

Lipo-prostaglandin E₁ modifies cognitive impairment in rats with vascular cognitive impairment by promoting angiogenesis via the VEGF/VEGFR pathway

BOHUI LIU¹, SUPING ZHANG¹, XIFENG XIONG², LI LING¹, RUI HE¹,
MUZHEN WANG¹, WANQING DENG¹, ZHIHE LIU² and YI LI¹

¹Department of Neurology; ²Guangzhou Institute of Traumatic Surgery, Guangzhou Red Cross Hospital, Medical College, Jinan University, Guangzhou, Guangdong 510220, P.R. China

Received September 6, 2016; Accepted May 16, 2017

DOI: 10.3892/mmr.2017.6984

Abstract. The pathological mechanism of vascular cognitive impairment (VCI) involves ischemic lesions in the hippocampus. Prostaglandin E₁ (PGE₁) serves roles in the promotion of vascular endothelial growth factor (VEGF) expression, angiogenesis and enhances blood flow to ischemic regions. However, the effect of PGE₁ on cognitive function in VCI rats and the underlying mechanism are unknown. In the current study, learning and memory function in VCI rats treated by lipo-PGE₁ injection was assessed through Morris Water Maze test. Furthermore, the histological alterations, blood vessel numbers in the hippocampal CA1 region and relative VEGF protein and mRNA expression were researched. The results confirmed that VCI rats treated with lipo-PGE₁ presented improved cognitive function, less neuronal cell loss, a greater number of blood vessels in the hippocampal region and higher VEGF protein and mRNA expression. However, the role of lipo-PGE₁ in VCI rats can be inhibited by SU5416 (a specific VEGFR2 antagonist). The results indicated that lipo-PGE₁ may alleviate cognitive deficits in VCI rats. The underlying mechanism may be associated with angiogenesis promoted by lipo-PGE₁, which may involve the VEGF/VEGFR pathway. These findings may have therapeutic implications for cognitive impairment induced by hypoperfusion or chronic ischemic lesions.

Introduction

Vascular cognitive impairment (VCI) is defined as progressive cognitive impairment associated with vascular diseases,

which is now recognized as a silent epidemic disease of the 21st century. In 2010, there were 36.5 million people suffering from dementia with a morbidity of 7.7 million new cases annually and a new case every 4 sec, of which VD accounted for ~15-20%, and the proportion reached nearly 50% in China and Japan (1). The prevalence of VCI may increase with prolonged life expectancy (2), which places a heavy burden on family, society and government. Now effective management strategies for VCI remain lacking and FDA-approved therapies are currently unavailable. Therefore, it is urgent to explore pathological mechanisms of VCI and develop potent therapeutic measures.

Although the definite cerebrovascular contributions to cognitive impairment and dementia are not fully understood due to the inherent heterogeneous nature of vascular pathology, it seems obvious that cerebrovascular diseases cause pathological damage and impair cognition. In the past decades, much progress has been made in the area of VCI pathophysiological mechanism. Many experts are beginning to focus on the role of cerebrovascular dysfunction, microvascular damage, brain capillary degeneration, vascular oxidative stress following cerebrovascular disease on the initiation and progression of VCI. Cerebral blood flow (CBF) reduction has been elucidated to participate in the process of cognitive impairment for its induced ischemic damage, mitochondrial dysfunction, oxidative stress and neurovascular environment disorders in clinical and experimental studies (3-5). However, increasing expression of endogenous and exogenous pro-angiogenic factors following ischemic damage may promote vascular endothelial cell proliferation together with neovascularization and collateral circulation formation, which is beneficial to improve CBF disorders (6,7). Therefore, therapeutics targeted at vasoactive agents may help to attenuate cognitive deficits in VCI.

As a potent angiogenesis promoter, VEGF stimulates endothelial cell proliferation and neovascularization as well as contributes to vasodilation and substantial improvement of microcirculatory permeability. Previously, it has been validated that VEGF protected neurons and nerves against ischemic damage in brain lesions (8). In addition, studies also declared its properties of stimulating neurogenic signals to release from endothelial cells, promoting neurogenesis, as well as adult

Correspondence to: Dr Suping Zhang, Department of Neurology, Guangzhou Red Cross Hospital, Medical College, Jinan University, 396 Tongfu Zhong Road, Guangzhou, Guangdong 510220, P.R. China
E-mail: honghui2535@163.com

Key words: lipo-PGE₁, angiogenesis, vascular endothelial growth factor, vascular endothelial growth factor receptor antagonist, cognitive impairment

neurons survival and improving cognitive performance as well as modulating neurodegeneration in the CNS (9).

Prostaglandin E₁ (PGE₁) has been widely used to manage peripheral artery occlusive diseases, microvascular disorders and thrombogenesis during transplantation for its properties such as vasodilation, antiplatelet, anti-inflammation and improvement of microcirculation (10,11). Previous evidence suggested that alprostadil (lipo-PGE₁) upregulated the VEGF expression and the number of VEGF coupled with VEGFR in neurons, thus alleviating peripheral nerve lesions and accelerating neural functional rehabilitation (12). Correspondingly, a former study of the authors and a separate previous study have also revealed that PGE₁ had therapeutic effect on cerebral ischemic rat model through angiogenesis and neurogenesis (13,14). However, the role of lipo-PGE₁ in VCI remains unknown.

Therefore, the authors proposed whether lipo-PGE₁ exerted protective effects against ischemic damage caused by chronic cerebral hypoperfusion (CCH) in VCI rats. The probable underlying mechanism was further discussed.

Materials and methods

Animals. A total of 60 adult male Sprague-Dawley rats (weighing 300 g) were obtained from Sun Yat-Sen University of Medical Sciences (quality certificate no. 44008500002363). All rats were bred and housed in the Traumatic and Surgical Research Center in Guangzhou Red Cross Hospital (Guangzhou, China) at constant temperature (23±2°C) with free access to food and water. The current study was conducted according to recommendations in the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA). The protocols were approved by the Committee on the Ethics of Animal Experiments of Guangzhou Red Cross Hospital (Guangzhou, China).

Experimental design. Following a 1 week adaptation period to become accustomed to the laboratory environment, rats were randomly allocated into three groups: Normal group, sham group, surgical group [subjected to bilateral common carotid artery occlusion (BCCAO)], before the surgical group was subsequently divided randomly into PGE₁, PGE₁+SU5416 and saline groups following pre-selection).

VCI model. The BCCAO method was adopted to create a CCH-induced VCI model (15). Rats were anesthetized with 10% chloral hydrate (3.5 ml/kg, intraperitoneal administration; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Following righting reflex disappeared, a 1.0-1.5 cm cut was made in the midline of ventral neck. Subsequently, carotid arteries were bilaterally ligated with a size 4 surgical suture following blunt dissection of surrounding muscles and tissues and careful separation from cervical sympathetic and vagal nerves. Rats were revived in 1 h following surgery, and no motor dysfunction was discovered. A total of 4 animals died by the 2nd day following surgery, which was considered to result probably from surgical injury. At the 15th day following surgery, rats were given a screening trial for 5 days (part of the MWM test) as previously described. Rats without cognitive deficits were eliminated if the screening ratio (SR) was <20%. SR was defined as (value 1 - value 2)/value 2×100%, where

value 1 represents escape latency of each surgical rat and value 2 represents mean escape latency of all rats.

Pharmacological treatment. PGE₁ and PGE₁+SU5416 groups were administered alprostadil (lipo-PGE₁; 10 µg/kg/d, intravenous; Ted Pharmaceutical Co., Ltd., Beijing, China) for 7 consecutive days (13). PGE₁+SU5416 group was simultaneously injected with VEGFR antagonist SU5416 (25 mg/kg/d, intraperitoneal; Sigma-Aldrich; Merck KGaA). The saline group was treated with a tail vein injection of saline group.

MWM test. MWM test was conducted to investigate learning capacity and spatial memory as described in former literature (15), which consisted of 5 days of position navigation testing and 1 day of spatial exploration testing. During 5 days of the position navigation test, swimming distance and escape latency from the moment that rats were placed into water to that they climbed onto the platform were recorded. The average latency and distance in four different quadrants of each rat every day represented their learning scores.

On the 6th day, a spatial exploration test was performed with rats placed into a pool at the opposite position from original platform quadrant with platform being removed. Times that rats swam across the previous platform location were recorded.

Sample preparation and collection. On the 35th day, following anesthesia with 10% chloral hydrate (3.5 ml/kg, intraperitoneal), five rats from each group were sacrificed with chest opened, descending aorta clipped and heart perfused by 4% paraformaldehyde (Beyotime Institute of Biotechnology, Haimen, China) following 4°C heparinized saline (75 IU/l) perfusion until the neck, head and chest were stiff. Then skulls were opened with brains carefully removed. Subsequently, brain tissues were fixed in 4% paraformaldehyde for 6-12 h before dehydrated in 10-30% sucrose and 0.01 M PBS. Brains were cut into coronal sections (10 µm-thick) frozen sections by Microtome-Cryostat (Leica Microsystems GmbH, Wetzlar, Germany) for immunohistochemistry.

The remaining rats of each group were decapitated following deep anesthesia under RNase-free conditions. Both sides of hippocampus were isolated and frozen immediately in liquid nitrogen. The left hippocampus was used for western blot analysis, whereas the right side for reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Histological detection. Hematoxylin and eosin staining protocol was applied to observe hippocampal histological changes. Following incubation at room temperature for 20 min, brain coronary sections were stained with hematoxylin (Beyotime Institute of Biotechnology) for 5 min, rinsed with running water for 15 sec, denatured with 10% hydrochloric acid alcohol for 3 sec, washed for 20 min and counterstained with eosin for 30 sec before dehydrating with an alcohol gradient and rendered transparent with xylene. The sections were observed immediately using a Zeiss confocal microscope (Zeiss GmbH, Jena, Germany).

Immunohistochemical analysis of blood vessels. Based on the mouse brain atlas (16), five coronal cryosections

(10 μm -thick) from each animal (-2.8 to -3.8 mm, posterior to bregma) were obtained. Coronal sections were mounted on glass slides. Antigen retrieval was performed before immunohistochemical technique for staining lamina antigen on the basement membrane of blood vessel was carried out according to instructions of Super Sensitive™ IHC Detection System kit (mouse/rabbit; Bioworld Technology, Inc., St. Louis Park, MN, USA). And then the slides were counterstained with hematoxylin and covered with Permount™ Mounting medium (Beyotime Institute of Biotechnology).

In each coronal brain slice, three different region of interests with dense vessels in the hippocampus CA1 region were set and scanned under a x10 objective lens. Then the full-focused images were generated from the laser scanning confocal microscope (Zeiss GmbH) under a x40 objective lens. Capillaries were identified by their single layer of flattened endothelial cells with lamina and hematoxylin immunostaining. Vessels (<12 μm) that were separated from adjacent vessels was regarded as a single capillary and counted in number. This number was added to the number of vascular branch points (number of vessel bifurcations) to sum up as the number of capillaries (17).

Western blot analysis. A total of 100 mg hippocampal tissue from each sample was dissociated, homogenized followed by 10 min of centrifugation in a high-speed refrigerated centrifuge (15,521 \times g; 4°C; Sigma-Aldrich; Merck KGaA). Subsequently, 20 μl supernatant was used to determine protein concentration using bicinchoninic acid assay protein assay kit (Beyotime Institute of Biotechnology), whereas the residual supernatant mixed with 5X SDS gel loading buffer. Western blot analysis was conducted as previously described (18). Proteins were separated by 12% SDS-PAGE (Beyotime Institute of Biotechnology), transferred onto polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA) before membranes were blocked and incubated by primary antibodies (VEGF, 1:500, cat no. AP0741; Bioworld Technology, Inc.; GAPDH, 1:1,000, cat no. 2118; Cell Signaling Technology, Inc., Danvers, MA, USA) at 4°C overnight. Then membranes were developed with chemiluminescent assay kit (Invitrogen; Thermo Fisher Scientific, Inc. Waltham, MA, USA) and analyzed by ChemiDOC™ XRS+ imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) following incubation with horseradish peroxidase-conjugated secondary antibody (anti-rabbit-HRP IgG, 1:5,000; Cell Signaling Technology, Inc.) at room temperature for 1 h.

Total RNA extraction. Under RNase-free conditions, approximately 100 mg hippocampal tissue was ground and homogenized in 1 ml TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at ambient temperature. Following addition of chloroform, samples were shaken for 15 sec, incubated for 3 min and centrifuged in a refrigerated centrifuge for 15 min (13,325 \times g; 4°C). The supernatant was mixed with an equal volume of isopropanol, centrifuged as previously described before incubation at -20°C. The supernatant was then discarded when precipitate was washed with 800 μl 75% ethanol in diethyl pyrocarbonate water. Finally, samples were centrifuged (5,166 \times g; 4°C) for 5 min and the RNA pellet was re-suspended in DEPC water.

(RT-qPCR) analysis. Following RNA extraction, cDNA synthesis was performed using the PrimeScript™ RT Reagent kit (Takara Bio, Inc., Otsu, Japan). The thermocycling parameters were as follows: An initial 2 min denaturation at 93°C, followed by 40 cycles of denaturation at 93°C for 15 sec, annealing at 55°C for 25 sec and extension at 72°C for 25 sec. Following each reaction, cycle threshold (Ct) was noted and calculated.

Primers for rat VEGF (forward, 5'-TCCTGTGTGCCCCAATGC-3' and reverse, 5'-ACGCACTCCAGGGCTTCA T-3') and GAPDH (forward, 5'-GTATGACTCTACCCACGG CAAGT-3' and reverse, 5'-TCTCGCTCCTGGAAGATG GT-3') were designed using Primer PremierVersion 2.0 software (Invitrogen; Thermo Fisher Scientific, Inc.) and their efficiency was confirmed by sequencing their conventional PCR products. The $2^{-\Delta\Delta C_t}$ method (19) was employed to analyze relative mRNA expression using GAPDH as a reference gene (20).

Statistical analysis. All data were analyzed with SPSS statistical software (version, 19.0; SPSS Inc., Chicago, IL, USA) and described as means \pm standard deviation. The behavioral data in navigation trial was analyzed by two-way repeated-measures analysis of variance. Others were analyzed by one-way analysis of variance or Student's t-test. If homogeneity of variance or sphericity assumptions were violated, a lower-bound or Huynh-Feldt correction was used. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Lipo-PGE₁ improves learning and spatial memory deficits in VCI rats induced by CCH. In place navigation test, escape latency decreased as trial progressed ($P < 0.01$). PGE₁ group spent significantly less time in finding the platform than saline and PGE₁+SU5416 groups ($P < 0.05$). Escape latency in the saline group was longer than that in the sham group ($P < 0.05$; Fig. 1A).

In spatial probe test, swim patterns indicated that PGE₁, normal and sham groups swam immediately towards the previous platform location in an attempt to find the platform and shuttled back and forth with 'shuttle type' swimming behavior. In contrast, saline and PGE₁+SU5416 groups swam in large loops randomly and aimlessly along the pool (Fig. 1B). Times in PGE₁ group was much more than that in saline and PGE₁+SU5416 groups ($P < 0.01$); Meanwhile, it was much more in normal and sham groups compared with the saline group ($P < 0.01$; Fig. 1C).

Lipo-PGE₁ alleviates structural damage in hippocampal CA1 region associated with CCH. Morphological alterations in hippocampal CA1 region were observed following HE staining. As demonstrated in Fig. 2, greater cell loss, karyopyknosis and dark-stained nuclei were observed in saline and PGE₁+SU5416 groups compared with the PGE₁ group. Furthermore, cells were more disordered in saline and VEGFR antagonist groups. Meanwhile, no infarction-like focal necrotic lesions were observed in BCCAO rats.

Lipo-PGE₁ increases the vessel number in hippocampal CA1 region. Vessels in hippocampal CA1 region were marked and

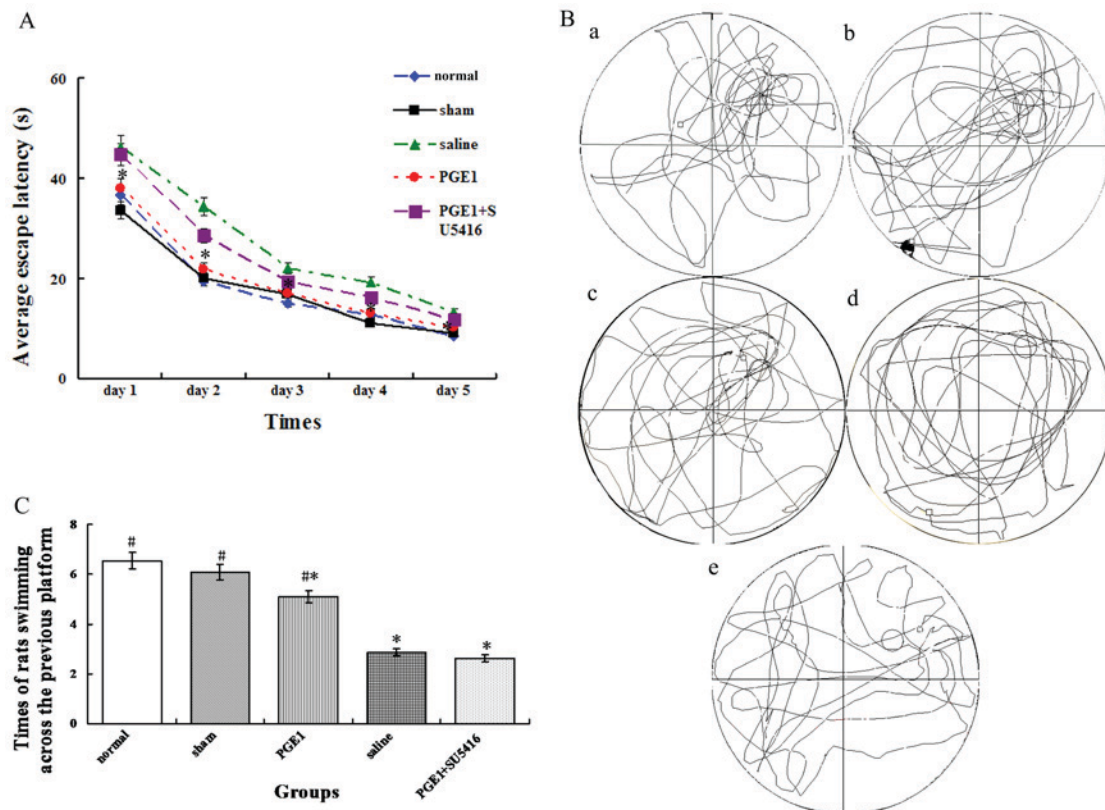


Figure 1. Learning and spatial memory deficits in VCI rats induced by CCH. (A) Performance of rats during 5 days navigation trial (n=10 for each group). The escape latency in PGE₁ group was less compared with saline and PGE₁+SU5416 groups ($P<0.05$) and it was longer in saline group than that of normal and sham groups ($P<0.05$). (B) Swimming trajectory in the spatial probe to test swimming patterns (n=10 for each group). (B-a) Normal group; (B-b) sham group; (B-c) PGE₁ group; (B-d) saline group; (B-e) PGE₁+SU5416. B-a, -b and -c presented a 'shuttle type' searching strategy with rats swimming back and forth in the platform. B-d and -e exhibited a 'marginal type' of searching strategy with rats swimming randomly across each quadrant in large loops. (C) Number of times that rats swam across the former location of the platform (n=10 for each group). # $P<0.01$ vs. saline group; * $P<0.01$ vs. PGE₁ group. VCI, vascular cognitive impairment; CCH, chronic cerebral hypoperfusion; PGE₁, prostaglandin E₁.

stained brown with anti-lamina antibody (Fig. 3A). Vessel number in PGE₁ group was significantly higher than that in saline and PGE₁+SU5416 groups ($P<0.05$). Furthermore, a significant increase in vessel number was observed in saline group compared with normal and sham groups ($P<0.01$; Fig. 3B).

Lipo-PGE₁ stimulates VEGF protein and mRNA expression in the hippocampus. Relative VEGF protein expression and VEGF mRNA expression in hippocampus was significantly higher in PGE₁ and PGE₁+SU5416 groups compared with the saline group ($P<0.05$, $P<0.01$). Whereas relative VEGF protein expression and VEGF mRNA expression in the saline group was higher than that in normal and sham groups (both $P<0.01$). In addition, there was no remarkable difference between PGE₁ and PGE₁+SU5416 groups (both $P>0.05$; Figs. 4 and 5).

Discussion

Cognition is acquired on the basis of past experience and future goals as well as environmental conditions and influence behavior correspondingly. Cognitive abilities in humans are considered to be involved in a series of domains including attention, executive function, memory and visuospatial processing. Each domain has its preferential functional area in the brain, and is interacted with others. VCI disease encompasses a wide

spectrum of cerebrovascular-driven cognitive impairment, from mild cognitive impairment to fully developed dementia, which is characterized by slowing of motor performance and information processing, with impairments in attention, executive function and memory (21). The formation of long-term episodic memories relies on the hippocampus and associated structures in the medial temporal region and limbic connections (22). The hippocampus damage is correlated with impaired learning and memory function (23). As brain neurons have hardly any energy reserves, oxygen and glucose are the primary components for the generation of energy. Hence, they are sensitive to cerebrovascular dysfunction, especially those in the hippocampus, which is especially vulnerable to ischemic damage. With the capacities to result in cerebrovascular dysfunction, brain capillary degeneration and vascular oxidative stress, hypoperfusion is supported to be one of the initial mechanistic factors in the process of development of cognitive impairment (4).

In order to explore the pathophysiological mechanism of hypoperfusion in the process of VCI initiation and the potential beneficial therapeutic methods, many different methods were employed to make the models of hypoperfusion, such as rat BCCAO, gerbil bilateral carotid stenosis, mouse bilateral carotid stenosis, UCCAO mice, rat 4-VO, gerbil 2-VO and mouse 2-VO as well as mouse middle cerebral artery occlusion (24). With the advantage of long-lasting chronic low CBF

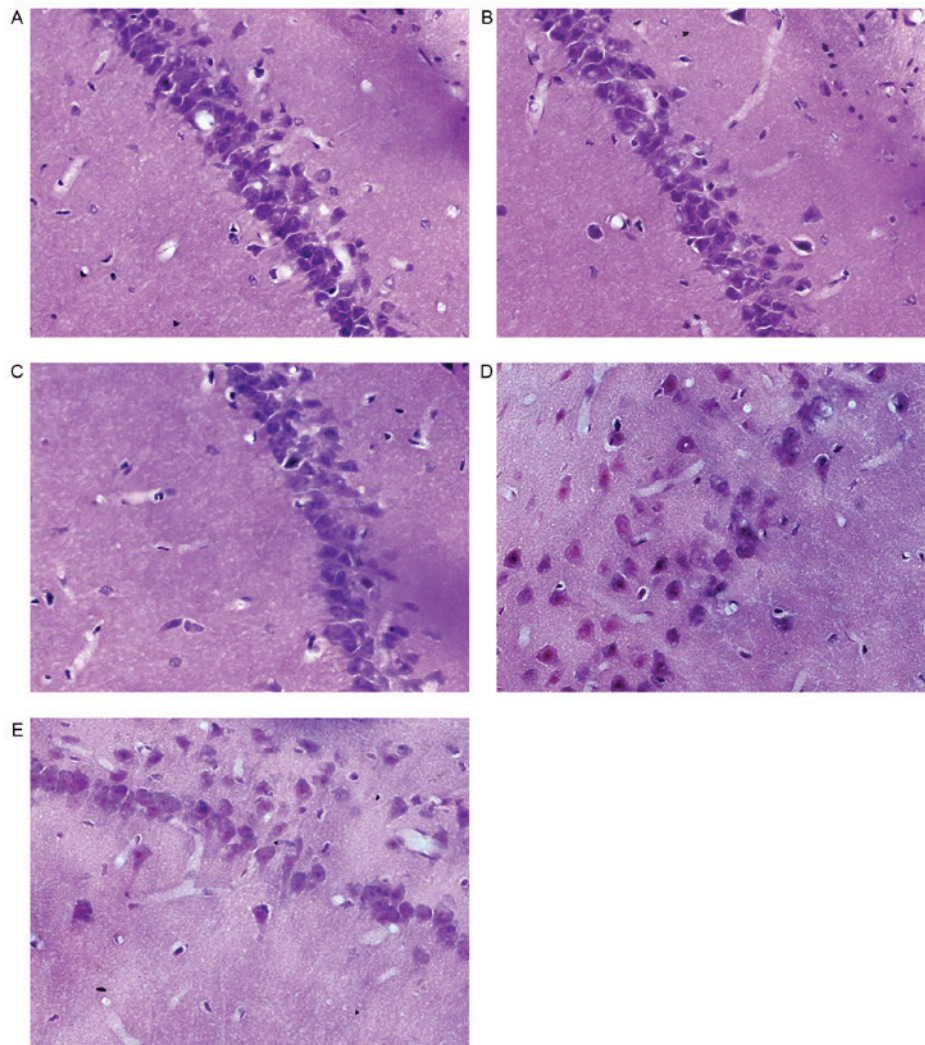


Figure 2. Histological alterations in the hippocampus CA1 region. (A) Normal group; (B) sham group; (C) PGE₁ group; (D) saline group; (E) PGE₁+SU5416 group (n=5 for each group). Brain coronal sections were stained with hematoxylin and eosin staining and observed immediately using a confocal microscope (magnification, x400). Cells in D and E were sparse and disorganized, and partial cell nuclei became pyknotic, karyorrhectic and karyolytic. PGE₁, prostaglandin E₁.

and less severity in CBF reduction than 4-VO methods, the rat BCCAO model results in cognitive impairments similar to those seen in clinical VCI patients, thus has been considered the most common and well-established paradigm (25). In the present study, the rat BCCAO method was employed. The data demonstrated that rats in saline control group performed significantly worse in WMW trial 4 weeks following surgery, which suggested impaired spatial learning capacity. The MWM trial is a test of learning and memory in rodents with primarily spatial/visual cues, and aversive motivation. In the majority of studies, water maze performance reflected spatial learning and memory, which is associated with hippocampal integrity (24). Simultaneously, the authors reported that the hippocampus CA1 was sparse with less neurons in the saline group. The CA1 subfield in the hippocampus was intricately involved in the acquisition and maintaining of learning and memory function and specific susceptible to both risk of ischemia and hypoperfusion, hence hippocampal CA1 region may be likely damaged in a cerebral hypoperfusion state and disturb the formation of cognitive function (26). Altogether, the results confirmed that rat BCCAO method may impair

learning and memory function as well as promote neuronal loss, which was consistent with a former study (27).

Previous evidence has also demonstrated that chronic cerebral hypoperfusion following BCCAO in rats induced deficits in spatial learning and memory and hippocampus damage (28). Qu *et al* (29) argued that BCCAO method damaged both the hippocampus dependent spatial learning and memory as assessed by MWM test, cortex-dependent working memory as assessed by the delayed alternation task, and contextual memory as assessed using the step-through passive avoidance task (29). Furthermore, learning and memory impairment may be apparent in MWM tasks even at 7 days post-surgery (30). Some hippocampal pathological changes appeared from 4 weeks, primarily with loss of CA1 neurons, axons and activation of astrocytes with increased astrocyte density. In addition, the vasculopathy can also be discovered 12 months following occlusion (25).

PGE₁, an important member of the prostaglandin family, is a product of cyclooxygenase-mediated arachidonic acid metabolism. PGE₁-induced improvement in circulation has been recognized as an effective treatment for skin ulcers

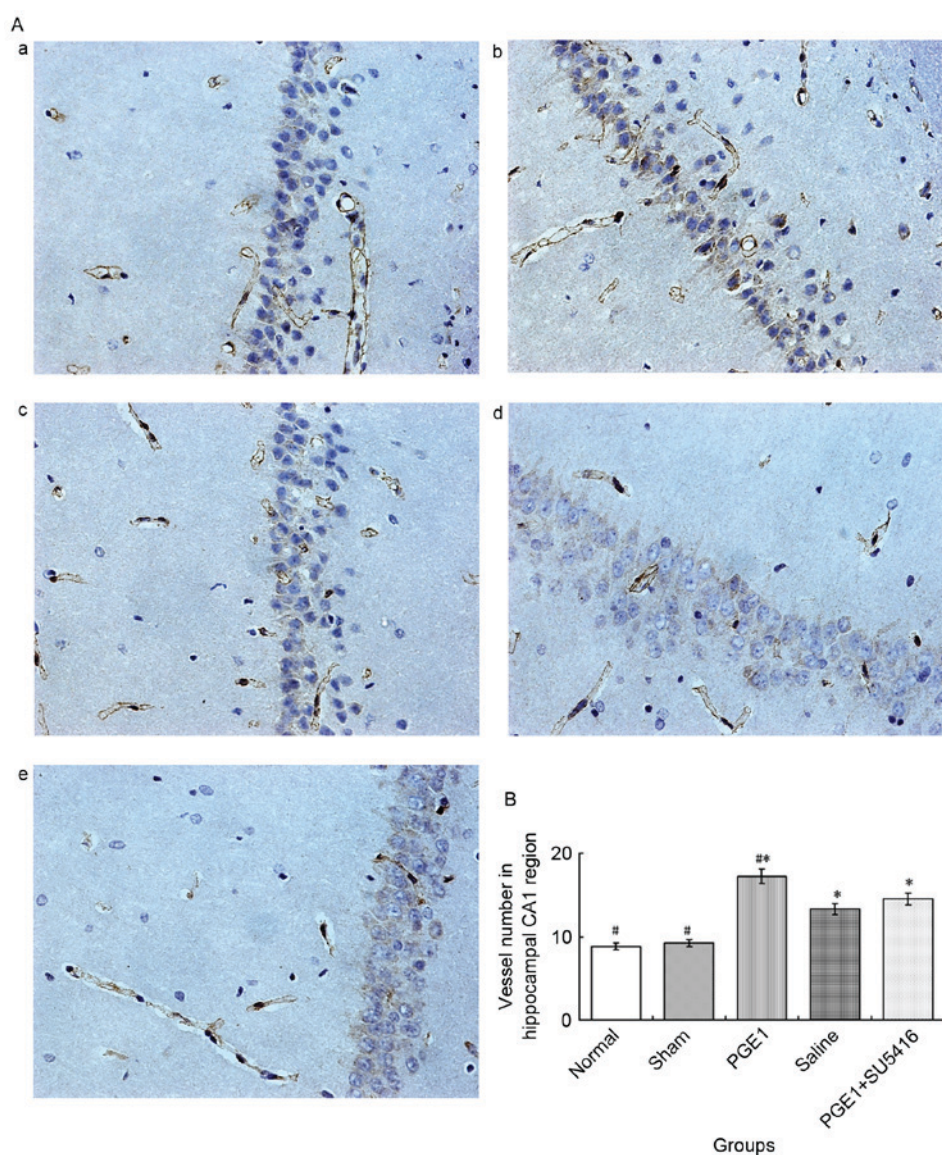


Figure 3. Blood vessels staining of different groups in the hippocampal CA1 region. (A) Blood vessels stained using anti-laminin antibody (n=5 for each group). (A-a) Normal group; (A-b) sham group; (A-c) PGE₁ group; (A-d) Saline group; (A-e) PGE₁+SU5416 group. Blood vessels were stained using a laminin antibody and observed immediately using a confocal microscope (magnification, x400). (B) Blood vessels number in the hippocampus. *P<0.01 vs. saline group. **P<0.05 vs. saline and PGE₁+SU5416 groups. PGE₁, prostaglandin E₁.

and arteriosclerosis obliterans through vasodilation. It was identified that PGE₁ reduced ischemic region in mouse ears, enhanced mature capillary vessel formation by facilitating VEGF expression (31), bridged chronic heart failure patients for heart transplantation because of its actions on neovascularization (14) and augmented cerebral blood perfusion (32). Additionally, a previous study of the authors confirmed that lipo-PGE₁ could reduce infarction volume and accelerate neuronal function recovery through angiogenesis at a dosage of 10 μ g/kg/d (13). Furthermore, Sheng *et al* (33) declared that both lipo-PGE₁ dosage of 22.6 nmol/kg (~8 μ g/kg) and 45.2 nmol/kg (~16 μ g/kg) could reduce infarct volume and exerted neuroprotective effects in a rat model of cerebral ischemia compared with control group, but there are no studies concerning the effect of lipo-PGE₁ on cognitive impairment rat model until now, as well as the accurate dosage managed in VCI rats. Considering the authors' previous study and other studies

focusing on the effect and the dosage of PGE₁, alprostadil (lipo-PGE₁), at a dosage of 10 μ g/kg/d was used to research the effect of lipo-PGE₁ on VCI. Alprostadil (lipo-PGE₁), whose primary active ingredient is PGE₁, is a vasoactive agent for venous injection with a lipid microspheres as the drug carrier. It has the advantage of a long half-time period, control release, less side-effects, as well as tendency to vascular lesion compared with PGE₁ powder. In the present study, the data demonstrated that hippocampus-based learning and memory capacity was improved in the lipo-PGE₁ group as well as more vessel numbers and less neuron loss in the hippocampus CA1 region, contrasted with the saline group and PGE₁+VEGFR antagonist. To the best of the authors' knowledge, the current study's results confirmed that lipo-PGE₁ protected VCI rats against chronic ischemic hypoxic damage, which was likely associated with its beneficial role in promoting angiogenesis, considering the corresponding increase of vascular density in

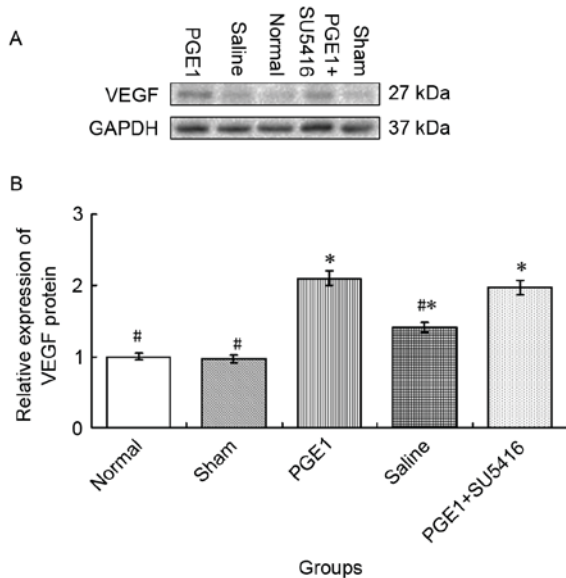


Figure 4. VEGF protein expression of different groups in the hippocampus. (A) VEGF expression of hippocampal tissues was detected by western blot analysis. GAPDH acted as a loading control (n=5 for each group). (B) Relative expression of VEGF protein in hippocampus. *P<0.01 vs. saline group. **P<0.05 vs. PGE₁ and PGE₁+SU5416 groups. VEGF, vascular endothelial growth factor; PGE₁, prostaglandin E₁.

the hippocampus. However, the neuroprotective and proangiogenic effects may be hampered by the VEGFR2 (Flk-1) antagonist, SU5416, which acted through specifically blocking the combination of VEGF and Flk-1 (34).

The underlying mechanism of angiogenesis may be associated with VEGF expression, which is an important stimulator of neovascularization. Among the VEGF family, VEGFA is essential during both the developmental period and adulthood of angiogenesis. VEGFA-induced signal transduction is mediated by the activation of two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR, flk-1). However, its vasorelaxation and angiogenesis effects are primarily mediated by flk-1. In addition to its role in stimulating neovasculature, VEGFA also contributes to maturation of newly formed capillary tubes (35) and enhancement of vascular perfusion (9). The current study illustrated that both VEGF protein and VEGF mRNA expressions were increased in groups treated with lipo-PGE₁ and PGE₁+SU5416. Although VEGF expression was not influenced by SU5416, the blood vessel density was lower in the PGE₁+SU5416 group compared with the PGE₁ group, indicating that lipo-PGE₁-induced angiogenesis may be inhibited by SU5416 through preventing VEGFA-KDR interaction.

Furthermore, lipo-PGE₁ may exert additional neuroprotective effects on neural restoration through promoting VEGF expression. The probable mechanisms involve VEGFR existing in both endothelial cells and other cells, such as neurons. VEGF may exert beneficial effects on endothelial cells by combining to the corresponding receptor. The presence of microvascular endothelial cells is crucial for newly formed neuron survival through secretion of growth factors such as brain-derived neurotrophic factor (BDNF), which is involved in neurogenesis besides learning and memory

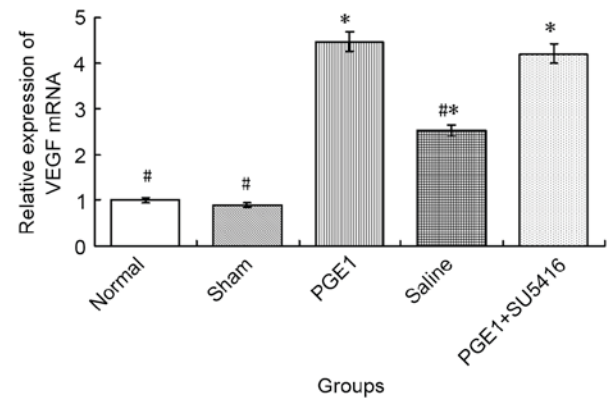


Figure 5. Relative expression of VEGF mRNA in the hippocampus. *P<0.01 vs. saline group. **P<0.01 vs. PGE₁ and PGE₁+SU5416 groups (n=5 for each group). VEGF, vascular endothelial growth factor; PGE₁, prostaglandin E₁.

improvement (36). However, VEGF may bind to VEGFR expressed by neurons and protect them against stress and oxidative damage directly (37). Secondly, VEGF has been reported to stimulate neurogenesis in both the subependymal zone and hippocampus, which may lead to improvement in learning and memory deficits in a manner distinct from increased angiogenesis in hippocampus (38). In addition, reduced VEGF expression negatively affects brain repair accompanied by notable apoptosis (39). Thirdly, nerves and blood vessels are structurally intertwined and share several developmental features and cell signaling pathways, such as the navigation of classic axon guidance cues in blood vessels and VEGF signaling in neurons (40). Interactions between cerebral endothelium and neuronal precursor cells enable angiogenesis and neurogenesis in normal growth and following ischemic injury. Thus, lipo-PGE₁ may provide new approaches for CCH-induced neurodegenerative disease by facilitating the neuroprotective effect of the endothelium.

However, further studies are warranted to study more neuroprotective mechanisms of PGE₁ in VCI, such as trials to measure CBF following lipo-PGE₁ treatment, and trials to evaluate whether lipo-PGE₁ facilitates neurogenesis and promotes neurovascular factors such as BDNF expression in VCI rats.

In conclusion, the present study demonstrated that lipo-PGE₁ protected VCI rats against chronic ischemic injury, alleviated pathological lesions and reduced cognitive impairment. The mechanism involved may be associated with angiogenesis mediated by lipo-PGE₁ via the VEGF/VEGFR pathway, suggesting that lipo-PGE₁ may have therapeutic potential in VCI management.

Acknowledgements

The current study was supported by the Science and Technology Bureau Foundation of Guangzhou (grant no. 2014J4100077) and the Medical Science and Technology Research Foundation of Guangdong (grant no. B2016018). The authors are grateful to the staff of Traumatic Surgery Institute in Guangzhou Red Cross Hospital and the Sun Yat-Sen University of Medical Sciences (Guangzhou, China).

References

1. Fratiglioni L, Launer LJ, Andersen K, Breteler MM, Copeland JR, Dartigues JF, Lobo A, Martinez-Lage J, Soininen H and Hofman A: Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. *Neurology* 54 (11 Suppl 5): S10-S15, 2000.
2. Chan KY, Wang W, Wu JJ, Liu L, Theodoratou E, Car J, Middleton L, Russ TC, Deary IJ, Campbell H, *et al*: Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990-2010: A systematic review and analysis. *Lancet* 381: 2016-2023, 2013.
3. Østergaard L, Engedal TS, Moreton F, Hansen MB, Wardlaw JM, Dalkara T, Markus HS and Muir KW: Cerebral small vessel disease: Capillary pathways to stroke and cognitive decline. *J Cereb Blood Flow Metab* 36: 302-325, 2016.
4. Liu H and Zhang J: Cerebral hypoperfusion and cognitive impairment: The pathogenic role of vascular oxidative stress. *Int J Neurosci* 122: 494-499, 2012.
5. Helman AM and Murphy MP: Vascular cognitive impairment: Modeling a critical neurologic disease in vitro and in vivo. *Biochim Biophys Acta* 1862: 975-982, 2016.
6. Lu H, Wang Y, He X, Yuan F, Lin X, Xie B, Tang G, Huang J, Tang Y, Jin K, *et al*: Netrin-1 hyperexpression in mouse brain promotes angiogenesis and long-term neurological recovery after transient focal ischemia. *Stroke* 43: 838-843, 2012.
7. Wegener S, Gottschalk B, Jovanovic V, Knab R, Fiebach JB, Schellinger PD, Kucinski T, Jungehülsing GJ, Brunecker P, Müller B, *et al*: Transient ischemic attacks before ischemic stroke: Preconditioning the human brain? A multicenter magnetic resonance imaging study. *Stroke* 35: 616-621, 2004.
8. Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH and Hermann DM: The phosphatidylinositol-3 kinase/Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. *FASEB J* 20: 1185-1187, 2006.
9. Ferrara N: Vascular endothelial growth factor: Basic science and clinical progress. *Endocr Rev* 25: 581-611, 2004.
10. Schror K and Hohlfield T: Mechanisms of anti-ischemic action of prostaglandin E1 in peripheral arterial occlusive disease. *Vasa* 33: 119-124, 2004.
11. Fang W, Li H, Zhou L, Su L, Liang Y and Mu Y: Effect of prostaglandin E1 on TNF-induced vascular inflammation in human umbilical vein endothelial cells. *Can J Physiol Pharmacol* 88: 576-583, 2010.
12. Tang J, Hua Y, Su J, Zhang P, Zhu X, Wu L, Niu Q, Xiao H and Ding X: Expression of VEGF and neural repair after alprostadil treatment in a rat model of sciatic nerve crush injury. *Neurol India* 57: 387-394, 2009.
13. Ling L, Zhang S, Ji Z, Huang H, Yao G, Wang M, He R, Deng W and Fang L: Therapeutic effects of lipo-prostaglandin E1 on angiogenesis and neurogenesis after ischemic stroke in rats. *Int J Neurosci* 126: 469-477, 2016.
14. Inoue H, Aihara M, Tomioka M and Watabe Y: Specific enhancement of vascular endothelial growth factor (VEGF) production in ischemic region by alprostadil-potential therapeutic application in pharmaceutical regenerative medicine. *J Pharmacol Sci* 122: 158-161, 2013.
15. Zhang ZH, Shi GX, Li QQ, Wang YJ, Li P, Zhao JX, Yang JW and Liu CZ: Comparison of cognitive performance between two rat models of vascular dementia. *Int J Neurosci* 124: 818-823, 2014.
16. Paxinos G and Franklin K (eds): Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates. 4th edition. Academic Press, San Diego, CA, 2001.
17. Fujita Y, Ihara M, Ushiki T, Hirai H, Kizaka-Kondoh S, Hiraoka M, Ito H and Takahashi R: Early protective effect of bone marrow mononuclear cells against ischemic white matter damage through augmentation of cerebral blood flow. *Stroke* 41: 2938-2943, 2010.
18. Xie W, Zhu D, Ji L, Tian M, Xu C and Shi J: Angiotensin-(1-7) improves cognitive function in rats with chronic cerebral hypoperfusion. *Brain Res* 1573: 44-53, 2014.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
20. Ma C, Wu G, Wang Z, Wang P, Wu L, Zhu G and Zhao H: Effects of chronic sleep deprivation on the extracellular signal-regulated kinase pathway in the temporomandibular joint of rats. *PLoS One* 9: e107544, 2014.
21. Rodríguez García PL and Rodríguez García D: Diagnosis of vascular cognitive impairment and its main categories. *Neurologia* 30: 223-239, 2015 (In English, Spanish).
22. Henke K: A model for memory systems based on processing modes rather than consciousness. *Nat Rev Neurosci* 11: 523-532, 2010.
23. Chen H, Iinuma M, Onozuka M and Kubo KY: Chewing maintains hippocampus-dependent cognitive function. *Int J Med Sci* 12: 502-509, 2015.
24. Jiwa NS, Garrard P and Hainsworth AH: Experimental models of vascular dementia and vascular cognitive impairment: A systematic review. *J Neurochem* 115: 814-828, 2010.
25. Miki K, Ishibashi S, Sun L, Xu H, Ohashi W, Kuroiwa T and Mizusawa H: Intensity of chronic cerebral hypoperfusion determines white/gray matter injury and cognitive/motor dysfunction in mice. *J Neurosci Res* 87: 1270-1281, 2009.
26. Buzsáki G and Moser EI: Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nat Neurosci* 16: 130-138, 2013.
27. Liu HX, Zhang JJ, Zheng P and Zhang Y: Altered expression of MAP-2, GAP-43, and synaptophysin in the hippocampus of rats with chronic cerebral hypoperfusion correlates with cognitive impairment. *Brain Res Mol Brain Res* 139: 169-177, 2005.
28. Xi Y, Wang M, Zhang W, Bai M, Du Y, Zhang Z, Li Z and Miao J: Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion. *Neurobiol Learn Mem* 109: 7-19, 2014.
29. Qu J, Zhou Q, Du Y, Zhang W, Bai M, Zhang Z, Xi Y, Li Z and Miao J: Rutin protects against cognitive deficits and brain damage in rats with chronic cerebral hypoperfusion. *Br J Pharmacol* 171: 3702-3715, 2014.
30. Wang J, Zhang HY and Tang XC: Huperzine A improves chronic inflammation and cognitive decline in rats with cerebral hypoperfusion. *J Neurosci Res* 88: 807-815, 2010.
31. Slevin M, Kumar P, Gaffney J, Kumar S and Krupinski J: Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potential. *Clin Sci (Lond)* 111: 171-183, 2006.
32. Cao M, Ke K, Sun H and Robertson A: Effects of prostaglandin E1 on perihematomal tissue after hypertensive intracerebral hemorrhage. *Acta Neurol Taiwan* 20: 172-181, 2011.
33. Sheng R, Zhang LS, Han R, Gao B, Liu XQ and Qin ZH: Combined prostaglandin E1 and lithium exert potent neuroprotection in a rat model of cerebral ischemia. *Acta pharmacol Sin* 32: 303-310, 2011.
34. Keskin U, Totan Y, Karadağ R, Erdurmuş M and Aydin B: Inhibitory effects of SU5416, a selective vascular endothelial growth factor receptor tyrosine kinase inhibitor, on experimental corneal neovascularization. *Ophthalmic Res* 47: 13-18, 2012.
35. Webber MJ, Tongers J, Newcomb CJ, Marquardt KT, Bauersachs J, Losordo DW and Stupp SI: Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. *Proc Natl Acad Sci USA* 108: 13438-13443, 2011.
36. Zaccagna S, Lambrechts D and Carmeliet P: Neurovascular signalling defects in neurodegeneration. *Nat Rev Neurosci* 9: 169-181, 2008.
37. Störkebaum E, Lambrechts D, Dewerchin M, Moreno-Murciano MP, Appelmanns S, Oh H, Van Damme P, Rutten B, Man WY, De Mol M, *et al*: Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat Neurosci* 8: 85-92, 2005.
38. Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D and During MJ: VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 36: 827-835, 2004.
39. Schänzer A, Wachs FP, Wilhelm D, Acker T, Cooper-Kuhn C, Beck H, Winkler J, Aigner L, Plate KH and Kuhn HG: Direct stimulation of adult neural stem cells in vitro and neurogenesis in vivo by vascular endothelial growth factor. *Brain Pathol* 14: 237-248, 2004.
40. Carmeliet P: Blood vessels and nerves: Common signals, pathways and diseases. *Nat Rev Genet* 4: 710-720, 2003.