Original Paper

Hydrogen Sulfide-Preconditioning of Human Endothelial Progenitor Cells Transplantation Improves Re-Endothelialization in Nude Mice with Carotid Artery Injury

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Key Words

Endothelial progenitor cells • Preconditioning • Re-endothelialization

Abstract

Background/Aims: The aim of present study was to test the hypothesis that preconditioning with sodium hydrosulfide (NaHS) could enhance the capacity of migration, adhesion and proliferation of endothelial progenitor cells (EPCs) *in vitro*, and also could improve the efficacy of EPCs transplantation for re-endothelialization in nude mice with carotid artery injury. The paper further addressed the underlying mechanisms. **Methods:** EPCs were isolated from peripheral blood mononuclear cells of healthy male volunteers and the markers of EPCs were analyzed by flow cytometry. Thereafter, different concentrations of NaHS (25, 50, 100, 200 and 500 μ M) were used for preconditioning EPCs. *In vitro* and *in vivo* migration, adhesion and proliferation as well as nitric oxide (NO) production of EPCs were evaluated. Carotid artery injury model was produced in nude mice and thereafter, NaHS-preconditioned EPCs were transplanted in order to evaluate their capacity of re-endothelialization. **Results:** Cellular immuno-staining showed that isolated cells expressed the key markers of EPCs. *In vitro*, EPCs proliferation rates and NO production were gradually increased in a NaHS-concentration dependent manner, while these benefits were blocked at a concentration of 500 μ M NaHS. Similarly, the migration and adhesion rates of EPCs were also increased the most prominently at a concentration of 200

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μM NaHS. *In vivo*, compared to the control group, treatment with NaHS-preconditioned EPCs significantly enhanced the capacity of re-endothelialization of EPCs. Fluorescent microscope revealed that there were more EPCs homing to the injury vessels in the NaHS-preconditioned EPCs group than the non-preconditioned group. With the administration of AMPK or eNOS inhibitors respectively, the above benefits of NaHS-preconditioning were abrogated. **Conclusion:** These results suggested that NaHS-preconditioning enhanced the biological function and re-endothelialization of EPCs through the AMPK/eNOS signaling pathway.

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Introduction

Cardiovascular diseases including coronary heart disease (CHD) and peripheral arterial disease remain a major cause of morbidity and mortality worldwide. Percutaneous coronary intervention (PCI) is an important treatment of CHD. Compared to bare-metal stents (BMSs) implantation, drug-eluting stents (DESs) significantly reduces targeted vessels restenosis as well as improves long-term outcomes. However, late and very late in-stent thrombosis remain the key issues of stent implantation [1, 2]. Several studies have shown that endothelial dysfunction is involved in atherosclerotic cardiovascular disease development [3-5]. The balance between endothelial injury and repair plays an important role in atherogenesis [5]. Notably, the favorable effects of DESs are due to its effect on preventing vascular smooth muscle cells (VSMCs) proliferation. However, vascular endothelial repair would also be compromised which in turn causes delayed re-endothelialization and late stent thrombosis [6]. Thus, promoting re-endothelialization may play a role in preventing DES thrombosis and sustaining vascular homeostasis [7, 8].

Cell-based therapy is a promising strategy for endothelial repair in vascular diseases [9]. Endothelial progenitor cells (EPCs), mobilizing from bone marrow into peripheral blood, have been showed to play an important role in vascular repair in response to injury [10, 11]. However, owing to the low retention and survival rates in the injured vascular wall, the clinical benefits of EPCs transplantation for vascular diseases are limited [12, 13]. Therefore, to promote transplanted EPCs homing to and retaining in the injured vascular walls are the key prerequisites for improving the efficiency and efficacy of re-endothelialization and vascular repair.

Hydrogen sulfide (H_2S), the third member of the gaso-transmitter family, is a key signaling molecule which has a variety of biological benefits for vascular repair [14, 15]. Endogenous H_2S is produced from the main substrate of L-cysteine through catalyzing cystathionine beta-synthase (CBS) and cystathionine-gamma-lyase (CSE). Exogenous H_2S stimulates endothelial cells proliferation and migration, which in turn contribute to angiogenesis, coronary collateral vessel growth, and protection of ischemia-reperfusion (IR) injury [16]. In diabetic rats, H_2S can accelerate diabetic wound repair by promoting angiogenesis [17]. It has also been demonstrated that preconditioning with H_2S could promote mesenchymal stem cells survival and improve myocardial repair in rats with myocardial infarction [18]. Nevertheless, questions regarding the efficacy and associated mechanisms of H_2S preconditioning for vascular repair are unresolved. Therefore, the aim of present study was to test the hypothesis that preconditioning with sodium hydrosulfide (NaHS), a H_2S donor, could enhance the migration, adhesion and proliferation rates of EPCs *in vitro*, and also could improve the efficacies of EPCs transplantation for re-endothelialization in rats with carotid artery injury, and the underlying mechanisms were also be explored.

Materials and Methods

EPCs culture and characterization

EPCs were isolated and cultured in accordance to previous described methods [19-21]. Briefly, peripheral blood mononuclear cells (PBMNCs) were isolated from healthy volunteers (20 to 40 years old

male after informed consent was obtained) and cultured on fibronectin-coated plates using endothelial basal medium-2 (Lonza, Basel, CC-4176) supplemented with EGM-2 Bullte Kit (Lonza) and 20 % Fetal bovine serum (FBS, GIBCO). After 4 days' culture, the non-adherent cells were discarded and adherent cells were cultured for another 7 days and then used for the following experiments. According to previous described [22, 23], early EPCs were defined as positive staining for both 1, 1'-dioctadecyl-3, 3,3',3'-tetramethylindocarbocyanine (DiI)-acetylated low density lipoprotein (ac-LDL) (20 µg/ml; Invitrogen, Carlsbad, CA, USA) and FITC-labeled BS-1 lectin (10 µg/ml; Sigma-Aldrich, St. Louis, MO, USA). The expressions of endothelial markers of cultured EPCs were examined using flow cytometry analysis with the following antibodies: phycoerythrin (PE)-labeled monoclonal mouse anti-human antibodies recognizing CD31 (BD Pharmingen), von Willebrand factor (vWF) (BD Pharmingen), kinase-insert domain receptor (KDR) (R&D Systems), and CD14 (BD Pharmingen). Based on the flow cytometry findings, the adherent mononuclear cells were identified as early EPCs which were consistent with previous reports [24, 25].

Tube formation assay

In vitro capillary-like structure formation of EPCs was evaluated using growth factor reduced Matrigel (BD Biosciences). Briefly, Matrigel (50ul/well) was added to 96-well plates and incubated at 37°C for 30 min. The cells (2×10^4 cells per well) were seeded on Matrigel and cultured for 8h. Tube formation was quantified using an inverted microscope (Olympus BX51).

Cells proliferation assay

H₂S was administrated by the form of sodium hydrosulfide (NaHS; Sigma-Aldrich). The concentration and incubation time of NaHS were determined based on our pilot study. The effects of NaHS on EPCs viability was assessed by CCK8 (Dojindo Molecular Technologies, Kumamoto, Japan). The cells were treated with a concentration of NaHS from 25 to 500 µM for 24h. To explore whether the AMPK-eNOS pathway contributed to the cyto-protection of NaHS, EPCs were treated with 10 µM compound C (an inhibitor of AMPK, purchased from Sigma-Aldrich) or 100 µM L-NAME (an inhibitor of eNOS, Calbiochem) for 30min before exposure to NaHS for 24h.

Western blot analysis

EPCs were lysed with cell lysis buffer (Cell Signaling Technology, Boston, MA, USA) according to the manufacturer's instructions. Cell lysates were quantified by BCA methods according to the manufacturer's instructions (Sangon Biotechnology, Shanghai, China). Totally, 50 µg proteins were subjected to SDS-PAGE and then transferred to polyvinylidene fluoride membranes. The following antibodies were used: rabbit anti-AMPKα antibody (1:1000; Cell Signaling Technology), Phospho-AMPKα (Thr 172) rabbit mAb (1:1000; Cell Signaling Technology), Phospho-eNOS (Ser1177) rabbit mAb (1:1000; Cell Signaling Technology), eNOS (49G3) rabbit mAb (1:1000; Cell Signaling Technology), and GAPDH (14C10) rabbit mAb (1:1000; Cell Signaling Technology). Proteins were visualized with HRP-conjugated anti-rabbit IgG (1:5000; Cell Signaling Technology). To detect the effect of stimulation of PB-derived EPCs with H₂S on the phosphorylation of AMPK and eNOS, EPCs were incubated with 200 µM NaHS for 12h before proteins were obtained.

Nitric oxide (NO) production

Measurement of NO production. NO production of EPCs was measured as the generation of nitrite. The cells were cultured with EBM-2 (growth factor-free) for 24h after NaHS stimulation. The supernatants were used to determine NO level using a NO assay kit by the nitrate reductase method (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

Intracellular NO production of EPCs. Intracellular NO production of EPCs was determined by staining with 4-amino-5methylamino-2, 7,-difluorofluorescein (DAF-FM; Molecular Probes, Carlsbad, CA, USA) and assessed by laser scanning confocal microscope as previous described [26].

In vitro EPCs migration assays

EPCs migration assays were performed using a Transwell system (Corning Costar, Tewksbury, MA, USA) with 8µM polycarbonate filter inserts in 24-well plates as described in our previous study [21]. Briefly, a total of 2×10^4 EPCs were suspended in 250ul EBM-2 medium in the upper chamber, and the lower chamber was placed in a 24-well culture dish containing 500ul EBM-2 supplemented with 50ng/ml VEGF.

After incubation at 37°C for 24h, cells were fixed with 4 % paraformaldehyde and stained by DAPI. The migrated cells were counted by independent investigators who were blinded to the treatment groups.

In vitro EPCs adhesion assays

A monolayer of human umbilical vein endothelial cells (HUVECs) was prepared for 48h before placing 2×10^5 cells in each well of a four-well plate. The HUVECs were pre-treated with 1 ng/ml tumor necrosis factor- α (TNF- α , Peprotech) for 12h. Thereafter, 1×10^5 CM-DiI-labeled EPCs were added to each well, and the cells were incubated at 37°C for 3h. The non-attached cells were gently removed with PBS, and the adherent EPCs were fixed in 4 % of PFA and randomly counted by an independent investigator who was blinded to the treatments.

Animal model production and in vivo re-endothelialization assay

The carotid artery injuries model and EPCs transplantation were performed in accordance to previous described methods [20, 21, 27]. Six to eight weeks old male NRM1 nu/nu athymic nude mice (obtained from the Laboratory Animal Center of Sun Yat-sen University, Guangzhou, China) were used in current study. All animals were raised in the Experimental Animal Center of Sun Yat-sen University. All experimental procedures and protocols were complied with the Animal Care and Use Ethics Committees of Sun Yat-Sen University. The animals were anaesthetized with Ketamine (100mg/kg IP) and xylazine (5mg/kg IP). The surgeries were performed under a stereoscopic microscope. The left carotid artery was exposed via a midline incision on the ventral side of the neck. The bifurcation of the carotid artery was located, and two ligatures were placed around the external carotid artery, which was then tied off with the distal ligature. An incision hole was made between the ligatures to introduce the denudation device. A curved flexible wire (0.35-mm diameter) was introduced into the common carotid artery and passed over the lining of artery for three times so as to denude the endothelium. The wire was then removed, and the external carotid artery was tied off proximal to the incision hole with the proximal ligature. EPCs (1×10^6) that had been cultured for 7 days were re-suspended in 100 μ l of pre-warmed PBS (37°C) and were transplanted 3h after carotid artery injury via tail vein injection with a 27 G needle. The same volume of PBS was injected into sham-operation mice as a control. Three days after carotid artery injury, endothelial regeneration was evaluated by staining denuded areas with 50 μ l of 5 % Evans Blue dye via tail vein injection. To examine the homing of transplanted EPCs to the site of the injured carotid vessel, labeled EPCs (1×10^6) were incubated with CM-DiI (Cell Tracker™ CM-DiI, Invitrogen) in accordance to the manufacturer's instructions. CM-DiI-labeled EPCs incorporated in the injured vessels were quantitatively analyzed under a fluorescence microscope (Olympus BX51).

Statistical analysis

All of the data are reported as the mean $\pm$ SD. Statistical significance was evaluated by Student's t-test. A value of $P < 0.05$ was considered to statistical significance. All statistical analyses used SPSS statistical software (SPSS version 13.0).

Results

Early EPCs characterization

EPCs were isolated from fresh human PBMCs by density gradient centrifugation and grown in EGM-2 media for 7 days. As presented in the Figure1A, cellular immuno-staining showed that the adherent cells were able to uptake of DiI-ac-LDL, bind FITC-lectin and form capillary-like structures on Matrigel (Fig.1C). Meanwhile, early EPCs expressed endothelial markers (CD31, vWF and KDR) and a monocyte marker CD14 at a comparable level (Fig.1B). These results were consistent with previous studies [20, 28] and confirmed that EPCs had been successfully isolated using this approach.

Effect of preconditioning on EPCs proliferation and NO production

The effect of NaHS on EPCs proliferation was assessed by CCK8. After 7 days, cultured EPCs from healthy subjects were pre-incubated with 25, 50, 100, 200 and 500uM of

Fig. 1. Characterization of EPCs derived from human PBMNCs. (A) Representative photographs of EPCs took up DiI-ac-LDL and bound FITC lectin. Scale bar: 100 μ m. (B) Markers of EPCs detected by flow cytometry analysis using CD31, vWF, KDR and CD14 (IgG isotype control was shown in blue, n = 4 per group). (C) EPCs formed capillary-like networks when seeded onto Matrigel surfaces. Scale bar: 100 μ m.

NaHS, respectively. As shown in the Fig. 2A, cells proliferation gradually increased from a concentration of 25 μ M to 200 μ M. Nevertheless, cells proliferation was inhibited at 500 μ M.

It was speculated that NO was imperative for H₂S to exert protective effects [29, 30], therefore, we evaluated NO level in the conditioned media at 48h of NaHS stimulation. As shown in the Figure 2B, NaHS promoted NO production in a dose-dependent manner. Nonetheless, at a concentration of 500 μ M, NO production was reduced which was further confirmed by DAF-FM fluorescence dye. We also found that the intracellular NO level in NaHS-preconditioning EPCs (200 μ M NaHS) was up-regulated most significantly compared to the control group (Fig. 2C). Based on these findings, NaHS concentration of 200 μ M was defined as the maximum dose for the following study.

NaHS-preconditioning enhanced EPCs migration, adhesion and repair capacity

The transwell was used to evaluate EPCs migration and the results showed that the numbers of migrating cells after NaHS-preconditioned were significantly increased in a dose-dependent manner (Fig. 3A and B). Similarly, after 7 days of culture, the adhesion was also significantly promoted in a dose-dependent manner (Fig. 3C and D). The ability of endothelial repair by EPCs was assessed in a carotid artery injury mode in nude mice (Fig. 4A). To investigate whether NaHS-preconditioning EPCs transplantation could accelerate re-endothelialization, Evans Blue staining was performed. Notably, compared to PBS infusion, treatment with non-preconditioned EPCs and NaHS-preconditioned EPCs (*in vitro* 200 μ M of NaHS treatment for 24h) significantly enhanced re-endothelialization capacity of EPCs *in vivo* (P < 0.01, Fig. 4B). To determine whether the transplanted EPCs were incorporated at sites of injury vessels, each nude mouse was transplanted with 1 * 10⁶ CM-DiI-labeled EPCs after carotid artery injury. Fluorescent microscope revealed that the transplanted EPCs were incorporated at the sites of injury. Data showed that there were more EPCs homing to the

Fig. 2. Effects of NaHS on EPCs proliferation and NO production. (A) EPCs proliferation was measured using CCK8. EPCs proliferation after 24h treatment with various NaHS concentrations (0-500uM) (n = 5 per group, *P<0.05 vs control). (B) NO production was assayed using the nitrate reductase method with NaHS-preconditioning for 24h. NO production of EPCs with various NaHS concentrations (0-500uM) (n = 5 per group, *P<0.05 vs control). (C and D) Intracellular NO level was measured by DAF-FM. (C), Intracellular NO production in EPCs. (D), Relative quantification of NO level.

Fig. 3. Effect of NaHS on EPCs migration and adhesion. (A and B) Representative photographs (A) and Quantitative analyses (B) showed EPCs migration after pre-treated with different levels of NaHS in vitro (*P<0.05 vs control, n = 5 per group). (C and D) Representative photographs (C) and Quantitative analyses (D) showed the DiI-labeled EPCs adhesion to HUVECs (*P<0.05 vs control, n = 5 per group).

Fig. 4. Effect of NaHS of EPCs for carotid artery repair. (A) Evans blue staining was used to identify injured carotid artery. Representative photograph showed injured and contralateral uninjured carotid artery. Dil-labeled EPCs were identified as red fluorescent cells, and green fluorescence represented the FITC-lectin positive endothelial layer. (B) EPCs transplantation after pre-treatment with 200uM of NaHS for 24h resulted in improvement of re-endothelialization (* $P < 0.01$ vs. the PBS group, # $P < 0.01$ vs. EPCs group, $n = 5$ per group). (C) Larger number of NaHS-preconditioned EPCs was detected (* $P < 0.01$ vs EPCs group, $n = 5$ per group). (D) EPCs tracing in vivo: an enface view of the injured carotid arteries Showed the CM-Dil-labeled EPCs attached to the vascular injured site on day 3.

injury vessels in the NaHS-preconditioned EPCs group than the non-conditioned EPCs group (Fig. 4C and D).

AMPK/eNOS signaling pathway involved in in vitro and in vivo repair capacity of EPCs

The activations of AMPK and eNOS are essential to EPCs proliferation and functioning. We therefore examined whether the important roles of NaHS in the proliferation, NO production, migration and adhesion of EPCs were mediated by AMPK/eNOS signaling pathway. We used inhibitors including compound C (an AMPK inhibitor) and L-NAME (an eNOS inhibitor) to inhibit the AMPK/eNOS signaling pathway, respectively. Following stimulation with NaHS (200 uM) for 24h, phosphorylation levels of AMPK and eNOS were enhanced in the NaHS-preconditioned EPCs. However, the increases in AMPK and eNOS phosphorylation were inhibited by pre-incubation with compound C and L-NAME (Fig. 5A and B). In addition, the migration and adhesion activity of EPCs were also suppressed with pre-incubation of compound C and L-NAME (Fig. 5C and D). We also investigated whether the AMPK/eNOS signaling pathway was associated with the re-endothelialization capacity of NaHS-preconditioned EPCs *in vivo*. Consist with the *in vitro* results, NaHS treatment significantly increased the re-endothelialization capacity of EPCs, but Compound C and L-NAME abolished the efficacy (Fig. 5E and F). Collectively, these results suggested that NaHS-preconditioning enhanced the re-endothelialization capacity of EPCs through the AMPK/eNOS signaling pathway.

Fig. 5. NaHS improved EPCs function in vitro and re-endothelialization in vivo by activating AMPK/eNOS pathway. (A and B) Representative photographs and quantitative analyses of protein. NaHS significantly increased AMPK and eNOS phosphorylation in EPCs (* $P < 0.01$ vs. control group, # $P < 0.01$ vs. NaHS group, $n = 5$ per group). (C and D) Quantification analysis of migration and adhesion to HUVECs with NaHS pretreatment (* $P < 0.01$ vs the PBS group, # $P < 0.01$ vs EPCs group, $n = 5$ per group). (E and F) Representative photographs and quantitative analyses of re-endothelialization with 200 μ M NaHS-preconditioning of EPCs in response to AMPK or eNOS inhibitors (* $P < 0.01$ vs the control group, # $P < 0.01$ vs. NaHS group, $n = 5$ per group).

Discussion

Re-endothelialization plays an important role in tissue regeneration and repair. Implantation of DES impairs endothelial function which in turn leads to delayed re-endothelialization and increased risk of late or very late in-stent thrombosis. Therefore, accelerating re-endothelialization is a significant therapeutic means for vascular repair, thrombosis prevention, and excessive neo-intimal hyperplasia suppression [31, 32].

Cell-based therapy has become a promising therapeutic strategy for vascular diseases, and re-endothelialization with stem cells transplantation has also become a new method to prevent vessel re-stenosis after stent placement. However, majority of transplanted cells were necrotic and apoptotic due to poor environment. We used a novel pharmacological preconditioning strategy to increase transplanted EPCs survival rate. Notably, H_2S has been considered as the third gaso-transmitter next to NO and carbon monoxide [33]. The principal findings of the present study are as follows: preconditioning of EPCs with NaHS significantly

promotes the migration, adhesion and proliferation of EPCs as well as increases NO production *in vitro*; and *in vivo* study, NaHS treatment markedly up-regulates the AMPK/eNOS signaling pathway as well as enhances re-endothelialization capacity of EPCs. Our findings for the first time demonstrate the potential effects of NaHS on improving the efficacy of EPCs for repair of endothelial injury.

Previous studies have showed that accelerating re-endothelialization is an important therapeutic means for repairing vascular injury and maintaining vascular homeostasis [34, 35]. EPCs transplantation represents a promising therapeutic strategy for restoration of the integrity of vascular endothelium. However, previous strategies of EPCs transplantation yielded modest effects on vascular repair due to acute-phase of transplanted cells necrosis and apoptosis [36]. The lower survival rate significantly influenced the efficacy of stem cells therapy [37-39]. The reasons for low survival rate were multifactorial including ischemic injury and associated inflammation and oxidation [40-44].

In recent years, researchers focused on strategies to improve cells survival rate after transplantation. Numerous studies suggested that genetically modified EPCs with protective genes such as Akt, Bcl-2, β 2AR, CXCR4, CXCR7, BH4, FOX2, eNOS could improve survival rate which in turn could contribute to promote therapeutic efficacies [20-21, 25, 27]. Medications, including statins and angiotensin converting enzyme inhibitors, have also been used to evaluate their effects on EPCs mobilization, and compared to gene-engineering, preconditioning of EPCs with chemical agents were more clinically plausible.

H₂S is an important signaling molecule with multiple biological properties. In mammalian cells, endogenous H₂S is predominantly synthesized by CBS, CSE, cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur-transferase (MST). Recently, evidence suggests that H₂S plays important roles on vascular homeostasis, stem/progenitor cells proliferation and differentiation, and inflammation and oxidation amelioration [39, 45, 46]. Our current study indicated that 500 μ M NaHS has no additional benefit for EPCs, and based on previous report, we considered that the potential mechanisms might be related to the absence of any detectable Ca²⁺ signal with a higher concentration of NaHS as reported by prior study [4]. In addition, high concentration of NaHS might also result in cytotoxicity which in turn also led to NO production and proliferation decrease.

The main purpose of present study was to investigate the efficacy of NaHS-preconditioning of EPCs for vascular repair. We firstly investigated the cyto-protective effect of NaHS on EPCs isolated from peripheral blood of healthy donors *in vitro*, and it showed that 500 μ M NaHS caused a reduction in cell proliferation, which was consistent with the results of Potenza et al. [15]. Therefore, we used NaHS with a concentration of 25-200 μ M in subsequent studies. Based on previous report [15], we postulated that the potential mechanism associated with inhibitory effect of 500 μ M NaHS might be related to dys-regulation of calcium dependent signaling pathway and cellular toxicity. Importantly, we found that NaHS-preconditioning significantly improved EPCs function with a maximal concentration of 200 μ M. Specifically, our results showed that NaHS-preconditioning promoted EPCs migration, adhesion, proliferation and NO production compared to the control group.

With the favorable findings from *in vitro* study, we further investigated the effects of NaHS-preconditioning for improving the efficacies of EPCs for re-endothelialization in a nude mouse carotid artery injury model. As shown in the Fig. 4 B and D, more infused DiI-labeled EPCs in NaHS-preconditioned group were found incorporated into the FITC-lectin positive endothelial layer compared to the non-preconditioned EPCs group. A significantly larger area of re-endothelialization was also observed which might be attributed to increasing NO production with NaHS stimulation [47]. Furthermore, NaHS stimulation increased AMPK/eNOS phosphorylation, which also contributed to improvement of proliferation, migration, and adhesion of EPCs *in vitro* and accelerated re-endothelialization *in vivo*. In contrast, suppressing AMPK/eNOS signaling pathway by compound C and L-NAME abrogated the vascular repair effect of NaHS *in vivo*. The migration, adhesion and NO production *in vitro* were also reduced. Collectively, these findings confirmed that NaHS/AMPK/eNOS signaling pathway involved in the improvement of efficacies of EPCs transplantation.

Conclusion

In summary, our present study shows that transplantation of NaHS-preconditioned EPCs accelerates EPCs homing and re-endothelialization thorough the AMPK/eNOS signaling pathway. NaHS-preconditioned EPCs transplantation may be a novel and promising cell-based therapeutic approach for vascular diseases.

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Disclosure Statement

None.

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