Thrombosis Research 135 (2015) 990–999

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

# Therapeutic effectiveness of bone marrow-derived mesenchymal stem cell administration against acute pulmonary thromboembolism in a mouse model

Xiaochun Peng [a,b], Jiansha Li [b,c], Xiangyuan Yu [a,b], Rubin Tan [a,b], Liping Zhu [a,b], Jun Wang [d], Rongshuai Wang [e], Guoqiang Gu [e], Qian Liu [e], Liang Ren [e], Chen Wang [f,*], Qinghua Hu [a,b,**]

[a] Department of Pathophysiology, Tongji School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[b] Key Laboratory of Pulmonary Diseases of Ministry of Health, Tongji School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[c] Institute of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[d] Department of Physiology, Capital Medical University, Beijing 100730, China
[e] Department of Forensic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[f] Department of Respiratory Medicine, Beijing Hospital, Ministry of Health, Beijing 100730, China

ARTICLE INFO

*Article history:*
Received 26 July 2014
Received in revised form 31 January 2015
Accepted 8 February 2015
Available online 12 February 2015

*Keywords:*
Bone marrow-derived mesenchymal stem cell
Acute pulmonary thromboembolism
Serial sectioning
Glyceraldehyde-3-phosphate dehydrogenase
Gender difference

ABSTRACT

*Instruction:* Acute pulmonary thromboembolism (APTE) is a common clinical condition associated with significant morbidity and mortality. Although promising, bone marrow-derived mesenchymal stem cell (BMSC) treatment for thrombus resolution remains controversial. The therapeutic effectiveness of BMSC against APTE has not been evaluated. This study aims to determine whether BMSCs administration is effective in mouse model.
*Materials and Methods:* Therapeutic efficacy of female and male BMSCs were evaluated by applying serial sectioning analysis method for the whole lungs of APTE mice and calculating each thrombus size in volume. Plasmid construction and stable transfection were used to manipulate expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in both genders of BMSCs. Western blot were performed to detect GAPDH and urokinase plasminogen activator expression in BMSCs.
*Results:* Our data showed, 1) compared with non-serial sectioning method, the serial sectioning method detected more thrombi, larger size ranges of thrombus area, and the volume of each individual thrombus. 2) BMSCs significantly decreased the thrombi size in APTE mice, with female BMSCs superior to male ones. 3) female BMSCs showed a higher GAPDH protein level and manipulations of GAPDH expression in female or male BMSCs profoundly affected their therapeutic efficacies as well as urokinase plasminogen activator expression.
*Conclusion:* This study indicates serial-sectioning analysis method is necessary for evaluating APTE and provides strong evidences for BMSCs possessing therapeutic effectiveness against APTE, with female BMSCs superior to male counterparts. GAPDH played a critical role in the superior function of female BMSCs, possibly by regulating the expression of urokinase plasminogen activator.


## Introduction

Acute pulmonary thromboembolism (APTE) is a life-threatening emergency, associated with significant morbidity and mortality [1]. However, because of bleeding complications, fragmentation and some clinical dilemmas, therapeutic efficacy of the available treatments including anticoagulation and thrombolytic management is unsatisfactory [2]. Therefore, developing new treatment strategies for APTE is required. To develop and evaluate new treatment approach, appropriate animal models as well as analysis methods for APTE are fundamental and necessary.

To the best of our knowledge, there are only a few mouse models for APTE. It was reported that APTE mouse models induced by a single intravenous injection of a thrombogenic stimulus (collagen plus epinephrine) resulted in high mortality [3]. Contrarily, our previous work showed that thrombi injection was a stable and feasible way to create APTE mouse model [4]. Though there is a conventional method for determining the size of solitary deep vein thrombus by calculating

*Abbreviations:* APTE, acute pulmonary thromboembolism; BMSCs, bone marrow-derived mesenchymal stem cells; GAPDH, glyceraldehyde-3-phosphate dehydrogenas; u-PA, urokinase plasminogen activator; M-BMSCs, male BMSCs; F-BMSCs, female BMSCs; OE-GAPDH, GAPDH-overexpression plasmid; OE-vector, overexpression vector; siRNA-GAPDH, GAPDH-specific siRNA plasmid; siRNA-vector, GAPDH-specific siRNA vector.

** Correspondence to: Q. Hu, Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. Tel.: +86 27 6331 4909; fax: +86 27 8369 2608.
* Correspondence to: C. Wang, Department of Respiratory Medicine, Beijing Hospital, Beijing 100730, China. Tel.: +86 10 85136810; fax: +86 10 65220179.
*E-mail addresses:* cyh-birm@263.net (C. Wang), qinghuaa@mails.tjmu.edu.cn (Q. Hu).

http://dx.doi.org/10.1016/j.thromres.2015.02.009

the sum of the cross-sectional area of all selected sections cut at 300-μm intervals [5,6], few studies investigated the size of multiple PTEs. Therefore, we performed serial sectioning of all pulmonary lobes and determined each thrombus size using volume instead of area. We further compared this analysis method with its conventional counterpart. The data demonstrated that the serial sectioning analysis method provided more objective and exhaustive data on the number, as well as size of thrombi in APTE mice.

Stem cell therapy is a novel and important treatment modality in clinical medicine that can be used against many diseases [7]. Meanwhile, there is growing concern regarding the safety of stem cell therapy. Several clinical studies indicated that the intravascular administration of stem cells may increase the risk for developing PTE [8,9]. A recent investigation in a mouse model indicated significant procoagulation and resulting PTE after stem cell administration [10]. However, there are also studies indicating that stem cells could facilitate thrombus resolution [11–13]. Modarai B et al reported that bone marrow-derived endothelial progenitor cells (EPC) were recruited into resolving venous thrombi [11]. These cells could contribute to thrombus recanalisation by expressing a variety of proangiogenic cytokines or by lining the new vessels that appeared within the thrombus [12]. A later investigation in 28 athymic nude rats with thrombus induction showed that intravenous administration of EPC resulted in significantly enhanced thrombus neovascularization and therefore facilitated thrombus resolution [13]. To our knowledge, the effect of bone marrow-derived mesenchymal stem cells (BMSCs) transplantation on previously existed PTE has not been evaluated.

Moreover, gender differences of stem cells on therapeutic efficiency had aroused much attention in recent years. Some studies showed superiority of female stem cells on fat augmentation [14] or against ischaemia/reperfusion injury [15]. However, another important study demonstrated a better therapeutic potential of male stem cells in an articular cartilage defect model [16]. Our latest work reported superior efficacy of female BMSCs from C57BL/6 J mice against monocrotaline-induced pulmonary hypertension. Furthermore, in that study we accidently found a significantly higher expression of glyceraldehyde-3-phosphate dehydrogenas (GAPDH) in female BMSCs and verified GAPDH played a critical role in the superior therapeutic efficacy of female BMSCs against pulmonary hypertension [17]. The discrepancies about gender difference on stem cell therapy implied that it might be disease or tissue specific [17]. Up to now, there is no report about sex differences of BMSCs treatment on APTE.

Facing these contradictions and unknown issues, in this study, we developed an analysis method using the serial sectioning of the entire lung in mouse models to determine whether BMSCs can aggravate or ameliorate the formation of existing, multiple PTEs and, to detect whether there were gender differences about BMSCs treatment against APTE. The urokinase-type plasminogen activator (u-PA) production was critically involved in bone marrow transplantation-associated early thrombus resolution [11], we further explored if u-PA can be the mechanism underlying the potential gender difference of BMSCs as the downstream target of GAPDH.

## Materials and Methods

### *Ethics Statement and the Animals Used*

C57BL/6 J mice were used for stem cell isolation and transplantation as well as APTE models. All animal experiments were approved by the Institutional Animal Care and Use Committee.

### *Primary Culture and Identification of BMSCs*

The isolation, identification and maintenance of BMSCs were performed as we described before [17]. Briefly, BMSCs were obtained from the bone marrow of 3- to 4-week-old female and male C57BL/6 J mice by flushing the femur and tibia diaphyses with Dulbecco's modified Eagle's medium (DMEM) and were cultured in DMEM supplemented with 20% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin. BMSCs at passage 3 were homogenous and appeared in long rhombus shape. Then the cells were trypsinized (0.25% trypsin) and adjusted to the density of $2 \times 10^6$/ml, and then incubated with fluorescence-conjugated antibodies for CD29, CD34, CD45 and their isotype controls in a black chamber at 4 °C for 30 min, respectively; after washing with PBS, cells analysis was performed with a flow cytometry (Becton Dickenson, USA) for further characterization of BMSCs.

### *In Vivo Experimental Protocol*

One day before the experiment, autologous thrombi were prepared with the following procedures. 200 μl plasma was collected from C57BL/6 J mouse tail and mixed with 50 μl thrombin $CaCl_2$ mixtures (10 U/ml bovine thrombin containing 0.5 mmol/L $CaCl_2$). By aspirating the mixture into a glass catheter (1.2 mm in inner diameter), the autologous thrombus was made and then stabilized for 10 min at room temperature, 30 min at 37 °C water bath, and stored at 4 °C overnight. The thrombus was cut into 1 mm long pieces (autologous thrombi) before use.

Under anaesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection, female and male C57BL/6 J mice, 20-23 g, 6-8 weeks old, received an intravenous injection of 30 autologous thrombi with 0.4 ml saline via the right jugular vein to produce the APTE model [4,18]. Then, the APTE mice were either not treated or treated with female or male BMSCs ($2 \times 10^6$ cells in 0.5 ml PBS via tail vein) in a sex-matched or a sex-mismatched way at 4 h, 8 h, or 16 h after thrombi injection. The experimental groups studied in either gender mice and each time point were as follows: 1) PTE mice treated with PBS (control group), 2) PTE mice treated with female BMSCs (F-BMSCs group), and 3) PTE mice treated with male BMSCs (M-BMSCs group). One day after thrombi injection, the mice were sacrificed, and the lungs were collected for further analysis.

### *Measurement of Thrombus Size*

After the lungs were fixed in 10% formalin and embedded in paraffin, 5-μm-thick serial sections were obtained from whole lung lobes and stained with hematoxylin and eosin (H&E). Digital images of all thrombi in the sections were captured at × 100 magnification (Nikon Imaging). The acquisition, spatial calibration, and analysis of the captured images were achieved using Image-Pro Plus analysis software (Media Cybernetics UK, Berkshire, UK). As for the serial sectioning analysis method, the size of each thrombus was calculated and recorded by volume. Briefly, we collected all the serial H&E pictures of each thrombus. The total area of one thrombus in all the serial pictures multiplying by section thickness (5 μm) equals the volume of this thrombus (Fig. 1A). For comparison, we also recorded the size of thrombi by calculating the sum of the thrombus areas in all selected sections cut at 300-μm intervals as the conventional method previously reported [5,6].

### *Lung Biopsy of Patients*

The study protocol using lung biopsy of patients was approved by the Institutional Research Ethics Committee. 3 biopsy embedded blocks, one for each of the three patients of pulmonary infection with suspected PTE were collected from the forensic department for the current study. The blocks of ~ 1.0 cm × 1.0 cm × 0.2 cm size for each were embedded in paraffin and the 5 μm thick serial sections obtained were stained with H&E. The thrombus number, thrombus size, and size distributions from serial analysis method were compared with those from non-serial analysis counterpart.