Propantheline Attenuates the Peripheral Side Effects of Donepezil without Affecting Its Antiamnestic Properties in Cerebral Ischemic Mice

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Cholinergic deficits are found in both vascular dementia and Alzheimer's disease (AD). Cholinesterase inhibition (CHI) is the only strategy that has been proven to have beneficial effects in patients, but may cause a broad spectrum of adverse events such as nausea, vomiting, and diarrhea, *etc*. To investigate how to attenuate the peripheral side effects of CHI agents without affecting their efficacy, the effects of propantheline bromide (PB) co-administered with donepezil on the gastric emptying (GE), gastrointestinal transit (GIT), brain cholinesterase (ChE) activities, and maze tasks of mice with memory impairment induced by transient ischemia were observed. The results indicated that PB decreased the increase in GE and GIT, but did not change brain acetylcholinesterase (AChE) activity or the latency of escape in cerebral ischemic mice treated with donepezil. These findings suggest that PB is nearly unable to penetrate the blood-brain barrier and could attenuate the peripheral side effects of CHI agents in the peripheral nervous system and without affecting their therapeutic effects in the central nervous system.

Key words —— donepezil, propantheline, side effects, cerebral ischemia, memory impairment

INTRODUCTION

With the increasing number of elderly in the world population, dementia, characterized by progressive loss of memory and higher cortical functions, has given rise to an enormous socioeconomic burden. Dementia is now the fourth leading cause of death after cardiovascular diseases, cancer, and cerebrovascular disease. Alzheimer's disease (AD) and vascular dementia (VD) are the two main causes of dementia. The incidence of VD, resulting from ischemic injury or sustained oligemia to brain regions associated with cognitive function, memory, and behavior, is the second most common form of dementia in the elderly after AD.[1–3] In keeping with the current predictions of increasing incidence of stroke and heart disease, VD could become the most common cause of senile dementia.

Cholinergic neurons are the predominant class which degenerates in the early stages of AD, leading to a significant decrease in acetylcholine (ACh) levels. A number of studies suggest that patients with VD may also exhibit cholinergic deficits. Observations from both postmortem examinations and clinical studies in VD patients suggest that cholinergic changes are associated with VD.[4–7] Therefore the current first-line therapeutic approach is to inhibit acetylcholinesterase (AChE) to maximize the potential of the released neurotransmitters.[8] Cholinergic deficits in VD are due to ischemia of basal forebrain nuclei and of cholinergic pathways and can be treated with cholinesterase inhibitors. Controlled clinical trials with cholinesterase inhibitors, such as donepezil, galantamine, and rivastigmine, in VD, as well as in patients with AD plus VD, have demonstrated improvements in cognition, behavior and activities of daily living.[9–11]

However, cholinesterase inhibitors may cause a broad spectrum of adverse events such as nausea, vomiting, and diarrhea, *etc*.[9–14] These adverse events, which make many patients stop taking cholinesterase inhibition (CHI) agents, are generally recognized as due to parasympathetic nervous system activity, while cholinesterase inhibitors ameliorate dementia by inhibiting AChE in central nervous system (CNS). Anticholinergics have been used to treat urinary incontinence in patients tak-

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ing cholinesterase inhibitors for dementia.[15] In this paper, we investigated whether propantheline bromide (PB), a muscarinic receptor blocker that is nearly unable to penetrate the blood-brain barrier, co-administered with donepezil, a CHI agent used in the treatment of dementia, could attenuate the peripheral side effects of donepezil without affecting its therapeutic effects.

MATERIALS AND METHODS

Animals —— Swiss albino mice were provided by the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs, and the certificate number was 200106003. The animals were maintained at a constant temperature of 24 ± 2°C and were fed a standard laboratory diet for 1 week. The animals were supplied with food and water *ad libitum*, housed in groups of 5 per cage on a 12-hr light/dark cycle (lights on 08:00–20:00 hr). To avoid the effects of circadian rhythm, each experiment was carried out at the same time of day. The experiments were performed in accordance with the Guideline for Care and Use of Experimental Animals and approved by the Experimental Animal Research Committee of Yantai University.

Chemicals —— Donepezil was purchased from Eisai Co., Ltd. (Suzhou, China). PB was the product of Shijiazhuang Pharmaceutical Company. 5-5′-Dithio *bis*-(2-nitrobenzoic acid) (DTNB) was from Sigma Co., Ltd. (St. Louis, MO, U.S.A). Other reagents are of analytic grade.

All drugs were dissolved in carboxymethyl cellulose sodium salt (CMC-Na) solution in saline. The solution was administered orally at 10 ml/kg in each experiment, while the vehicle was given orally at 10 ml/kg in the corresponding control group.

Measurement of Gastrointestinal Transit in Mice —— The experiment on gastrointestinal transmit (GIT) was carried out following the method of Matsuda *et al.*[16–18] To determine when the maximal effect of donepezil on GIT occurred, 50 male mice weighing 18–22 g were divided randomly into 5 groups containing 10 animals each and fasted for 24 hr before the experiment. The control group was given CMC-Na solution, and donepezil 0.625 mg/kg was administered for 30, 60, or 90 min prior to the intragastric administration of a charcoal meal, which contained a solution of 1.5% CMC-Na and 5% charcoal as a marker. The prokinetic drug domperidone as reference drug was given 60 min before the charcoal meal.

To investigate the effects of donepezil plus PB, 70 male mice weighing 18–22 g were randomly divided equally into 7 groups and fasted for 24 hr before the experiment. The control and reference drug groups were treated as above, and donepezil 0.625 mg/kg and donepezil (0.625 mg/kg) plus PB 1, 2, or 4 mg/kg was administered 30 min prior to the administration of a charcoal meal.

Thirty minutes after the charcoal meal, the mice were killed by cervical dislocation. The abdominal cavity was opened, and the gastrointestinal tract was removed. The distance traveled by the charcoal plug from the pylorus to cecum was determined and expressed as a percentage of the total length of the small intestine.

Measurement of Gastric Emptying (GE) —— GE was determined with the phenol red method.[16–18] Fifty male mice weighing 18–22 g were randomly divided into 5 equal groups and fasted for 24 hr before the experiment. The control group was given CMC-Na solution and the reference group was administered domperidone. Donepezil 0.625 mg/kg, PB 2 mg/kg, or donepezil (0.625 mg/kg) plus PB 2 mg/kg were then administered. A solution of 1.5% CMC-Na containing 0.05% phenol red as a marker was given intragastrically (10 ml/kg body weight) to conscious mice 60 min after the drugs were administered. Thirty minutes later, the mice were killed by cervical dislocation. The abdominal cavity was opened, and the gastroesophageal junction and pylorus were clamped. Then the contents of stomach were removed, weighed, placed in 14 ml of NaOH 0.1 M, and homogenized. The suspension was allowed to settle for 1 hr at room temperature, 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v), and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 ml of NaOH 0.5 M, and the amount of phenol red was determined based on the absorbance of the sample read at 560 nm. Phenol red recovered from animals killed immediately after administration of the test meal was used as the standard (0% emptying). GE ability, expressed as residual phenol red (%) in the 30 min period was calculated according to the following equation: GE ability = amount of test sample/amount of standard × 100%.

Transient Cerebral Ischemia in Mice —— Mice were subjected to transient cerebral ischemia induced by bilateral common carotid occlusion.[19,20] In brief, the mice were anesthetized with urethane (1.5 g/kg, ip). The bilateral common carotid arteries

were exposed, carefully separated from the adjacent veins and sympathetic nerves, and then occluded with artery clips (Gaobeidian Experimental Instrument Factory, Beijing, China) for 20 min. While the arteries were clamped, 0.3 ml of blood was withdrawn from the tail vein. Then the artery clips were removed and cerebral blood flow was restored. The skin incision was closed. The mice were kept at 37 ± 1°C with a heating lamp and a heating pad during the operation and then put in a constant temperature compartment at the same temperature until they were awake. Sham-operated mice were subjected to the same procedure without carotid clamping and withdrawal of blood.

Morris Water Maze Task —— Mice alive 24 hr after operation were used in the Morris water maze task. Each group consisted of 12 mice, except that the ischemia model control had 10 mice. From the first day after operation, the sham and ischemia model groups were given saline, and the other groups were given drugs as in the GE experiment 1 hr before the time arranged for trials. On the third day, before the start of learning, mice were given a pretraining session in which they were allowed to swim freely in a pool (90 cm diameter, with a water depth of 19 cm) for 60 sec without an escape platform. The pool was placed in a large dimly lit test room and surrounded by visual cues. In the learning block, from day 4 to day 8, the pool was filled to a depth of 19 cm with water maintained at 25 ± 1°C. A platform (5-cm diameter) was situated 1 cm below the surface of the water. The pool was divided into four quadrants with the platform in a fixed position in one quadrant. Daily learning consisted of four trials in which the mouse was placed in the water from four different starting points and the latency of escape onto the platform was recorded. This was conducted for 5 consecutive days. A maximum of 60 sec was allowed during which the mouse had to find the platform and climb onto it.[19, 20] When the mouse reached the platform, it was allowed to remain on it for 20 sec. If the mouse failed to find the platform within 60 sec, it was removed from the water and placed on the platform for 20 sec. All latency for a given day was calculated by averaging the four trials.

Assay of Cholinesterase in Mice Brain —— For cholinesterase activity assay, mice were killed by decapitation after the Morris water maze task had been carried out. The cerebral cortex from the right hemisphere was dissected on ice and homogenized in 10 (w/v) volumes of ice-cold sodium phosphate buffer (75 mM, pH 7.2). Cholinesterase activity in the homogenates, diluted with sodium phosphate buffer (1/20, v/v), was measured using the spectrophotometric methods of Ellman *et al.*[21] and Dong[22] at 37°C with acetylthiocholine bromide as the substrate. In the last procedure, DTNB was added and the absorbance at 405 nm was monitored in a microplate reader (Bio-TEK Corporation, Burleigh QId, Australia).

Statistical Aanalysis —— Results obtained are expressed as mean ± S.D. Multigroup comparisons of means were carried out using the one-way analysis of variance (ANOVA) test. Dunnett's test was used to compare the differences between two groups. A p value of less than 0.05 was considered significant.

RESULTS

Effect of Donepezil and Propantheline on GIT in Mice

GIT was accelerated significantly by donepezil 0.625 mg/kg during 30–60 min after it was given (Table 1). The maximal acceleration of 26% was achieved 60 min after administration. Thus in subsequent experiments the effects of donepezil were observed 60 min after it was administered. Donepezil increased GIT, but GIT was inhibited by PB. Furthermore, when donepezil and PB were administered simultaneously, PB attenuated the GIT promoted by donepezil. At a dose of 2 mg/kg, PB restored GIT to the same level as in the saline group (Table 2). This dose was equivalent to the therapeutic one in patients based on body surface area. Thus in the measurement of GE, the dose of PB was 2 mg/kg.

Effects of Donepezil and PB on GE in Mice

Donepezil 0.625 mg/kg accelerated GE. PB co-

Table 1. Effects of Donepezil on GIT in Mice in Different Time after Administration

Group	Time (min)	Dose (mg/kg)	GIT (%)	Acceleration (%)
Saline		—	57.7 ± 6.7	
Domperidone	60	5	67.6 ± 5.3**	17
Donepezil	30	0.625	67.9 ± 9.4**	18
Donepezil	60	0.625	72.7 ± 4.0**	26
Donepezil	90	0.625	63.7 ± 3.9*	12

Each value is mean ± S.D. Values are given for each group (n = 10). *$p > 0.05$, **$p < 0.05$ compared with saline.

Table 2. Effect of PB Co-administered with Donepezil on GIT and GE in Mice

Group	Dose (mg/kg)	GIT (%)	GE (%)
Saline	—	57.7 ± 6.7	51.6 ± 12.3
Domperidone	5	73.1 ± 11.3**	32.2 ± 9.4**
Donepezil	0.625	72.7 ± 4.0**	33.1 ± 10.3**
PB	2	43.9 ± 9.4**	66.2 ± 14.1**
Donepezil + PB	0.625 + 1	66.2 ± 13.2*,△	
Donepezil + PB	0.625 + 2	63.3 ± 10.4*,△△	49.8 ± 11.4*,△△
Donepezil + PB	0.625 + 4	60.1 ± 14.5*,△△	

Each value is mean ± S.D. Values are given for each group (n = 10). $^{*}p > 0.05$, $^{**}p < 0.05$ compared with saline; $^{\triangle}p > 0.05$, $^{\triangle\triangle}p < 0.05$ compared with donepezil.

Table 3. Effect of PB Co-administered with Donepezil on Escape Latency in Cerebral Ischemic Mice

Group	Dose (mg/kg)	Escape latency (sec)
Sham	—	25 ± 11
Ischemia model	—	41 ± 15**
Donepezil	0.625	24 ± 10*,△△
PB	2	47 ± 11
Donepezil + PB	0.625 + 2	19 ± 12*,△△,▲

Each value is mean ± S.D. Data show the escape latency on the 5th learning day. Values are given for each group (n = 12, except ischemia model control n = 10). $^{*}p > 0.05$, $^{**}p < 0.05$ compared with sham; $^{\triangle\triangle}p < 0.05$ compared with ischemia model; $^{\blacktriangle}p > 0.05$ compared with donepezil.

Table 4. Effect of PB Co-administered with Donepezil on the AChE Activity of Brain in Cerebral Ischemic Mice

Group	Dose (mg/kg)	AChE activity (IU/mg·protein)
Sham	—	0.66 ± 0.021
Ischemia model	—	0.70 ± 0.029*
Donepezil	0.625	0.32 ± 0.011△△
PB	2	0.72 ± 0.033△
Donepezil + PB	0.625 + 2	0.39 ± 0.014△△,▲

Each value is mean ± S.D. Data show AChE activity in the brain. Values are given for each group (n = 12, except ischemia model control n = 10). $^{*}p > 0.05$ compared with sham; $^{\triangle}p > 0.05$, $^{\triangle\triangle}p < 0.05$ compared with ischemia model; $^{\blacktriangle}p > 0.05$ compared with donepezil.

administered with donepezil attenuated the increase of in GE. At dose of 2 mg/kg, PB restored GE to nearly normal level (Table 2).

Effects of PB Co-administered with Donepezil on Escape Latency of Mice with Memory Impairment

About 30% of mice with transient cerebral ischemia were died within 24 hr after operation. Mice subjected to transient cerebral ischemia took a longer time to locate the hidden platform than the sham-operated control mice during the learning trials, although the ischemia did not affect the swimming ability of the mice in the pretraining trial in the water maze. Treatment of mice with donepezil significantly shortened the latency of escape onto the platform during the learning, especially on the 5th learning day, as compared with the ischemia model control. PB alone did not have any effect on the latency. Co-administration of PB and donepezil did not change the effects of donepezil on the latency of escape onto the platform in the water maze performance, showing that PB had no effect on the therapeutic effect of donepezil (Table 3).

Effects of PB Co-administered with Donepezil on AChE Activity in the Brain of Cerebral Ischemic Mice

After administration of donepezil or both donepezil and PB for 8 days, AChE activity in the brain was decreased compared with that in the ischemia model mice (Table 4). However, there was no difference between the donepezil alone and donepezil plus PB groups. This suggested that PB could not change the ability of donepezil to penetrate through the blood-brain barrier and the inhibitory effects of donepezil on brain AChE activity.

DISCUSSION

Cholinesterase inhibitors can increase ACh levels throughout the body, including the peripheral nervous system, putting patients at risk of adverse cholinergic effects in addition its therapeutic effect. The most common side effects of cholinesterase inhibitors are gastrointestinal. CHI over enhancing gastrointestinal motility may lead to diarrhea, nausea, and vomiting. Clinical trials have con-

sistently shown higher rates of adverse gastrointestinal effects in patients treated with rivastigmine, donepezil, and galantamine than in those receiving placebo.[9–14] Thus package inserts for galantamine, rivastigmine, and donepezil all warn that cholinesterase inhibitors are associated with gastrointestinal events. The most severe adverse effects occur when patients receive the agents in the early period or higher doses of cholinesterase inhibitors, when patients have low body weight, and during upward dose titration. Although forced titration schedules for galantamine and donepezil were carried out, the withdrawal rate was still up to 19%.[23, 24]

GE and GIT are the markers of gastrointestinal motility. CHI could increase gastrointestinal motility.[25, 26] Our data showed that this occurred in the early period after administration. However anticholinergic medication can result in reverse effects and may have the risk of cognitive impairment due to receptor specificity and to the ability of these medications to cross the blood-brain barrier. Our results indicated that PB decreased the increase in GE and GIT. Restoring them to normal levels, in turn, may decrease or avoid the occurrence of diarrhea, and may be possible to suppress nausea and vomiting, suggesting that PB attenuates the adverse effects of CHI agents in the gastrointestinal tract.

In the Morris water maze task, transient cerebral ischemia prolonged the latency of escape onto the platform in the water maze in mice, indicating that ischemia resulted in memory impairment. Donepezil significantly shortened the latency of escape onto the platform, suggesting that CHI could increase the ability of learning and memory in cerebral ischemic mice. PB alone did not have any effect on the latency, and co-administration with donepezil did not change the effects of donepezil on the latency, suggesting that PB had no effect on the therapeutic effects of donepezil. The inhibition of cholinesterase activity is an important efficacy marker of cholinesterase inhibitors in treating patients with dementia. Although a diversity of cholinesterase activity was observed in different ischemia model and even at different times after ischemia in animals, it was confirmed that cholinesterase inhibitors are effective in attenuating dementia in almost all animal experiments.[27–32] In our experiment, donepezil inhibited AChE activity in the brain, which yielded therapeutic effects. PB did not change the cholinesterase activity inhibited by donepezil in the brain, suggesting that PB may not change the amount of donepezil entering the brain. These findings suggest that the muscarinic receptor blocker, which is barely able to penetrate the blood-brain barrier, may not only attenuate the side effects of cholinesterase inhibitors in the peripheral nervous system, but also has no effect on their therapeutic effect in the CNS for treating dementia in patients with VD.

Anticholinergic drugs that are quaternary amines, such as PB, are fully ionized at physiologic pH and do not enter the CNS in any significant amounts.[15, 33] Tertiary amines are less likely to be ionized at physiologic pH, and their ability to cross the blood-brain barrier depends on their relative lipid and water solubility.[34] Thus tertiary amines cannot be co-administered with cholinesterase inhibitors to decrease the peripheral side effects to avoid deteriorating the cognition of patients with dementia.

In conclusion, the muscarinic receptor blocker PB is unable to penetrate the blood-brain barrier and when co-administered with a cholinesterase inhibitor attenuates its side effects without affecting its therapeutic effects, and thus may be beneficial for patients with VD.

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