Potential Therapeutic Strategy in Chronic Obstructive Pulmonary Disease Using Pioglitazone-Augmented Wharton's Jelly-Derived Mesenchymal Stem Cells

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Background: A recent study reported that mesenchymal stem cells possess potential cellular therapeutic properties for treating patients with chronic obstructive pulmonary disease, which is characterized by emphysema. We examined the potential therapeutic effect of Wharton's Jelly-derived mesenchymal stem cells (WJMSCs), following pretreatment with pioglitazone, in lung regeneration mouse emphysema models.

Methods: We used two mouse emphysema models, an elastase-induced model and a cigarette smoke-induced model. We intravenously injected WJMSCs (1×10^4/mouse) to mice, pretreated or not, with pioglitazone for 7 days. We measured the emphysema severity by mean linear intercepts (MLI) analysis using lung histology.

Results: Pioglitazone pretreated WJMSCs (pioWJMSCs) were associated with greater lung regeneration than non-augmented WJMSCs in the two mouse emphysema models. In the elastase-induced emphysema model, the MLIs were 59.02±2.42 μm (n=6), 72.80±2.87 μm (n=6), for pioWJMSCs injected mice, and non-augmented WJMSCs injected mice, respectively (p<0.01). Both pioWJMSCs and non-augmented WJMSCs showed regenerative effects in the cigarette smoke emphysema model (MLIs were 41.25±0.98 [n=6] for WJMSCs and 38.97±0.61 μm [n=6] for pioWJMSCs) compared to smoking control mice (51.65±1.36 μm, n=6). The mean improvement of MLI appeared numerically better in pioWJMSCs than in non-augmented WJMSCs injected mice, but the difference did not reach the level of statistical significance (p=0.071).

Conclusion: PioWJMSCs may produce greater lung regeneration, compared to non-augmented WJMSCs, in a mouse emphysema model.

Keywords: Pioglitazone; Mesenchymal Stem Cells; Emphysema

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airflow limitation associated with chronic bronchitis and emphysema, and is known as one of the leading causes of death worldwide[1-3]. Although the etiology of COPD remains unclear, there is a clear relationship between smoking and chronic airway inflammation, alveolar destruction by extracellular matrix proteolysis, and ineffective repair of resident lung cells[4-7]. Recent reports describe the effects of stem cell treatments on tissue regeneration in organs like the heart, brain, liver, and lungs[8-12]. Several reports indicated that mesenchymal stem cells (MSCs) from bone marrow had therapeutic effects in an experimental elastase-induced emphysema model and a cigarette smoke-induced model[13-15]. Other reports examined use of Wharton's Jelly-derived mesenchymal stem cells (WJMSCs) and their potential clinical cell therapy applications in various disease models[16-20]. In this study, we isolated WJMSCs and then examined the effect of WJMSCs injection on two emphysema mouse models, an elastase-induced mouse model and a cigarette smoke-induced mouse model.

Our recent research showed that pioglitazone pretreatment of adipose-derived MSCs increased the production of growth factors, and the resultant therapeutic effects, in an emphysema mouse model, compared to non-treated adipose-derived stem cells[21]. We applied this method in an attempt to improve the efficacy of WJMSCs in two emphysema mouse models. Additionally, we sought to identify the distribution of intravenously injected WJMSCs pretreated with pioglitazone (pioWJMSCs), prior to human application.

Materials and Methods

1. Cell sources and pretreatment with pioglitazone

Following the receipt of parental consent, we obtained Wharton's Jelly from the umbilical cord of a baby born at Asan Medical Center in Seoul, Korea. WJMSCs were isolated by the following method. After removal of blood vessels, we dissected the Wharton's jelly, using a scalpel, into small segments. The dissected tissue segments were cultured in a 100-mm cell culture dish with minimum essential medium, alpha modification containing 10% fetal bovine serum and antibiotics in a humidified 37℃, 5% CO_2 incubator. After 1 week, the cultured tissue segments were treated with 0.05% trypsin-EDTA (Gibco Life Technologies, Grand Island, NY, USA) and then passed through a 0.45-μm cell strainer to obtain WJMSCs. For pio-WJMSCs (pioglitazone, Sigma-Aldrich, St. Louis, MO, USA), the WJMSCs culture medium was treated with 3 μmol/L pioglitazone for 1 week.

2. Mice

Female C57BL/6 mice, aged 7 weeks, were purchased from Orient Bio (Seongnam, Korea) and maintained under specific pathogen-free conditions in the animal facility of the Institutional Animal Care and Use Committee of Asan Medical Center.

3. Induction of two mouse emphysema models

As described previously[22], we induced an experimental elastase-induced emphysema model by intratracheal injection of 0.6 U of porcine pancreatic elastase (Sigma-Aldrich) at day 0. The mice were injected with 1×10^4 of pioWJMSC or WJMSC by intravenous injection on day 7. The animals were killed on day 14, after which the lungs were removed and prepared for histological analysis.

As described previously[14], for the experimental smoke-induced emphysema model we exposed mice to cigarette smoke 5 days per week for 6 months using commercially available cigarettes that contained 8.0 mg of tar and 0.6 mg of nicotine (Camel, R. J. Reynolds Tobacco Company, Winston-Salem, NC, USA). After exposure to cigarette smoke for 6 months, the mice were injected with 1×10^4 of pioWJMSCs or WJMSCs by intravenous injection and then killed on day 7 immediately prior to lung removal and preparation.

4. Histology and quantification of emphysema

Lung tissue was inflated with 0.5% low melting agarose, fixed in 4% formalin, embedded in paraffin, cut into 6-μm thickness, and stained with hematoxylin and eosin. Histological assessment of the sections was determined using the mean linear intercepts (MLI) method[23].

5. Fluorescence optical imaging

For injected pioWJMSC tracking in the lung, we performed fluorescence optical imaging analysis, as described previously[24]. In brief, pioWJMSCs were labeled using the quantum dots (QD) (Q-Tracker 800) Cell Labeling Kit (Invitrogen, Carlsbad, CA, USA). PioWJMSCs (1×10^6) were suspended in 200 μL of complete growth medium and labeled with 10 nM of QDs labeling solution. After 60 minutes, QD-labeled cells were washed twice with complete growth medium. We suspended 3×10^5 QDs 800-labeled pioWJMSCs in 100 μL saline and injected into the mice through the tail vain. The mice were sacrificed after 1, 4, 24, and 72 hours and image of the lung was taken using the IVIS Spectrum Pre-clinical In vivo Imaging System (PerkinElmer, Waltham, MA, USA).

6. WJMSC tracking using human-specific Alu sequence

We suspended 3×10^5 pioWJMSCs in 100 μL saline, then injected this solution into the mice through the tail vain. The mice were sacrificed after 1, 4, 24, and 72 hours, after which the lungs were removed to extract genomic DNA using a genomic DNA extraction kit (Qiagen, Duesseldorf, Germany). To verify the number of detected lung cells, we performed a standard curve using the pioWJMSCs. A quantitative polymerase chain reaction (qPCR) was carried out as previously described[24]. In brief, polymerase chain reactions (PCRs) were amplified by 40 cycles at 95°C for 15 seconds and 70°C for 1 minute using the LightCycler 480 SYBR Green I Master (Roche, Mannheim, Germany). PCR was carried out using the LightCycler 480 (Roche) and software. Primer sequences were as follows: Alu, 5′-CGAGGCGGGTGGATCAT-GAGGT-3′ and 5′-TCTGTC-GCCCAGGCCGGACT-3′.

7. Ethical statement

This study was approved by the institutional review board of the Asan Medical Center (approval No. 2015-0303). The receipt of parental consent was obtained.

Results

1. Increased therapeutic effects of pioWJMSCs on lung regeneration in mice with emphysema

1) Elastase-induced emphysema model

Experimental mice with emphysema are widely used to study COPD and the effects of MSCs. To evaluate the effect of pioWJMSCs, we used an elastase-induced emphysema model with intravenous injection of pioWJMSCs or WJMSCs (Figure 1A). Intratracheal injection of elastase produced severe lung destruction with MLI, an emphysema severity index, increasing from 43.55±1.9 to 92.57±4.56 μm (p<0.0001) (Figure 1B,
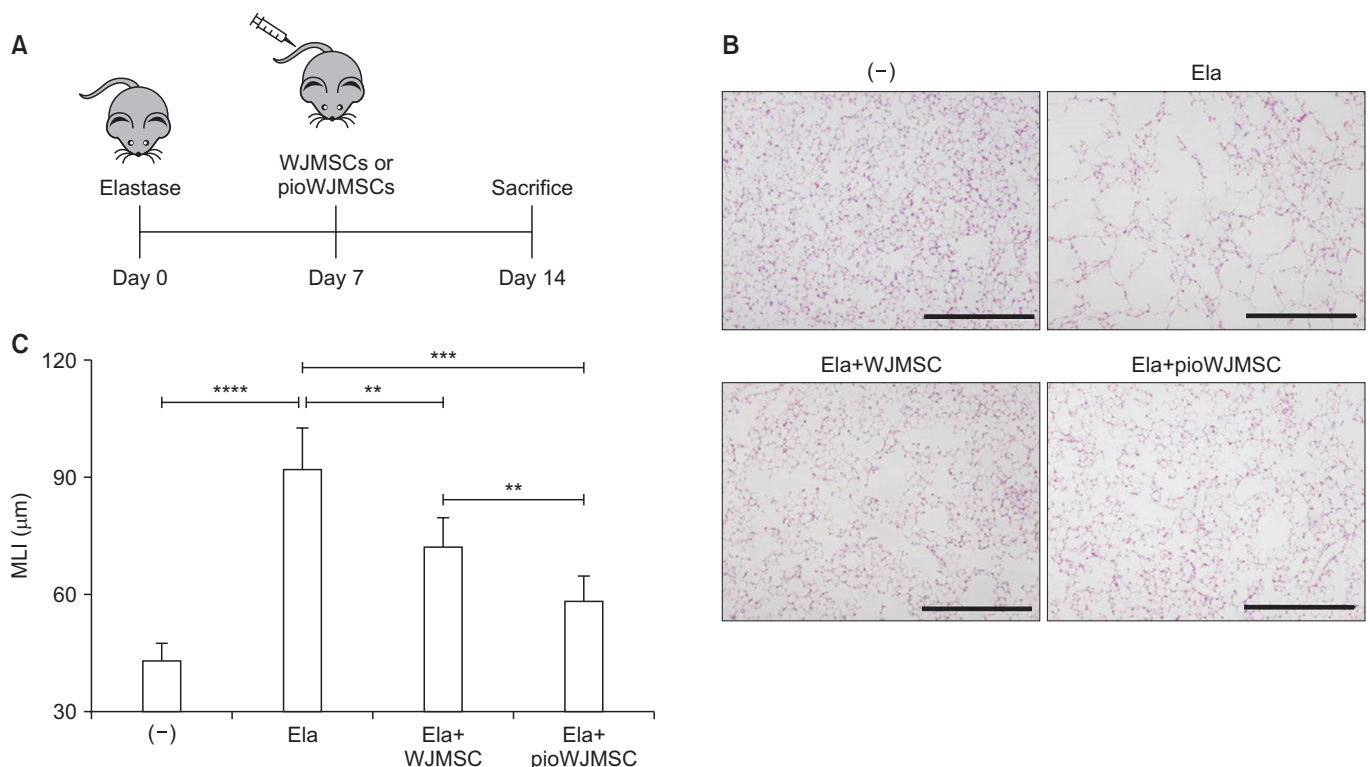

Figure 1. The therapeutic effects of pioglitazone-augmented Wharton's Jelly-derived mesenchymal stem cells (pioWJMSCs) on lung regeneration in mice with elastase-induced emphysema. C57BL/6 mice were intratracheally injected with 0.6 U of elastase (Ela) at day 0 and then intravenously injected with Wharton's Jelly-derived mesenchymal stem cells (WJMSCs) or pioWJMSCs on day 7. (A) Experiment scheme. (B) Hematoxylin and eosin (H&E) stained lung tissue sections on day 14. (−) control: no elastase; Ela: 0.6 U of elastase; Ela+WJMSC: elastase+1×10^4 WJMSC; Ela+pioWJMSC: elastase+1×10^4 pioWJMSC. Scale bars=0.5 mm. (C) Morphometric analysis of the mean linear intercept (MLI). The values represent the mean±standard error of mean (n=6). Statistically significant differences are indicated. **p<0.01, ***p<0.001, ****p<0.0001.

C). In contrast, both the pioWJMSCs and WJMSCs injection groups exhibited lung regeneration (Figure 1B, C). The mice injected with WJMSCs showed decreased MLI (72.80±2.87 µm) and the MLI of pioWJMSCs (59.02±2.42 µm) was significantly decreased, even more than the WJMSCs group (p<0.01). These results suggested that pioWJMSCs may exhibit augmented regenerative activity in an elastase-induced emphysema model.

2) Cigarette smoke-induced emphysema model

We observed similar results in the smoke-induced emphysema model (Figure 2A–C). The smoking (SM) group showed lung destruction with MLI increases from 35.43±0.8 to 51.65±1.36 µm (p<0.0001) (Figure 2B, C). Both the pioWJMSCs and WJMSCs injected groups showed significantly decreased MLI (WJMSCs 41.25±0.98 µm, pioWJMSCs 38.97±0.61 µm; p<0.001) compared to the SM group that was not treated, but the difference did not rise to the level of statistical significance (p=0.071).

2. Tracking of pioWJMSCs after intravenous injection into the mice

1) QDs-labeled fluorescence image

To track the distribution of intravenously injected pioWJM-SCs in the lung, the pioWJMSCs were labeled with QDs. QDs-labeled pioWJMSCs were intravenously injected into the mice, and the fluorescence levels in the lung were analyzed using an optical imaging system at 1, 4, 24, and 72 hours after injection. The fluorescent signal was detected in the lung up to 4 hours (Figure 3A) and gradually decreased as time passed (Figure 3B). In the control group, the fluorescent signal was undetectable in the lungs at all times. After 72 hours postinjection, we detected no fluorescent signals in the pioWJMSCs group.

2) Tracking using a human-specific Alu sequence

To quantify the relative amount of intravenously injected pioWJMSCs, we performed qPCR with a human-specific Alu sequence using extracted genomic DNA in pioWJMSCs injected, and non-injected, mice lungs. For analysis using human Alu-specific sequences qPCR data, the regression r^2 value curve was determined by 10 ng of genomic DNA from
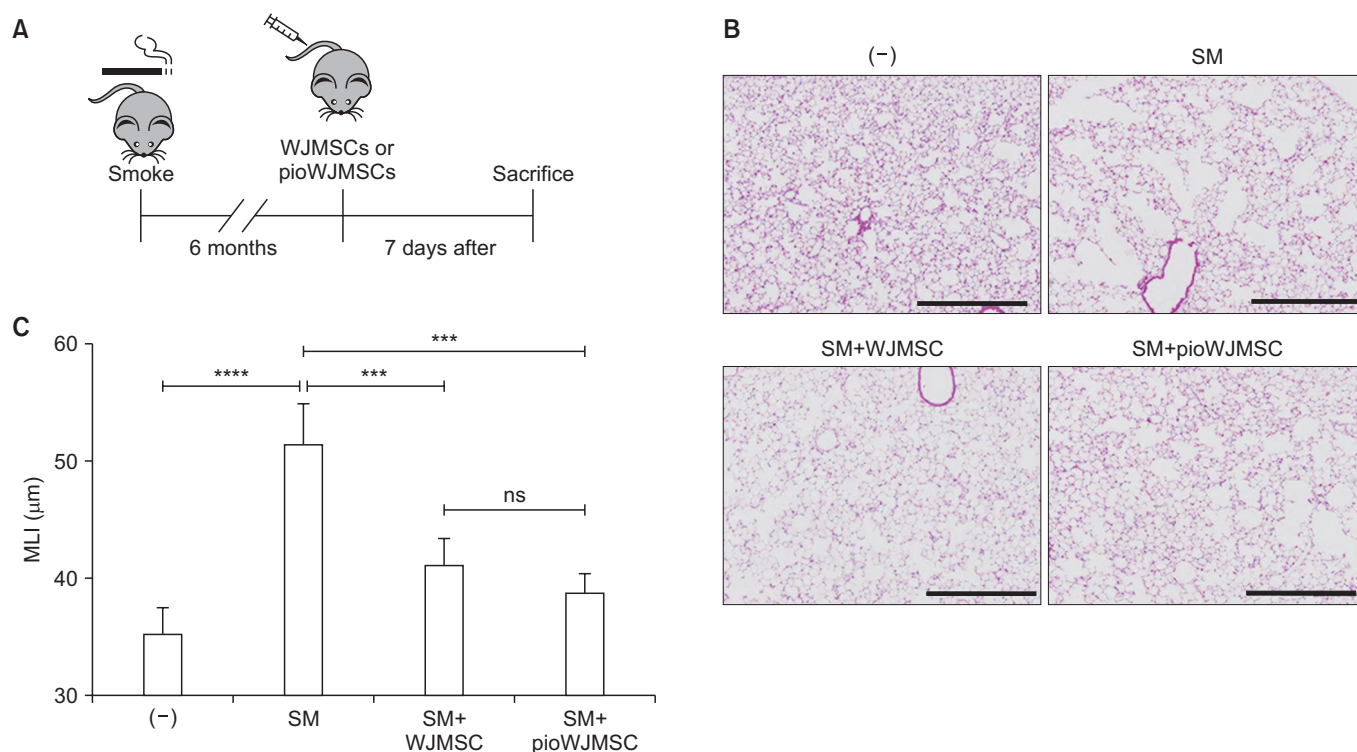

Figure 2. The therapeutic effects of pioglitazone-augmented Wharton's Jelly-derived mesenchymal stem cells (pioWJMSCs) on lung regeneration in mice with smoke-induced emphysema. C57BL/6 mice were exposed to cigarette smoke (SM) for 6 months and then intravenously injected with Wharton's Jelly-derived mesenchymal stem cells (WJMSCs) or pioWJMSCs. (A) Experiment scheme. (B) Hematoxylin and eosin (H&E) stained lung tissue sections after 7 days of injection. (−) control: no smoke; SM: smoke only; SM+WJMSC: smoke+1×10⁴ WJMSC; SM+pioWJMSC: smoke+1×10⁴ pioWJMSC. Scale bars=0.5 mm. (C) Morphometric analysis of the mean linear intercept (MLI). The values represent the mean±standard error of mean (n=6). Statistically significant differences are indicated. ***p<0.001, ****p<0.0001. ns, not significant.
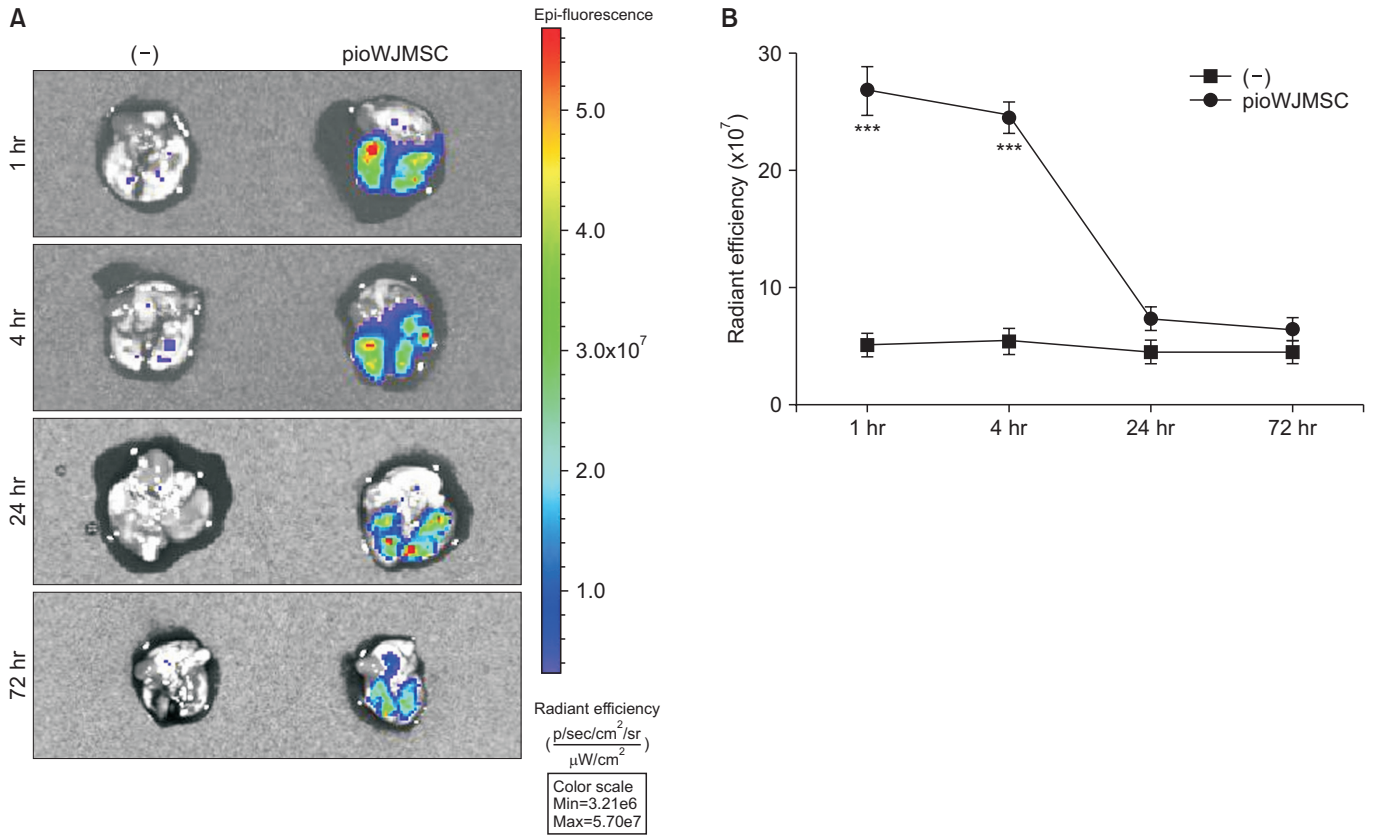
Figure 3. Tracking of pioglitazone-augmented Wharton's Jelly-derived mesenchymal stem cells (pioWJMSCs) after intravenous injection in the lung. (A) The fluorescence images of the lung from (−) control mice or quantum dots (QDs) labeled pioWJMSCs-intravenously injected mice at 1, 4, 24, and 72 hours. Representative images are shown (n=3). (B) Radiant efficiency of the lung from (−) control mice or QDs labeled pioWJMSCs-intravenously injected mice at 1, 4, 24, and 72 hours. Statistically significant differences are indicated. ***p<0.001.
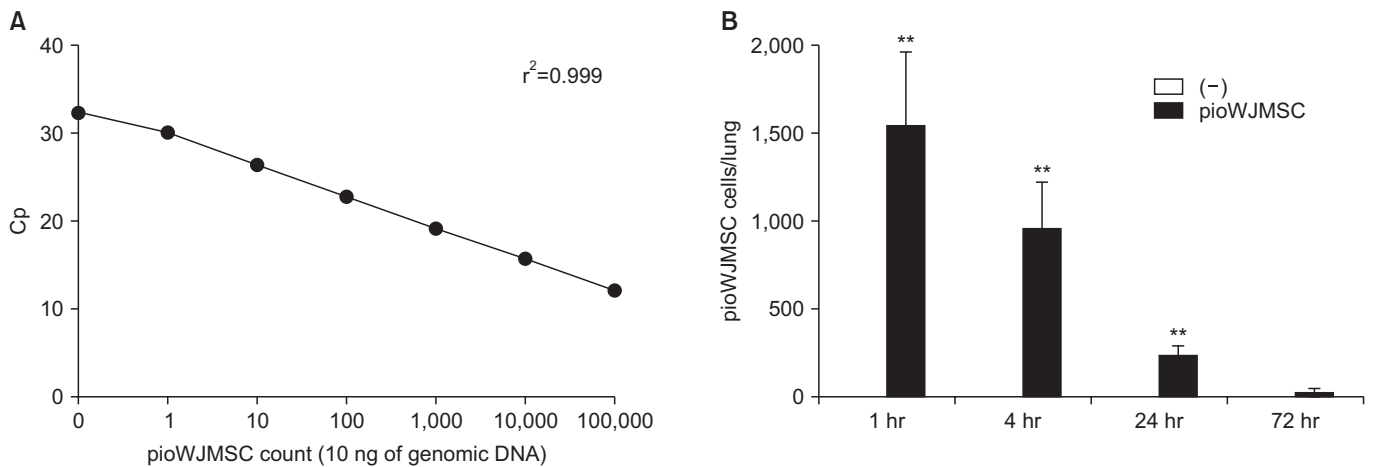

Figure 4. The quantification of the amount of intravenously injected pioglitazone-augmented Wharton's Jelly-derived mesenchymal stem cells (pioWJMSCs) in the lung. (A) Standard curve to evaluate the determination of pioWJMSCs in the lung. (B) The amount of pioWJMSCs in the lung from pioWJMSCs-injected mice or control mice was calculated based on panel A. The values represent the mean±standard error of mean (n=3). Statistically significant differences are indicated. **p<0.01.

10, 10^2, 10^3, 10^4, and 10^5 numbers of pioWJMSCs (Figure 4A). We used this curve as a standard curve for determining the number of intravenously injected pioWJMSCs in the lungs. After injection, there were approximately 1,500 pioWJMSCs at 1 hour, and we continued to detect pioWJMSCs for up to 24 hours in the lung (Figure 4B). PioWJMSCs were undetectable at 72 hours after injection.

Discussion

In this study, we found that the pioWJMSCs were associated with significantly more lung regeneration in an emphysema mouse model, compared to non-treated WJMSCs. We also examined intravenously injected pioWJMSCs using fluorescence optical imaging and human Alu-specific sequence qPCR for pioWJMSCs tracking in the lung. These data may suggest potential clinical therapeutic effects of pioWJMSCs in COPD.

Previous studies showed that MSCs from other sources like bone marrow have a therapeutic effect in an experimental mouse emphysema model[13-15]. In our recent report, adipose-derived stem cells (ASCs) and ASCs-derived nanovesicles exhibit therapeutic lung regeneration effects and increased production of growth factors in mouse models of emphysema[21,25].

WJMSCs are a source of MSCs for therapeutic applications. The isolation efficiency of WJMSCs[26] was greater than that of bone marrow[27] or adipose tissue[28]. WJMSCs also exhibit higher proliferative capacity[29], abundant production of growth factors[30], and lack of immunogenicity[31,32].

In COPD, the regeneration mechanisms of stem cells are not clearly identified; however, several studies have achieved regeneration secondary to the paracrine effects of MSCs[33-35], and growth factors such as vascular endothelial growth factor in emphysema mouse models[36,37]. Additionally, fibroblast growth factor-2 enhances stem cell regeneration in canine emphysema models[38].

There remain disadvantages to stem cell therapy that must be overcome. Bone marrow is best known as a source of MSCs. As donor age increases, the number and proliferative capacity of the stem cells decrease[39]. To address this issue, we isolated WJMSCs which are characterized by their ability to differentiate into osteocytes, chondrocytes, and adipocytes[40,41], better than bone marrow or adipose-derived stem cells within *in vitro* cultures[41]. WJMSCs are relatively undifferentiated cells, compared to stem cells derived from adipose tissue or bone marrow[42]. WJMSCs also express CD29, CD44, CD73, CD90, and CD105, similar to bone marrow or other tissue-derived mesenchymal stem cells, but the hematopoietic stem cell markers CD34, CD45, and histocompatibility antigen CD14, CD31, and CD33 are not expressed[43,44]. These features may facilitate the use of WJMSCs as cell therapy agents. In this study, we attempted to enhance the efficacy of WJMSCs using piogli-tazone in an emphysema mouse model.

For future clinical trials of pioWJMSCs in COPD patients, we needed to identify the distribution of intravenously injected pioWJMSCs. To accomplish this in mouse lung tissue, we performed fluorescence optical image analysis using a QD labeling kit and human-specific Alu sequence-based qPCR. Using optical imaging, it was easy to track the distribution of QDs-labeled cells in tissues. QDs are non-toxic to live cells, have high stability, high fluorescence sensitivity, and possess clinical applications[45,46]. Also, we evaluated the quantity of injected pioWJMSCs in the lung using qPCR with a human-specific Alu primer. Human Alu-sequences are commonly found in introns and present in the human genome at an extremely high copy number (~500,000 copies)[47]. This method is used to detect the human genome in the mouse lung and features highly sensitive, specific, and fast analysis[48,49].

In conclusion, the results of this study agree with previous findings regarding the regenerative effects of MSCs. pioWJMSCs were more potent, and may serve as a basis for clinical trials with patients in the near future.

Authors' Contributions

Conceptualization: Oh YM. Methodology: Park JS, Kim HK. Formal analysis: Park JS, Kim HK. Data curation: Park JS, Kang EY, Cho R. Validation: Park JS, Kim HK. Investigation: Park JS, Kang EY, Cho R. Writing - original draft preparation: Park JS. Writing - review and editing: Oh YM. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflicts of interest relevant to this article are reported.

Acknowledgments

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