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Relief of carotid stenosis improves impaired cognition in a rat model of chronic cerebral hypoperfusion

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To investigate how cognitive impairment is affected by the relief of bilateral carotid stenosis, chronic cerebral hypoperfusion was established through stenosis of the bilateral carotid common artery in adult Sprague-Dawley rats. Subsequently, the model rats received the intragastric placebo, donepezil (5 mg/kg), or surgery to relieve carotid stenosis after bilateral carotid common artery stenosis. After carotid stenosis was relieved, the cerebral blood flow values significantly increased, and P300 latency and escape latency in the Morris water-maze were significantly shortened. The concentrations of acetylcholine and norepinephrine in the dorsal hippocampus increased after carotid stenosis was relieved. Furthermore, P300 latency and escape latency were shortened in the relief-treated group compared to the drug-treated group, and acetylcholine levels in the relief-treated group were higher than the drug-treated group. No significant difference was found for the norepinephrine levels in the dorsal hippocampus between the relief-treated and drug-treated groups. Cognitive impairment can be significantly reduced by bilateral carotid stenosis relief, and the effect of relieving stenosis on cognitive dysfunction is superior to the effect of administering an acetylcholinesterase inhibitor.

Key words: acetylcholine, carotid artery stenosis, carotid endarterectomy, monoamine neurotransmitter, vascular cognitive impairment

INTRODUCTION

Vascular cognitive impairment (VCI) is the second most prevalent form of dementia and presents a heterogeneous pathology (Jellinger 2008). The cognitive syndrome of VCI includes all levels of cognitive decline from the earliest deficits to severest broad dementia (Erkinjuntti and Gauthier 2009, Rojas-Fernandez and Moorhouse 2009). Conventional vascular risk factors (e.g., hypertension, diabetes, generalized atherosclerosis, and smoking) are risk conditions for stroke, carotid stenosis, and dementia alike (Meyer et al. 2000). Recently, wealth of experimental data have suggested that carotid stenosis acts as a marker for intracerebral or generalized atherosclerosis. More importantly, symptomatic or asymptomatic carotid stenosis may be an independent risk factor for cognitive impairment and decline (Johnston et al. 2004, Mathiesen et al. 2004, Sztriha et al. 2009).

Progress in understanding VCI has resulted in promising symptomatic and preventive treatments. Cholinergic deficits are well documented in VCI and are independent of any signs of a concomitant Alzheimer's disease pathology (Gottfries et al. 1994). Several controlled clinical trials have shown that an acetylcholinesterase inhibitor (ACEI), including donepezil, shows promising effects on cognitive improvement in VCI patients. Furthermore, the safety and efficacy of donepezil was studied in the largest clinical trial of pure VCI patients (Black et al. 2003, Wilkinson et al. 2003, Gill and Rochon 2004). However, current pharmacotherapeutic approaches, including ACEI, do not significantly mitigate the clinical course of neurodegeneration or the process of aging, and they offer limited and transient benefits to many patients (Kavirajan and Schneider 2007, Mendez et al. 2007). Curative treatments of vascu-

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lar dementia are yet to be discovered. Because high-grade stenosis of the carotid artery is an independent risk factor for cognitive impairment, treatment of this cerebrovascular risk factor may be more appropriate. It is widely accepted and widespread used that interventional therapeutic approaches to the treatment of carotid artery stenosis, such as carotid endarterectomy (CEA) or carotid artery stenting (CAS), are characterized by a restoration of cerebral blood flow (CBF) and a well-known prophylactic effect against cerebrovascular diseases (NASCETC 1991). Now, the effects of carotid stenosis on cognitive impairment have been the subject of many studies. However, the overall effects still remain unclear (Aleksic et al. 2006, De Rango et al. 2008, Mlekusch et al. 2008, Soinne et al. 2009).

Apart from the cholinergic system, monoamine neurotransmitters are also involved in cognitive function (Schuck et al. 2002). In addition, numerous studies have suggested that an association exists between the development of cognitive impairment and a significant change in the monoamine neurotransmitter metabolism (Tohgi et al. 1992, Parnetti et al. 1994, Wallin et al. 1996). Brain monoamine transmitter deficits may also exacerbate impaired cognition function. Thus, changes in monoamine neurotransmitters are an indicator of impaired cognition.

In the current study, the effect of relieving carotid stenosis on cognitive impairment, which is induced via bilateral carotid stenosis in rats, was evaluated and compared with the result of administering an ACEI as a pharmacological therapy. The changes of CBF were measured by a laser-Doppler flowmetry after carotid stenosis was relieved. Cognitive function was measured by the P300 auditory event-related potential and the Morris water-maze test after the administration of ACEI or after carotid stenosis was relieved. Furthermore, *in vivo* microdialysis and high-performance liquid chromatography with electrochemical detection (HPLC-ED) were used to measure the extracellular concentrations, in the hippocampus of the rats, of acetylcholine (ACh), norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (DA).

METHODS

Animal group and preparation

Adult male Sprague-Dawley rats weighting 245 - 275 g (Experimental Animal Center, Third Military Medical University, China) were housed in groups of six per cage in temperature-controlled conditions, with food and water supplied *ad libitum*. All experiments were performed with the approval of the Third Military Medical University Animal Ethics Committee. Efforts were made to minimize animal suffering and to keep the number of animals used at a minimum.

The experimental animals were randomly allocated into four groups: (1) the sham-operated group, which underwent the same surgical procedure without ligation of the common carotid arteries, (2) the drug-treated group, which intragastrically received donepezil (5 mg/kg once a day; Kosasa et al. 2000) for 2, 4 and 6 weeks, (3) the saline group, which was administered the same volume of normal saline as the drug-treated group but was not administered the drugs, (4) and the relief-treated group, which underwent an operation to relieve carotid stenosis. All interventions were performed on day 14 after occurrence of carotid stenosis.

In the drug-treated group, donepezil was tested at doses from 2.5 to 10 mg/kg because this dose range is reported to be well tolerated and to reversibly inhibit cholinesterase activity in the adult rat brain by approximately 20 - 70% (Kosasa et al. 2000). The CBF recordings were obtained prior to, at 2 hours after and 1, 3, 7, and 14 days after the donepezil administration. In the relief-treated group, the rats were given an operation to relieve carotid stenosis at 14 day after occurrence of carotid stenosis. The CBF recordings were also obtained before, at 2 hours, and 1, 3, 7, and 14 days after the carotid stenosis relief.

Cerebral blood flow measurement

The animals were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg), and the skin overlying the right skull was reflected. A plastic guide cannula (with an outer diameter of 3 mm, an inner diameter of 2 mm, and a length of 4 mm) for the laser-Doppler flowmetry probe (PROBE 418-1, Master Probe) was fixed perpendicularly to the skull at 1 mm posterior and 2.5 mm lateral to the bregma using dental resin. The CBF was recorded by placing a 2.0 mm straight probe (PROBE 418-1 Master Probe) through the guide cannula. The CBF values from the rats in the sham-operated and saline groups were obtained by laser-Doppler flowmetry (PF5010LDPM Perimed AB, Sweden) prior to, at 2 hours after, and 1, 3, 7, 14, and 30 days after the surgery. The CBF values were

expressed as a percentage of the baseline value.

Surgical procedure

A method described previously (Kim et al. 2001) was used in this experiment with a slight modification. Briefly, the rat was anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). Through a midline cervical incision, the skin and muscles were bluntly dissected, and the bilateral common carotid artery was exposed and freed from its sheath. The bilateral common carotid artery was bound with a stainless microtube with a diameter of 0.45 mm and a length of 0.5 cm. Subsequently, the bilateral common carotid artery was ligated using a 4-0 suture line soaked in dexamethasone at the proximal portion that was 0.5 cm from the bifurcation of the internal and external carotid artery. During the surgical procedure, a rectal temperature was maintained between 36.5 and 37.5°C. Rats were given aspirin as an anticoagulant (30 mg/L) in their drinking water 3 days after surgery. In the sham-operated group, the bilateral common carotid artery was exposed, but no ligature was made.

Detection of the P300 auditory event-related potential

At 2, 4, and 6 weeks after both treatments (relief-treatment or drug treatment), the P300 auditory event-related potential (ERP) was detected (n=6 at each time point in every group). Prior to the testing or training, each subject was implanted with two stainless steel bone screws (6×1 mm). The recording screw was located at the right bregma association area (1 mm caudal to the bregma and 1 mm lateral to the midline), and the monopolar recording was referenced to the stainless screw placed on the nasal bone. P300 can be elicited through the use of classical conditioning to infrequent ('oddball') auditory stimuli (Medtronic, Denmark). Rats were presented with a randomized sequence of two discriminative stimuli (two different tones at 1 and 2 Hz, for a period of 50 ms and an amplitude of 85 dB). Tone bursts were delivered once per second. The low-frequency (1 Hz) tone, with a relative probability of 0.01, was the target sound for the 'oddball' stimulus, and after 600 ms, it was followed by an electric shock (80 V) applied to the copper floor. The high-frequency (2 Hz) tone was the background sound. Rats were trained for this classical aversive conditioning task for 10 min daily for more than 1 week up to the day before the ERP measurement. After training, the ERP measurement was carried out. During the ERP measurement, the rats were not anesthetized, but they were paralyzed with pancuronium (0.1 mg/kg) and ventilated. The P300 wave was recorded and documented in charts. At the end of detection, the rats in every group were intraperitoneally anesthetized with sodium pentobarbital (65 mg/kg) to determine the neurotransmitter levels in the hippocampus.

Morris water-maze

At 2, 4, and 6 weeks after both treatments (i.e., relief-treatment or drug treatment), the rats (n=8 at each time point in every group) were subjected to a water maze test, which consisted of a large circular pool (diameter: 2 m, height: 1.5 m) filled with water (25±1°C), that was rendered opaque by the addition of a quantity of milk that prevented the rats from seeing an underwater platform (5 cm diameter) that was 3.5 cm below the water surface. The day before the start of the training, the rats were acclimated by being allowed to swim freely in a pool.

To measure the acquisition learning behavior, 10 navigation trials were performed daily (2 trials per day) for 5 consecutive days for each rat. The rats were trained to swim to a hidden platform that was situated below the surface of the water and was placed at the center of one of the four quadrants throughout training. During the four trials, each rat was randomly placed in four starting positions. The rats were given 2 minutes to find the platform and to remain on it for 15 s. If the animals failed to find the platform within the given time, they were gently guided to the platform and were allowed to stay on it for 15 s. The rats were trained to find the hidden platform in the pool over 10 trials.

To evaluate the rat's spatial retention ability, the space probe trails were carried out on the day 6. The platform was removed, and the total swimming distance traveled and the swimming distance in the target quadrant for 2 minutes were recorded by the tracking system.

Determination of ACh and monoamine neurotransmitter levels in the hippocampus

In vivo microdialysis

We performed *in vivo* microdialysis experiments 2, 4, and 6 weeks after both treatments (relief-treatment or drug-treatment). Under sodium-pentobarbital anes-

thesia, rats (n=6 at each time point in every group) were implanted with the guide cannula in the hippocampus according to the stereotaxic atlas of Paxinos and Watson (1986). Seventy-two hours following surgery, a concentric-style microdialysis probe (Scipro Inc., part no. MAB 4.15.3. PES for the hippocampus, outer diameter of 0.2 mm) was inserted through the guide cannula into the dorsal hippocampus (AP −3.6 mm, ML +3.2 mm, DV −4.0 mm from the bregma). The dialysis probes were connected to an infusion pump that was set to deliver artificial cerebrospinal fluid containing 136 mM NaCl, 1.7 mM KCl, 1.2 mM $CaCl_2$, 6 mM Na_2HPO_4, 5 mM glucose, and 100 nM neostigmine at a constant rate of 1.5 μL/min. After an equilibration period of 2 h, dialysis samples were collected every 30 min and refrigerated. After the experiments, the brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

Acetylcholine analysis

ACh in the dialysate was directly assayed by reversed-phase HPLC with a post-column enzyme reaction and electrochemical detection. An aliquot (20 μl) of the sample was directly injected into a SepStik ACh/Ch analytical column (inner diameter 530 mm × 1 mm × 10 μm; MF-8904). ACh and choline were separated before entering a SepStik ACh/Ch immobilized enzyme reactor (IMER) (50 mm × 1 mm inner diameter) (MF-8903) containing immobilized ACh esterase and choline oxidase. ACh was hydrolyzed to acetate and choline, and choline was oxidized to hydrogen peroxide and betaine. Hydrogen peroxide corresponding to ACh was then detected using a 6-mm downstream glassy carbon electrode held at 750 mV versus Ag/AgCl (BAS, Indiana, USA). The mobile phase consisted of 50 mM phosphate buffer (pH 8.0 – 8.5) that contained 10 mM NaCl, 1.0 mM Na_2EDTA, and 0.5% v/v ProClinTM, which was pumped at a rate of 100 μl/min by a HPLC pump (LB-1 Xingda, Beijing, China). The quantity of ACh was calculated based on a known standard curve. The standard curve was constructed across a concentration range from 50 to 100 fmol/20 μL. The curve was linear across this range of concentrations. The lower limit of detection using these assay conditions was approximately 10 fmol/20 μL.

Analysis of monoamine neurotransmitter

At 2, 4, and 6 weeks after both treatments (relief-treatment or drug treatment), the endogenous levels of norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (DA) in the hippocampus were also determined by HPLC-ED as described previously (n=6 at each time point in every group; Liang et al. 2006). After the rats were intraperitoneally anesthetized with sodium pentobarbital (65 mg/kg), the brains were rapidly removed under controlled temperature conditions (0°C). The dorsal hippocampi were dissected, weighed, and processed for HPLC. Dissected hippocampal tissues were homogenized with 50 μl of 0.1 M perchloric acid/mg of hippocampal tissue. After centrifugation (15 000 g, 10 min, 4°C), 30 μl of supernatant was injected into a C18 reverse-phase HR-80 catecholamine column (ESA, Bedford, MA, USA). NE, 5-HT, and DA were quantified by HPLC via electrochemical detection. The mobile phase (pH 2.9) consisted of 90% 75 mM sodium phosphate, 275 mg/L octane sulfonic acid solution, and 10% methanol. The flow rate was 1 ml/min. Peaks were detected by an ESA Coulochem II with a model 5010 detector (E1=50 mV, E2=400 mV). Data were collected and processed using the Chromeleon computer system (Gynkotek, Gemering, Germany).

Statistical analysis

All results are expressed as mean ± SD. The escape latencies and the percentage of the swimming distance in the platform quadrant of every group during the Morris water-maze test were analyzed by a repeated-measures ANOVA followed by a *post-hoc* Dunnett test. Statistical analyses of all other data were performed using a one-way ANOVA followed by Tukey's tests, $p>0.05$ was considered statistically significant.

RESULTS

Changes of CBF in the experimental groups

Changes of CBF in the rats after the bilateral carotid stenosis

Figure 1A shows the mean CBF values of the rats in the sham-operated and saline groups. In the sham-operated group, the mean CBF after the sham operation

varied from 98.6 to 102.4% and did not show any significant changes between any time intervals (one-factorial ANOVA, $p>0.2$). In contrast, the CBF values decreased significantly compared to the pre-operative baseline after the surgery in the saline group. At 2 hours, the CBF values were significantly reduced to 28.6±8.4% in the saline group. On day 1, the CBF values in saline group began to recover but remained significantly lower than the sham-operated group until 14 days ($p=0.003$). At day 30, the CBF values were still lower ($p=0.01$) than the sham-operated group.

Changes of CBF in the rats after bilateral carotid stenosis was relieved

Figure 1B shows the mean CBF values of the rats in the drug-treated and relief-treated groups following donepezil administration and carotid stenosis relief. On day 1, the CBF values in the relief-treated group significantly increased compared to the drug-treated group ($p=0.002$). At 14 days, the CBF values in the relief-treated group were still higher than the drug-treated group ($p= 0.009$).

P300 auditory event-related potential

After 4 and 6 weeks of treatment, the P300 latencies of the relief-treated and drug-treated groups were shorter than the values prior to treatment ($p=0.012$). Compared with the saline group, the P300 latencies of the relief-treated groups were significantly shorter (363.63±18.52 ms *vs.* 319.64±22.18 ms at 4 weeks and 369.48±25.74 ms *vs.* 317.15±16.31 ms at 6 weeks; all $p<0.05$). The P300 latencies of the relief-treated and drug-treated groups were significantly shorter at 4 and 6 weeks after both treatments compared with the saline group (all $p<0.01$). The P300 latency of the relief-treated group was significantly shorter than that of the drug-treated group at 4 and 6 weeks after treatment ($p=0.007$ and 0.005, respectively), but no significant change was apparent at 2 weeks (Fig. 2).

Morris water-maze

In the current experiments, the escape latency in every group was observed at 2, 4, and 6 weeks following the donepezil administration and carotid stenosis relief. The escape latency in both the relief-treated and the drug-treated groups tended to decrease over time. A group comparison revealed that the relief-treated group presented a shorter latency to find the platform than the drug-treated group at 4 weeks (9.46±0.44 s *vs.* 17.37±2.2 s, $p=0.024$; the tenth navigation trial) and 6 weeks (11.87±1.58 s *vs.* 17.46±2.57 s, $p=0.01$; the tenth navigation trial), but no difference was found at 2

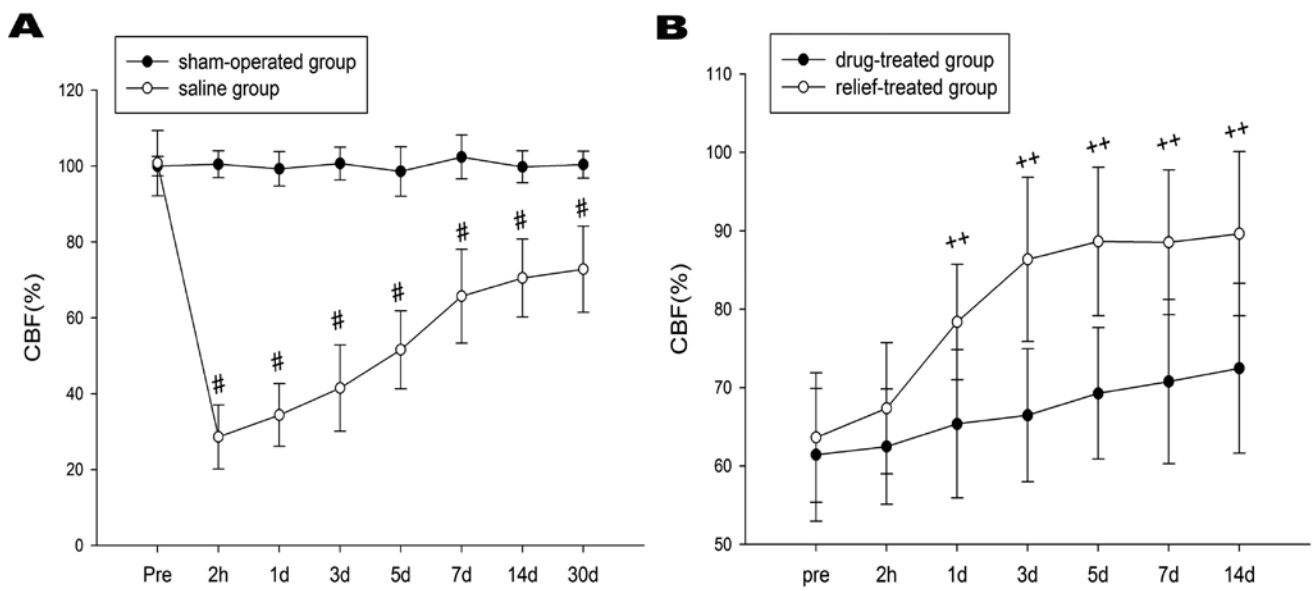

Fig. 1. Changes in CBF in the experimental groups. (A) Changes of CBF in the sham-operated and control groups; (B) Changes of CBF in the relief-treated and drug-treated groups. Values are means ± SD, $n=7$ per group. $^{\#}p<0.05$ compared to the sham-operated group and $^{++}p<0.05$ compared to the drug-treated group.

weeks (17.62±5.48 s *vs.* 19.56±4.67 s, p=0.22; the tenth navigation trial).

Compared with the saline group, the swimming distance percentage in the target quadrant was significantly increased in the relief-treated groups (33.85±9.46% *vs.* 42.37±10.11% at 2 weeks; 32.19±8.58% *vs.* 47.16±14.06% at 4 weeks; 33.75±6.97% *vs.* 48.92±10.54% at 6 weeks; all p<0.01). Furthermore, after 4 and 6 weeks of treatments, a significant increase in the swimming distance percentage in the target quadrant was detected in the relief-treated group compared to the drug-treated group (31.37±4.66% *vs.* 26.37±4.48% at 4 weeks; 33.37±5.16% *vs.* 28.38±5.26% at 6 weeks; all p<0.01). However, no significant difference was observed at 2 weeks (24.37±4.38% *vs.* 25.38±4.47%).

Hippocampal ACh levels

The basal concentration of ACh in the dorsal hippocampus was 92.85±1.27 fmol/20 μL. The effects of the systemic administration of donepezil and carotid stenosis relief on the extracellular concentrations of ACh in the dorsal hippocampus are depicted in Fig. 3. The extracellular concentrations of ACh in both the drug-treated and relief-treated groups significantly (all p<0.05) increased following weeks 2, 4 and 6 of interventions. Compared with the saline group, extracellular levels of ACh in both the drug-treated and relief-treated groups also increased at weeks 2, 4 and 6 after both treatments (all p<0.025). Furthermore, significant differences in extracellular levels of ACh in dorsal hippocampus were observed between the drug-treated and relief-treated groups at weeks 2 and 6 after both treatments (all p<0.01).

Levels of monoamine neurotransmitters in the hippocampus

Figure 4 shows the levels of monoamine neurotransmitters in the hippocampus of each group. Compared with the sham-operated group, the levels of NE and 5-HT were reduced in the normal saline group on postoperative day 15. The amount of DA in the normal saline group was significantly reduced 6 weeks after carotid stenosis (from 453.41±38.28 to 303.78±36.65; p=0.005) compared with the sham-operated group. The levels of NE in the relief-treated and drug-treated groups were 486.86±46.14 pg/mg and 487.36±31.58 pg/mg, respectively, before treatment. After two weeks of treatments, the levels of NE in the relief-treated and drug-treated groups were increased to 557.96±38.63 pg/mg and 550.57±35.62 pg/mg, respectively (all p< 0.004). Compared with the normal saline group, the level of NE was increased in the relief-treated and drug-treated

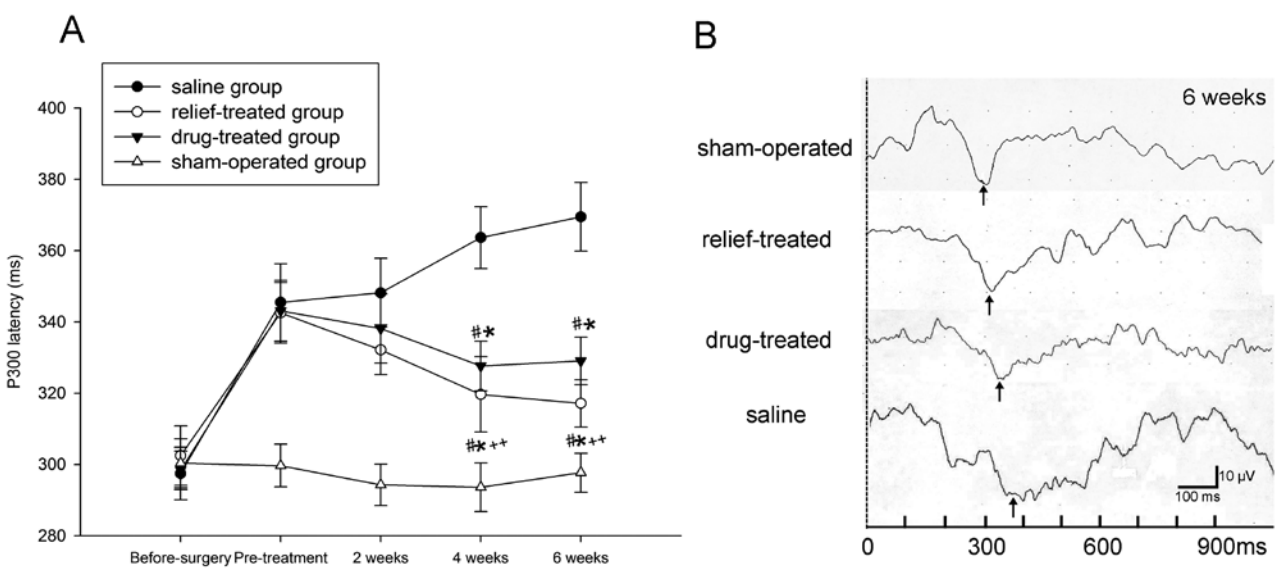

Fig. 2. Chart of changes of P300 latency in each group at 2, 4, and 6 weeks following both treatments. (A) P300 latencies in the relief-treated and drug-treated groups were much shorter than the latencies in the saline group. During weeks 4 and 6, the P300 latency in the relief-treated group was much shorter than the drug-treated group. (B) The representative waveform of P300 (arrows) at 6 weeks.. Values are means ± SD, n=6 per group. $^{\#}p<0.05$ compared to pre-treatment; $^{*}p<0.05$ compared to the saline group; $^{++}p<0.05$ compared to the drug-treated group.

groups at weeks 2, 4 and 6 after both treatments (all $p<0.05$); and the level of 5-HT was also higher at week 6 after both treatments (all $p<0.05$). No significant difference was found in the monoamine neurotransmitter levels between the relief-treated and drug-treated groups.

DISCUSSION

VCI increases exponentially with the aging of the population. However, no approved effective treatments exist for VCI (Launer and Hofman 2000, Erkinjuntti et al. 2004). The present study used the detection of the P300 auditory event-related potential and the Morris water maze test and demonstrated that the cognitive decline induced by carotid stenosis was markedly improved by both the relief of bilateral carotid stenosis and the administration of donepezil. Bilateral carotid stenosis relief was found to produce a more pronounced cognitive improvement. Furthermore, we have shown that concentrations of ACh and NE in the dorsal hippocampus detected by HPLC-ED were significantly increased following both treatments.

Originally, the approved intervention for VCI is a medical management aimed at symptomatic treatments, including antithrombotics, ergot alkaloids, nootropics, *Ginkgo biloba* extracts, plasma viscosity drugs and hyperbaric oxygen (Roman 2000). Recently, evidence has shown that donepezil, a core member of ACEI, effectively attenuates the cognitive impairment of experimental animal models and randomized controlled clinical studies (Wilkinson et al. 2003, Lee et al. 2007). Our study indicated that the donepezil-treated group showed a statistically significant improvement in cognitive function, which was measured with the Morris water-maze test and the detection of P300 event-related potential. However, evidence has shown that the symptomatic improvement with donepezil does not prevent a stepwise progression of cognitive deficits and may be accompanied by significant adverse events, such as diarrhea, nausea, arthralgia, leg cramps, and headache (Dichgans et al. 2008).

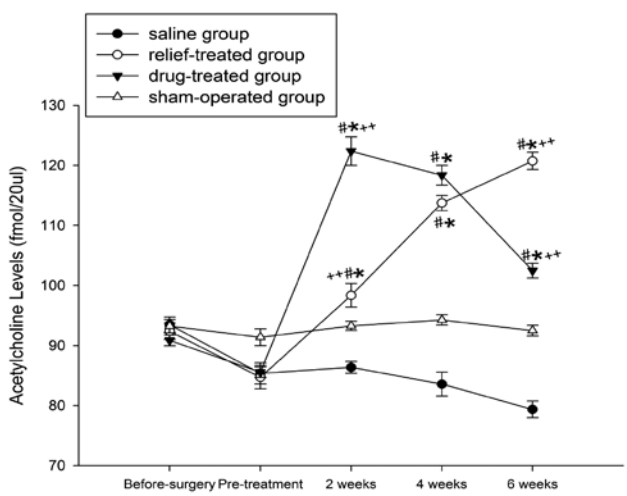

Fig. 3. Time course of the effect of donepezil administration and carotid stenosis relief on acetylcholine extracellular levels in the dorsal hippocampus. The extracellular concentrations of ACh in both the drug-treated group and the relief-treated group increased significantly. ACh levels were higher in the relief-treated group than in the drug-treated group. Values are means ± SD, $n=6$ per group. $^{\#}p<0.05$ compared to pre-treatment; $^{*}p<0.05$ compared to the saline group; $^{++}p<0.05$ compared to the drug-treated group.

Currently, there is a great need for prospective studies to develop appropriate measures for the prevention and treatment of VCI. Surgical management with interventional radiology, such as microcatheterization, is a reliable and beneficial method of treating carotid stenosis, which is an independent risk factor of VCI (Johnston et al. 2004, Sztriha et al. 2009). In the current study, the relief-treated group surgically had their bilateral carotid stenosis relieved at day 14, and our data indicated that the mean CBF values significantly increased after carotid stenosis was relieved. Consequently, it is unknown whether carotid revascularization, with the assistance of surgical intervention, is responsible for the improvement in the cognitive impairment caused by carotid stenosis. In the present experiment, we found that cognitive impairment of the relief-treated group was strikingly recovered, as detected by both the P300-evoked potential system and the Morris water-maze. Several clinical studies have demonstrated an improvement in cognitive performance following CEA (Fearn et al. 2003, Moftakhar et al. 2005, Lal 2007), and carotid revascularization may be a preventative therapy for progressive cognition deficits induced by carotid artery stenosis.

VCI remains of interest to clinicians and researchers because it has high morbidity rates and may be preventable (Rojas-Fernandez and Moorhouse 2009). In the current study, the effects on cognitive performance of medical management with donepezil or surgical relief of carotid stenosis were also compared. Our results clearly indicated that the beneficial effect of relieving carotid stenosis on cognitive decline was greater than the effect obtained through treatment with

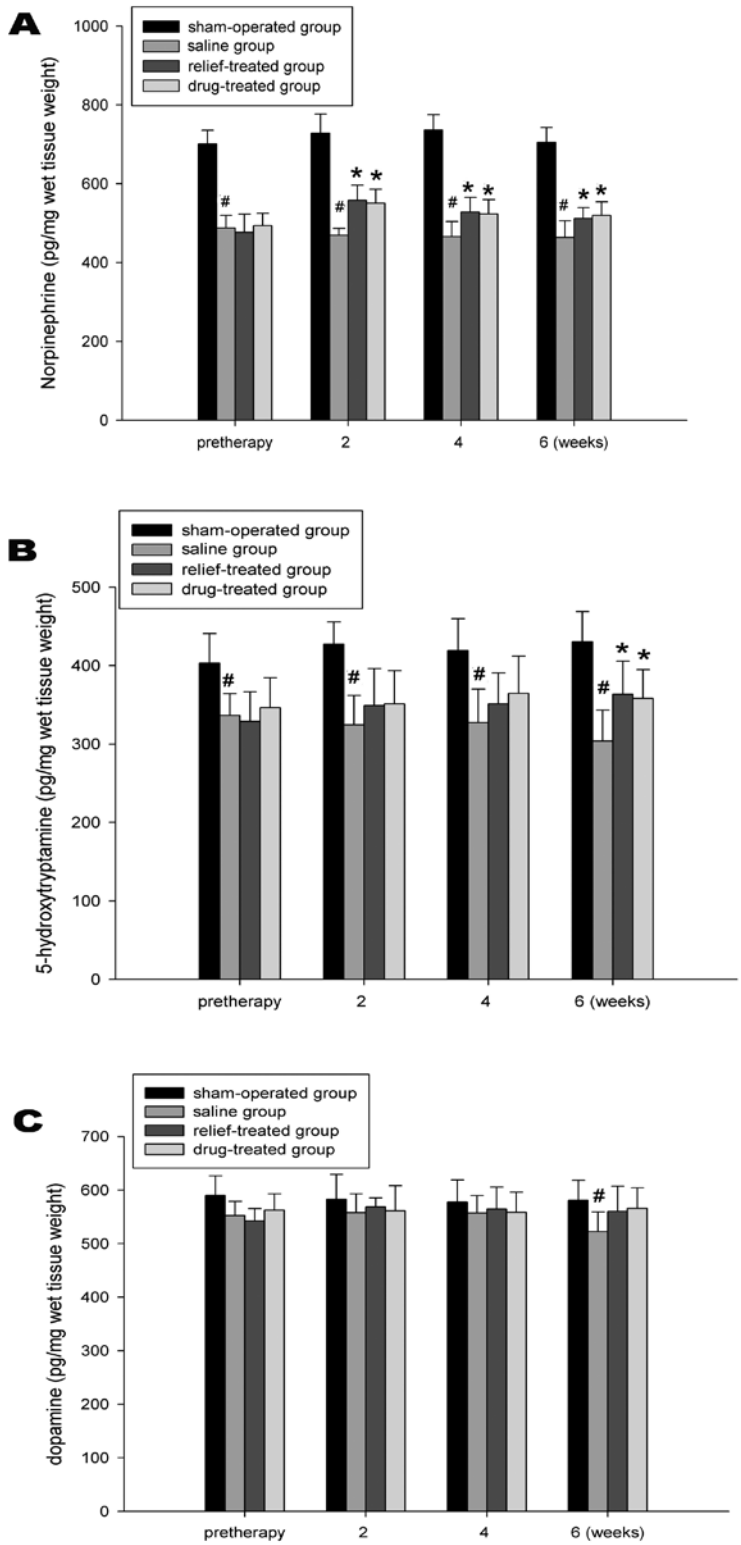

Fig. 4. The effect of donepezil administration and carotid stenosis relief on the concentrations of monoamine neurotransmitters in the dorsal hippocampus. (A) Time course of the concentrations of norepinephrine in every group. (B) Time course of the concentrations of 5-hydroxytryptamine in every group. (C) Time course of the concentrations of dopamine in every group, n=6 per group. $^{\#}p<0.05$ compared to the sham-operated group; $^{*}p<0.05$ compared to the saline group.

donepezil. The preliminary results of our study imply that carotid revascularization may be a prospective and safe method for treating cognitive decline induced by carotid stenosis in the short term.

Although clinical evidence has shown that carotid revascularization (CEA or CAS) for carotid stenosis significantly improves cognitive performance, the detailed mechanisms have not yet been elucidated. Apart from silent brain infarction, the cognitive impairment in carotid stenosis could be due to microembolization or regional hypoperfusion. As concerns the mechanisms involved in the cognitive changes associated with carotid interventions, several studies have demonstrated that an improvement in cognitive function following CEA or CAS may be expected from a reduced embolism and improved hemodynamics (van Laar et al. 2006, Sanchez-Arjona et al. 2007). In the present study, our data showed that the mean CBF values in relief-treated group were higher than in the drug-treated group after carotid stenosis relief. In addition, we also found a significant difference between the results of the Morris water-maze test in the saline group compared with the sham-operated group. Originally, the Morris water-maze test was specifically designed to evaluate hippocampus-dependent cognitive function (Morris 1984). Thus, our data suggest that hippocampal disorders may be involved in the cognitive impairment that is caused by carotid stenosis. It is recognized that hippocampal activity is shaped by many neuromodulators and neurotransmitters (Rebola et al. 2008). Furthermore, acetylcholine activity, a well-established neurotransmitter that plays a key role in higher cognitive processes, is severely reduced in the hippocampus of patients with VCI (Wilkinson et al. 2003). Our results show that the concentration of ACh in the dorsal hippocampus detected by HPLC-ED is markedly decreased after carotid stenosis. Furthermore, concentration of ACh in the dorsal hippocampus in the drug-treated group was significantly increased compared with the saline group at weeks 2, 4, and 6 and increased to a peak at 2 weeks following donepezil administration. We also found that the concentration of ACh in the dorsal hippocampus in the relief-treated group was significantly increased compared with the saline group at 2, 4, and 6 weeks following the relief of carotid stenosis. Our data indicated that the increase in extracellular acetylcholine levels that was caused by the administering of donepezil gradually declined in the drug-treated group. Conversely, the acetylcholine extracellular levels in the relief-treated group gradually increased within 6 weeks following the relief of carotid stenosis. Thus, increased extracellular acetylcholine levels may be associated with the improved cognitive function after relief treatment.

Furthermore, recent studies have related changes in monoamines neurotransmitters to cognitive deficit (Court and Perry 2003, Chamberlain et al. 2006). However, the exact mechanism of monoamines, a series of classical neurotransmitter systems that are related to cognition, has not yet been elucidated. The present study demonstrated that the concentrations of monoamines, including NE, DA, and 5-HT, in the dorsal hippocampus were significantly reduced after bilateral carotid stenosis. The most remarkable change was found for the concentration change of NE. Similar evidence in humans has also confirmed that the production of monoamines is reduced in VCI (Wallin et al. 1996). Therefore, the decrease in the monoamines neurotransmitters is thought to be paralleled by the development of cognitive deficits. Furthermore, our experiment showed that monoamine extracellular levels were significantly increased after bilateral carotid stenosis relief. Consequently, the recovery of monoamine abnormalities may contribute to the attenuation of cognitive failure. Intriguingly, the results from our study revealed that, among the neurotransmitters, the most pronounced change was in the concentration of NE. Our data is in agreement with a recent study by Gliebus and others (Gliebus and Lippa 2007), which demonstrated that adrenergic transmission plays a pivotal role in the retrieval of contextual and spatial memories in the hippocampus.

CONCLUSIONS

The principal findings of the current study are that cognitive deficits can be reversed by relieving carotid stenosis. Furthermore, the effects of the surgical carotid stenosis relief on reduced cognition were better than those of pharmacotherapy with donepezil. The increased ACh and NE extracellular levels may be associated with the improved cognitive function following carotid stenosis relief. Nevertheless, the present study was a preliminary exploration of the cognitive improvement caused by carotid revascularization in an animal model, which has been poorly addressed in the previous literature. The results and mechanism of this effect requires further investigation. Further studies with different time intervals, more refined testing, and perfusion-weighted imaging are needed.

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