Beneficial effects of platelet-derived growth factor on hemorrhagic shock in rats and the underlying mechanisms

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Liu L, Zhang J, Zhu Y, Xiao X, Peng X, Yang G, Zang J, Liu S, Li T. Beneficial effects of platelet-derived growth factor on hemorrhagic shock in rats and the underlying mechanisms. *Am J Physiol Heart Circ Physiol* 307: H1277–H1287, 2014. First published August 29, 2014; doi:10.1152/ajpheart.00006.2014.—Studies have shown that local application of platelet-derived growth factor (PDGF) can be used for the treatment of acute and chronic wounds. Further study indicated that PDGF increased the activity of Rho kinase and PKC, and Rho kinase, PKC, and Cx43 play very important roles in the regulation of PDGF on vascular reactivity and hemodynamics. The improvement of PDGF-BB in shock, which are closely related to the improvement of vascular reactivity and hemodynamics. The improvement of PDGF-BB in vascular reactivity is vascular endothelium and myoendothelial gap junction dependent. Cx43, Rho kinase, and PKC play very important role in this process. These findings suggest that PDGF may be a potential measure to treat acute clinical critical diseases such as severe trauma, shock, and sepsis.

**PLATELET-DERIVED GROWTH FACTOR (PDGF)** is an important member of a family of VEGFs. It is expressed in many tissues and cells, including vascular endothelial cells (VECs), hepatocytes, renal cells, macrophages, mononuclear cells, and so on (12, 31). Some studies have shown that PDGF can accelerate tissue repair and wound healing for acute injury or some chronic nonhealing wounds, such as radiation-induced chronic nonhealing wounds and diabetic foots (2, 11, 15, 30, 41). Nevertheless, it is not clear if PDGF has beneficial effects in acute critical conditions such as severe trauma, shock, and sepsis.

Studies have demonstrated that vascular function is severely impaired after severe trauma, shock, sepsis, or associated multiple-organ dysfunction syndrome (1, 7, 25). This impaired vascular function, especially vascular hyporeactivity, severely interferes with the occurrence, development, and treatment of these diseases, and it is the important reason for the refractory hypotension in these critical conditions. Whether PDGF can play beneficial effects in these acute critical conditions via improving vascular reactivity (the responses of blood vessels to vasoactives) and hemodynamics are not known.

PDGF is one of the main substances released by VECs. Some studies have shown that PDGF regulates cell proliferation and differentiation through its association with the phosphatidylinositol 3-kinase pathway (35). Some studies have also shown that PDGF inducing the proliferation of vascular smooth muscle cells (VSMCs) is closely related to the gap junction protein connexin (Cx)43 and Cx40, PKC, and Rho kinase were observed. PDGF-BB (1–15 μg/kg iv) significantly improved the hemodynamics and blood perfusion to vital organs (liver and kidney) as well as vascular reactivity and improved the animal survival in hemorrhagic shock rats. PDGF recovering shock-induced vascular hyporeactivity depended on the integrity of the endothelium and myoendothelial gap junction. Cx43 antisense oligodeoxynucleotide abolished these improving effects of PDGF, whereas Cx40 oligodeoxynucleotide did not. Further study indicated that PDGF increased the activity of Rho kinase and PKC as well as vascular Ca2+ sensitivity, whereas it did not interfere with the intracellular Ca2+ concentration in hypoxia-treated vascular smooth muscle cells. In conclusion, systemic application of PDGF-BB may exert beneficial effects on hemorrhagic shock, which are closely related to the improvement of vascular reactivity and hemodynamics. The improvement of PDGF-BB in vascular reactivity is vascular endothelium and myoendothelial gap junction dependent. Cx43, Rho kinase, and PKC play very important role in this process. These findings suggest that PDGF may be a potential measure to treat acute clinical critical diseases such as severe trauma, shock, and sepsis.

**MATERIALS AND METHODS**

**Ethic Approval**

The present study was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, China). The protocol conformed with guidelines of the ethical use of animals. Efforts were made to minimize animal suffering and to reduce the number of animals used. This investigation conforms with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011).

**Materials**

PDGF-BB, norepinephrine (NE), Y-27632, staurosporine, fura-2, 18c-glycyrrhetic acid (18c-GA), and Cx43 antibody were obtained from Sigma (St. Louis, MO). Cx40 antibody was purchased from...
Santa Cruz Biotechnology (Santa Cruz, CA). Phosphorylated (p-)myosin phosphatase-targeting protein (MYPT) antibody, MYPT antibody, and nitrocellulose membranes were purchased from Millipore (Bedford, MA). Cx43 antisense oligodeoxynucleotide (AODN) and Cx40 AODN were obtained from Invitrogen (Carlsbad, CA). Horseradish peroxidase-conjugated secondary antibody, the enhanced chemiluminescence substrate kit, Western blot stripping buffer, and the bicinchoninic acid protein assay kit were obtained from Pierce (Appleton, WI).

Animal Management

Wistar rats (200–250 g) were fasted for 12 h but allowed water ad libitum before the experiment. Rats were first anesthetized with pentobarbital sodium (30 mg/kg body wt ip). This agent was added until rats had no response to a needle stimulus, but the total amount of pentobarbital sodium used was ≤50 mg/kg. The right femoral arteries and veins were catheterized with polyethylene catheters (outer diameter: 0.965 mm and inner diameter: 0.58 mm) to monitor mean arterial pressure (MAP) and PDGF administration (PDGF-BB). The left ventricle (LV) of the heart was catheterized with a polyethylene catheter via the right carotid artery to monitor hemodynamic parameters, including LV systolic pressure (LVSP) and maximal change rates of LV pressure (±dP/dtmax). After completion of the catheterization, rats were allowed to stabilize for 20–30 min and then hemorrhaged from the right femoral arterial catheter until MAP was ≤40 mmHg; rats were maintained at this MAP level for 2 h to complete the production of the hemorrhagic shock model.

Preparation of Blood Vessels and Hypoxia

SMAs were obtained from shock or sham-operated rats. After removal of the connective tissue, SMAs were cut into 2- to 3-mm-long arterial rings and prepared as endothelium-intact and endothelium-denuded arteries for measurements of vascular reactivity and Ca2+ sensitivity and for other experiments. According to the experimental design, SMA rings underwent or did not undergo hypoxia treatment. Briefly, the hypoxia procedure involved SMA rings being placed into a hypoxia culture compartment, 95% N2 and 5% CO2 were bubbled through at 10 l/min for 15 min, and rings were allowed to equilibrate for 10 min. These procedures were repeated three times until the O2 concentration was <0.2% and then maintained for 2 h.

Experimental Protocol and Methods

Effects of PDGF on hemodynamics and survival in hemorrhagic shock rats. Ninety-six Wistar rats were randomly divided into six groups (n = 16 rats/group): hemorrhagic shock control, lactated Ringer solution (LR), and PDGF (1, 3.5, 7 and 15 μg/kg). At the end of the hypotensive period (2 h), rats in the LR group received 2 ml LR (of shed blood) infusion, and rats in the PDGF (1, 3.5, 7, and 15 μg/kg) groups received a PDGF (1, 3.5, 7, or 15 μg/kg) infusion in 2 ml LR for ~30 min. Hemodynamic parameters, including LVSP and ±dP/dtmax, were determined at baseline, at the end of the hypotensive period, and at 1 h and 2 h after PDGF administration with a Polygraph Physiologic Recorder (Power Lab, AD Instruments, Castle Hill, NSW, Australia). The dosages of PDGF used in the present study were based on our pilot study. After all measurements, all catheters were removed, and the incisions were sutured. Rats were put back to their cages to observe 24-h survival. The zero time of survival observation was the onset of PDGF infusion. To relieve pain after the operation, Jingsongleng [xydlinothiozole (0.15 mg/kg)] was injected intramuscularly.

Effects of PDGF on blood flow and mitochondrial function in the liver and kidney. One hundred sixty Wistar rats were randomly divided into five groups (n = 8 rats/group at each time point): LR and PDGF (1, 3.5, 7, and 15 μg/kg). Animal management and PDGF administration were the same as described above. Blood flow in liver and kidney tissues was measured by a Laser-Doppler Blood Flowmeter (Periflux system 5000, Primed, Stockholm, Sweden) at baseline, at the end of the hypotensive period, and at 1 h and 2 h after PDGF administration. After the measurement of blood flow at each time point, 5 g of liver and kidney tissues were sampled for the measurement of mitochondrial function using a Mitochondrial Function Analyzer (MT 200, Strathkelvin, Scotland). Mitochondrial function was reflected by the respiration control rate (35, 42). Briefly, samples of liver or kidney tissues were put into 20 ml ice-cold isolation buffer (0.25 mol/l sucrose, 0.1 mmol/l Na2EDTA, and 0.01 mol/l Tris, pH 7.6). Tissue with isolation buffer was homogenized and then centrifuged at 1,600 g for 12 min at 4°C. The supernatant was centrifuged twice at 25,000 g for 15 min at 4°C. The pellet was collected and resuspended in 1 ml isolation buffer. The concentration of mitochondrial protein was measured by the Lowry method; 1.4 ml of measurement buffer [0.2 mol/l Tris (pH 7.6), 15 mmol/l KCl, 15 mmol/l KH2PO4, 1 mmol/l Na2EDTA, 5 mmol/l MgCl2, and 0.25 mol/l sucrose] warmed to 30°C were added into the reaction chamber and equilibrated for 2 min. Then, 0.2 ml of 3 mg/ml of a mitochondrial mixture were put into the reaction chamber and equilibrated for 20 s. Ten microliters of 0.5 mol/l sodium malate (C4H4Na2O5·H2O) and sodium glutamate (C4H8NNaO4) as well as 5 μl ADP (400 mmol/l) were added in succession. The rate of O2 consumption was determined using a Mitochondrial Function Analyzer.

Beneficial effects of PDGF on vascular reactivity in vivo and in vitro. In vivo. Fifty-six Wistar rats were randomly divided into seven groups (n = 8 rats/group): sham operated, shock, LR, and PDGF (1, 3.5, 7, and 15 μg/kg). After the completion of catheterization and laparotomy, SMAs were exposed. The hemorrhagic shock model and PDGF administration were as described above. The pressor response of NE (3 μg/kg iv bolus injection) and the contractile response of SMAs to NE were measured at baseline, at the end of the hypotensive period, and at 10, 20, 30, and 40 min after the administration of PDGF. SMA diameter was measured with an Intravital Video System (S6D, Leica, Wetzlar, Germany). The pressor effect of NE was expressed as the increased value of MAP before and after NE administration. The contractile response of SMAs to NE in vivo was expressed as a reduced percentage of SMA diameters after NE administration.

In vitro. Forty-eight Wistar rats were divided into sham-operated (n = 8) and hemorrhagic shock (n = 40) groups. After sham operation or hemorrhagic shock (40 mmHg, 2 h) procedure, SMAs were isolated and made into SMA rings. SMA rings from hemorrhagic shock rats were further divided into five groups (n = 8 rings/group): shock control and shock + PDGF (40, 60, 80, and 100 ng/ml). SMAs in PDGF groups were incubated with PDGF (40, 60, 80, and 100 ng/ml) for 30 min, and the responsiveness of SMAs to NE (10−9–10−3 mol/l) was determined using an isolated vascular tension measurement system (AD Instruments) as previously described (18, 20). Briefly, artery rings were mounted on wire, suspended between a force transducer and a post attached to a micrometer, and then immersed into a 10-ml isolated organ chamber (Power Lab, AD Instruments) containing Krebs-Henseleit solution, which was continuously bubbled with 95% O2 and 5% CO2. The temperature was maintained at 25°C. A 1.5-g preload was given, and the Krebs-Henseleit solution was replaced every 20 min. The tension of the artery ring was determined by the Power Lab system via a force transducer. After 2 h of equilibration, the response of SMA to NE (10−9, 10−8, 10−7, 10−6, and 10−5 mol/l) was determined. The maximal contraction (Emax) and pD2 (−log [EC50] of NE) were obtained from the concentration-response curves and used to compare vascular reactivity.

Relationship of PDGF regulation of vascular reactivity to the endothelium and MEGJs in hemorrhagic shock rats. Thirty-two SMA rings from hemorrhagic shock rats (40 mmHg, 2 h) were divided into two groups (n = 16 rings/group): shock and shock + PDGF. Sixteen
1. SMA rings from sham-operated rats were used as a control group. Each group was further divided into endothelium-intact and endothelium-denuded groups (n = 8 rings/group). The endothelium of SMAs was denuded by gentle scraping. The responsiveness of endothelium-intact or endothelium-denuded SMAs to a series concentration of NE (10⁻⁹–10⁻⁵ mol/l) was determined. SMA rings in the shock + PDGF group were incubated with PDGF (100 ng/ml) for 30 min. The integrity of the endothelium of SMAs was checked by determining its vasodilation ability to Ach (10⁻⁶ mol/l, Sigma). If Ach (10⁻⁶ mol/l) induced the relaxation of SMA precontracted with NE (10⁻⁴ mol/l) by >75%, the endothelium of SMAs was considered intact (18, 28).

2. To further investigate if the protective effect of PDGF on the vascular contractile response was via the endothelium and MEGJs, SMAs were first incubated with 18s-GA (10⁻⁵ mol/l), an inhibitor of MEGJs, for 30 min followed by PDGF (100 ng/ml) for another 30 min. The contractile response of SMAs to a series concentration of NE (10⁻⁹–10⁻⁵ mol/l) was determined as described above.

3. **Effect of PDGF on Ca²⁺ concentration and Ca²⁺ sensitivity in VSMCs of SMAs.** Forty SMAs obtained from normal rats were divided into five groups (n = 8 SMAs/group): normal control, 2-h hypoxia, PDGF, Cx AODN, and Cx AODN + PDGF. The Ca²⁺ image (to relatively reflect the concentration of Ca²⁺) in VSMCs of SMAs was measured using a Confocal Laser Scanning Fluorescence Microscope (Leica Instruments) after incubation with fura-2 (40 nmol/l) for 1 h. The phosphorylation level of 20-kDa myosin light chain (MLC₂₀) in SMAs was measured using a glycerol-PAGE with MLC₂₀ antibody as previously described. Briefly, PDGF (100 ng/ml)- and Cx AODN-treated SMAs were mixed with urea sample buffer and incubated for 1 h at room temperature. The homogenate was centrifuged, and supernatant protein was collected and subjected to 10% glycerol-PAGE. The phosphorylation level of MLC₂₀ (Sigma, 1,200) was determined by Western Blot.

4. **Effect of PDGF on Ca²⁺ sensitivity by 10.22±0.33 on June 10, 2017 http://ajpheart.physiology.org/ Downloaded from**
In vitro. The contractile response of SMAs to NE was significantly decreased at 2 h after hemorrhagic shock, and its cumulative response curve was significantly shifted to the right (Fig. 2D). PDGF (40–100 ng/ml) significantly improved the contractile response of SMAs to NE in a dose-dependent manner, and their cumulative response curves were significantly shifted to the left ($P < 0.05$ or $0.01$; Fig. 2D). Maximum effects for the agonist ($E_{max}$) were significantly increased (Fig. 2, D and E).
vascular reactivity during shock. The results showed that Cx40 and/or Cx43 are involved in the protective effect of PDGF on shock (21). These experiments aimed to discover if Cx40/Cxs that take part in the regulation of vascular reactivity during shock are involved in the protective effect of PDGF on shock.

Relationship of PDGF Regulation of Vascular Reactivity to the Endothelium and MEGJs in Hemorrhagic Shock Rats

To further investigate if the protective effect of PDGF on the vascular contractile response is via the endothelium and MEGJs, endothelium-intact and endothelium-denuded SMAs and an inhibitor of MEGJ, 18α-GA, were used in these experiments. PDGF (100 ng/ml) incubation significantly enhanced the contractile response of endothelium-intact SMAs to NE (the increase rate was 147.6% compared with the shock group), whereas the same concentration of PDGF only slightly increased the contractile response of endothelium-denuded SMAs (the increase rate was only 15.0%; Fig. 3, A–D). 18α-GA (10−5 mol/l) antagonized the beneficial effect of PDGF on the vascular reactivity of SMAs (Fig. 3, E and F).

Relationship Between the Regulatory Effect of PDGF on Vascular Reactivity and Cx40/Cx43

MEGJ comprises different gap junction proteins called Cxs. Research has shown that Cx40 and Cx43 are the main types of Cxs that take part in the regulation of vascular reactivity during shock (21). These experiments aimed to discover if Cx40 and/or Cx43 are involved in the protective effect of PDGF on vascular reactivity during shock. The results showed that Cx40 and Cx43 AODN incubated with SMAs for 24 h significantly inhibited their own protein expression but did not affect the protein expression of the other Cx (Cx40 AODN inhibited the expression of Cx40 but did not inhibit the expression of Cx43, and Cx43 AODN inhibited the expression of Cx43 but did not inhibit the expression of Cx40). Inhibition rates were 79.9% for Cx43 and 78.0% for Cx40 (Figs. 4, A–C). Inhibition of expression of Cx43 with Cx43 AODN decreased the improving effect of PDGF on vascular reactivity of hypoxia-treated SMAs, but inhibition of expression of Cx43 with Cx40 AODN did not interfere with this effect of PDGF on vascular reactivity of SMAs (Fig. 4, D and E).

Effects of PDGF on Vascular Ca2+ Sensitivity and Ca2+ Concentration in SMAs

The contraction of VSMCs is modulated via Ca2+ concentration-dependent and Ca2+ concentration-independent (Ca2+ sensitivity) pathways that regulate MLC20 phosphorylation and contraction. To ascertain if the improving effect of PDGF on vascular reactivity after shock is via Ca2+ concentration-dependent or Ca2+ concentration-independent (Ca2+ sensitivity) pathways, we observed the effects of PDGF on the Ca2+ concentration and Ca2+ sensitivity of VSMCs with SMAs and the phosphorylation of MLC20.

Fig. 2. Effects of PDGF-BB on vascular reactivity after hemorrhagic shock in vivo and in vitro. A: changes of mean arterial blood pressure (MAP) after PDGF administration in vivo. B: increased MAP after norepinephrine (NE) bolus infusion (3.5 μg/kg) after PDGF administration in vivo. C: contractile responses of superior mesenteric arteries (SMAs) to bolus NE (3.5 μg/kg) after PDGF administration in vivo. D: contractile responses of SMAs to a cumulative concentration of NE after incubation with PDGF in vitro. E: maximal contractile response (Emax) of SMAs to a cumulative concentration of NE in vitro. Sham, sham-operated group. PDGF40, PDGF60, PDGF80, and PDGF100 indicate PDGF (40, 60, 80, and 100 ng/ml)-treated groups. Data are means ± SD of 8 observations. *P < 0.05 and **P < 0.01 compared with the SHK group; +P < 0.05 and +++P < 0.01 compared with the LR group.
Changes in the concentration and sensitivity of Ca^{2+}. The intracellular Ca^{2+} concentration of VSMCs in SMA after 2 h hypoxia was slightly increased. PDGF (100 ng/ml) did not increase the intracellular Ca^{2+} concentration in VSMCs in hypoxia-treated SMAs. The vascular Ca^{2+} sensitivity (the contractile response of SMAs to different concentrations of Ca^{2+}) was significantly decreased after 2 h of hypoxia compared with the control group. PDGF (100 ng/ml) increased the Ca^{2+} sensitivity of hypoxia-treated SMAs. Cx43 AODN antagonized the increasing effect of PDGF on the Ca^{2+} sensitivity of hypoxia-treated SMAs (Fig. 5, A–D).

Changes in MLC_{20} phosphorylation. After 2-h hypoxia, the MLC_{20} phosphorylation level in SMAs was significantly decreased compared with the control group; the decrease rate was 11.3%. PDGF (100 ng/ml) increased the MLC_{20} phosphorylation level in hypoxia-treated SMAs; this increase rate was 7.9%. This increasing effect of PDGF on the MLC_{20} phosphorylation level was antagonized by Cx43 AODN (Fig. 5, E and F).

Relationship of the Regulatory Effect of PDGF on Vascular Reactivity and Ca^{2+} Sensitivity to PKC and Rho Kinase

The above results showed that PDGF regulation of vascular reactivity (the response of blood vessels to vasoactives) is not via a Ca^{2+} concentration-dependent pathway but via a Ca^{2+} sensitivity pathway (Ca^{2+} concentration-independent pathway). Our previous studies (19, 40) have shown that Rho kinase and PKC are the main regulatory molecules for the Ca^{2+} sensitivity of VSMCs. Hence, these experiments aimed to investigate the relationship of the regulatory effect of PDGF on vascular reactivity and Ca^{2+} sensitivity to Rho kinase and PKC.

Effects of PKC and Rho kinase inhibitors on the regulatory effect of PDGF on vascular reactivity and Ca^{2+} sensitivity. The results showed that staurosporine (an inhibitor of PKC) and Y-27632 (an inhibitor of Rho kinase) antagonized the upregulated effect of PDGF on vascular reactivity (Fig. 6, A and B) and Ca^{2+} sensitivity (Fig. 6, C and D) of SMAs after shock. Inhibition values of Y-27632 in vascular reactivity and Ca^{2+} sensitivity were 82.01% and 90.97%, respectively, and inhibition values of staurosporine in vascular reactivity and Ca^{2+} sensitivity were 34.12% and 42.46%, respectively. These results suggest that PKC and Rho kinase took part in the regulation of PDGF on vascular reactivity and Ca^{2+} sensitivity after shock and that Rho kinase may have the main effect (Fig. 6, A–D).

Effects of PDGF on the activity of Rho kinase and PKC and its relationship with Cx43. Two hours of hypoxia inhibited the activities of Rho kinase (reflected by the phosphorylation level of MYPT) and PKC [reflected by the phosphorylation level of the PKC substrate peptide PepTag C1 (amino acid sequence: PLTSRTLSVAAK)] of SMAs. PDGF (100 ng/ml) significantly increased the phosphorylation of MYPT and PepTag C1 in hypoxia-treated SMAs. Inhibition of Cx43 with its Cx40 AODN antagonized the effect of PDGF on the phosphorylation of MYPT and PepTag C1 (Fig. 6, E–H).
DISCUSSION

PDGF can accelerate tissue repair and wound healing for acute or some chronic nonhealing wounds (2, 11, 15, 30, 41). The present study showed that PDGF has beneficial effects in acute critical conditions such as traumatic hemorrhagic shock. Administration of exogenous PDGF (1–7 μg/kg) could significantly improve animal survival as well as increase tissue blood flow and mitochondrial function of vital organs in traumatic hemorrhagic shock rats. These beneficial effects of PDGF in shock may result from hemodynamic stabilization by improving vascular reactivity. The mechanism is related to gap junction protein Cx43-mediated Rho kinase and PKC activation.

The gap junction is the main structure enabling substances and messages to be exchanged directly between cells. It plays an important part in the genesis, development, and death of normal tissues as well as the maintenance of body homeostasis. The gap junction between VECs and VSMCs is the MEGJ (14, 16, 34). Studies have shown that there are 21 Cx members in the human gene profile and 20 Cx members in the mouse gene profile. They are broadly distributed in all tissues. They can pass small-molecular substances to participate in the regulation of cell functions. VECs may regulate vascular tone and motion in the entire blood vessel via MEGJs. The main Cx molecules in the cardiovascular system are Cx37, Cx40, Cx43, and Cx45 (36, 37).

Our previous study (22) found that Cx40 and Cx43 participate in the regulation of vascular reactivity after shock. The present study found that PDGF (40–100 ng/ml) significantly increased vascular reactivity in endothelium-intact SMAs in a dose-dependent manner, whereas the same concentration of PDGF only slightly increased vascular reactivity in endothelium-denuded SMAs. 18-OA (an inhibitor of MEGJs) antagonized the increasing effect of PDGF on vascular reactivity. Inhibition of expression of Cx43 with Cx43 AODN decreased the effect of PDGF on vascular reactivity, whereas inhibition of expression of Cx40 with Cx40 AODN did not interfere with the effect of PDGF on vascular reactivity. These findings suggest that the protective effect of PDGF on vascular reactivity was through the MEGJ after shock. The main Cx type that participated in this mediation effect of PDGF was Cx43. Other studies (4, 6) have also demonstrated that PDGF activates Cx43. The mechanism by which PDGF activates Cx43 after shock is not clear. It may be closely related to the phosphorylation of Cx43, since Johnston et al. (13) found PDGF can phosphorylate Cx43 by MAPK to promote smooth muscle cell proliferation. The
precise mechanism by which PDGF activates Cx43 in the shock condition, however, needs further investigation.

Ca$_{2+}$-dependent and Ca$_{2+}$-independent pathways (also called Ca$_{2+}$ sensitivity pathways) are involved in the regulation of VSMC contraction. The Ca$_{2+}$-dependent pathway is mainly dependent on the concentration of intracellular free Ca$_{2+}$ and activity of MLC kinase in VSMCs. The Ca$_{2+}$-independent pathway is not dependent on the concentration of intracellular Ca$_{2+}$ but is mainly dependent on the activity of MLC phosphatase and the phosphorylation level of MLC$_{20}$ in VSMCs (21). Research has shown that the key molecules that participate in the regulation of the Ca$_{2+}$-independent pathway include PKC and Rho kinase. Our present study indicated that PDGF did not increase the Ca$_{2+}$ concentration of VSMCs after hypoxia but did increase the Ca$_{2+}$ sensitivity and MLC$_{20}$ phosphorylation of VSMCs in SMAs (21).

PDGF comprises five isoforms, including four homoisoforms (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and one heteroisoform (PDGF-AB). A previous study (32) has shown that PDGF-BB has a stronger vascular contraction effect than other isoforms (PDGF-AA and PDGF-AB) in rat aorta rings. Thus, the isoform that we used in the present study was PDGF-BB, but whether other types of PDGF are also beneficial to the treatment of traumatic shock needs further study. The PDGF receptor (PDGFR) comprises of two tyrosine kinase receptor chains, which are combined to different PDGFR types: homodimeric PDGFR-αα and PDGFR-ββ and heterodimeric PDGFR-αβ. Different PDGF isoforms have different binding specificity and affinity to PDGFRs. Research has demonstrated that PDGF-BB may bind to PDGFR-αα, PDGFR-ββ, and PDGFR-αβ (39); however, via which PDGFRs PDGF-BB improves the vascular reactivity in the shock state need further investigation.

PDGF and PDGFR expression or activity would change during some diseases. Research has shown that substrate stiffness significantly enhances PDGFR activity, and stiffening of the vessel wall could actively promote the pathogenesis of vascular disease by enhancing PDGFR signaling to drive VSMC growth and survival (3). In experimental atherosclerosis, the expression of PDGFR-AA was increased (29). However, whether PDGF and its receptor are altered in shock condition is not known. There are several studies that have demonstrated that PDGF and its receptors are increased after chronic hypoxia and ischemia-induced neovascularization, pulmonary arterial hypertension, vascular remodeling, and so on (9, 43). Hence, we presume that PDGF and its receptors may be increased after hemorrhagic shock, but this needs further confirmation.

It is well known that PDGF is a major stimulus for the abnormal migration and proliferation of VSMCs, and this contributes to atherosclerosis or organ fibrosis. Many studies have shown that several PDGF subtypes participate in the...
development of atherosclerosis and organ fibrosis. PDGF-DD is upregulated in endothelial cells exposed to atherosclerosis-prone flow patterns (38). PDGF-BB participates in the occurrence of diabetic atherosclerosis (5). PDGF-CC may participate in the occurrence and development of kidney fibrosis (27). Whether PDGF-BB would induce similar side effect after application in hemorrhagic shock is not known. It is presumed that it would not, because the application duration was not long. However, the precise action needs to be further explored.

Our previous study (22) demonstrated that PDGF at a lower concentration (20 ng/ml) could decrease the vascular reactivity of SMAs through inhibition of Rac1, a family member of small G proteins. Other studies (33, 44) have also demonstrated that PDGF at lower concentrations (20–30 ng/ml) can activate Rac1. Rac1 could inhibit MLC kinase activity via p21-activated kinase and then decreases the phosphorylation level of MLC. Our present study found PDGF at concentrations of 40–100 ng/ml improved vascular reactivity in the hemorrhagic shock state via a Ca\(^{2+}\)/H\(^{+}\) concentration-independent pathway. However, the strength of PDGF activation of MLC\(_{20}\) phosphorylation is not big. This result demonstrates that there may be other mechanism responsible for PDGF regulation of vascular reactivity besides MLC\(_{20}\) phosphorylation regulation. Our previous study found that PDGF improving vascular reactivity is also via non-MLC\(_{20}\) phosphorylation pathway when PDGF is over some concen-

![Diagram](image1)

**Fig. 6.** Relationship of PDGF-BB regulation of vascular reactivity and Ca\(^{2+}\) sensitivity to Rho kinase and PKC. **A** and **B**: changes of vascular reactivity of SMAs after incubation with PDGF (100 ng/ml). **C** and **D**: changes of Ca\(^{2+}\) sensitivity of SMAs after incubation with PDGF (100 ng/ml). **E** and **F**: effect of PDGF on PKC activity in SMAs (reflected by the PKC substrate peptide PepTag C1). **G** and **H**: effect of PDGF on Rho kinase activity in SMAs (reflected by the phosphorylation level of myosin phosphatase-targeting protein (MYPT)). Cx43 AODN completely suppressed the phosphorylation of MYPT in PDGF-treated SMAs. Stp, staurosporine; p-MYPT, phosphorylated MYPT. Data are means ± SD of 8 observations. *P < 0.05 and **P < 0.01 compared with the Ctl group; #P < 0.05 and ##P < 0.01 compared with the Hyp group; @P < 0.05 and @@P < 0.01 compared with the PDGF group.
tration (>60 ng/ml). This study found that PDGF at 40–100 ng/ml increased the vascular reactivity after shock in a concentration-dependent manner, whereas the phosphorylation of MLC\textsubscript{20} was not increased as the concentration of PDGF increased. Over 60 ng/ml PDGF did not further increased MLC\textsubscript{20} phosphorylation (26). These results suggest that PDGF regulates vascular reactivity after shock not only via a MLC\textsubscript{20} phosphorylation pathway but also via non-MLC\textsubscript{20} pathway.

There was an interesting phenomenon in the present study in that the blood flow of the liver was increased over the baseline level after PDGF application, whereas the blood flow in the kidney never returned to baseline after PDGF application. The detailed reason is not known. The reason may be related to the fact that there is more PDGFR expression in the liver than other organs (8, 10), except that the liver and kidney have different structures and the liver has more blood perfusion in the basal state than in the kidney. The precise reason needs further investigation.

The present study had some limitations. First, the animal we used was only the rat, the model we used was only hemorrhagic shock, and whether these results can be extrapolated to the human and other critical conditions need further investigation. Second, the half-life of PDGF in circulation is only 2–3 min, we observed the beneficial effect of short-time infusion of PDGF (30 min), and whether prolonging the infusion time can further increase the beneficial effect of PDGF is not known. Third, Cx37, Cx40, Cx43, Cx45, and Cx46 are expressed in the cardiovascular system; however, we only studied the role of Cx40 and Cx43 in the present study. Whether other Cx molecules participate in the protective effect of PDGF on vascular reactivity is not clear. Finally, whether and by which PDGFR does PDGF improve vascular reactivity in the shock state need further investigations.

In conclusion, systemic application of PDGF-BB may exert beneficial effects on hemorrhagic shock, which is closely related to the improvement of vascular reactivity and hemodynamics. The improvement of PDGF-BB in vascular reactivity is vascular endothelium and MEGJ dependent. Cx43, Rho kinase, and PKC play very important roles in this process. This finding suggests that PDGF may be a potential measure to treat acute clinical critical diseases such as severe trauma, shock, and sepsis.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: L.L., J. Zhang, Y.Z., X.X., X.-y.P., G.-m.Y., J. Zhang, S.-q.L., and T.L. performed experiments; L.L., J. Zhang, Y.Z., X.X., J. Zhang, and S.-q.L. analyzed data; L.L. and T.L. interpreted results of experiments; L.L., J. Zhang, X.-y.P.; G.-m.Y., and T.L. prepared figures; L.L., J. Zhang, and T.L. drafted manuscript; L.L., J. Zhang, and T.L. approved final version of manuscript; T.L. conception and design of research; T.L. edited and revised manuscript.

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