Original Paper

Protective Effects of Bone Marrow-Derived Endothelial Progenitor Cells and *Houttuynia Cordata* in Lipopolysaccharide-Induced Acute Lung Injury in Rats

Da-Sheng Cai   Heng Zhou   Wei-Wei Liu   Ling Pei

Department of Anaesthesiology, the First Hospital of China Medical University, Shenyang City, P. R. China

Abstract
Background/Aims: Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is a serious health problem, and an effective treatment is needed for use in the clinical setting. *Methods:* In this study, we first constructed ALI models in Adult Sprague-Dawley rats. We then used an herbal medicine, *Houttuynia cordata* (HC), to enhance the effect of endothelial progenitor cells (EPCs) on ALI. *Results:* (1) HC improved the therapeutic effects of EPCs on lipopolysachharide-induced ALI in the rat model; (2) HC down-regulated the anti-inflammatory response by suppressing inflammatory cytokines; (3) the combination of EPC and HC reduced expression of iNOS and ET-1 and subsequently prevented lung injury. *Conclusion:* Combined EPC and HC therapy was more effective than either therapy alone. EPC and HC could be used in the clinical treatment of ALI.

Copyright © 2013 S. Karger AG, Basel

Introduction

Cell transplantation is a novel strategy that has been used in the treatment of incurable human diseases [1-3]. Endothelial progenitor cells (EPCs), a heterogeneous subpopulation of bone marrow mononuclear cells, are thought to contribute to vascular homeostasis and endothelial repair [4]. EPCs were first isolated from the mononuclear cell fraction of human peripheral blood [5]. In adults, EPCs may move to sites of injury or a site of neovascularization and then differentiate into mature endothelial cells, thus contributing to re-endothelialization and neo-vascularization [6, 7]. An increased number of EPCs is often observed in patients

Dr Ling Pei

Department of Anaesthesiology, the First Hospital of China Medical University
Nanjing North Street 155, Heping District, Shenyang City 110001
Liaoning Province (P. R. China)
E-Mail lling_pei@163.com

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586

DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1578

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

who present with an acute coronary syndrome such as acute myocardial infarction [8] or unstable angina [9], suggesting the mobilization of EPCs during acute ischemic events.

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is a serious health problem because it is correlated with high mortality and morbidity [10]. The pathomechanism of ALI/ARDS is characterized by a severe acute inflammatory response and diffuse endothelial damage [11]. Therefore, regeneration of pulmonary endothelium has been suggested as potential therapeutic target for ALI/ARDS. Although research on EPCs in ALI/ARDS is at the stage, previous studies demonstrated that could engraft into the injured lung parenchyma and differentiate into lung epithelial cells to repair the injured lungs [12, 13]. We used an herbal medicine, *Houttuynia cordata* (HC), to inhibit the inflammatory response induced by lipopolysaccharide (LPS) in rats. HC is a flowering plant native to southeastern and northeastern Asian countries, and it is effective for treating anaphylaxis, cancer, and viral infection [14, 15].

We first studied the effects of *H. cordata* on EPCs, and we did not detect any changes in the proliferation and differentiation of EPCs after *H. cordata* treatment (data not shown). We combined these two therapies for two reasons: (1) *H. cordata* has no toxic effects on EPCs; and (2) previous studies showed the anti-inflammatory properties of *H. cordata*. The pathomechanism of ALI/ARDS is characterized by a severe acute inflammatory response. Therefore, in this study we explored the effects of transplantation of bone marrow-derived EPCs and HC on lung injury and evaluated their underlying mechanisms of action in a rat model with LPS-induced lung injury.

Materials and Methods

Animals

Adult Sprague-Dawley rats (4 weeks old, 250–300 g) were obtained from the Laboratory Animal Center of China Medical University (Shenyang, China). All animals used in this study were inbred, thus they had the same genetic background. All experimental procedures performed were approved by the China Medical University Ethics Committee.

Herbal material

Dried leaves and stems of *H. cordata* Thunb were purchased from Xuzhou Pharmaceutical Corporation (Xuzhou, China). The air-dried material was extracted with water (100 mg material per ml distilled H_2O) for 4 h in a 45 °C water bath. The water extract was filtered through a 0.2 μm filter and then lyophilized into dry powder. The powder was stored at –80 °C and reconstituted with sterile water before use.

LPS-induced lung injury

LPS from *Escherichia coli* serotype 055:B5 was obtained from Sigma-Aldrich (Carlsbad, CA, USA). Induction of lung injury was performed as previously described [16]. Briefly, recipient rats were anesthetized by ketamine hydrochloride. While the rats were anesthetized, intranasal insufflation was conducted by inhalation of LPS (30 μg/rat in PBS) through one nostril. Rats were divided into five experimental groups (20 rats in each group): (A) untreated group, (B) LPS treatment followed by vehicle (PBS) treatment, (C) LPS treatment followed by EPC treatment, (D) LPS treatment followed by HC treatment, (E) LPS treatment followed by both EPC and HC treatment.

Isolation and culturing of EPCs

Following the method of Lee et al. [17] and Mao et al. [18], EPCs were isolated from male rat bone marrow and engrafted in female recipients. Briefly, mononuclear cells were separated from the marrow of the tibia and femur of male Sprague-Dawley rats (4 weeks old) by density gradient centrifugation with 1.083 g/ml Histopaque (Sigma). Isolated cells were maintained in DMEM (Sigma) containing heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. After 4 days in culture, nonadherent cells were removed by washing with PBS, and new medium was applied thereafter. The medium was subsequently changed every 3 days for further cultivation. On day 7 of culture, the adherent cells (known as early EPCs) were harvested by trypsinization for analysis or transplantation.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586

DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1579

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

Characterization of EPCs

EPCs were characterized by intake of 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (Dil-ac-LDL) and fluorescein isothiocyanate-labeled *Ulex europaeus* agglutinin-1(FITC-UEA-1). Cells were sequentially incubated with Dil-ac-LDL (10 µg/ml, Molecular Probes, Eugene, OR, USA) and FITC-UEA-1(10 µg/ml, Sigma). Cells were examined using inverted fluorescence microscopy (Olympus, Tokyo, Japan), and double-positive cells were identified as EPCs.

Flow cytometer (FCM) detection of surface markers on EPCs

Adherent cells were digested into a single-cell suspension, blocked for 30 min, and incubated with FITC-conjugated CD34 (BD Biosciences, Baltimore, MD, USA), PE-conjugated VEGFR2 (R&D Systems Inc., Minneapolis, MN, USA), FITC-conjugated CD146 (BD Biosciences), FITC-conjugated vWF (R&D), CD133 (Abcam, Cambridge, MA, USA), or PE-conjugated anti-human kinase insert domain-conjugating receptor (KDR; R&D) for 30 min at 4 °C. They then were washed twice with PBS. Suspensions were immediately analyzed by a FACSCalibur machine (BD Biosciences).

Treatment procedures

A 1.5-cm longitudinal incision was made on the ventral surface of the neck of rats, and the right external jugular vein was exposed. Rats were injected with PBS or EPCs (approximately 5×10^6 in 200 µl PBS). After surgery, rats were sutured and sterilized according to normal procedure and allowed to recover spontaneously from the anesthesia. Rats with or without EPC transplantation were injected with HC (50 mg/g) via the tail vein every day.

Blood gas analysis

Seven days after treatment, arterial blood was drawn from the ear artery of rats with ALI under room-air conditions. Oxygen saturation (Sa_{O2}) and Po2 (Pa_{O2}) in the arterial blood were analyzed using a blood gas analyzer (ABL 520, Radiometer, Copenhagen, Denmark).

Plasma cytokine measurements

Plasma samples were obtained from rats 7 days after treatment. TNF-α and IL-10 levels were measured in plasma samples using ELISA kits (R&D).

Determination of bronchoalveolar lavage (BAL) protein

BAL was performed by intratracheal injection of 10 mL PBS followed by gentle aspiration. Recovered fluid was pooled and centrifuged at 500 g for 10 min at 4 °C. Supernatants were removed and stored at –20 °C until testing was performed. Total protein was quantified in BAL using the bicinchoninic acid method as provided by the manufacturer (Pierce, Rockford, IL, USA).

Lung wet-to-dry ratio

Seven days after treatment, the intermediate lobe of the right lung was excised and weighed immediately. Lung tissues were dried in an oven at –80 °C for 12 h and reweighed. The lung wet-to-dry ratio was obtained by dividing the mass of the initial specimen by the mass of the dried specimen.

RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) of the sry gene

Total RNA was isolated form the intermediate lobe of the right lung using an RNeasy Mini Kit (Biomed, Beijing, China). First strand cDNA was reverse transcribed with 1 µg of total RNA using the TaKaRa Reverse Transcription Kit (TaKaRa, Dalian, China) and oligo (dT) 15 primers (TaKaRa). The *sry* primers were 5'-GCCTCATCGAAGGGGTTAAAGTG-3' (forward primer) and 5'-GAGGACTGGTGTGCAGCTCTA-3' (reverse primer). The housekeeping gene *GAPDH* was used as an internal control for normalization of the results. The *GAPDH* primers were 5'-AGAAGGCTGGGGCTCATTTG-3' (forward primer) and 5'-AGGGGCCATCCACAGTCTTC-3' (reverse primer). PCR amplification of cDNA was performed in 20 µl mixtures. Finally, amplicons were electrophoresed in 2% agarose gel with ethidium bromide and visualized under UV illumination.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586

DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1580

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

Histopathologic analysis

Hematoxylin-eosin (HE) was used to stain the intermediate lobe of the right lung, which then was fixed with 10% formaldehyde in PBS for 24 h, dehydrated in a graded ethanol series, embedded in paraffin, and sliced at 5 μm. Paraffin sections were stained with HE for histopathologic analysis. The sections were assessed for the presence of airway epithelial necrosis, intra-alveolar edema, hyaline membranes, hemorrhage, and the recruitment of inflammatory cells to the airspace.

Gelatin zymography

Fifty micrograms of protein were applied to 10% polyacrylamide gels with 1% gelatin incorporated as a substrate for gelatinolytic protease analysis. After running the gel the SDS was removed by washing twice in 2.5% Triton X-100 for 30 min. The gels were incubated overnight in zymography development buffer containing 50 mM Tris-HCl (pH = 7.4), 2 mM NaN_3, and 5 mM $CaCl_2$. After development the gels were stained for 3 h in 45% methanol/10% glacial acetic acid containing 1% (w/v) Coomassie Blue R-250; the gels subsequently were partially destained with the same solution without dye. The gelatinolytic activity of each matrix metalloproteinase (MMP) was qualitatively evaluated as a clear band against the blue stained gelatin background.

Western blotting analysis

Tissues from the intermediate lobe of the right lung were lysed in lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 1% Triton-X100) containing a protease inhibitor cocktail (Sigma). Equivalent amounts of protein (60 μg) were separated using 10% SDS-PAGE and transferred to a PVDF membrane (Millipore Corporation, Billerica, MA, USA). Western blotting was performed using the following primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA): iNOS (sc-8310), ET-1 (sc-21625), MMP-2 (sc-8835), MMP-9 (sc-12759), TIMP-1 (sc-5538), TIMP-2 (sc-365671), and β-actin (sc-130657,). The binding of each specific antibody was detected with horseradish peroxidase-conjugated respective secondary antibodies (Amersham Biosciences, Amersham, UK) and ECL solutions (Amersham Biosciences). The intensity of each Western blot band was semi-quantified using Quantity One software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Statistical analyses were performed using GraphPad PRISM 4 software (San Diego, CA, USA). Overall survival of rats was analyzed using the Kaplan-Meier method, and the significance of differences in survival rates was estimated using the log rank test. Values of $P < 0.05$ were considered to be significant.

Results

Characterization of EPCs

EPCs isolated from rat bone marrow displayed characteristic EPC phenotypes, as indicated by their uptake of Dil-acetyl-LDL and expression of UEA-I (Fig. 1A). The EPCs also displayed expression of CD34, vWF, CD146, VEGFR2, CD133, and KDR on the plasma membrane (Fig. 1B).

Engraftment of EPCs to the injured lung

Following the methods of Mao et al. [18], the engraftment of male donor cells in female recipients was assessed by RT-PCR of the Y chromosome-specific gene sry. As shown in Figure 1C, sry gene expression was detected using RT-PCR in the recipient female lung tissues after treatment.

Combined application of EPC and HC treatment attenuated the severity of lung injury and improved the survival rate of rats

Figure 2A shows histological sections of rat lung tissues. There were no obvious lesions on the lung tissues of untreated and EPC-treated rats. In the PBS-treated group, the size of the pulmonary alveolus cavity decreased and thickening of the alveolar wall was observed. The EPC-treated group showed reduced damage, including decreased formation of a hyaline
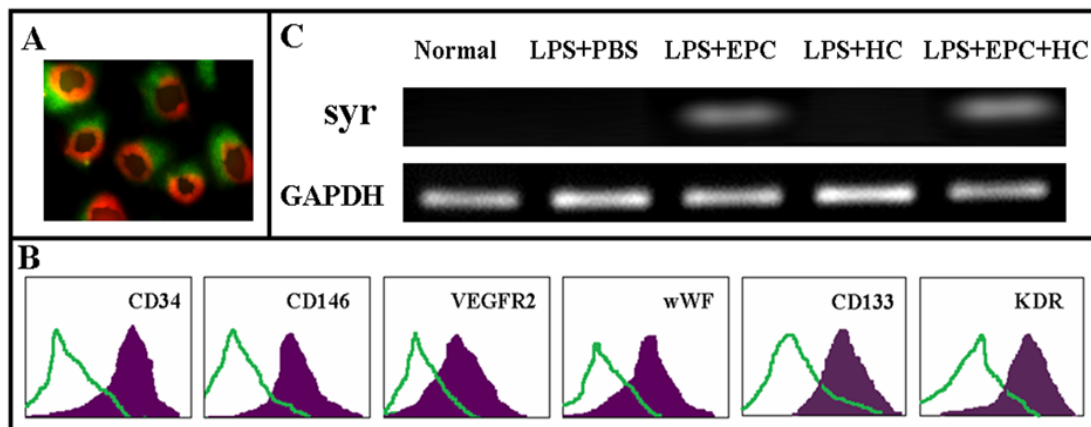
Fig. 1. Characteristics of isolated EPCs from rat bone marrow. A) Red color represents fluorescent staining of DiI-Ac-LDL and green color represents fluorescent staining of FITC-conjugated UEA-I. B) Representative profiles based on flow cytometric analysis of immunolabelled cells with a control antibody (green line) and specific antibodies (violet line) to EPC-related markers (CD34, CD146, VEGFR2, and wWF). C) Recipient female rats that had been treated with LPS showed expression of the sry gene (determined by RT-PCR).

membrane, reduced thickness of the alveolar wall, and lower number of inflammatory cells in the lung interstitium. Compared with the other groups, the combined application of EPC and HC had the best effect (Fig. 2A).

Pulmonary edema is a hallmark of ALI, and it consists of various degrees of water and proteins [19]. Disruption of the alveolar-capillary barrier was assessed by measuring the water content in the lung. The lung wet-to-dry ratio was reduced in the EPC-treated rats ($31.5 \pm 4.24\%$ vs. $45.3 \pm 4.35\%$ in the LPS group; Fig 2B, $P < 0.05$). EPC and HC-treated rats ($24.9 \pm 2.96\%$) showed a lower lung wet-to-dry ratio than the EPC-treated group ($31.5 \pm 4.24\%$) or the HC-treated group ($32.7 \pm 3.52\%$) (Fig. 2B, $P < 0.05$).

Bronchoalveolar lavage (BAL) protein is a marker of endothelial and epithelial permeability [20]. Concentration of the BAL protein was significantly reduced in the EPC- and HC-treated group (189.6 ± 33.7 ng/ml) compared with the EPC-treated group (356.7 ± 39.6 ng/ml), the HC-treated group (348.6 ± 32.5 ng/ml), and the LPS group (758.3 ± 36.8 ng/ml) (Fig. 2C, $P < 0.05$). In comparison with the LPS group, Pa_{O2} and Sa_{O2} were significantly improved in rats receiving EPC transplantation (Fig. 2D, $P < 0.05$). Unfortunately, HC treatment had no effect on Pa_{O2} and Sa_{O2}. Rats in both the EPC-treated group and the HC-treated group showed a significantly higher survival rate compared with the LPS group (Fig. 2E, $P < 0.01$). Moreover, the survival rate of rats treated with EPC and HC was higher than that of rats treated with either EPC or HC alone (Fig. 2E, $P < 0.01$).

EPC and HC treatment altered cytokine responses

The level of proinflammatory cytokine TNF-α in the plasma was about 2-fold higher in the LPS group compared with the EPC-treated group (65.3 ± 4.86 pg/ml vs. 33.2 ± 4.25 pg/ml, $P < 0.05$) (Fig. 3A). The TNF-α level was significantly reduced in the HC-treated group (14.8 ± 4.89 pg/ml) compared with the EPC-treated group and the LPS group ($P < 0.05$). Rats in the LPS group had a significantly higher level of IL-10 than those in the untreated group (58.8 ± 3.25 pg/ml vs. 13.6 ± 1.05 pg/ml, $P < 0.05$) (Fig. 3B). There was no significant difference of the plasma level of IL-10 between the LPS group and the EPC-treated group (58.8 ± 3.25 pg/ml vs. 56.9 ± 3.65 pg/ml). Interestingly, the HC-treated group (18.6 ± 3.98 pg/ml) had a lower IL-10 concentration compared with the LPS group and EPC-treated group.

Mechanism(s) of action EPC and HC on ALI

The expression levels of ET-1 and iNOS in the LPS group were higher than the levels in rats treated with EPC, HC, or the combination of EPC and HC (Fig. 4B, $P < 0.05$). Western

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586

DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1582

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

Fig. 2. EPC and HC improved symptoms of LPS-induced lung injury. A) The lung interstitium of the treated group showed reduced cavities of pulmonary alveoli, formation of hyaline membranes, and hemorrhage. EPC and HC significantly decreased the lung wet/dry ratio B) and BAL protein concentration C). D) The P_{O_2} (Pa_{O_2}) and oxygen saturation (Sa_{O_2}) in arterial blood were significantly improved in rats receiving EPC and HC treatment. E) Kaplan-Meier survival curves of the groups described in the Material and Methods section. Compared with the PBS-treated rats, rats that received EPC and HC treatment had a significantly higher survival rate.
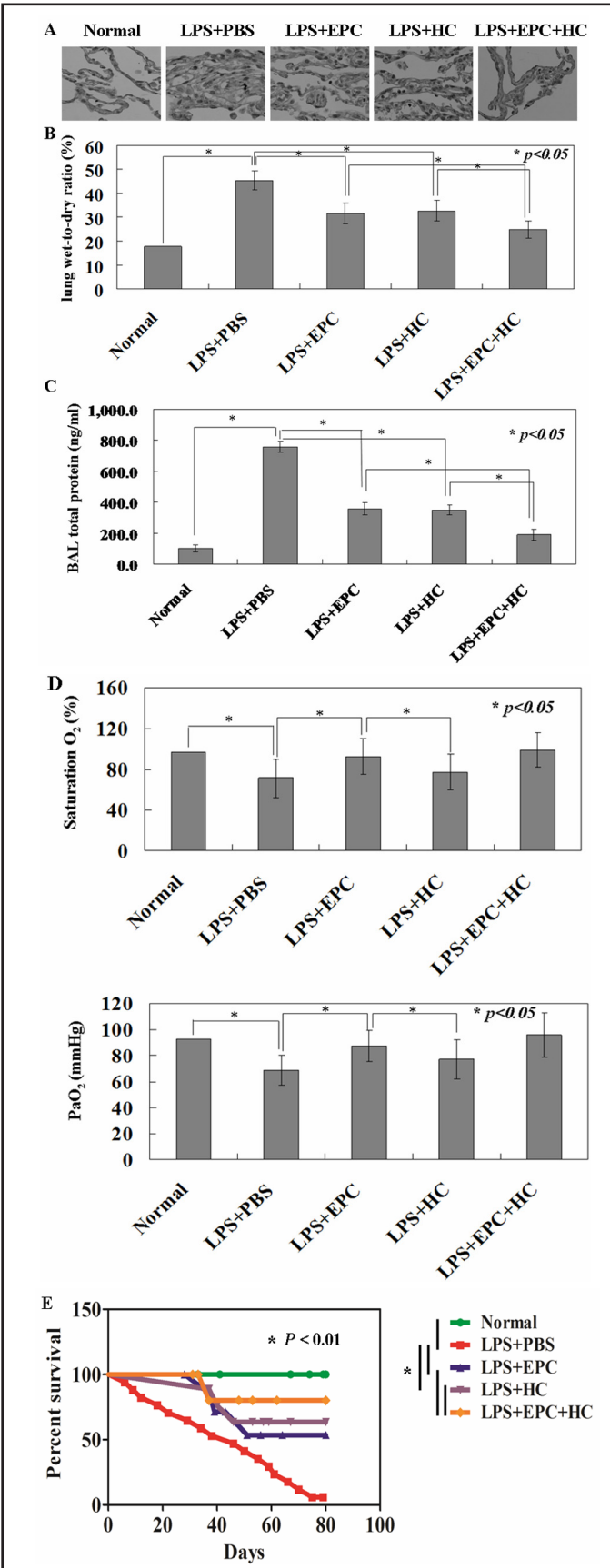
Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586
DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1583

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

Fig. 3. The ELISA kit was used to detect IL-10 and TNF-α in the plasma of rats. After treatment, levels of IL-10 and TNF-α were significantly lower than those in the PBS-treated group.
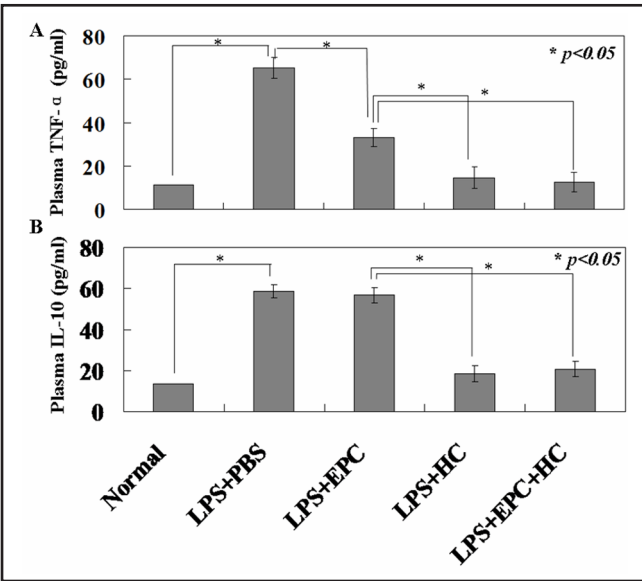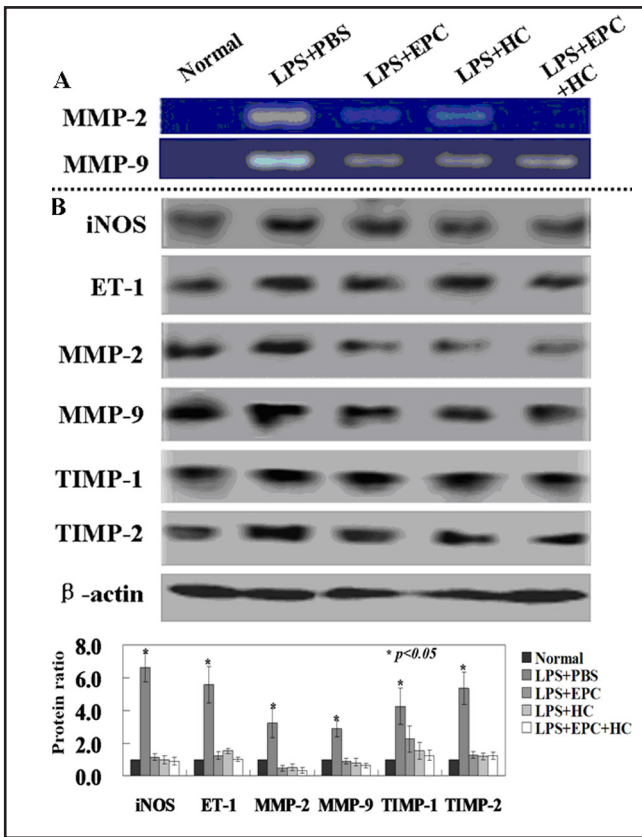

Fig. 4. A) Gelatinolytic activity of secreted active MMP-2 and MMP-9 was analyzed by zymography. B) The protein concentration of iNOS, ET-1, MMP-2/9, and TIMP-1/2 extracted from the lung tissues was evaluated using Western blotting and was normalized against β-actin. The bar graph shows significant differences between each group (P < 0.05).

blotting analysis revealed that the levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the LPS group were also significantly higher than those in the untreated group (Fig. 4). However, rats in the EPC- and HC-treated groups had lower levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 than rats in the LPS group. Consistent with the results of Western blotting analysis, the activities of MMP-2 and MMP-9 in the LPS group also were inhibited by EPC or HC treatment (Fig. 4A). The activities of MMP-2 and MMP-9 were lowest in the combination of EPC and HC group.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2013;32:1577-1586

DOI: 10.1159/000356594
Published online: December 03, 2013

© 2013 S. Karger AG, Basel
www.karger.com/cpb

1584

Cai/Zhou/Liu/Pei: Combined Effects of EPC and HC on Acute Lung Injury

Discussion

The pathology of ALI and ARDS is caused by transmural alveolar wall damage [11, 21]. In previous studies, EPC transplantation has been demonstrated to be a useful therapeutic method with clinical practical value for treating ALI/ARDS [22, 23]. In this study, we confirmed that EPCs could repair lung damage induced by LPS. ALI and ARDS are characterized by diffuse pulmonary infiltration, increased pulmonary capillary permeability, and development of noncardiogenic pulmonary edema [24]. Transplantation of EPCs significantly reduced the lung wet-to-dry ratio in rabbits with endotoxin-induced ALI. We also found that EPC reduced the lung wet-to-dry ratio in our LPS-induced ALI rat models. These results suggested reduced pulmonary water content in the EPC-treated animals.

The main purpose of this study was to identify the role of HC in LPS-induced ALI. *H. cordata* is known to be an effective herbal medicine for treating allergic inflammation [15], and we found that HC is effective in the treatment of pulmonary edema. Combined EPC and HC therapy was more effective than treatment with either EPC or HC alone, as evidenced by the expression levels of iNOS and ET-1. Both iNOS and ET-1 have been shown to be important biomarkers of the ALI disease state and have been used to elevate the inflammatory state via a proinflammatory effect [18]. Consistent with previous studies [18, 25], expressions of iNOS and ET-1 were significantly up-regulated in the lung tissues of rats with ALI, indicating the presence of oxidative and inflammatory stresses in the injured lung. However, the levels of iNOS and ET-1 were significantly lower in rats after treatment with HC and EPC compared to untreated rats.

In regards to the mechanism of action of HC and EPC for the treatment of ALI, our results demonstrated that these treatments (EPC, HC, or EPC + HC) have potent inhibitory effects on the inflammatory response and the MMP axis. Cytokine IL-10 has been suggested to mediate the anti-inflammatory response via its ability to internalize and thus act as a scavenger of free endotoxin [26]. TNF-α, which is a major proinflammatory cytokine, is produced by many different cell types, including endothelial cells during inflammation [18]. In this study, we found that HC could inhibit IL-10 and TNF-α in LPS-induced ALI. Lee et al. [27] described the beneficial therapeutic effects of HC on Th2-mediated or allergic skin disorders. Consistent with their study, we also found that HC could inhibit inflammatory reactions in ALI. The MMP axis is known to play a crucial role in extracellular matrix remodeling and vascular homeostasis [28]. Early studies showed that increased MMP activity and expression enhanced vascular remodeling in ALI [29, 30]. We confirmed that both MMP-2/9 and TIMP-1/2 were inhibited by the combination of HC and EPC.

The main findings of this study are as follows: (1) HC improved the therapeutic effects of EPCs on LPS-induced ALI in the rat model; (2) HC down-regulated the anti-inflammatory response by suppressing inflammatory cytokines; (3) the combination of EPC and HC reduced expression of iNOS and ET-1 and subsequently prevented lung injury.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgements

Reference

1   Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001;98:10344-10349.

2   Sato Y, Araki H, Kato J, Kawano Y, Kobune M, Sato T, Miyanishi K, Takayama T, Takahashi M, Takimoto R, Iyama S, Matsunaga T, Ohtani S, Matsuura A, Hamada H, Niitsu Y: Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood 2005;106:756-763.

3   Qian H, Yang H, Xu W, Yan Y, Chen Q, Zhu W, Cao H, Yin Q, Zhou H, Mao F, Chen Y: Bone marrow mesenchymal stem cells ameliorate rat acute renal failure by differentiation into renal tubular epithelial-like cells. Int J Mol Med 2008;22:325-332.

4   Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434-438.

5   Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM: Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-967.

6   Akeson AL, Wetzel B, Thompson FY, Brooks SK, Paradis H, Gendron RL, Greenberg JM: Embryonic vasculogenesis by endothelial precursor cells derived from lung mesenchyme. Dev Dyn 2000;217:11-23.

7   Iwatsuki K, Tanaka K, Kaneko T, Kazama R, Okamoto S, Nakayama Y, Ito Y, Satake M, Takahashi S, Miyajima A, Watanabe T, Hara T: Runx1 promotes angiogenesis by downregulation of insulinlike growth factor-binding protein-3. Oncogene 2005;24:1129-1137.

8   Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T: Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001;103:2776-2779.

9   George J, Goldstein E, Abashidze S, Deutsch V, Shmilovich H, Finkelstein A, Herz I, Miller H, Keren G: Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. Eur Heart J 2004;25:1003-1008.

10 Frutos-Vivar F, Ferguson ND, Esteban A: Epidemiology of acute lung injury and acute respiratory distress syndrome. Semin Respir Crit Care Med 2006;27:327-336.

11 Ware LB, Matthay MA: The acute respiratory distress syndrome. N Engl J Med 2000;342:1334-1349.

12 Yamada M, Kubo H, Kobayashi S, Ishizawa K, Numasaki M, Ueda S, Suzuki T, Sasaki H: Bone marrow-derived progenitor cells are important for lung repair after lipopolysaccharide-induced lung injury. J Immunol 2004;172:1266-1272.

13 Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, Brigham KL: Bone marrow-derived mesenchymal stem cells in repair of the injured lung. Am J Respir Cell Mol Biol 2005;33:145-152.

14 Li GZ, Chai OH, Lee MS, Han EH, Kim HT, Song CH: Inhibitory effects of Houttuynia cordata water extracts on anaphylactic reaction and mast cell activation. Biol Pharm Bull 2005;28:1864-1868.

15 Lu HM, Liang YZ, Yi LZ, Wu XJ: Anti-inflammatory effect of Houttuynia cordata injection. J Ethnopharmacol 2006;104:245-249.

16 Szarka RJ, Wang N, Gordon L, Nation PN, Smith RH: A murine model of pulmonary damage induced by lipopolysaccharide via intranasal instillation. J Immunol Methods 1997;202:49.

17 Lee DY, Cho TJ, Kim JA, Lee HR, Yoo WJ, Chung CY, Choi IH: Mobilization of endothelial progenitor cells in fracture healing and distraction osteogenesis. Bone 2008;42:932-941.

18 Mao M, Wang SN, Lv XJ, Wang Y, Xu JC: Intravenous delivery of bone marrow-derived endothelial progenitor cells improves survival and attenuates lipopolysaccharide-induced lung injury in rats. Shock 2010;34:196-204.

19 Schläpfer M, Leutert AC, Voigtsberger S, Lachmann RA, Booy C, Beck-Schimmer B: Sevoflurane reduces severity of acute lung injury possibly by impairing formation of alveolar oedema. Clin Exp Immunol 2012;168:125-134.

20 Pauluhn J: Acute inhalation toxicity of polymeric diphenyl-methane 4,4'-diisocyanate in rats: time course of changes in bronchoalveolar lavage. Arch Toxicol 2000;74:257-269.

21 Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K: Acute lung injury review. Intern Med 2009;48:621-630.

22   Lam CF, Roan JN, Lee CH, Chang PJ, Huang CC, Liu YC, Jiang MJ, Tsai YC: Transplantation of endothelial progenitor cells improves pulmonary endothelial function and gas exchange in rabbits with endotoxin-induced acute lung injury. Anesth Analg 2011;112:620-627.

23   Gao X, Chen W, Liang Z, Chen L: Autotransplantation of circulating endothelial progenitor cells protects against lipopolysaccharide-induced acute lung injury in rabbit. Int Immunopharmacol 2011;11:1584-1590.

24   Ashbaugh DG, Bigelow DB, Petty TL, Levine BE: Acute respiratory distress in adults. Lancet 1967;2:319-323.

25   Fujii Y, Magder S, Cernacek P, Goldberg P, Guo Y, Hussain SN: Endothelin receptor blockade attenuates lipopolysaccharide-induced pulmonary nitric oxide production. Am J Respir Crit Care Med 2000;161:982-989.

26   Chen CT, Foo NH, Liu WS, Chen SH: Infusion of human umbilical cord blood cells ameliorates hind limb dysfunction in experimental spinal cord injury through anti-inflammatory, vasculogenic and neurotrophic mechanisms. Pediatr Neonatol 2008;49:77-83.

27   Lee JS, Kim IS, Kim JH, Kim JS, Kim DH, Yun CY: Suppressive effects of Houttuynia cordata Thunb (Saururaceae) extract on Th2 immune response. J Ethnopharmacol 2008;117:34-40.

28   Chelladurai P, Seeger W, Pullamsetti SS: Matrix metalloproteinases and their inhibitors in pulmonary hypertension. Eur Respir J 2012;40:766-782.

29   Keck T, Balcom JH 4th, Fernández-del Castillo C, Antoniu BA, Warshaw AL: Matrix metalloproteinase-9 promotes neutrophil migration and alveolar capillary leakage in pancreatitis-associated lung injury in the rat. Gastroenterology 2002;122:188-201.

30   Ma B, Zhou PY, Ni W, Wei W, Ben DF, Lu W, Xia ZF: Inhibition of activin receptor-like kinase 5 induces matrix metallopeptidase 9 expression and aggravates lipopolysaccharide-induced pulmonary injury in mice. Eur Rev Med Pharmacol Sci 2013;17:1051-1059.