

Original Articles

Effect of Do-In (*Prunus persica* L. BATSCH) Water Extract (PPE) on Concentration of Extracellular Acetylcholine in the Rat Hippocampus

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Objective : This study was designed to examine the effects of orally administered *Prunus persica* water extract (PPE), which is used as herbal medicine, for treatment of Yu Xue (stasis of blood) and tacrine on the basal concentration of extracellular acetylcholine in the hippocampus of rats.

Methods : To investigate the effects of PPE and tacrine on concentration of extracellular acetylcholine in the hippocampus of rats, the microdialysis technique, under the same experimental conditions, was used. And we used male Wistar rats which were 7 weeks of age and 210-290 g. PPE was extracted with boiling water, and the rats were anesthetized with pentobarbital Na. Their skulls were exposed and a hole was drilled for implantation of a microdialysis probe. In order to increase the recovery of acetylcholine, a probe with a long membrane was used. One day after surgery, the microdialysis probe was perfused with Ringer's solution at a flow rate of 1.5 l/min. The acetylcholine concentration in dialysis samples was measured by high-performance liquid chromatography (HPLC) with electrochemical detection. AChE activity was measured using the radiometric method, as described by Sherman.

Results : The comparative effects of PPE and tacrine on hippocampal extracellular acetylcholine concentration was that these cholinesterase inhibitors produced dose-dependent increases in the extracellular acetylcholine concentration. And the effect of PPE and tacrine on rat brain AChE activity was that PPE produced maximal inhibition at 1 h after administration, when AChE activity was 44% of the intact level. AChE activity gradually recovered thereafter, and reached 78% of the intact level at 12 h after administration.

Conclusion : In this study, PPE has a potent activity and a long-lasting effect on the central cholinergic system, in terms of the basal concentration of extracellular acetylcholine in the hippocampus and the AChE activity in the brain of rats. And oral administration of PPE increased dose-dependently the basal concentration of extracellular acetylcholine in the hippocampus of rats. PPE may be one of the more useful cholinesterase inhibitors for the treatment of Alzheimer's disease.

Key Words: Do-In (*Prunus persica* L. BATSCH); Acetylcholinesterase inhibitor; Acetylcholine; Microdialysis; Tacrine

Introduction

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Semen Persicae (*Prunus persica* L. BATSCH) water extract (PPE) has long been used as an agent of degenerative disorders in China, Japan and Korea. Extract of the seed of *Prunus persica* has been used in

the treatment of the degenerative disorders such as hypermenorrhea, dysmenorrhea, leiomyoma and infertility^{1,2)}.

Pharmacological studies in vitro have revealed that PPE is comprised of constituents having a reversible and non-competitive cholinesterase inhibitory activities such as an inhibitor of acetylcholinesterase (AChE), relative to butyrylcholinesterase. It produces marked and long-lasting inhibition of brain AChE and increases the brain content of acetylcholine in vivo. Tacrine, which compared with PPE, is the first drug approved by the U.S. FDA for the treatment of Alzheimer's disease, although it has adverse effects related to its actions on the peripheral nervous system and to hepatic toxicity^{3,4)}.

Alzheimer's disease is a progressive neurodegenerative disease characterized by deficits in memory and cognitive function. One of the most pronounced changes in the brains of Alzheimer's disease patients occurs in the cholinergic systems. Cholinesterase inhibitors are the only class of drugs currently approved for the treatment of Alzheimer's disease.

Since the extracellular acetylcholine concentration measured by the intracerebral microdialysis technique reflects the acetylcholine concentration in the synaptic cleft, it is a useful parameter with which to compare the potential efficacy of various cholinesterase inhibitors in the central cholinergic system. The effects of PPE and tacrine on extracellular acetylcholine concentration have been studied by microdialysis.

In the present study, we have examined the effects of orally administered PPE and tacrine on the basal concentration of extracellular acetylcholine in the hippocampus of rats under the same experimental conditions and they were compared. Moreover, in order to validate the microdialysis data, we measured the inhibition of brain AChE and the brain concentrations of these drugs.

Materials and methods

1. Animals

Male Wistar rats (7 weeks of age, 210-290 g) were housed at a room temperature of $23 \pm 1^\circ\text{C}$ and relative humidity of $55 \pm 10\%$, under a 12-h light/dark cycle (start at 07:00 h) for at least 1 week before the experiments. The animals were given free access to food and water. All experiments were approved by the Animal Care and Use Committee of Dongguk.

PPE extract preparation from *Prunus persica* L. BATSCH

Prunus persica L. BATSCH water extract (300 g) was obtained from the Oriental Medical Hospital, Dongguk University College of Oriental Medicine, and was extracted with boiling water for 3 hr. Then, the extract was evaporated to under reduced pressure by 75%, 85%, 95% ethanol solutions. The last extracts were diluted by 0.9% NaCl and filtered. The extract solution was stored at 4°C .

Tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride) by Sigma (St. Louis, MO, USA). All other chemicals were commercial products of reagent grade. PPE and tacrine were dissolved in distilled water and administered by gavage.

2. Implantation of microdialysis probe

Rats were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and placed in a stereotaxic frame (Narishige, Tokyo, Japan). The skull was exposed and a hole was drilled for implantation of a microdialysis probe. In order to increase the recovery of acetylcholine, a probe with a long membrane (5.0 mm membrane length, 0.5 mm diameter; I-4-05 Eicom, Kyoto, Japan) was used. The probe was implanted into the right lateral hippocampus at 4.7 mm lateral and 5.3 mm posterior to the bregma and to a depth of 8.5 mm from the brain

surface according to the atlas of Paxinos and Watson⁹. The probe was fixed with dental cement. After surgery, the rats were housed in their home cage. Placement of the probe within the hippocampus was confirmed by visual inspection of the probe track at the end of the experiment.

3. Microdialysis

One day after surgery, the microdialysis probe was perfused with Ringer's solution (147 mM NaCl, 4.7 mM KCl, 0.6 mM MgSO₄, 2.5 mM CaCl₂, 5.0 mM HEPES) at a flow rate of 1.5 l/min. The perfusate was discarded during the first 1 h of perfusion and then collected at 20-min intervals into microtubes containing 5 ul of 0.1 M phosphate buffer (pH 3.5). Tacrine was orally administered after three fractions had been collected. The perfusate was collected until 6 h after administration.

4. Acetylcholine assay

Acetylcholine concentration in dialysis samples was measured by high-performance liquid chromatography (HPLC) with electrochemical detection⁹. As an internal standard, 500 fmol of isopropylhomocholine was added to the sampling tubes, and the mixture was subjected to HPLC. The HPLC system (DSA-300, Eicom) consisted of a degasser, an HPLC pump system, an autosampler, a column temperature controller, and an electrochemical detector with a platinum electrode (5 mm Φ). A guard column and an enzyme reactor (3.0 mm Φ \times 4.0 mm; AC-Enzymapak, Eicom) were placed before and after the analytical column (2.0 mm Φ \times 160 mm; Shimpak AC-Gel, Shimadzu CO., Tokyo, Japan), respectively. Following its separation from the analytical column, acetylcholine was converted to hydrogen peroxide inside the enzyme reactor. The analytical and enzyme columns and the electrode were kept at 30°C in a column temperature controller. The mobile phase was

0.1 M phosphate buffer (pH 8.3) containing 200 mg/l sodium 1-decanesulfonate, 65 mg/l tetramethylammonium chloride and 50 mg/l EDTA \cdot 2Na. The flow rate of the mobile phase was 0.15 ml/min. Peaks were recorded on a Powerchrom integrator (Eicom). Acetylcholine in the dialysate sample was quantified by the internal standard method. The data are expressed as percentages of the pre-level (average of three samples prior to administration).

5. Measurement of AChE activity

Rats were decapitated at 0.5, 1, 2, 4, 8 and 12 h after receiving PPE (2.5 g/kg) and tacrine (10 mg/kg) by oral administration. Control animals received no treatment. The whole brain, excluding cerebellum and olfactory bulb, was removed and the two hemispheres were split for assays of AChE inhibition and brain concentrations of the drugs.

AChE activity was measured using the radiometric method, as described by Sherman (1991)^{6,7}. [³H]acetylcholine iodide (Amersham Korea, Seoul, Korea) was used as a substrate. In order to minimize the dilution effect which is seen in ex vivo assays of the inhibition by reversible cholinesterase inhibitors, the brain hemisphere was homogenized in four volumes of buffer, and finally 10 mg of brain tissue was diluted in 100 ul of assay solution. Data are presented as percentages of the intact level.

6. Statistical analysis

The data are expressed as means \pm S.E.M. Microdialysis data were analyzed using a repeated measures analysis of variance with dose and fraction as factors. If a significant dose \times fraction interaction existed, post-hoc analysis using Dunnett's multiple comparison test was applied between doses for individual fractions. Cholinesterase inhibition data were analyzed with Dunnett's multiple comparison test. A P

value of less than 0.05 was considered significant. Statistical analysis was conducted using the software package SAS ver. 6.12 (SAS Institute Japan, Tokyo, Japan), available on the statistical analysis support system.

Results

Comparative effects of PPE and tacrine on hippocampal extracellular acetylcholine concentration

We examined the effects of PPE and tacrine on the extracellular acetylcholine concentration in the hippocampus of rats. These cholinesterase inhibitors produced dose-dependent increases in the extracellular acetylcholine concentration (Fig. 1, Fig. 2). PPE had a significant effect at a dose of 2.5 g/kg. The maximum increase produced by this dose was seen at about 1.5 h, when acetylcholine reached 465% of the pre-treatment level. The duration of the acetylcholine increase

produced by the drug at this dose was more than 6 h (Fig. 1). The increase in the extracellular acetylcholine concentration produced by tacrine at 5 mg/kg was maximum at 2 h, when the level was 418% of the pre-treatment level. The acetylcholine-increasing action at this dose continued for more than 6 h (Fig. 2).

Effects of PPE and tacrine on rat brain AChE activity

Fig. 3 shows the effects of PPE(2.5 g/kg) and tacrine (10 mg/kg) on rat brain AChE activity *ex vivo*. PPE produced maximal inhibition at 1 h after administration, when AChE activity was 44% of the intact level. AChE activity gradually recovered thereafter, and reached 78% of the intact level at 12 h after administration. The maximal inhibition of AChE by tacrine was observed at 1-2 h after administration, although AChE inhibition remained at a plateau for 0.5-8 h after administration (0.5 h, 41%; 1 h, 40%; 2 h, 41%; 4 h, 42%). The AChE activity was 73% of the intact level at 12 h after administration. Acetylcholine elevation in the

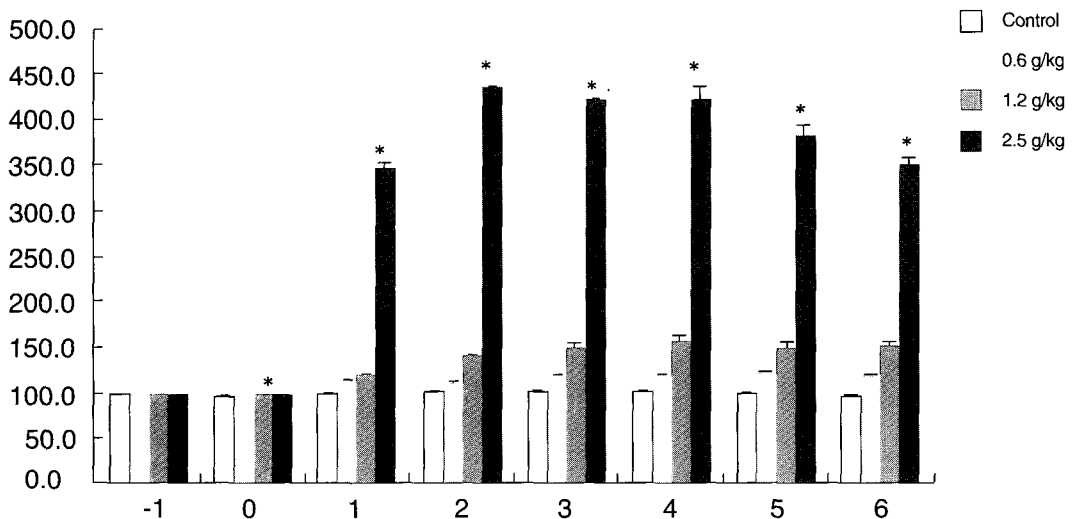


Fig. 1. Effect of oral administration of PPE on the basal concentration of extracellular acetylcholine in the hippocampus of rats.

Data are expressed as percentages of the pre-levels (average of three samples prior to administration=100%). Values are means \pm S.E.M. $n=6$. Pre-levels were: control: 102.6 ± 19.1 ; 0.625 g/kg: 75.4 ± 6.3 ; 1.25 g/kg: 88.2 ± 17.1 ; 2.5 g/kg: 73.2 ± 6.2 fmol/tube. * $p < 0.05$ vs. control (Dunnett's multiple comparison test).

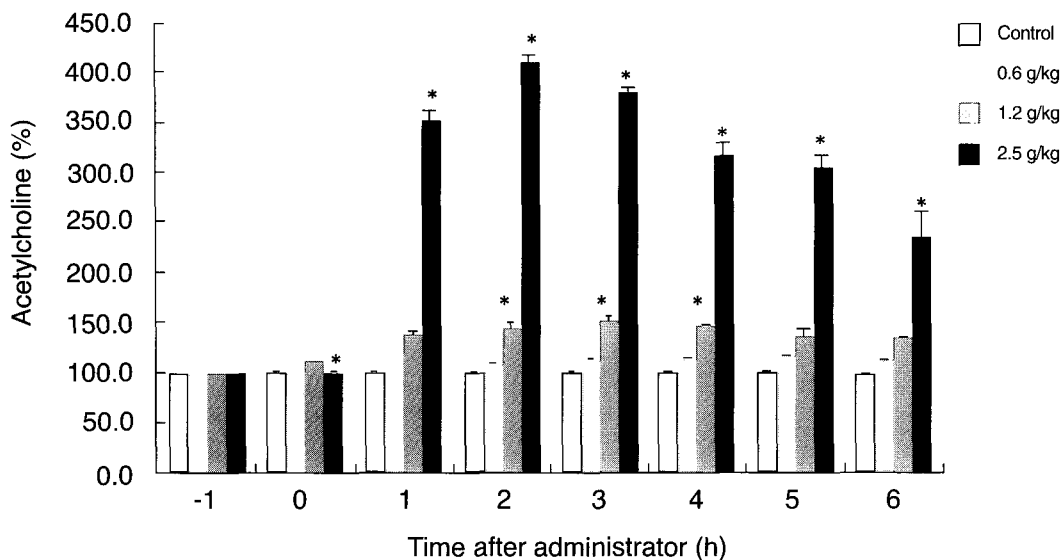


Fig. 2. Effect of oral administration of tacrine on the basal concentration of extracellular acetylcholine in the hippocampus of rats.

Data are expressed as percentages of the pre-levels (average of three samples prior to administration=100%). Values are means \pm S.E.M. n=6. Pre-levels were: control: 101.5 ± 15.4 ; 1.25 mg/kg: 90.2 ± 9.5 ; 2.5 mg/kg: 81.3 ± 7.5 ; 5 mg/kg: 74.2 ± 12.1 fmol/tube. * $p < 0.05$ vs. control (Dunnett's multiple comparison test).

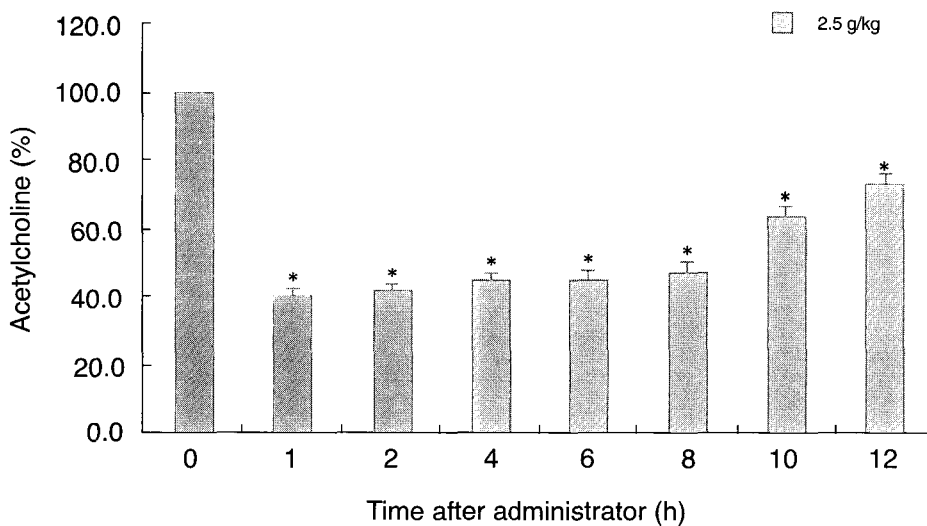


Fig. 3-A. Comparison of the hippocampal extracellular brain AChE inhibition induced by PPE.

Extracellular acetylcholine data are expressed as percentages of the pre-level (average of three samples prior to administration=100%). Values are means \pm S.E.M. n=6. AChE activity is expressed as a percentage of the intact level shown at 0 h (100%). Values are means \pm S.E.M. n=5. * $p < 0.05$ vs. intact (Dunnett's multiple comparison test).

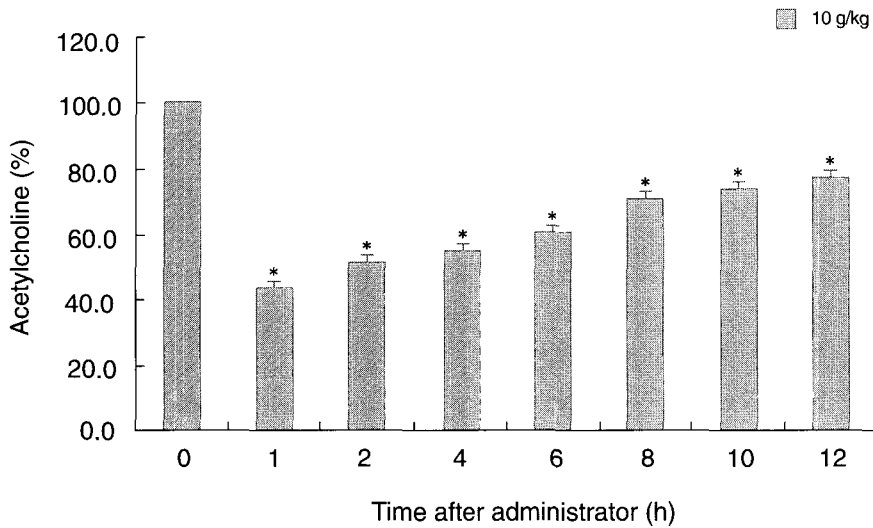


Fig. 3-B. Comparison of the hippocampal extracellular brain AChE inhibition induced by Tacrine.

Extracellular acetylcholine data are expressed as percentages of the pre-level (average of three samples prior to administration=100%). Values are means \pm S.E.M. n=6. AChE activity is expressed as a percentage of the intact level shown at 0 h (100%). Values are means \pm S.E.M. n=5. * p <0.05 vs. intact (Dunnett's multiple comparison test).

hippocampus was a mirror image of AChE inhibition.

Discussion

The results in this study clearly demonstrate that PPE has a potent activity and a long-lasting effect on the central cholinergic system, in terms of the basal concentration of extracellular acetylcholine in the hippocampus and the AChE activity in the brain of rats. Oral administration of PPE dose-dependently increased the basal concentration of extracellular acetylcholine in the hippocampus of rats. PPE caused a marked and dose-dependent increase in extracellular acetylcholine concentration. Our data show that extracellular acetylcholine is increased by PPE even when the oral administration route is used. PPE at a dose of 2.5 g/kg was somewhat more potent than tacrine at a dose of 5 mg/kg. Kawashima et al. (1994)[8] also examined the

effect of tacrine (1.65 and 5 mg/kg, i.p.) under the same experimental conditions.

The acetylcholine-increasing action in the rat hippocampus produced by PPE and tacrine was well-correlated to the ex vivo AChE inhibition in the rat brain at the same dose of each drug. Both the acetylcholine-increasing action and the AChE inhibition of PPE at 2.5 g/kg were somewhat less potent than those of tacrine at 10 mg/kg. Moreover, PPE and tacrine showed long-lasting effects on both the extracellular acetylcholine concentration and the brain AChE activity. These results are consistent with the idea that the effect of these compounds on the extracellular acetylcholine concentration is based on the inhibition of AChE in the brain.

Conclusion

The findings of this study demonstrated that centrally acting cholinesterase inhibitors, PPE and tacrine, potently increase the extracellular acetylcholine concentration in the synaptic cleft of the hippocampus of rats mostly through AChE inhibition, and that PPE has a potent activity and a long-lasting effect on the central cholinergic system. PPE may be one of the more useful cholinesterase inhibitors for the treatment of Alzheimer's disease.

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