ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Nov; 20 (11): 987-990
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Effects of ligustrazine, tanshinone ⅡA, ubiquinone, and idebenone on mouse water maze performance

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KEY WORDS maze learning; ligustrazine; tanshinone ⅡA; ubiquinone; idebenone; scopolamine; pyrazines; benzoquinones

ABSTRACT

AIM: To observe the effects of four drugs, ligustrazine (Lig), tanshinone ⅡA (Tan), ubiquinone (Ubi) and idebenone (Ide), on learning and memory of mouse. **METHODS**: Mouse water maze was used to evaluate nootropic effect. **RESULTS**: In comparison with the defective model (only scopolamine 3 $mg \cdot kg^{-1}$, Tan 20 $mg \cdot kg^{-1}$, ig) shortened the escape latency dramatically from (36 ± 19) s to (11 ± 5) s ($P < 0.01$) and reduced errors from 7 ± 5 to 1.5 ± 1.3 ($P < 0.05$). Ubi 20 $mg \cdot kg^{-1}$ ig decreased the escape latency from (37 ± 18) s to (17 ± 12) s and errors from 8 ± 5 to 2.1 ± 2.7 ($P < 0.01$). Ide 120 $mg \cdot kg^{-1}$ (ig) reduced the errors from 8 ± 6 to 3.4 ± 2.9 ($P < 0.05$), but had no remarkable effect on the escape latency. Lig did not exhibit marked effect on the deficit. **CONCLUSION**: Tan, Ubi, and Ide improved scopolamine-caused spatial performance defects in mouse.

INTRODUCTION

Alzheimer disease (AD) is a neurological disorder identified clinically by progressive memory loss and dementia. AD has been recognized as the most common form of adult-onset dementia and estimated to affect nearly 10 percent of the population over 65[1]. The main pathological changes of AD exist in the brain, including dystrophy of the cortex and hippocampus, etc, and primary degeneration of various kinds of neurons, especially cholinergic neurons. The cholinergic hypothesis of AD was proposed[2] and drugs that improve cholinergic functions, as well as those dilate brain blood vessels, have been proved potent and typical among the various strategies in AD therapy.

Ligustrazine (Lig), extracted from Chinese herb *Ligusticum* chuanxiong, and tanshinone ⅡA (Tan), one of the main hydrophobic components of *Salvia miltiorrhiza*, yielded anticoagulation and thrombolysis. Lig dilated blood vessels and thus increased blood flow in dog brains[3]. Tan was utilized to treat coronary heart disease in clinic. As an important component of the mitochondrial respiratory chain, ubiquinone (Ubi) has been widely applied in cardiovascular diseases. Its analogue idebenone (Ide) also activated motichondrial function of brain cells and therefore, improve brain metabolisms and psychiatric conditions in dementia[4,5]. It is necessary to focus on the behavioral pharmacology of these drugs, which might provide valuable hints for the development of promising therapeutics for AD.

Water maze is a simple and practical method frequently performed to study impact of nootropics on learning and memory[6]. In this study, we employed a modification of this model system[7] to observe the effects of the above four drugs on mouse performance.

MATERIALS AND METHODS

Mice Kunming strain mice of either sex, weighed (20.0 ± *s* 2.0) g, were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate No 005).

Materials Tan was prepared and kindly gifted by Prof MIN Zhi-Da, Department of Phytochemistry,

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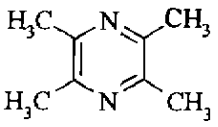
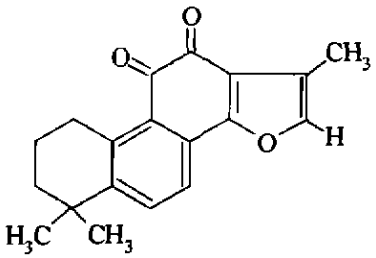
Received 1998-12-02 Accepted 1999-06-10

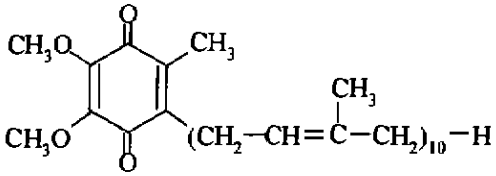
ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Nov; 20 (11)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629



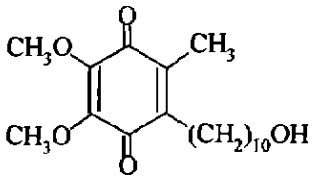
Ligustrazine



Tanshinone ⅡA



Ubiquinone



Idebenone

China Pharmaceutical University. Ligustrazine phosphate (Lig) was purchased from Limin Pharmaceuticals, Guangzhou, China; Ubi from Taizhou Biochemical Pharmaceuticals, Taizhou, China; and Ide was purchased from Takara Corporation, Japan. Other chemicals were of reagent grade.

Behavioral test Mice were kept in a 12-h light/dark cycle and permitted to eat and drink freely. Following the period of 2-d habituation, each mouse received 3 training sessions daily for successive 7 d. A plastic water maze (80 cm × 50 cm × 20 cm) was utilized to assay mouse performance. The depth of water was 10 cm and temperature (23.0 ± 0.5) ℃. The mouse was placed in the water maze, with its nose towards the wall of starting point, and trained to find the platform. Mice were trained to a criterion of reaching the platform within 20 min and <2 errors of entering non-exits. Once a mouse reached this criterion, training was reduced to one session daily until all mice were qualified. Then trained mice were randomly assigned to groups. All pharmaceuticals were suspended in 0.5 % sodium carboxymethylcellulose (CMC-Na). Different doses of drugs were administered orally 1 h and scopolamine (3 mg·kg^{-1}) was injected intraperitoneally 30 min before behavioral test. The time of reaching the platform and the number of errors were recorded.

Statistical analysis Data of behavioral test were expressed as $x \pm s$ and with t-test. ANOVA followed by Duncan's multiple-range test was used for comparison between the groups.

RESULTS

Mice injected with scopolamine (3 mg·kg^{-1}, ip) prolonged the latency of escaping to the platform and showed increased errors of entering the non-exits. Lig had no remarkable effect on the defect (Fig 1).

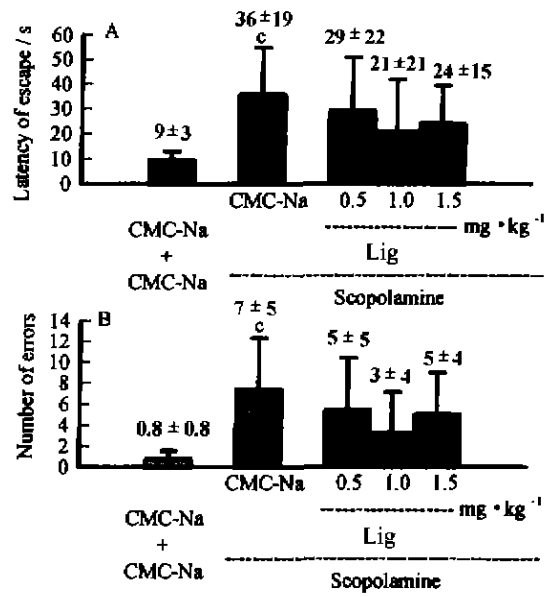


Fig 1. Effects of Lig on scopolamine-induced spatial performance deficits in mouse water maze. A) Latency of finding the platform; B) Number of entering non-exits. $n = 10$ mice, $\bar{x} \pm s$. $^c P < 0.01$ *vs* CMC-Na + CMC-Na ($n = 14$ mice). Lig had no remarkable effect on the defect (CMC-Na + scopolamine, $n = 18$ mice).

Tan, Ubi, and Ide attenuated the scopolamine-induced deficits to different degrees. Tan (20 mg·kg^{-1}, ig) markedly shortened the escape latency ($P < 0.01$) and reduced the errors ($P < 0.05$, Fig 2).

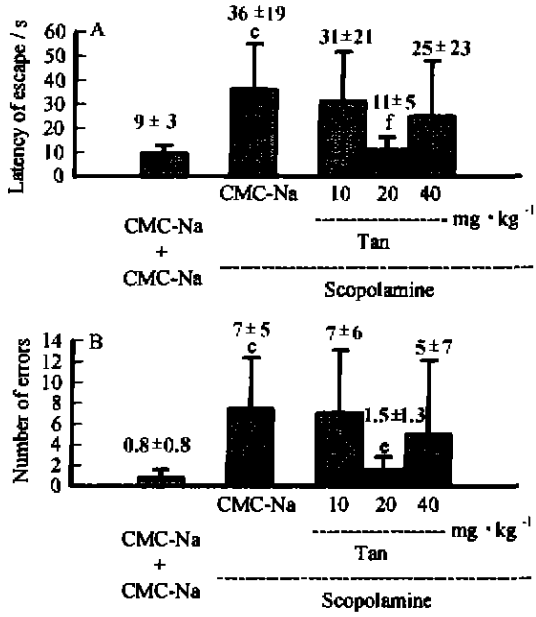


Fig 2. Effects of Tan on scopolamine-induced spatial performance deficits in mouse water maze. A) Latency of finding the platform; B) Number of entering non-exits. $n = 8$ mice. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* CMC-Na + CMC-Na ($n = 14$ mice). $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* CMC-Na + scopolamine ($n = 18$ mice).

Ubi (20 mg·kg^{-1}, ig) resulted in very remarkable reduction of both the escape latency and errors ($P < 0.01$, Fig 3).

Ide (120 mg·kg^{-1}, ig) prominently decreased the errors, but not the escape latency ($P < 0.05$, Fig 4).

DISCUSSION

While Lig has been reported to enhance cognitive ability of rats[8], our data did not support significant enhancement in mice. This discrepancy might be ascribable to difference in animals or testing models.

Our results showed that Tan, Ubi, and Ide improve scopolamine-induced spatial learning defects in mouse water maze to different extents. It is worthwhile to find whether the three drugs promote learning and memory of mouse through the common cholinergic pathway. Particularly, their influences on

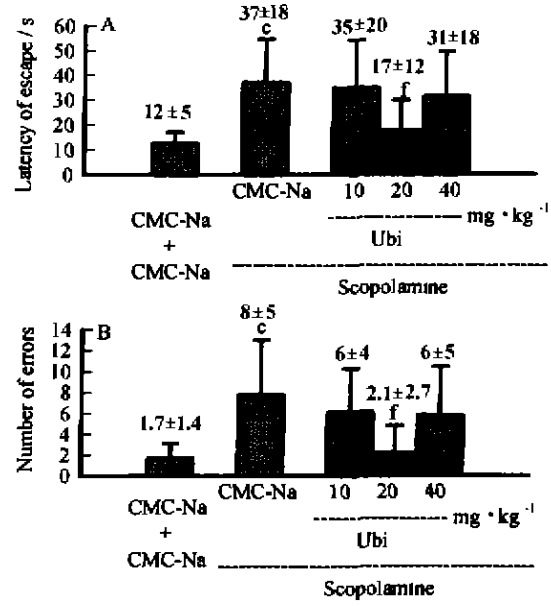


Fig 3. Effects of Ubi on scopolamine-induced spatial performance deficits in mouse water maze. A) Latency of finding the platform; B) Number of entering non-exits. $n = 10$ mice. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* CMC-Na + CMC-Na ($n = 10$ mice). $^{f}P < 0.01$ *vs* CMC-Na + scopolamine ($n = 16$ mice).

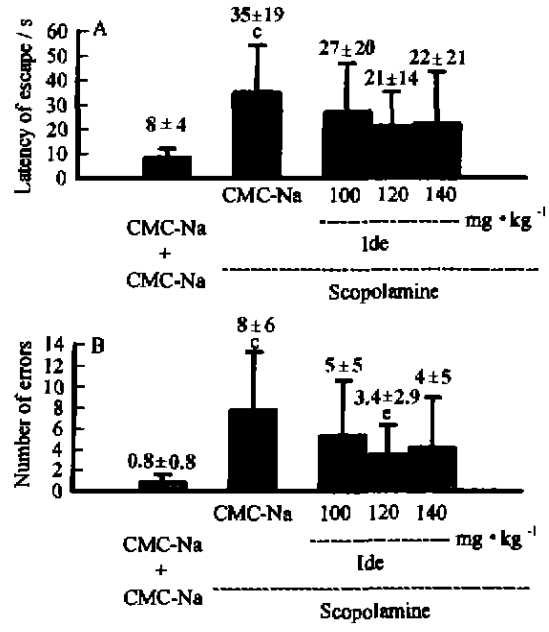


Fig 4. Effects of Idebenone on scopolamine-induced spatial performance deficits in mouse water maze. A) Latency of finding the platform; B) Number of entering non-exits. $n = 10$ mice. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* CMC-Na + CMC-Na ($n = 10$ mice). $^{e}P < 0.05$ *vs* CMC-Na + scopolamine ($n = 16$ mice).

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E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

activities of the key enzymes in this pathway, cholinesterase (ChE) and choline acetyltrans-ferase (ChAT), remain to be investigated.

Nitta A *et al*[9] reported that Ide (20 mg·kg^{-1} administered successively for 21 d) elevated the gene expression of hippocampus NGF in rats with basal forebrain lesions, in addition to the improvement in leaning and memory and the increase in ChAT activity. Preliminary result of our research suggested that Ubi (10 mg·kg^{-1} bid for 15 d) enhanced NGF mRNA level in mouse hippocampus (unpublished data). Further efforts should be made to verify the effects of Ubi and Ide on NGF gene expression *in vivo*. Studies in this aspect is of great importance not only for illuminating the mechanisms of certain nootropics, but also for developing potential anti-AD drugs. Behavioral pharmacological methods, such as water-maze procedure, can be employed as powerful tools in primary screening of active AD therapeutics, especially from traditional Chinese medicines.

ACKNOWLEDGMENTS To Prof TANG Xi-Can, Ms WANG Hong, and Ms LIU Jin (Shanghai Institute of Materia Medica, Chinese Academy of Sciences) for their valuable advice and help.

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川芎嗪、丹参酮ⅡA、泛醌和艾地苯醌对小鼠水迷宫操作的影响

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关键词 迷宫学习；川芎嗪；丹参酮ⅡA；泛醌；艾地苯醌；东莨菪碱；吡嗪类；苯醌类

目的：观察川芎嗪、丹参酮ⅡA、泛醌和艾地苯醌四个药物对小鼠学习记忆的影响. **方法**：采用小鼠水迷宫评价促智作用. **结果**：与东莨菪碱模型组相比，丹参酮ⅡA (20 mg·kg^{-1}、ig)到达平台的时间从(36±19) s 缩短至(11±5) s，进入死角错误从(7±5)次减少至(1.5±1.3)次；泛醌(20 mg·kg^{-1}，ig) 也能缩短时间从(37±18) s 至(17±12) s 和减少错误次数从 8±5 至 2.1±2.7；艾地苯醌(120 mg·kg^{-1}，ig) 只能显著缩短时间从(8±6) s 至(3.4±2.9) s. 川芎嗪不能缩短时间和减少错误. **结论**：丹参酮ⅡA、泛醌和艾地苯醌能不同程度地改善小鼠因东莨菪碱所致的记忆损害.

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