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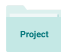

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ORIGINAL ARTICLE

Immunomodulatory Properties of Mesenchymal Stem Cells Can Mitigate Oxidative Stress and Inflammation Process in Human Mustard Lung

Amir Nejad-Moghaddam[1] · Sohiela Ajdary[2] · Eisa Tahmasbpour[1] · Farhad Riazi Rad[2] · Yunes Panahi[1] · Mostafa Ghanei[1]

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Abstract Oxidative stress and inflammation are one of the main pathological consequences of sulfur mustard on human lungs. Unfortunately, there is no effective treatment to mitigate pathological effects of sulfur mustard in mustard lungs. Here, we aimed to evaluate potential efficacy of systemic mesenchymal stem cells administration on expression of oxidative stress- and inflammation-related genes in sulfur mustard-exposed patients. Our patient received 100 million cells per injection, which was continued for four injections within 2 months. Sputum samples were provided after each injection. Oxidative stress was evaluated by determining sputum levels of malondialdehyde and glutathione. Furthermore, changes in expression of several oxidative stress- (metallothionein 3, glutathione reductase, oxidative stress responsive 1, glutathione peroxidase 2, lacto peroxidase, forkhead box M1) and inflammation-related genes (matrix metallopeptidase 2, matrix metallopeptidase 9, transforming growth factor-β1, vascular endothelial growth factor, metallopeptidase inhibitor 1, metallopeptidase inhibitor 2) were also evaluated using real-time PCR after treatments. Two-lung epithelial-specific proteins including Clara cell protein 16 and Mucin-1 protein levels were measured using enzyme immunoassay method. No significant differences were found between serum levels of Clara cell protein 16 and serum Mucin-1 protein in patient before and after cell therapy. Most of the oxidative stress responsive genes, particularly oxidative stress responsive 1, were overexpressed after treatments. Expressions of antioxidants genes such as metallothionein 3, glutathione reductase and glutathione peroxidase 2 were increased after cell therapy. Upon comparison of inflammation-related genes, we observed upregulation of vascular endothelial growth factor and

✉ Mostafa Ghanei
m.ghanei@bmsu.ac.ir

[1] Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Mollasadra Street, 19945–546, Tehran, Iran

[2] Department of Immunology, Pasteur Institute of Iran, Tehran, Iran

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matrix metallopeptidase 9 after mesenchymal stem cells therapy. Additionally, a trend for increased value of glutathione and decreased levels of malondialdehyde was observed from baseline to final evaluation times. Mesenchymal stem cells administration mitigates oxidative stress and inflammation in sulfur mustard-exposed patients.

Keywords Adipose-derived mesenchymal stem cells · Inflammation and oxidative stress genes · Sulfur mustard · Airway remodeling · Lung epithelial proteins

Introduction

Sulfur mustard (SM) or bis(2-chloroethyl) sulfide is a potent vesicating blistering chemical warfare agent, which was first used in World War I and then by Iraq against Iran in 1980s (Razavi et al. 2013). During the Iran–Iraq war, over 100,000 people were injured and more than 30,000 of them are still suffering from the late effects of SM (Balali-Mood et al. 2008). SM primarily affects skin, eyes and lungs (Khateri et al. 2003). Changes in morphological structure of injured lungs following exposure to SM in patients with chronic obstructive airways disease (COPD) can be also observed (Beheshti et al. 2006). In addition, remodeling of airways along with thickness of bronchial wall and narrowing of the lumen have been reported (Ghanei et al. 2004).

It is well known that oxidative stress (OS) and inflammation induced by free radicals are one of the major mechanisms for pathological effects of SM exposure on respiratory systems (Tahmasbpour et al. 2015). Current strategies for treatment of respiratory disorders among these patients include bronchodilators, *N*-acetyl cysteine and antibiotics (Poursaleh et al. 2012). Oxygen therapy (Ghanei et al. 2011), antibiotics, prednisolone (Ghanei et al. 2005), membrane stabilizers, antioxidants, macrolides and interferons (Panahi et al. 2005) are other popular drugs that used in these patients. These current therapies are not suitable for SM-exposed patients because airway structure is destroyed and they need a regeneration method for repairment. Recent investigations have focused on mesenchymal stem cells (MSCs) therapy in different models of lung disorders (Nejad-Moghaddam et al. 2015). Data from clinical trials indicate that transplantation of adipose-derived MSCs to patients is relatively safe and without toxic effects (Lalu et al. 2012). MSCs have been also demonstrated that have beneficial effects in both phase 1 and 2 of clinical trials such as graft-versus-host disease (GVHD) (Chen et al. 2012). Application of MSCs in COPD patients and individuals with idiopathic pulmonary fibrosis (IPF) has been also reported (Tzouvelekis et al. 2013, Weiss et al. 2013).

This article aims to evaluate therapeutic effects of MSCs on oxidative stress markers such as glutathione (GSH) and malondialdehyde (MDA) in sputum of a SM-exposed patient. Furthermore, changes in the expression of some oxidative stress- and inflammation-related genes will be considered after cell therapy.

Materials and Methods

Patient

This clinical trial (IRCT2015110524890N1) study was conducted at Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran (2014–2015) (Ethical code: IR.BMSU.REC.1393.32). In this study, we considered therapeutic effect of MSCs on expression of OS and inflammation-related genes in a SM-exposed male patient. Our patient had a documented encounter with SM during the Iran–Iraq war. The patient signed informed consent before the study. He was selected based on the following criteria: (a) the severity of lung injury was ranged from moderate ($50 < $ forced expiratory volume in 1 s (FEV1) < 65) to severe ($40 <$ FEV1 < 50); (b) absence of contraindications in spirometry (hemoptysis, cerebral arterial aneurysm or aortic, pulmonary embolism, uncontrolled blood pressure, recent pneumothorax, no doubt recent surgery/thoracic); and (c) no coagulation. The exclusion criteria for patient selection process were as follow: (a) simultaneous participation in another study; (b) smoking habitat; (c) the existence of pneumonia during the study; (d) the incidence of transfusion reaction; (e) other diseases (cardiovascular disease, hypertension, diabetes).

Adipose-Derived Mesenchymal Stem Cells: Isolation, Characterization, Analysis of Abnormality and Cell Injection

Isolation and Culture

200 ml of abdominal adipose tissue was obtained under local anesthesia by liposuction aspirates protocol. The lipoaspirate was washed with PBS to remove the tissue debris. 100 ml PBS containing 0.1 % w/v collagenase A type I (Sigma) was added to isolated tissue and then incubated at 37 °C for 1 h. Collagenase activity was neutralized using DMEM medium (Gibco) impregnated with 10 % fetal bovine serum (FBS, Gibco). Cell pellets were resuspended in culture medium after centrifugation at 2000 rpm for 10 min and then transferred to culture flasks for 72 h at 37 °C in 5 % CO_2 condition. The medium of the culture flasks changed every 3 days, and cells were passaged twice. Cells viability was measured using trypan blue staining method.

Characterization of MSCs with Flow Cytometry

To analyze the cell surface antigen expression, 5×10^5 fresh cells from the third passage were harvested by trypsin–EDTA. Cells were centrifuged at $100\times g$ for 1 min, resuspended in stain buffer (PBS, 2 % FBS) and incubated on ice for 10 min. Trypsin was neutralized by centrifugation and isolated cells were washed twice with PBS and finally resuspended in stain buffer. Cells were incubated for 30 min in dark environment. After incubation, the cells were labeled with anti-human monoclonal antibodies (MAbs) conjugated to fluorochromes. These antibodies were as follow:

anti-CD90-fluorescein isothiocyanate (FITC), CD73-phycoerythrin (PE), CD11b-FITC, CD34-FITC, CD44-FITC, CD45-PE, CD105-PE (Sigma-Aldrich, USA). The frequencies of all immunolabeled cells were analyzed by FACS Canto II flow cytometer (BD Biosciences, USA), in which approximately 500,000 events were assessed and the data were analyzed with FlowJo software (version 10.0).

Karyotyping

Standard Giemsa staining procedure was performed and chromosome preparations were obtained from 80 % confluent cells (Borgonovo et al. 2014). Cells were treated with Colcemid solution (Invitrogen) to stop microtubule formation. The mitotic arrested cells were harvested using trypsin–EDTA solution. Extracted cells were immersed in 75 mM KCl for 30 min at room temperature and they were obtained by centrifugation. The supernatant was replaced with fixative solution and the resulting suspension was spread over slides for visual observation and imaging. At least 15 metaphase spreads were analyzed. The karyotypes were imaged with light microscope (Nikon) using a CytoVision software.

Storage of Adipose-Derived Mesenchymal Stem Cells (ADMSCs)

ADMSCs were harvested at 90 % confluency and prepared for freezing before injection. To collect cells, culture medium was replaced with sterile PBS and after 3 min PBS was replaced with trypsin–EDTA solution and incubated at 37 °C for 5 min. 10 ml of complete medium (DMEM with 10 % FBS) was added to inactivate trypsin, and centrifuged at 1500×*g* for 5 min. Cell pellet was resuspended in cryopreservation medium (80 % FBS, 10 % dimethylsulfoxide and 10 % MEM medium) with final concentration of 5 million cells per milliliter and aliquoted into cryovials. After overnight storage at −80 °C, vials were transferred into a liquid nitrogen container for long-term storage.

Injection of Stem Cells

Our patient received four injections of 100 million cells every 20 day and followed up for 6 months. MSCs were injected intravenously along with 300 ml normal saline into the patient at a maximum rate of 2×10^6 cells/min. Each infusion took approximately 30 min. Patient was evaluated 20 days before the first infusion and 2 days before the injections 2, 3 and 4, respectively. The assessment of patient was performed for 90 and 150 days after the first injection.

Lung Epithelium-Specific Proteins Assay

8 ml of whole blood was collected into vacutainer tubes with EDTA and serum was isolated by centrifugation at 1500×*g* for 15 min. Serum was collected and stored at −80 °C until future examinations. Serum Clara cell protein 16 (CC16) and Mucin-1 protein (KL-6) level was measured using enzyme immunoassay method (Human Clara Cell Protein 16 ELISA Kit; DL-CC16-Hu and Human Krebs Von den Lungen

6 Elisa kit; E01K0061, respectively), according to the manufacturer's instructions. Concentration of CC16 and KL-6 proteins was determined using standard curve. The assay had a validated range of 0.5–10 ng/ml for KL-6 and 62.5–4000 pg/ml for CC16 based on applied ELISA kit. The intensity of color was measured spectrophotometrically at 450 nm in a microplate reader (BioTek model).

Molecular Analysis for Inflammation and Oxidative Stress Genes

Sputum Induction and Processing

Sputum was collected according to the method described by Pin et al. (1992). The patient washed his mouth and throat carefully with normal saline solution. After that, he collected sputa in a falcon 50 ml conical tube. Sputum samples were treated with freshly prepared 0.1 % dithiothreitol (DTT) solution (Sputolysin; Calbiochem Corp., San Diego, CA), in a ratio of 1:4 (sputum to DTT). Samples were mixed gently by vortex mixer and placed in a shaking water bath at 37 °C for 15 min and 100 µl were considered for cell counting. The remaining solution was centrifuged at $2000\times g$ for 5 min and stored at −80 °C for RNA extraction.

RNA Extraction and Real-Time PCR

Total RNA was isolated from processed sputum samples using RNX plus solution (SinaClon) according to the manufacturer's protocol. The quality and quantity of RNA were monitored using a NanoDrop 1000 spectrophotometer (Thermo scientific, USA). RNA was reversely transcribed using RevertAid Reverse Transcriptase (Thermo science, Germany) at 42 °C for 1 h and random hexamer (Thermo science, Germany) as primer. The amplifications were performed using a Rotor Gene 6000 (Corbett Research, Australia) thermocycler and Real Q-PCR 2× Master Mix Kit (Amplicon, Denmark) in 40 cycles. Each reaction contained 5 µl master mix, 100 nm primers for oxidative stress- (*MT3, GSR, OXSR, GPS, LPO, FOXM1*) and inflammation-related genes (*MMP2*, *MMP9*, *TGF-b*, *VEGF, TIMP1*, *TIMP2*) (primer sequences are shown in Table 1) and 1 µl template cDNA. Beta-2-microglobulin (*B2M*) was used as a reference gene for normalization of the gene expression. Delta Ct (ΔCT) was calculated using the following formula: [ΔCT = CT (target) − CT]. Gene expression level was determined by $2^{-\Delta Ct}$ method (Livak and Schmittgen 2001).

MDA Measurement

MDA level of sputum was assessed using the thiobarbituric acid method (Colagar et al. 2009). Briefly, 1 ml of sputum was centrifuged for 7 min at 2000*g*, and then, 100 µl of supernatants was added in 900 µl of distilled water into a glass tube. About 500 µl of thiobarbituric acid reagent (including 0.67 g of 2-thiobarbituric acid dissolved in 100 ml of distilled water along with 0.5 g NaOH and 100 ml glacial acetic acid) was added to each tube and then incubated in a boiling water bath for at least 1 h. After cooling in room temperature, each tube was centrifuged

Table 1 Primers sequence for oxidative stress- and inflammation-related genes

Genes		Sequence	Size (bp)
OXSR1 (oxidative stress responsive 1)	FW	AGTTCATTGTTTGCCCTGCT	95
	RV	GCAAAGGGAGTCTACCACACA	
FOXM1 (forkhead box M1)	FW	GCTGGCTGCACTATCAACAA	154
	RV	TCGAAGGCTCCTCAACCTTA	
GPX2 (glutathione peroxidase 2)	FW	GGTAGATTTCAATACGTTCCGGG	174
	RV	TGACAGTTCTCCTGATGTCCAAA	
MT3 (metallothionein 3)	FW	ACATGGACCCTGAGACCT	142
	RV	TTGGCACACTTCTCACACT	
GSR (glutathione reductase)	FW	TCACCAAGTCCCATATAGAAATC	116
	RV	TGTGGCGATCAGGATGTG	
LPO (lacto peroxidase)	FW	CGTGATGGAGACAGGTTCT	96
	RV	ACAGACAAGGCGTGAGA	
TGF-β1 (Transforming Growth Factor-β1)	FW	CCCAGCATCTGCAAAGCTC	101
	RV	GTCAATGTACAGCTGCCGCA	
MMP2 (matrix metallopeptidase 2)	FW	GGACACACTAAAGAAGATGCAGAACT	81
	RV	CGCATGGTCTCGATGGTATTC	
MMP9 (matrix metallopeptidase 9)	FW	GCACGACGTCTTCCAGTACC	124
	RV	CAGGATGTCATAGGTCACGTAGC	
VEGF (vascular endothelial growth factor)	FW	AAATGCTTTCTCCGCTCTGA	173
	RV	CCCACTGAGGAGTCCAACAT	
TIMP1 (metallopeptidase inhibitor 1)	FW	AAGGCTCTGAAAAGGGCTTC	105
	RV	GCAGGATTCAGGCTATCTGG	
TIMP2 (metallopeptidase inhibitor 2)	FW	ATGCACATCACCCTCTGTGA	177
	RV	CTCTGTGACCCAGTCCATCC	
B2M (beta-2-microglobulin)	FW	AATTGAAAAAGTGGAGCATTCAGA	135
	RV	GGCTGTGACAAAGTCACATGGTT	

Bp base pair, *FW* forward, *RV* reverse primers

for 10 min at 4000*g* and the absorbance of supernatants was recorded using UV spectrophotometric method at 534 nm. Each sample was assessed in triplicate.

GSH Measurement

GSH level was assayed according to the Tietz method (Tietze 1969). Briefly, 800 µl of 3 mM NaHPO along with 100 µl of 0.04 % 5,5-dithiobis 2-nitrobenzoic acid (DTNB) in 0.1 % sodium citrate was added to sputum fluids. The absorbance of the solution was monitored at 412 nm using a UV spectrophotometer. Each sample was assessed in triplicate.

Statistical Analysis

Basic and clinical characteristics along with PFTs are reported as mean ± SD. An independent *t* test was considered to compare the mean of parametric data between each evaluation. Data were analyzed using SPSS, version 19 and a probability of less than 0.05 was considered as significant.

Results

No statistically significant differences were observed in FEV1, FVC and FEV1/FVC during 6 month (Table 2). Our data revealed a reduced volume for diffusing capacity or transfer factor of the lung for carbon monoxide (Table 2). Furthermore, a statistically significant improvement was observed after injections in COPD Assessment Test (CAT), CASA-Q and St. George's Respiratory Questionnaire (SGRQ) scores during treatments (Table 3).

The existence of CD markers (CD73; CD90; CD105; and CD44) demonstrated that cultured cells were ADMSCs (Fig. 1). These CD markers are specific for ADMSCs, which were identified by specific monoclonal antibodies.

The karyotyping method was applied to consider any possible abnormalities in cells before MSCs injections; however, our data showed normal MSCs (Fig. 2). Injections would be canceled if chromosomal abnormalities were observed. In case of chromosomal abnormalities, the process of sampling was repeated.

Mean concentrations of sputum GSH and MDA concentrations in each evaluation time are shown in Fig. 3. Significant differences were found in mean concentrations of

Table 2 Pulmonary function tests during 6 month of MSCs therapy

Evaluation time (day)	FEV1	FVC	FEV1/FVC	TLC	RV	RV/TLC	Tlco (Hb)	MEF 25–75
0	52	80	74	111	179	154	119	33
20	46	66	70	92	160	162	99	29
40	47	65	76	96	168	167	106	25
60	45	69	68	105	185	168	115	20
90	52	78	67	115	190	161	85	21
150	51	81	72	124	209	157	69	23

Table 3 Respiratory questionnaire scores before and after MSCs therapy

Evaluation time (day)	SGRQ	CAT	CASA-Q	VAS
0	61	34	40	55
20	55	30	42	60
40	48	29	39	62
60	41	26	45	57
90	38	24	61	70
150	39	25	65	72

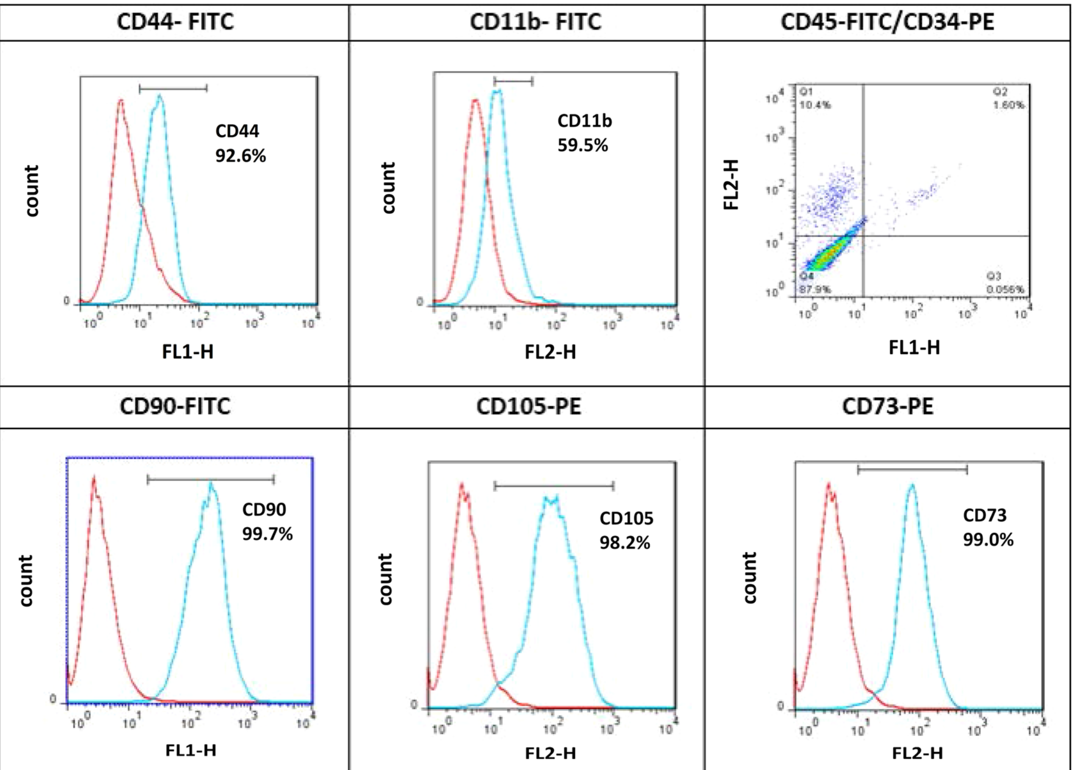

Fig. 1 Phenotype of mesenchymal stem cells was determined through cells surface markers [(CD90, CD73, CD44 and CD105)$^{+}$, (CD34, CD45)$^{-}$, and CD11b^{-}] by flow cytometric analysis. Data were analyzed with FlowJo software. The existence of CD markers (CD73; CD90; CD105 and CD44) demonstrated that cultured cells were ADMSCs

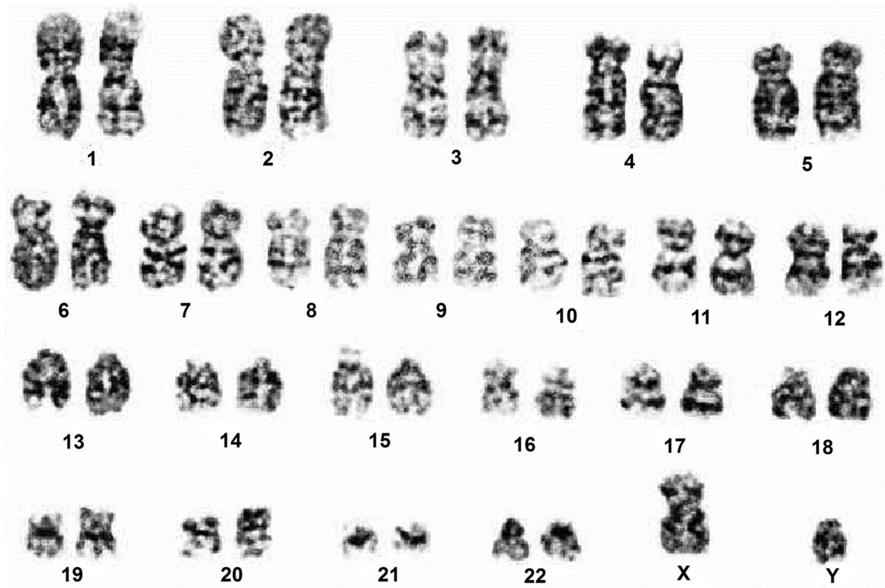

Fig. 2 Karyotyping of human adipose-derived MSCs. Karyotype analysis of ADMSCs at passage-3 before cell freezing. Karyotyping method shows MSCs are normal and can be used for injection

GSH ($p = 0.019$) and MDA ($p = 0.006$) from baseline to final injection. The mean of sputum MDA concentration in baseline was significantly higher than the final evaluation time (163.96 ± 23.07 nmol/l vs. 103.42 ± 11.22 nmol/l; $p = 0.002$). The ratio of sputum MDA value from baseline to final evaluation time was 1.58. Conversely, the mean of sputum GSH concentration in baseline was significantly lower than the final evaluation time (1.13 ± 0.2 vs. 2.83 ± 0.7 μM; $p = 0.006$).

No significant difference was found between serum levels of CC16 and serum levels of KL-6 in patient before and after cell therapy during the 6-month follow-up (Fig. 4a, b).

Table 4 shows changes in expression of oxidative stress- and inflammation-related genes after each period of evaluation. We found that most of the oxidative stress responsive genes, particularly *OXSR1*, were overexpressed after treatments. Expression of antioxidants genes such as MT3, *GSR* and *GPX* was increased after MSC therapy; however, we do not observe change in expression of *FOXM1* before and after treatments. Upon comparison of inflammation-related genes, we observed upregulation of *VEGF and MMP9* after MSCs therapy. There was no significant alteration in expression of *MMP2, TGF, TIMP1 and TIMP2* after MSC therapy.

Discussion

Recent reports have indicated that infusion of MSCs is an important therapeutic approach for treatment of lung injuries (Nejad-Moghaddam et al. 2015). Since MSCs of varying properties can be isolated from a different spectrum of organs, a strategic and rational approach in MSC sourcing for a particular application has yet

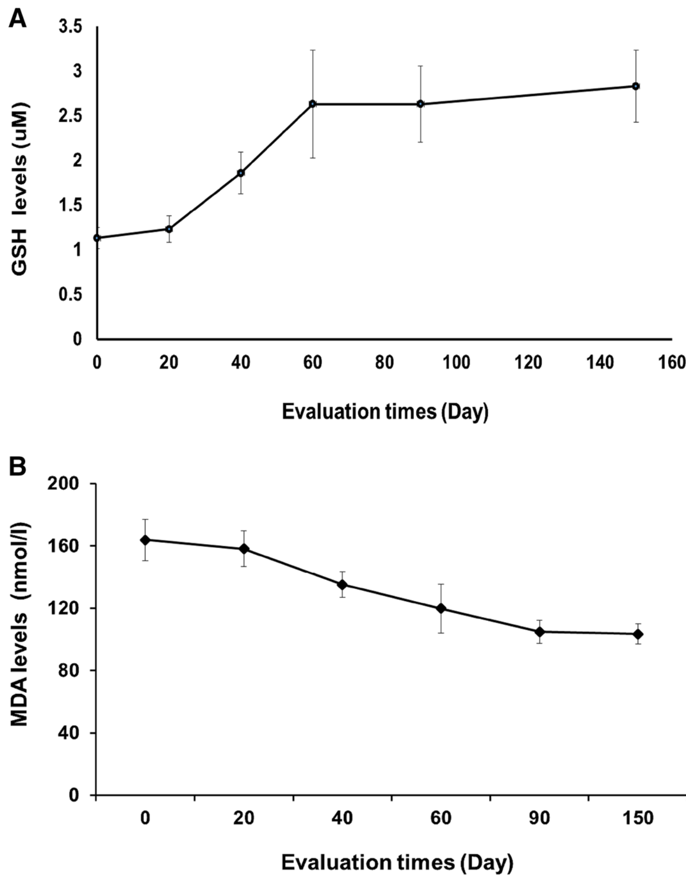

Fig. 3 Mean concentrations of sputum GSH (**a**) and MDA (**b**) concentrations in each evaluation time. Decreased levels of GSH along with increased value of MDA were found in sputum of the patient in baseline as these compared with final evaluation time. A trend for increased value of GSH and decreased levels of MDA was observed from baseline to final evaluation times

to be achieved (Wei et al. 2013). Although MSCs therapy has been used in different diseases, to the best of our knowledge there is no study on SM-exposed patients with lung injuries. In the current study, we considered for the first time therapeutic effect of MSCs on expression of several genes involved in OS and inflammation. Our data revealed that infusion of MSCs can increase expression of important antioxidants such as *MT3, GPX and GSR* in lung of the patient. These enzymes are very important to mitigate oxidative stress and free radicals. Since oxidative stress has been reported to occur in lung tissue of SM-exposed patients, MSCs therapy can be considered as a new strategy for treatment of these patients.

We found decreased levels of GSH along with increased value of MDA in sputum of our patient in baseline compared with the final evaluation time. This suggests an increased oxidative stress status caused by GSH depletion in sputum of the patient at baseline evaluation. However, a trend for increased value of GSH and

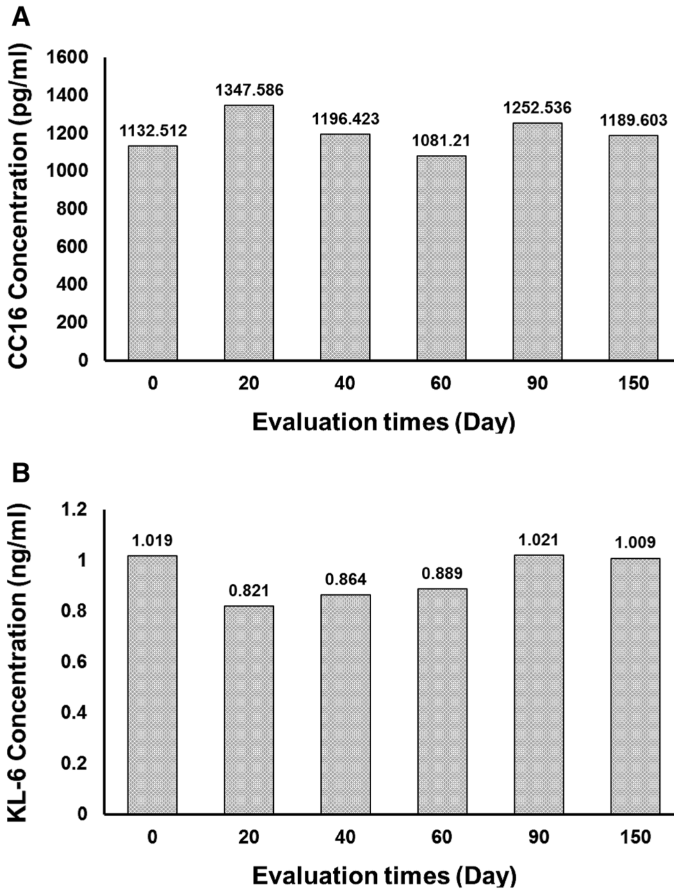

Fig. 4 CC16 (**a**) and KL-6 (**b**) levels before and after cell therapy. Our data show that CC16 and KL-6 levels are not significantly changed during the evaluation times. Significant changes in these biomarkers may be obtained after further evaluation times. The concentration of KL6 and CC16 is based on ng/ml and pg/ml, respectively

decreased levels of MDA was observed from baseline to final evaluation times. We speculate that GSR deficiency may be the main reason for GSH depletion in lungs of the patient because we found a significant reduction in expression of sputum *GSR*. GSR deficiency, which has been reported in lungs of SM-exposed patients, is associated with GSH depletion and consequently oxidative stress (Ghanei et al. 2008; Tahmasbpour et al. 2016).

We also detected overexpression of VEGF in our patient after each injection of the cell. VEGF protects the alveolar epithelium with a role in repair after lung injury, but causes fluid flux across the exposed endothelium if the alveolar capillary membrane is functionally breached (Chang et al. 2014). Increased expression of *VEGF* in our case suggests positive role of MSCs in treatment and healing of lung injuries in these patients. Recent studies have demonstrated that MSCs mediates an anti-apoptosis effect, which partly depends on an upregulation of VEGF

Table 4 Gene expression of oxidative stress- and inflammation-related genes after treatments with MSCs

Genes	20 days	40 days	60 days	90 days	150 days
Oxidative stress					
MT3	0.000375	0.003173	0.008609	0.473029	3.09513
GSR	0.016064	0.011924	0.018711	0.241484	4.316913
OXSR	0.004876	0.00982	0.05672	0.239816	4.924578
FOXM	0.00142	0.066064	0.07911	0.03983	0.049037
GPX	0.096055	0.111878	0.088388	0.154963	1.172835
LPO	0.013048	0.025916	0.543367	0.084788	1.094294
Inflammation					
VEGF	0.002036	0.234881	0.105112	0.095391	4.257481
MMP2	0.228458	4.287094	5.81589	0.103665	0.486327
MMP9	0.00148	0.028956	0.168404	0.269807	7.516182
TGF	0.00071	0.020448	0.204476	0.504476	0.90125
TIMP2	0.00491	0.010972	0.027776	0.438303	0.078021
TIMP1	0.82932	0.179244	0.993092	0.607097	0.707734
MMP2/TIMP2	46.5291	390.7304	209.3854	0.236514	6.233283
MMP9/TIMP1	0.00178	0.161545	0.169575	0.444421	10.62006

OXSR1 oxidative stress responsive 1, *FOXM1* forkhead box M1, *GPX2* glutathione peroxidase 2, *MT3* metallothionein 3, *GSR* glutathione reductase, *LPO* lacto peroxidase, *TGF-β1* transforming growth factor-β1, *MMP2* matrix metallopeptidase 2, *MMP9* matrix metallopeptidase 9, *TIMP2* metallopeptidase inhibitor 2, *VEGF* vascular endothelial growth factor, *TIMP1* metallopeptidase inhibitor 1, *B2M* beta-2-microglobulin. The expression profile of all related genes is presented as fold-change

(Tsubokawa et al. 2010). Since SM damages lung epithelial cells and causes releasing of proteases from neutrophils, these factors decrease the VEGF concentration in the alveolar compartment (Ghanei and Harandi 2011). Therefore, the protective effect of MSCs injections on lung tissues may be partly mediated by increasing *VEGF* expression and inhibiting the apoptosis of lung cells.

Expression of metalloproteinases (*MMPs*), especially MMP9, was also increased in our patient. MMPs are a member of proteolytic enzymes that have several critical roles including extra cellular matrix (ECM) remodeling via their activity in the proteolytic degradation of extracellular macromolecules such as collagens, facilitating cell migration, cleaving cytokines and activating defenses (Van Doren 2015). MMPs occur in development, wound healing and major inflammatory diseases (Churg et al. 2012). The balance of MMPs to tissue inhibitors of metalloproteinase (TIMPs) is crucial and determines matrix turnover, where either an excess of MMPs or a deficit of TIMPs may result in excess ECM degradation (Morimoto et al. 1997). MMPs play critical roles, given their broad effects on matrix remodeling and modulation of inflammation and cell signaling. Recent data have suggested that MMPs have the major roles in stimulating inflammation or shutting it down, as well as modifying the release of fibrogenic growth factors, processes that are important in the genesis of the various lesions of COPD (Eurlings et al. 2014). Because *MMP9* expression is declined in SM damage, increased expression of MMP9 after MSCs

treatment suggests returning the balance between TIMPs and MMPs in these patients. The result from a trend toward decreased expressions of *TIMP-1* and 2 factors indicate the accuracy of the process. By reducing the amount of TIMPs, we can see increased MMP9 levels and this results can be certified on initiation reduce inflammation in extracellular matrix in the airway.

Our findings also showed non-significant changes in serum levels of CC16 and of KL-6 proteins in patient before and after MSC therapy. If the repair is happen, KL-6 should be reduced, while CC16 levels should be increased. These data suggested that mesenchymal cells homing and niche formation in lungs need more time to be repaired. Therefore, this test should be followed for long terms.

In conclusion, MSCs therapy is a method that improves the respiratory quality by mitigating of OS and inflammation in SM-exposed patient. It also improves the balance of MMPs to TIMPs, as well as VEGF value in these patients. Therefore, further long-term studies involving larger number of patients are needed before MSCs therapy can be safely recommended for the management of lung injury in these patients.

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Author's Contribution AN-M, FRR and ET were contributed to the study concept and design, laboratory research, interpreted the data analysis, manuscript writing and review; SA and MG were involved in study concept and design and project supervisions and manuscript review; YP was responsible for stem cells injections at emergency center.

Compliance with Ethical Standards

Conflict of Interest All the authors have read and approved the final version of the manuscript. The authors confirm that there are no conflicts of interest.

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