Effects of ischemic preconditioning on PDGF-BB expression in the gerbil hippocampal CA1 region following transient cerebral ischemia

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Abstract. Ischemic preconditioning (IPC) is induced by exposure to brief durations of transient ischemia, which results in ischemic tolerance to a subsequent longer or lethal period of ischemia. In the present study, the effects of IPC (2 min of transient cerebral ischemia) were examined on immunoreactivity of platelet-derived growth factor (PDGF)-BB and on neuroprotection in the gerbil hippocampal CA1 region following lethal transient cerebral ischemia (LTCI; 5 min of transient cerebral ischemia). IPC was subjected to a 2-min sublethal ischemia and a LTCI was given 5-min transient ischemia. The animals in all of the groups were given recovery times of 1, 2 and 5 days and change in PDGF-BB immunoreactivity was examined as was the neuronal damage/death in the hippocampus induced by LTCI. LTCI induced a significant loss of pyramidal neurons in the hippocampal CA1 region 5 days after LTCI, and significantly decreased PDGF-BB immunoreactivity in the CA1 pyramidal neurons from day 1 after LTCI. Conversely, IPC effectively protected the CA1 pyramidal neurons from LTCI and increased PDGF-BB immunoreactivity in the CA1 pyramidal neurons post-LTCI. In conclusion, the results demonstrated that LTCI significantly altered PDGF-BB immunoreactivity in pyramidal neurons in the hippocampal CA1 region, whereas IPC increased the immunoreactivity. These findings indicated that PDGF-BB may be associated with IPC-mediated neuroprotection.

Introduction

Ischemic preconditioning (IPC) is induced by exposure to brief durations of transient ischema, resulting in ischemic tolerance to a subsequent longer or lethal period of transient ischemia (1). Kitagawa *et al* (2) initially introduced the concept of ischemic tolerance in the brain, and demonstrated that 2 min of transient cerebral ischemia, 1-2 days prior to a subsequent longer or lethal period of transient cerebral ischemia, exerted protective effects against ischemic injury in the gerbil hippocampal CA1 region. Further studies have demonstrated the protective effects of IPC in other animal models, including global and focal cerebral ischemia (1,3-5). Ischemic tolerance has also been demonstrated in human clinical practice; less severe strokes occured in patients that suffered from a prior transient ischemic attack within a short period of time (6,7). IPC-induced neuroprotection is considered a promising target for the development of a potential therapeutic strategy; however, the basic mechanisms underlying IPC-induced neuroprotection remain to be elucidated (1).

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Platelet-derived growth factor (PDGF) is a polypeptide that acts as a potent mitogen in several cell types, which consists of two homodimers (AA and BB) and one heterodimer (AB) (8,9). The biological activities of PDGF are mediated through binding to PDGF receptors (PDGFR), designated α and β. The α subunit can bind to either the -A or -B chain, whereas the β subunit can bind only to the-B chain (10-12). PDGF-BB is widely expressed in the central nervous system (13) and is upregulated in neurons following cerebral ischemia in animal models (14-17). Suppression of PDGF-BB mRNA expression enhances N-methyl-D-aspartate (NMDA)-induced excitotoxicity in the neonatal rat brain (18). Conversely, the intraventricular administration of PDGF-BB markedly promotes neuronal survival following cerebral ischemia in rats (19). These findings suggest that PDGF-BB may serve a crucial role in the protection of neurons against cerebral ischemic insults.

To the best of our knowledge, the expression pattern of endogenous PDGF-BB in the IPC-mediated hippocampus, following subsequent transient cerebral ischemia, has not been studied. Therefore, the present study aimed to investigate the effects of IPC on cellular localization and alterations in endogenous PDGF-BB in the IPC-induced hippocampus. The hippocampus is an important structure for the study of neuronal damage and its mechanism following transient cerebral ischemia, and the gerbil is considered a good animal for studying transient cerebral ischemia (20-23).

Materials and methods

Experimental groups and ischemic surgery. As previously described (20), 102 male gerbils were obtained from the Experimental Animal Center, Kangwon National University, Chuncheon, South Korea. The gerbils were 6 months old with a body weight of 65-75 g. The animals were housed in a conventional state under adequate temperature (23°C) and humidity (60%) control with a 12-h light/dark cycle, and provided with free access to water and food. The gerbils were used according to guidelines that are in compliance with the current international laws and policies (24). The present study was approved by the Institutional Animal Care and Use Committee at Kangwon National University (Chuncheon, South Korea; approval no. KW-160802-1).

The gerbils were divided into four groups (n=7 at each time point: 0, 1, 2 and 5 days in each group): i) Sham-operated group, both common carotid arteries were exposed; however, the gerbils were not exposed to ischemia (sham-operation); ii) ischemia-operated group was exposed to 5 min of transient cerebral ischemia (lethal transient cerebral ischemia, LTCI); iii) IPC + sham-operated group was subjected to 2 min sublethal transient ischemia prior to sham-operation; and iv) IPC + ischemia-operated group was subjected to 2 min of sublethal ischemia 1 day prior to 5 min of transient ischemia. The IPC paradigm has been proven to be effective at protecting neurons against ischemic damage in this ischemic model (25). The gerbils in the ischemia-operated and the IPC + ischemia-operated groups were given recovery times of 1, 2 and 5 days, since pyramidal neurons in the hippocampal CA1 region survive until 3 days and begin to die 4-5 days post-LTCI.

Surgery for ischemic insults. IPC and LTCI were developed according to our previously described method (20). Briefly, the experimental animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. Ischemia was induced by occluding the bilateral common carotid arteries with non-traumatic aneurysm clips (Yasargil FE 723K; Aesculap AG, Tuttlingen, Germany). After 2 or 5 min of occlusion, the aneurysm clips were removed from the arteries. The body temperature under free-regulating or normothermic (37±0.5°C) conditions was monitored using a rectal temperature probe (TR-100; Fine Science Tools, Foster City, Inc., CA, USA) and was maintained using a thermometric blanket prior to, during and after surgery until the animals completely recovered from anesthesia. Thereafter, the gerbils were maintained in a thermal incubator (temperature, 23°C; humidity, 60%; Mirae Medical Industry, Seoul, South Korea) to maintain the body temperature of animals until they were sacrificed at 1, 2 and 5 days following ischemia.

Spontaneous locomotor activity. In order to elucidate increased hyperactivity following ischemia-reperfusion (I-R), spontaneous locomotor activity was measured, according to a previously published procedure (26). Briefly, gerbils (n=7 at each time point in each group) were maintained in a Plexiglas cage (25x20x12 cm), located inside a soundproof chamber. Locomotor activity was recorded using a Photobeam Activity system-Home Cage (San Diego Instruments, San Diego, CA, USA). Spontaneous locomotor activity was monitored during 1 h, a total of 24 h after I-R and, simultaneously, the number of times each animal stood on its hind legs and the time (in sec) spent exhibiting grooming behavior were recorded. Each animal was observed continuously via a 4x8 photobeam. Scores were generated from live observations, and video sequences were used for subsequent re-analysis.

Tissue processing for histology. According to our previously published procedure (27), the gerbils were deeply anesthetized with pentobarbital sodium (40 mg/kg, i.p; JW Pharmaceutical Co., Ltd., Seoul, South Korea,) and were transcardially perfused with 4% paraformaldehyde. The brains were removed and the tissues cryoprotected by infiltration with 30% sucrose overnight at 4°C. Subsequently, their brains were serially sectioned into 30 µm coronal sections using a cryostat (Leica Microsystems GmbH, Wetzlar, Germany).

Cresyl violet (CV) staining and Fluoro-Jade B (F-J B) histofluorescence. To investigate neuronal damage in the hippocampus following I-R, CV and F-J B histofluorescence staining were performed, as previously described (28). Briefly, for CV staining, the sections were stained with 1.0% (w/v) CV acetate (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and dehydrated. The section were then mounted with Canada balsam (Kanto Chemical Co., Inc., Tokyo, Japan). For F-J B histofluorescence, the sections were immersed in a 0.0004% F-J B (Histochem, Inc., Jefferson, AR, USA) staining solution. After washing, the sections were examined using an epifluorescent microscope (Zeiss Carl, Göttingen, Germany) with blue (450-490 nm) excitation light and a barrier filter.

Immunohistochemistry for neuronal nuclei (NeuN) and PDGF-BB. For immunohistochemical staining, the sections were analyzed according to our previously described procedure (28). The brain sections were blocked with 10% normal goat serum (cat. no. S-1000; Vector Laboratories Inc., Burlingame, CA, USA) in 0.05 M PBS followed by staining with primary mouse anti-NeuN (a neuron-specific soluble nuclear antigen; cat. no. 574597; 1:1,000; EMD Millipore, Billerica, MA, USA) and rabbit anti-PDGF-BB (cat. no. ab21234; 1:200; Abcam, Cambridge, MA, USA) overnight at 4°C. The sections were then incubated with secondary antibodies (cat. no. I-1000; 1:250; Vector Laboratories Inc.) and were developed using Vectastain ABC (Vector Laboratories Inc.). The sections were visualized with 3,3'-diaminobenzidine (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in 0.1 M Tris-HCl buffer. In order to establish the specificity of the immunostaining, a negative control test was carried out with pre-immune serum, instead of primary antibody. The negative control resulted in the absence of immunoreactivity in any structures.

Western blot analysis. To examine alterations in the protein expression levels of PDGF-BB in the hippocampal CA1 region, western blotting was conducted, according to our previously published procedure (28). Briefly, tissues (n=7 at each time point) were homogenized, and the protein concentrations were determined in the supernatants using a Micro Bicinchoninc Acid Protein Assay kit with bovine serum albumin as the standard (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Aliquots containing 20 μg total protein were boiled and loaded onto a 12.5% polyacryamide gel. After electrophoresis, the gels were transferred to nitrocellulose transfer membranes (Pall Corporation, Port Washington, NY, USA). To reduce background staining, the membranes were incubated with 5% non-fat dry milk for 30 min at 4°C prior to incubation with rabbit anti-PDGF-BB (cat. no. ab178409; 1:1,000; Abcam) for 2 hrs at 4°C. Membranes were subsequently exposed to peroxidase-conjugated goat anti-rabbit immunoglobulin G (cat. no. A0545; 1:5,000; Sigma-Aldrich; Merck KgaA). Antibodies were visualized using an enhanced chemiluminescence kit (Pierce; Thermo Fisher Scientific, Inc.). Antibodies against β-actin were used as a loading control (cat. no. ab8227; 1:2,000; Abcam).

Data analysis. For cell counts, as previously described (20), sections were selected according to anatomical landmarks corresponding to anterior-posterior, from -1.4 to -1.8 mm of gerbil brain atlas. The number of NeuN-immunoreactive and F-J B-positive cells was counted in a 200x200 μm square, applied at the approximate center of the CA1 region, including the stratum pyramidale. Cell counts were analyzed as a percentage, with the sham group designated as 100%.

In order to analyze PDGF-BB immunoreactivity, we used our previously published method (28). Briefly, cellular immunoreactivity of PDGF-BB was graded in the hippocampal CA1 and CA3 regions. Digital images were captured using an AxioM1 light microscope (Zeiss Carl) equipped with a digital camera (Axiocam; Zeiss Carl) connected to a PC monitor. Semi-quantification of the immunoreactivity of PDGF-BB was evaluated using digital image analysis software (MetaMorph 4.01; Molecular Devices, LLC, Sunnyvale, CA, USA). The
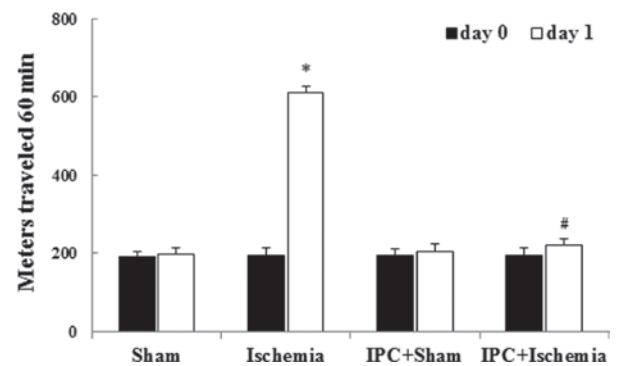

Figure 1. Locomotor activity in the sham-operated, ischemia-operated, IPC + sham-operated and IPC + ischemia-operated groups at days 0 and 1 after lethal transient cerebral ischemia. Spontaneous locomotor activity is significantly reduced in the IPC + ischemia-operated group compared with in the ischemia-operated group (n=7/group); *P<0.05, vs. the sham-operated group; #P<0.05, vs. the ischemia-operated group. Data are presented as the mean ± standard error of the mean. IPC, ischemic preconditioning.

staining intensity of PDGF-BB was evaluated on the basis of optical density (OD), which was obtained following transformation of the mean gray level using the formula: OD = log (256 / mean gray level). The background OD was subtracted from areas adjacent to the measured area. After the background density was subtracted, a ratio of the OD of an image file was calibrated in Adobe Photoshop 8.0 (Adobe Systems, San Jose, CA, USA) and was analyzed as a percentage, with the sham-operated group designated as 100% in NIH Image 1.59 (National Institutes of Health, Bethesda, MD, USA).

Protein expression levels were analyzed, according to our previously published method (29). Briefly, western blots were scanned and semi-quantification was conducted using Scion Image software version 2.0 (Scion Corp., Frederick, MD, USA), which was used to determine relative OD (ROD): A ratio of the ROD was calibrated as %, with the sham-operated group designated as 100 %.

Statistical analysis. All data are presented as the mean ± standard error of the mean. A multiple-sample comparison was applied to test the differences between groups and days. The differences between groups on the same day were assessed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. For analysis of time-dependent differences between the groups, two-way ANOVA was used with a Bonferroni post hoc test. P<0.05 was considered to indicate a statistically significant difference. All experiments were repeated twice.

Results

Spontaneous motor activity. Spontaneous motor activity was detected to investigate the alterations in motor behavior at day 0 (before ischemia) and day 1 (1 day after ischemia; Fig. 1) (30,31). Similar levels of locomotor activity were observed in all experimental groups on day 0. In the ischemia-operated group, locomotor activity was evidently increased compared with that in the sham-operated group 1 day after LTCI. In the IPC + ischemia-operated group, locomotor activity was not significantly increased
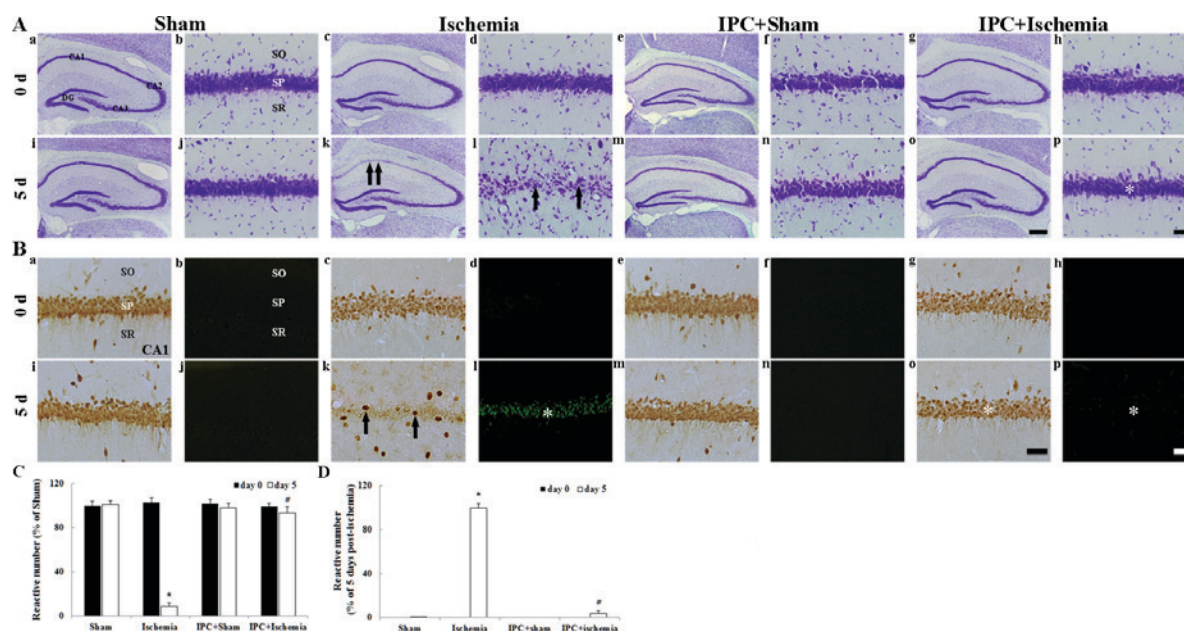
Figure 2. (A) CV, (B) NeuN and F-J B staining were conducted. (Aa-Ap) CV staining, (Ba, Bc, Be, Bg, Bi, Bk, Bm and Bo) NeuN immunohistochemistry and (Bb, Bd, Bf, Bh, Bj, Bl, Bn and Bp) F-J B histofluorescence staining in the hippocampus of sham-operated, ischemia-operated, IPC + sham-operated and IPC + ischemia-operated groups. In the ischemia-operated group, CV+ cells (arrows in Ak and Al) were damaged in the SP of the CA1 region 5 days after lethal transient cerebral ischemia. In addition, at this time point, few NeuN+ (arrows in Bk) and numerous F-J B+ (asterisk in Bl) cells were detected in the SP. However, the distribution of CV+ (asterisk in Ap), NeuN+ (asterisk in Bo) and F-J B+ (asterisk in Bp) cells in the IPC + ischemia-operated group was similar to that in the sham-operated group. Scale bar: (Aa, Ac, Ae, Ag, Ai, Ak, Am and Ao) 800 μm; (Ab, Ad, Af, Ah, Aj, Al, An, Ap and Ba- Bp) 50 μm. Quantitative analyses of (C) NeuN+ and (D) F-J B+ cells in all groups. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. the sham-operated group; #P<0.05 vs. the ischemia-operated group. SO, stratum oriens; SR, stratum radiatum; SP, stratum pyramidale; IPC, ischemic preconditioning; CV, cresyl violet; NeuN, neuronal nuclei; F-J B, Fluoro-Jade B.

compared with that in the IPC + sham-operated group 1 day after LTCI. Previous studies have demonstrated that hippocampal neuronal damage leads to locomotor hyperactivity (29,30).

CV+ cells. CV staining was used to determine the distribution of all cells in the hippocampus. In all experimental groups on day 0 after LTCI, CV+ cells were easily observed in all subregions of the hippocampus (Fig. 2Aa-Ah). In particular, pyramidal neurons in the stratum pyramidale were identified by their larger and pyramid-like or round shape (Fig. 2Ab, Ad, Af and Ah). In the ischemia-operated group, CV+ pyramidal neurons were markedly decreased in number in the CA1 region, but not in the CA2/3 region, 5 days after LTCI compared with in the sham-operated group (Fig. 2Ak, Al); at a high magnification, damaged CA1 pyramidal neurons were shrunken and contained dark and polygonal nuclei (Fig. 2Al). In the IPC + sham-operated group, the distribution pattern of CV+ cells in the CA1 region was similar to that in the sham-operated group (Fig. 2Am and An). In the IPC + ischemia-operated group, the morphology of CV+ pyramidal neurons in the CA1 region was similar to that in the IPC + sham-operated group (Fig. 2Ao and Ap).

NeuN+ and F-J B+ cells. IPC-mediated neuroprotection in the CA1 region was detected using anti-NeuN immunohistochemistry and F-J B histofluorescence staining (Fig. 2B). In all experimental groups on day 0 after LTCI, pyramidal neurons in the CA1 region were well stained with NeuN (Fig. 2Ba, Bc, Be, Bg and C) and no F-J B+ cells were observed (Fig. 2Bb, Bd,

Bf, Bh and D). In the ischemia-operated group, a significant loss of NeuN+ neurons, alongside numerous F-J B+ cells, was observed in the stratum pyramidale of the CA1 region 5 days after LTCI (Fig. 2Bk, C and D).

At day 5 following LTCI, in the IPC + sham-operated group, the pattern of NeuN+ CA1 pyramidal neurons was similar to that in the sham-operated group (Fig. 2Bm and C) and no F-J B+ cells were detected (Fig. 2Bn and D). The distribution of NeuN+ and F-J B+ cells in the stratum pyramidale of the IPC + ischemia-operated group was similar to that in the IPC + sham-operated group (Fig. 2Bo, Bp, C and D).

PDGF-BB immunoreactivity: CA1 region. PDGF-BB immunoreactivity in the CA1 region was detected by immunohistochemistry (Fig. 3). PDGF-BB immunoreactivity was easily detected in pyramidal neurons in the stratum pyramidale of the CA1 region in the sham-operated groups (Fig. 3A, E, I, M and Q). In the ischemia-operated group, PDGF-BB immunoreactivity began to be decreased in the CA1 pyramidal neurons from 1 day after LTCI and was hardly observed in CA1 pyramidal neurons 5 days after LTCI (Fig. 3B, F, J, N and Q).

In the IPC + sham-operated group, PDGF-BB immunoreactivity in CA1 pyramidal neurons was similar to that in the sham-operated group (Fig. 3C, G, K, O and Q). In the IPC + ischemia-operated group, PDGF-BB immunoreactivity in CA1 pyramidal neurons was not altered after LTCI compared with in the IPC + sham-operated group (Fig. 3D, H, L, P and Q).

PDGF-BB immunoreactivity: CA3 region. PDGF-BB immunoreactivity in the CA3 region was detected by
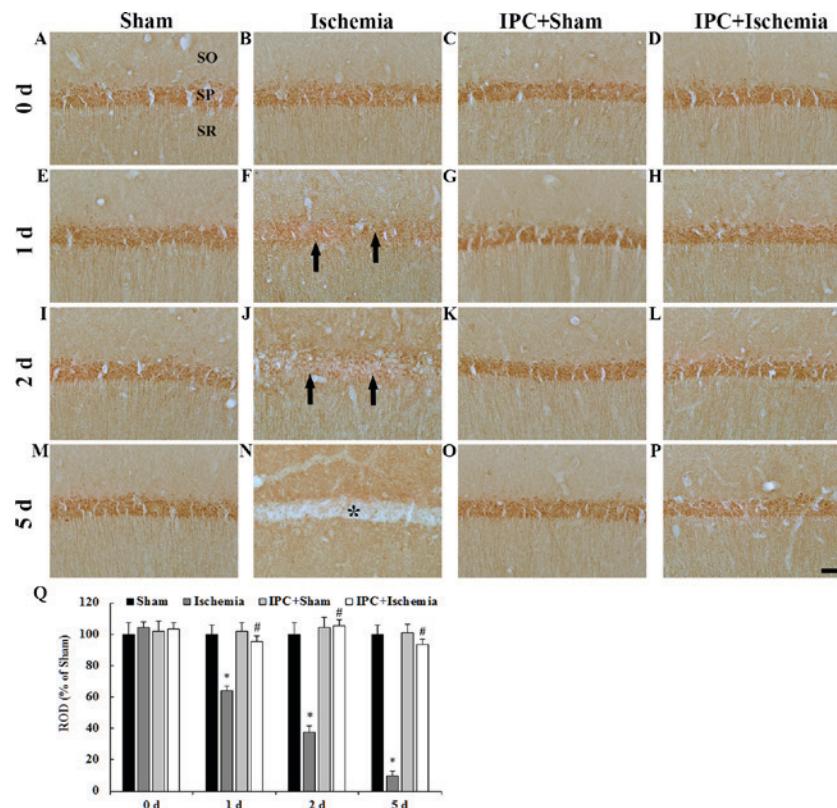
Figure 3. (A-P) PDGF-BB immunohistochemistry in the CA1 region of the (A, E, I and M) sham-operated, (B, F, J and N) ischemia-operated, (C, G, K and O) IPC + sham-operated and (D, H, L and P) IPC + ischemia-operated groups. In the ischemia-operated group, PDGF-BB immunoreactivity was markedly decreased in the SP (arrows in F, J) at 1 day post-ischemia. PDGF-BB immunoreactivity was barely detected in pyramidal neurons (asterisk in N) of the CA1 region 5 days after LTCI. In the IPC + sham- and IPC + ischemia-operated groups, PDGF-BB immunoreactivity was similar to that in the sham-operated group. Scale bar, 50 μm. (Q) Quantitative analysis of PDGF-BB immunoreactivity in the SP of all groups. The ratio of ROD was calibrated as a %, with the sham-operated group designated as 100%. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. the sham-operated group; #P<0.05 vs. the ischemia-operated group. SO, stratum oriens; SR, stratum radiatum; SP, stratum pyramidale; IPC, ischemic preconditioning; PDGF-BB, platelet-derived growth factor-BB; ROD, relative optical density.

immunohistochemistry (Fig. 4). In the CA3 region of the sham-operated group, PDGF-BB immunoreactivity was detected in neurons of the stratum pyramidale (Fig. 4A, E, I, M and Q). In the ischemia-operated group, PDGF-BB immunoreactivity was not significantly altered in the stratum pyramidale (Fig. 4B, F, J, N and Q). In the IPC + sham-operated group, PDGF-BB immunoreactivity in the stratum pyramidale was slightly increased 0 days after LTCI compared with in the sham-operated group (Fig. 4C); thereafter, the immunoreactivity was similar to that in the sham-operated group until 5 days post-ischemia (Fig. 4G, K, O and Q). In the IPC + ischemia-operated group, Changes in PDGF-BB immunoreactivity in the stratum pyramidale was similar to that in the IPC + sham-operated group (Fig. 4D, H, L, P and Q).

Protein expression levels of PDGF-BB. Western blot analysis indicated that the alterations in PDGF-BB protein expression in the CA1 region post-LTCI were similar to those observed by immunohistochemistry (Fig. 5). In the ischemia-operated group, PDGF-BB protein levels were significantly decreased 2 days after LTCI and the levels were lowest 5 days post-LTCI. In the IPC + sham-operated group, PDGF-BB protein expression was significantly increased compared with in the sham-operated group. In the IPC + ischemia-operated group, PDGF-BB protein levels were not significantly altered following LTCI (Fig. 5).

Discussion

Transient brain ischemia leads to selective damage/death of pyramidal neurons in the hippocampal CA1 region several days after I-R. This neuronal death is commonly referred to as 'delayed neuronal death', since it occurs very slowly for 4-5 days following 5 min of transient brain ischemia, which is a lethal type of ischemia for CA1 pyramidal neurons (27). Conversely, pyramidal neurons in the hippocampal CA3 region are much less vulnerable to ischemic insults (32). The present study examined the delayed neuronal death of CA1 pyramidal neurons using CV histochemistry, NeuN immuno-histochemistry and F-J B histofluorescence.

The results of the present study demonstrated that CA1 pyramidal neurons in the IPC-induced gerbil hippocampus did not die following LTCI. IPC, which is induced by exposure to brief durations of transient ischema, does not induce neuronal damage/death in ischemic areas and prevents ischemic injury following a subsequent longer or lethal transient ischemic insult (33). The first description of IPC in the brain was reported by Kitagawa *et al* (34) in a gerbil model, and similar findings have been reported in rats (35,36) and mice (37). In addition, IPC-mediated neuroprotection has been studied in brain slices (38), as well as in murine cell culture (39). In the present study, brief IPC (2 min of transient ischemia) was used to prevent neuronal death in the hippocampal CA1 region, and this
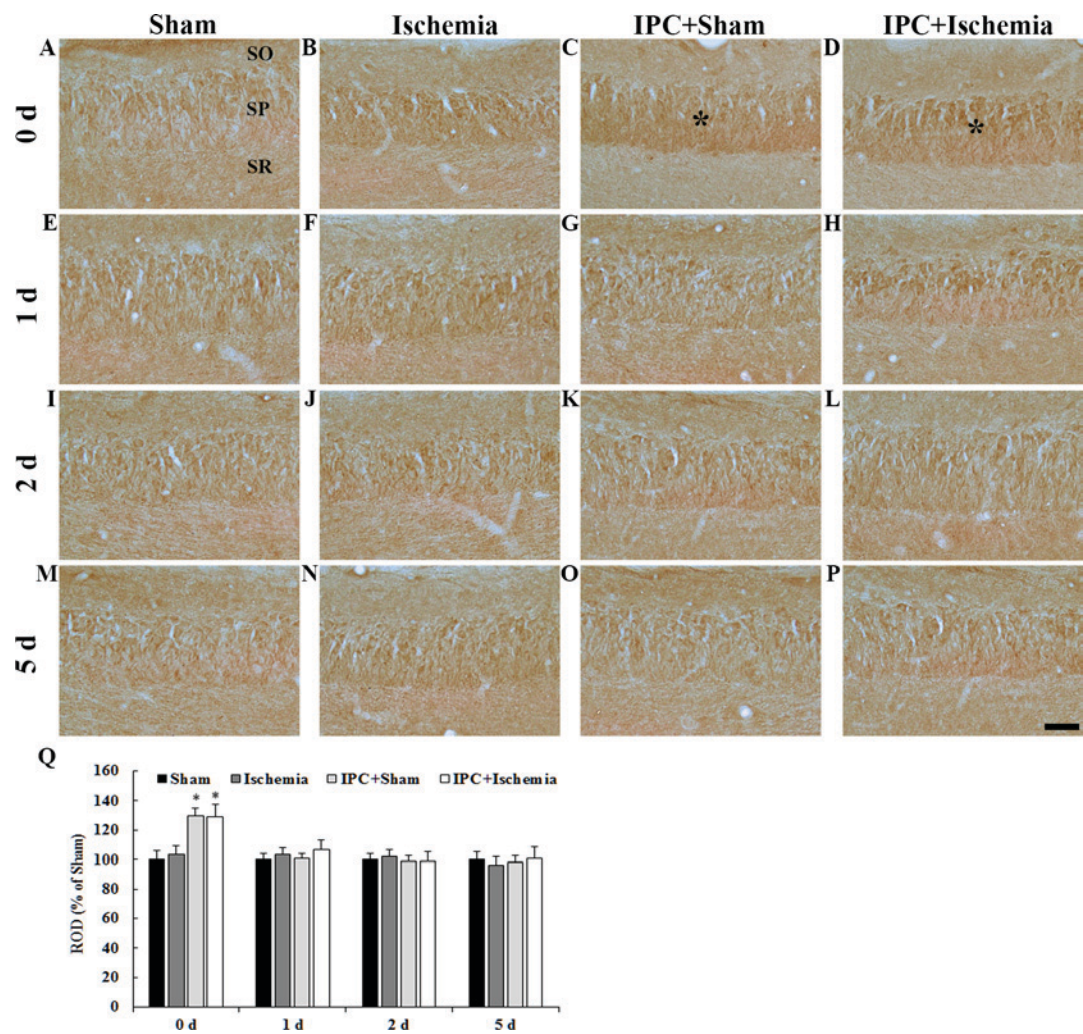
Figure 4. (A-P) PDGF-BB immunohistochemistry in the CA3 region of the (A, E, I and M) sham-operated, (B, F, J and N) ischemia-operated, (C, G, K and O) IPC + sham-operated and (D, H, L and P) IPC + ischemia-operated groups. PDGF-BB immunoreactivity was increased in pyramidal neurons of the SP (asterisks in C and D) at day 0 in the IPC + sham-operated and IPC + ischemia-operated groups. Scale bar=50 µm. (Q) Quantitative analysis of PDGF-BB immunoreactivity in the SP of all groups. The ratio of the ROD was calibrated as a %, with the sham-operated group designated as 100%. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. the sham-operated group. SO, stratum oriens; SR, stratum radiatum; SP, stratum pyramidale; IPC, ischemic preconditioning; PDGF-BB, platelet-derived growth factor-BB; ROD, relative optical density.
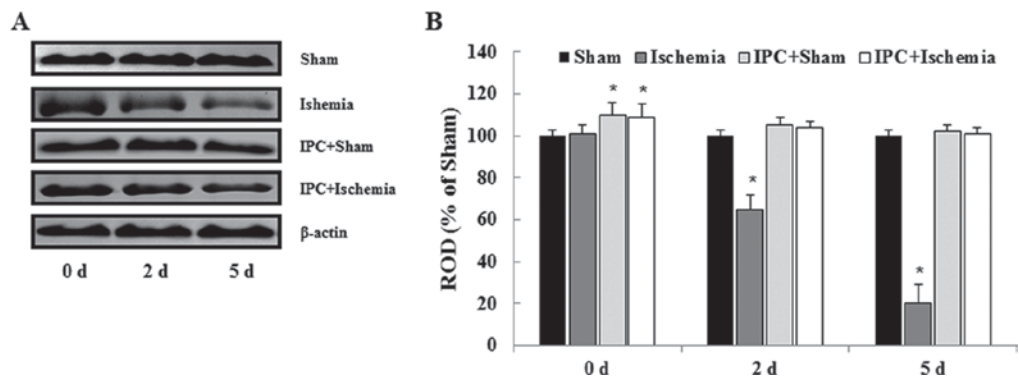

Figure 5. (A) Western blot analysis was conducted to detect PDGF-BB protein expression in the CA1 region. PDGF-BB levels were significantly increased in the IPC + sham-operated and IPC + ischemia-operated groups. (B) ROD values are presented as %, with the sham-operated group designated as 100%. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. the sham-operated group. IPC, ischemic preconditioning; PDGF-BB, platelet-derived growth factor-BB; ROD, relative optical density.

brief IPC stimulus did not induce neuronal damage, as assessed by CV, NeuN and F-J B staining, which is very sensitive to acute neuronal injury (40). The results indicated that CA1 pyramidal neurons exhibited normal features in the IPC-induced gerbil

brain 5 days after LTCI. To the best of our knowledge, although IPC provides marked neuroprotection against ischemic brain injury, its underlying mechanisms require further elucidation for the development of therapeutic strategies for the treatment of ischemic stroke, as the molecular mechanisms underlying IPC-induced ischemic tolerance are not fully understood (1).

It is well known that endogenous PDGF-BB is expressed in neurons (13) and its expression is altered after some brain insults (13,14). In particular, it has been reported that PDGF-BB may be considered a potent neuroprotective factor under cerebral ischemic conditions (14,19,41,42). Previous studies have demonstrated that the application of exogenous PDGF-BB protein may prevent neuronal cell death in the CA1 region after global brain ischemia, and may induce infarct tolerance against reversible focal brain ischemia (19,42,43).

Albers *et al* (44) previously demonstrated that NMDA antagonists may prevent neuronal injury in a gerbil model of transient cerebral ischemia by inhibiting the excitotoxicity induced by NMDA receptors. NMDA-induced excitotoxicity triggers several downstream cascades, including nitrosative and oxidative stress, mitochondrial dysfunction, and protease and phospholipase activation, which culminate in cell death (45). This NMDA-induced excitotoxicity is widely considered to mediate the delayed neuronal death of hippocampal CA1 pyramidal neurons following transient global cerebral ischemia (46). Therefore, inhibition of Ca^{2+} overload may be a potential mechanism underlying PDGF-mediated neuroprotection (47). Furthermore, the suppression of PDGF-BB mRNA expression has been reported to increase susceptibility of the brain to NMDA-induced excitotoxicity or ischemia (18,48,49). In concordance with these *in vivo* data, the activation of PDGFR-β by PDGF-BB strongly inhibited NMDA-induced excitotoxicity in cultured hippocampal neurons (50,51). These studies indicated that endogenously synthesized and exogenously applied PDGF-BB may exert neuroprotective effects, and that PDGFR-β expression in neurons may principally mediate this protective effect.

On the basis of these aforementioned studies, the present study compared alterations in PDGF-BB immunoreactivity in the CA1 region between the ischemia-operated and IPC + ischemia-operated groups. PDGF-BB immunoreactivity was significantly decreased in the CA1 pyramidal neurons following LTCI and was barely detected in the neurons 5 days after LTCI; however, IPC maintained PDGF-BB immunoreactivity in the CA1 pyramidal neurons in the IPC + sham- and IPC + ischemia-operated groups. These findings indicated that PDGF-BB may be associated with neuronal damage after ischemic insults and that the regulation of PDGF-BB expression may be affected by PDGFR-β.

In conclusion, the findings of the present study indicated that IPC (2 min of transient cerebral ischemia) increased PDGF-BB expression in the gerbil hippocampal CA1 pyramidal neurons following 5 min of LTCI. The present study provides evidence regarding the mechanism underlying IPC-mediated neuroprotection against transient cerebral ischemic injury.

Acknowledgements

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