dissected on ice into frontal cortex, hippocampus, corpus striatum, and one hemisphere. Each brain region was homogenized in 50, 50, 250, and 40 volumes of ice cold sodium phosphate buffer (75 mmol·L^{-1}, pH 7.0), respectively. For the determination of time course of butyrylcholinesterase (BuChE) activity after oral dose, the blood was taken from the orbital venous plexus. The serum was obtained after centrifugation (3500 × g, for 10 min).

ChE assay The brain homogenate was preincubated for 5 min with tetraisopropyl pyrophosphoramide (0.04 mmol·L^{-1}), a selective inhibitor of BuChE. For assay of AChE or BuChE activity, a reaction mixture of 4 mL containing acetylthiocholine iodide 0.3 mmol·L^{-1} or butyrylthiocholine iodide 0.4 mmol·L^{-1}, sodium phosphate buffer (0.1 mmol·L^{-1}, pH 7.4) 1 mL, and homogenate or serum 0.1 mL was incubated at 37 ℃ for 8 min. The reaction was terminated by adding 1 mL 3 % sodium lauryl sulfate, then 1 mL 0.2 % 5,5'-dithio-bis(2-nitrobenzoic acid) to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The rate of color production was measured spectrophotometrically at 440 nm[9]. All samples were assayed in duplicate. ChE activity was measured as absorbance (A) values/g protein. BuChE activity was determined only in ♪ rats.

Protein assay Protein concentration was measured with the Coomassie blue protein-binding method[10] using bovine serum albumin as standard.

Statistical analysis Data were expressed as % inhibition (vs saline control) ± s and compared with t-test.

RESULTS

Acute experiment Changes in ChE activity at 30 min in the 3 brain regions and serum were tested following ig with several doses of Hup A, E2020, and tacrine. There was a dose-dependent inhibition of ChE by all 3 ChEI. The most sensitive region was the cortex, since AChE inhibition ($P < 0.01$ vs control) was seen with Hup A 0.5 – 2.0 μmol·kg^{-1}, E2020 12 – 16 μmol·kg^{-1}, and tacrine 90 – 120 μmol·kg^{-1}. AChE activity was also inhibited in hippocampus and striatum. However, correlated to this, only E2020 and tacrine produced BuChE inhibition up to 30 % ($P < 0.05$, $P < 0.01$ vs control) in serum (Tab 1).

Compared with the results following ig, Hup A (1.0 μmol·kg^{-1}) injected ip exerted similar anticholinesterase effects in rats, while tacrine (60 μmol·kg^{-1}) resulted in a greater efficacy not only on AChE in 3 brain regions ($P < 0.01$ vs ig route), but also on BuChE in serum ($P < 0.05$ vs ig route), and

Tab 1. Anticholinesterase effects of huperzine A, E2020, and tacrine in rats*.
[b]$P < 0.05$, [c]$P < 0.01$ vs saline group.
[e]$P < 0.05$, [f]$P < 0.01$ vs acute experiment (ig).

ChEI/ mg·kg^{-1} (μmol·kg^{-1})	AChE inhibition/ % Cortex	Hippocampus ($n = 6$)	Striatum	BuChE inhibition/ % Serum ($n = 3$)
Acute experiment (ig)				
Huperzine A				
0.48 (2.0)	22 ± 7[c]	22 ± 8[c]	18 ± 8[c]	22 ± 4
0.36 (1.5)	20 ± 6[c]	17 ± 3[c]	18 ± 4[c]	18 ± 10
0.24 (1.0)	16 ± 6[c]	15 ± 3[c]	16 ± 8[c]	16 ± 14
0.12 (0.5)	10 ± 6[c]	8 ± 7	13 ± 10[b]	7 ± 12
E2020				
6.66 (16)	18 ± 6[c]	12 ± 5[c]	12 ± 8[b]	33 ± 7[c]
5.00 (12)	11 ± 6[c]	10 ± 4[c]	10 ± 6[b]	22 ± 11
3.33 (8)	9 ± 11	6 ± 8	8 ± 6	8 ± 10
Tacrine				
28.2 (120)	20 ± 6[c]	11 ± 10[b]	11 ± 10[b]	52 ± 5[c]
21.1 (90)	8 ± 6[c]	9 ± 6	8 ± 4[c]	40 ± 20[b]
14.1 (60)	7 ± 7	2 ± 2	2 ± 5	24 ± 17
Acute experiment (ip)				
Huperzine A				
0.24 (1)	18 ± 8	14 ± 9	14 ± 12	17 ± 14
E2020				
3.33 (8)	16 ± 8	14 ± 6[e]	13 ± 10	15 ± 12
Tacrine				
14.1 (60)	24 ± 6[f]	21 ± 5[f]	18 ± 8[f]	83 ± 15[f]
Subacute experiment (ig)				
Huperzine A				
0.24 (1)	19 ± 7	16 ± 2	16 ± 10	22 ± 8
E2020				
6.66 (16)	20 ± 10	12 ± 7	15 ± 12	22 ± 11
Tacrine				
21.1 (90)	5 ± 5	3 ± 3	8 ± 6	38 ± 6

*Values were expressed as % inhibition (vs saline control) ± s. Basal saline control values of cortex, hippocampus, striatum were 1 360 ± 70, 1 540 ± 150, 9 390 ± 880 A values/g protein, respectively ($n = 14$). Basal saline control value of serum was 23 ± 5 A values/g protein ($n = 7$).

E2020 (8 μmol·kg^{-1}) acted like tacrine, but change only showed in hippocampus ($P < 0.05$ vs ig route) (Tab 1). These data suggest that the efficacy of E2020 and tacrine with ig route are lower than that of Hup A.

Time course of ChE activity after ig medication Maximal AChE inhibition in whole brain reached at 60 min for Hup A (1.5 μmol·kg^{-1}), E2020 (16 μmol·kg^{-1}), and tacrine (120 μmol·kg^{-1}). Peak inhibition in cortex and serum were seen at 30 – 60 min for the 3 ChEI. Inhibition of AChE

activity in the cortex exceeding 10 % maintained between 15 and 240 min for Hup A, but from 15 to 180 min for E2020 and tacrine.

Peak inhibition of BuChE varied in magnitude for the 3 inhibitors, and the inhibition was greater for tacrine (65 %), moderate for E2020 (34 %) and Hup A (39 %). In case of Hup A, the BuChE activity recovered to control levels at 360 min, whereas, still 20 % and 46 % of inhibition existed for E2020 and tacrine, respectively (Fig 1).

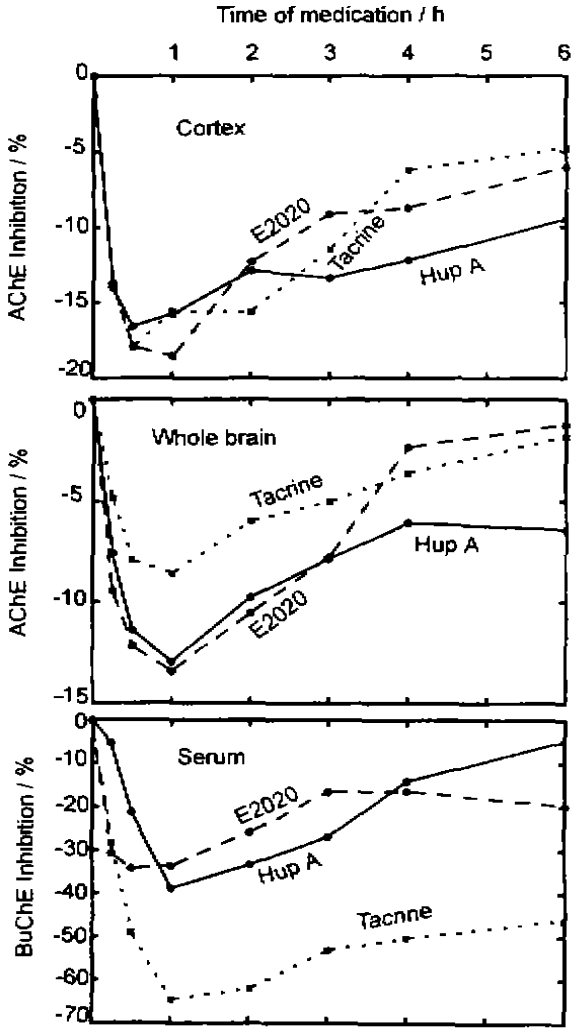

Fig 1. Time course of ChE inhibition following oral administration of Hup A (1.5 μmol·kg^{-1}), E2020 (16 μmol·kg^{-1}), and tacrine (120 μmol·kg^{-1}) in cortex, whole brain, and serum in rats. Values were expressed as % inhibition (*vs* saline control). *n* = 4 – 6. ChE was measured as *A* values/g protein. Basal saline control values of cortex and brain were 1 230 ± 60 and 1 840 ± 120 (*n* = 6). Basal saline control values of serum was 18 ± 3 (3 males).

Subacute experiment The subacute treatment (ig once daily for 8 d) with Hup A (1.0 μmol·kg^{-1}), E2020 (16 μmol·kg^{-1}), and tacrine (90 μmol·kg^{-1}) showed no significant difference as compared to that of acute treatment ($P > 0.05$ *vs* acute treatment) (Tab 1). These results indicated that no tolerance to drugs occured.

Side effects Side effects were most severe for tacrine and least severe for Hup A. The commonest side effect, seen after all 3 drugs, was fasciculations. With tacrine (120 μmol·kg^{-1}), side effects were clearly visible at 5 min following medication, reached maximum at 30 – 60 min, and lasted for at least 4 h. Other characteristic symptoms of cholinergic hyperactivity such as tremor, salivation, splay of hind limbs, and sedation also appeared. But all the rats survived. With E2020 (16 μmol·kg^{-1}), side effects were less frequent and less severe than with tacrine. But fasciculations or other cholinergic signs did not occur with Hup A (2 μmol·kg^{-1}) in rats. Subacute treatment with 3 ChEI induced less severe side effects than that of acute treatment.

DISCUSSION

The present data showed that Hup A, E2020, and tacrine all produced a dose-dependent inhibition of AChE activity following a single ig medication. Hup A exerted significant AChE inhibition in three brain regions, the relative inhibition potency of oral Hup A was found to be 11 and 80 times as potent as E2020 and tacrine based on the dosage of molecular weight. The inhibitory potency of AChE induced by Hup A was almost similar following ig and ip. However, E2020 and tacrine given ip showed much higher efficacy than those given ig. It had been reported that in the inhibition of AChE *in vitro*[11] or icv injection[12], the relative potency of E2020 was stronger than that of Hup A. The different potency induced by different routes of administration of Hup A, E2020, and tacrine was also found in the rat radial maze tasks, Hup A improved AF64A-induced working memory dificits at lower oral dose than that of E2020 and tacrine[12]. These results indicated that Hup A had higher efficacy with ig route and stronger potency to penetrate the blood brain barrier than E2020 and tacrine did.

Tacrine was more potent as an inhibitor of serum BuChE than that of brain AChE. The apparent inhibition constant (K_i value) for AChE is in the nmol $\cdot L^{-1}$ range *in vitro*[11], indicating that tacrine has high affinity for the enzyme. The higher dose of tacrine used ig might be explained by low bioavailability and/or rapid metabolism. The more peripheral adverse effects induced by tacrine may relate to its non-selective inhibition on ChE activity[13].

Our results indicated that tolerance didn't occur after consecutive treatment with ChEI even accompanied by the longer inhibition duration. This finding accorded with earlier results[14,15] and suggested that chronic administration of low doses of AChE inhibitors in AD might not attenuate the therapeutic efficacy.

The results obtained with Hup A suggested that the disadvantages of AChE inhibitors, such as short duration of action, low bioavailability and severe peripheral side effects, might be overcome by improving CNS selectivity. Therefore, Hup A fits more closely with the established criteria for an ideal cholinesterase inhibitor to be used in clinical studies.

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石杉碱甲，E2020 和他克林对大鼠胆碱酯酶的抑制作用

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关键词 胆碱酯酶抑制剂；胆碱酯酶类；石杉碱甲；E2020；他克林

目的：比较石杉碱甲、E2020 和他克林对大鼠胆碱酯酶的抑制作用. **方法**：比色法测定大鼠各脑区的 AChE 及血清中 BuChE 活力. **结果**：三药灌胃后对大鼠脑中 AChE 均产生剂量依赖性抑制. 以石杉碱甲作用最强. 他克林对 BuChE 的抑制显著强于 E2020 和石杉碱甲，其副反应最为明显. 单次经口给药后，石杉碱甲对脑内 AChE 的抑制作用长于 E2020 和他克林. 多次给药后，对胆碱酯酶的抑制作用三药均未有耐受性产生. **结论**：石杉碱甲是一种高选择性 AChE 抑制剂，具有口服活性高，作用时程长，副反应小的优点，适于临床应用.