Pre-treatment with mesenchymal stem cells reduces ventilator-induced lung injury

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ABSTRACT: Bone marrow-derived mesenchymal stem cells (MSCs) reduce acute lung injury in animals challenged by bleomycin or bacterial lipopolysaccharide. It is not known, however, whether MSCs protect from ventilator-induced lung injury (VILI).

This study investigated whether MSCs have a potential role in preventing or modulating VILI in healthy rats subjected to high-volume ventilation.

24 Sprague–Dawley rats (250–300 g) were subjected to high-volume mechanical ventilation (25 mL·kg⁻¹). MSCs (5 × 10⁶) were intravenously or intratracheally administered (n=8 each) 30 min before starting over-ventilation and eight rats were MSC-untreated. Spontaneously breathing anaesthetised rats (n=8) served as controls. After 3 h of over-ventilation or control the animals were sacrificed and lung tissue and bronchoalveolar lavage fluid (BALF) were sampled for further analysis.

When compared with controls, MSC-untreated over-ventilated rats exhibited typical VILI features. Lung oedema, histological lung injury index, concentrations of total protein, interleukin-1 β , macrophage inflammatory protein-2 and number of neutrophils in BALF and vascular cell adhesion protein-1 in lung tissue significantly increased in over-ventilated rats. All these indices of VILI moved significantly towards normalisation in the rats treated with MSCs, whether intravenously or intratracheally. Both local and systemic pre-treatment with MSCs reduced VILI in a rat model.

KEYWORDS: Acute lung injury, cell therapy, injurious ventilation, lung inflammation, lung oedema, mechanical ventilation

Acute lung injury (ALI) and its most severe evolution, acute respiratory distress syndrome (ARDS), are devastating non-cardiac clinical diseases characterised by acute respiratory failure and bilateral pulmonary infiltrates consistent with oedema resulting from the disruption of the lung alveolar–capillary membrane barrier [1]. Despite extensive research into the pathogenesis and management of ALI and ARDS, mortality still remains high, at approximately 40% [2].

The status of ALI/ARDS patients can be aggravated by a significant side effect of mechanical ventilation, which is an essential vital support in these patients. Indeed, experimental and clinical evidence indicates that mechanical ventilation may exacerbate the patient's pre-existing condition or even damage healthy lungs, by inducing an inflammatory response that contributes to extra-pulmonary organ dysfunction [3]. In fact, some features of ventilator-induced lung injury (VILI) resemble those of ALI/ARDS, and therefore act synergistically with them. VILI results from the action of mechanical forces on lung structures.

Over-stretching of alveolar walls causes endothelial and epithelial breaks and interstitial oedema. In particular, cyclic over-distension and the collapse/re-opening of airway units with each breath are two key pathophysiological mechanisms leading to VILI [3]. To reduce the potential injurious effects of mechanical ventilation, protective ventilator strategies have been established and recommended in clinical guidelines [4].

Given the considerable health burden associated with ALI/ARDS, research on new therapeutic strategies for this syndrome is very active. A cell therapy approach recently proposed as a potential treatment for ALI/ARDS is based on the use of bone-marrow derived mesenchymal stem cells (MSCs) [5–7]. The rationale for applying adult stem cells of this type derives from their immunosuppressive and anti-inflammatory properties and their freedom from host rejection, allowing them to be used in allotransplantation [8, 9]. Most of the experimental research carried out to date on the potential therapeutic function of MSCs in ALI/ARDS has been focused on the primary causes of lung injury. Specifically, data obtained in different

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Received:

Sept 06 2011

Accepted after revision:

Feb 14 2012

First published online:

March 22 2012

studies carried out on cell cultures, rodent models and *ex vivo* human lungs have shown that MSCs alleviate the severity of acute lung injury caused by bleomycin [10] or bacterial lipopolysaccharide (LPS) [11, 12] and act as potent antimicrobial treatment [13]. Whereas some authors injected the MSCs intravenously, others instilled the cells directly into the trachea [10, 11, 14], and apparently positive effects were achieved by both means.

However, whether MSCs could be effective for reducing or modulating VILI remains to be elucidated. Since VILI is characterised by lung inflammation and MSCs exhibit anti-inflammatory properties, the hypothesis of this work was that pre-treatment with these adult stem cells could attenuate VILI. Accordingly, the aim of this experimental research was to study the potential therapeutic effect of MSCs in an acute animal model of VILI induced by high tidal volume ventilation. Given that the best route for MSCs application for treating lung injury is unknown, we assessed whether the intravenous or intratracheal administration of these stem cells would result in the same potential reduction of VILI.

METHODS

Animals

The study was carried out on 38 pathogen-free male Sprague-Dawley rats (250–300 g; Criffa, Lyon, France) that were housed in light-dark cycle-regulated, air-conditioned (23°C) and air-humid (60%) animal quarters and had free access to standard food pellets (A04; Panlab, Barcelona, Spain) and tap water. This experimental work was approved by the Ethical Committee for Animal Research of the University of Barcelona.

MSCs

Lewis rat marrow stromal cells kindly provided by the Tulane Center for Gene Therapy (Tulane University, LA, USA) were used in this study. These cells are well characterised [15] and used in several published reports, being positive/negative for relevant MSC surface markers and able to develop osteogenic, chondrogenic and adipogenic differentiation. Cells (passage 6–11) were cultured in MEM- α (Gibco; Gaithersburg, MD, USA) supplemented with 20% of fetal bovine serum (FBS; Hyclone Cell Culture, Logan, UT, USA), 1% of antibiotic-antimycotic (10,000 U·mL⁻¹ penicillin G sodium, 10,000 μ g·mL⁻¹ streptomycin sulphate, 25 μ g·mL⁻¹ amphotericin B; Gibco) and 2% of L-glutamine (200 mM in 0.85% NaCl; Gibco). Cells were grown in an incubator (37°C, 5% CO₂, 100% humidity). Subconfluent cells were dissociated with 0.25% trypsin and 1 mM EDTA in Hanks' balanced salt solution (Gibco) and subcultured at low density in new culture flasks.

Administration of MSCs and induction of VILI

Animals were hydrated with subcutaneous injection of physiological saline solution and intraperitoneally anaesthetised with 10% urethane (5 mL per 300 g body weight; Sigma, St Louis, MO, USA). The rectal temperature of the rats was maintained within the range of 36.5–37.5°C throughout the procedure. The animals were randomly assigned to four groups (n=8 each). One control group was kept under spontaneous breathing for the entire duration of the experiment, while the other three groups of rats were tracheostomised and intubated (cannula 16GA BD Adsyte Pro, Becton Dickinson; Madrid, Spain). Subsequently, one group was kept under spontaneous breathing and the

animals from another two groups were subjected to the following interventions: 1) injection of 5×10^6 MSCs diluted in 0.3 mL of sterile Dulbecco's phosphate-buffered saline (DPBS) through the penile vein; 2) intratracheal instillation of 0.3 mL of DPBS containing 5×10^6 MSCs through the intubation cannula. During the intratracheal instillation experiments, the rats were maintained in a head-up tilted position to prevent MSC spillage. After 30 min of spontaneous breathing, the three groups of intubated rats were subjected to high-volume ventilation to induce VILI. To this end, the rats were paralysed by infusion of pancuronium bromide (0.4 mg·kg⁻¹ body weight; P1918, Sigma) into the penile vein and rapidly connected to a rodent ventilator (Harvard Apparatus, Model 683; South Natick, MA, USA). The animals were ventilated with room air at a rate of 80 breaths·min⁻¹ using a high tidal volume of 25 mL·kg⁻¹. After 3 h of high-volume ventilation (or spontaneous breathing in the control group) the rats were sacrificed by exsanguinations.

Obtaining and processing bronchoalveolar lavage fluid

After exsanguination, the left main bronchus was tied with a string at the left hilum. Bronchoalveolar lavage fluid (BALF) was obtained from the right lung by connecting a syringe to the cannula placed into the trachea and then flushing through it 3 mL of sterile phosphate-buffered saline four times. BALF recovery was always greater than 85%. Cells in the BALF were counted on a haemocytometer (Neubauer, Marienfeld, Lauda-Königshofen, Germany) from slides prepared by cytocentrifugation (Shandon Cytospin 4, Thermo Electron Corporation, Marietta, OH, USA) and Diff-Quick staining (Pancreac Quimica SAU; Castellar del Vallès, Spain). For each rat, approximately 500 cells were counted. Moreover, the BALF was centrifuged at $1,300 \times g$ for 10 min and the supernatant stored at -80°C for subsequent analysis.

Lung wet/dry weight ratio

The left lungs were dissected, immediately after exsanguination, for oedema assessment and the wet weight recorded. The lungs were then placed in an incubator at 55°C for 24 h, and the dry weight was determined.

Histological examination

The right lungs were removed and one lobe was fixed in 10% paraformaldehyde. The histological analysis of excised lung tissue was carried out by two independent experts, blinded to the treatment, using 4- μ m sections stained with haematoxylin and eosin (H&E). A computerised analysis was carried out by digitally capturing the image of each whole H&E-stained section with a Leica DMD108 Digital Microimaging Device (Leitz Microsystems; Wetzlar, Germany). A threshold analysis of whole lung sections, manually excluding non-parenchymal structures (e.g. large blood vessels, airway structural elements and connective structures), was performed by using the Metamorph image analysis software (Molecular Devices; Sunnyvale, CA, USA) by a blinded investigator. To this end, red-green-blue colour images were split into individual colour channels and analyses were performed using the green channel. The threshold was then adjusted to the highest point that would highlight all areas of the parenchyma while excluding background. The threshold area of total parenchyma was measured and recorded. Then the threshold was reset to highlight only areas of high pixel density correlating with lung damage (damage threshold), as

FIGURE 1. Lung oedema, quantified by the a) wet/dry weight ratio, b) histological index of lung injury and c) histological score in control rats and in rats subjected to high-volume ventilation with or without instillation of mesenchymal stem cells (MSC) intravenously (VEN) or intratracheally (TRA). Data are presented as mean $\pm$ SEM. *: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$

determined by microscopic regional image analysis. Equal threshold values were applied to all the images analysed in all the groups. A lung injury index was calculated as the ratio of damaged area relative to total parenchyma area, and this was reported as fold increase over control lungs (non-ventilated rats) [16]. In addition, a pathologist blinded to the rat's experimental

conditions scored the right lung tissue preparations according to the following scale: 0: normal lung; 1: septal congestion; 2: septal inflammatory infiltrates; 3: alveolar haemorrhage and/or hyaline membranes.

MSC staining

MSCs (5×10^6) were fluorescently-labelled with Vybrant -DiI (1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) cell labelling solution (Molecular Probes, Carlsbad, CA, USA) for 20 min at 37°C and then washed and resuspended with DPBS. After labelling, MSCs were intratracheally or intravenously applied in six additional rats as previously described. Right lungs from MSC pre-treated rats subjected to the high-volume ventilation were frozen in Tissue-Tek (Sakura Finetech, Tokyo, Japan) optical cutting temperature (OCT) compound by immersion in liquid nitrogen. Cryostat sections (10 μ m thick) were adhered to glass slides previously coated with poly-L-lysine, fixed in formalin for 10 min and analysed by a fluorescence microscope (Eclipse Ti; Nikon Instruments Inc, NY, USA)

Immunohistochemistry

Vascular cell adhesion protein (VCAM)-1 expression in lung tissues was assessed by using a monoclonal antibody (H-276: sc-8304; Santa Cruz Biotechnology Inc., CA, USA) following the manufacturer's protocol for paraffin-embedded tissues. Immunoreactivity was revealed by the labelled streptavidin biotin alkaline-phosphatase technique (Dako Cytomation, Glostrup, Denmark). Lung sections were counterstained with haematoxylin and read by an investigator blind to the group of the sample. VCAM-1 quantification was carried out by using a computerised analysis, as previously reported, and calculated as the ratio of VCAM-1 expression area relative to total parenchyma area.

RNA extraction and real-time PCR

Total RNA from lung tissue was isolated with the Prime script RT reagent kit (Takara Bio Inc, Otsu, Japan). Total RNA was then quantified and quality evaluated by using a spectrophotometer (Biophotometer; Eppendorf, Hamburg, Germany). A total of 100 ng μ L⁻¹ RNA was used for reverse transcriptase PCR using the Taqman Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Quantitative real time-PCR (Applied Biosystems) was employed to analyse the tissue distributions using the gene-specific primers (IDT; Integrated DNA Technologies, Germany), interleukin (IL)-1 receptor antagonist (IL-1RA) primer 1: 5'-AGCGGATGAAGG-TAAAGCG-3', primer 2: 5'-CTGTGCCTGTCTTGTGTCA-3'; keratinocyte growth factor (KGF) primer 1: 5'-CACAAATCCA-ACTGCCACAG-3' primer 2: 5'-GGATTGACAAACGAGGCA-AAG-3'; hepatocyte growth factor (HGF) primer 1: 5'-CAAAC-TAACCATCCACCCTACT-3', primer 2: 5'-ATTGCCCTATTTCCGTTGT-3'. The expression level of glyceraldehydes-3-phosphate dehydrogenase was used as the internal control; primer 1: 5'-GTAACCGGCGTCCGATAC-3', primer 2: 5'-GTTCTAGAGACAGCCGCATC-3'.

Cytokine and protein measurements

Total protein concentration in BALF was quantified using the Micro BCA protein assay kit (Pierce, Rockford, IL, USA). The concentration of IL-1 β and macrophage inflammatory protein-2 (MIP-2) in BALF was determined by ELISA (Quantikine ELISA Kit; R&D Systems, Minneapolis, USA).

FIGURE 2. Representative images of a–d) haematoxylin and eosin-stained and e, f) Vybrant Dil-stained lung tissue sections. In rats subjected to high-volume ventilation pre-treated with mesenchymal stem cells (MSC) either intravenously (VEN; c) or intratracheally (TRA; d), lung injury was notably reduced compared with over-ventilated rats (b). The arrow shows MSCs localised in the tissue sections. Original magnification $\times 200$. g) Quantification of the MSCs localised in lung tissue. Data are presented as mean $\pm$ SEM.

Statistical analysis

Results are reported as mean $\pm$ SEM. ANOVA with Fisher's protected least significant difference as a *post hoc* analysis was

used for multigroup comparisons (StatView 5.0.1; Abacus Concept, Berkeley, CA, USA). Statistical significance was established as $p < 0.05$.

FIGURE 3. Absolute a) neutrophil and b) macrophage counts in the bronchoalveolar lavage fluid in control rats and in rats subjected to high-volume ventilation, with or without instillation of mesenchymal stem cells (MSC) intravenously (VEN) or intratracheally (TRA). Data are presented as mean $\pm$ SEM. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

RESULTS

Lung oedema assessed by the wet/dry weight ratio method was significantly greater in animals subjected to over-ventilation than in controls. In over-ventilated animals, pre-treatment with MSCs reduced oedema to values very close to those of controls. A similar improvement was found for intratracheal and intravenous administration route of MSCs (fig. 1a). The results obtained from the two histological analyses of lung tissues, the computerised lung injury index and the visual score provided consistent results (fig 1b and c). Whereas lung injury was significantly detected in the over-ventilated MSC-untreated animals (figs 1b, c and 2b), in the rats pre-treated with MSCs, regardless of the administration route, lung injury was considerably reduced (figs 1b, c, 2c and d). Figure 2c–g indicates that MSCs were localised in the tissue sections only in case that these cells were applied intravenously.

Figure 3 shows the results of cell counting in BALF: neutrophils significantly increased and macrophages significantly decreased in the over-ventilated animals, while pre-treatment with MSCs, either intravenously or intratracheally, reduced

neutrophils and increased macrophages (fig. 3a and b, respectively). Data in figure 4 indicate that MSCs were localised in the BALF only in the cases that these cells were applied intratracheally. Total cell counts in BALF were almost normalised in the over-ventilated animals that were subjected to either intravenous or intratracheal pre-treatment with MSCs ($1.70 \pm 0.14 \times 10^4 \text{ cells mL}^{-1}$ and $1.72 \pm 0.27 \times 10^4 \text{ cells mL}^{-1}$, respectively) whilst they decreased in the BALF of over-ventilated rats compared with controls ($1.28 \pm 0.22 \times 10^4 \text{ cells mL}^{-1}$ and $1.94 \pm 0.19 \times 10^4 \text{ cells mL}^{-1}$, respectively; $p < 0.05$).

Figure 5a–c shows that the concentrations of total proteins, pro-inflammatory cytokine IL-1 β and MIP-2 in BALF were significantly higher in MSC-untreated animals subjected to high-volume ventilation than in controls. Application of MSCs, either intravenously or intratracheally, in over-ventilated rats markedly reduced the amount of total proteins, IL-1 β and MIP-2 in BALF (fig. 5a–c). Figure 5d also shows that real-time PCR showed a significant increase in mRNA levels of IL-1RA in lungs of MSC pre-treated groups compared to controls and to animals subjected to high-volume ventilation. mRNA analysis of HGF and KGF in the lung tissues did not reveal any significant difference among the groups (data not shown).

Expression of VCAM-1, evaluated by immunohistochemical examination of lung tissue, was found to be greater in the over-ventilated group (fig. 6b), whilst pre-treatment with MSCs, either intravenously or intratracheally, decreased VCAM-1 to levels close to control values (fig. 6a, c, d and e).

DISCUSSION

The results obtained in this experimental high-volume ventilation model confirm the hypothesis that MSCs prevent lung injury induced by high-amplitude mechanical ventilation. It was also found that the early potential therapeutic effect of MSCs was similar for both local (intratracheal) and systemic (intravenous) infusion. MSCs were localised in lung tissue only in case that these cells were injected intravenously, whilst MSCs applied intratracheally were only found in BALF.

This study was carried out with a common rat model of VILI, with typical magnitude of overstretch (25 mL kg^{-1}) and duration of the injurious mechanical stimulus (3 h) [17]. The animals subjected to lung over-distension exhibited the expected pattern of VILI; namely loss of lung barrier integrity and lung inflammation. Lung oedema, resulting from increased permeability of the alveolocapillary barrier, augmented from a normal value of lung wet/dry weight ratio to a figure typically found in animals subjected to over-ventilation [17]. Accordingly, the histopathological tissue analysis showed considerable lung injury in the animals receiving high-volume ventilation. Moreover, cell and protein analysis of BALF showed a clear pattern of VILI in the animals subjected to overstretch: 1) increased number of neutrophils, caused by augmented endothelial and epithelial permeability [1, 18]; 2) reduced counts of alveolar macrophages, consistent with the rapid decline in BALF macrophages found in other works in response to high-volume ventilation [19] and attributed to their fast inflammatory activation [20]; 3) increased total protein concentration, a hallmark of leakage in the lung barrier [1, 17]; 4) augmented concentration of cytokines MIP-2 and IL-1 β , among the most biologically active cytokines in the lungs of patients with ALI [21, 22]; 5) in lung tissue, increased VCAM-1 expression,

FIGURE 4. Images of a–d) cytopsin preparations and e, f) Dil staining of bronchoalveolar lavage fluid (BALF) from a) control rats and rats subjected to high-volume ventilation b) without or c, e) with administration of mesenchymal stem cells (MSC) intravenously (VEN) or d, f) intratracheally (TRA). The arrows show the presence of MSCs in BALF of rats intratracheally instilled with MSCs. Original magnification $\times 400$. g) Number of MSCs recovered in BALF. Data are presented as mean $\pm$ SEM.

having an important role in neutrophil and lymphocyte sequestration in VILI [23]. Taken together, these common biomarkers of lung injury indicate that the animals subjected to high-volume ventilation and not treated with MSCs developed VILI.

Whereas some studies on the protective effects of adult stem cells in lung injury have been carried out using bone marrow-derived mononuclear cells, including both mesenchymal and hematopoietic stem cells [4–6], in this work we focused on MSCs

FIGURE 5. a) Total proteins, b) cytokine interleukin (IL)-1β and c) macrophage inflammatory protein-2 (MIP-2) concentrations in bronchoalveolar lavage fluid (BALF) in control rats and in rats subjected to high-volume ventilation with or without instillation of mesenchymal stem cells (MSC) intravenously (VEN) or intratracheally (TRA). d) IL-1 receptor antagonist (IL-1RA) mRNA expression in lung tissue. Data are presented as mean ± SEM. *: $p < 0.05$; *** $p < 0.001$; #: $p < 0.0001$.

exclusively. We used well-characterised MSCs [15] and applied a number of cells in keeping with the different doses reported in the literature when these stem cells were applied, either intravenously or intratracheally, in rats and mice [10, 24].

Pre-treatment with MSCs was effective in significantly reducing the development of VILI in the rats subjected to over-ventilation. Our results clearly indicate that all the variables assessed in the animals pre-treated with MSCs exhibited a tendency to recover the control values, *i.e.* oedema and histological index of injury in lung; neutrophils and macrophages cell counts and biomolecules concentration in BALF and in lung tissue increased mRNA IL-1RA expression. Taken together, these data provide solid evidence that the local inflammatory process and the associated loss of alveolocapillary membrane integrity induced by high-volume ventilation were considerably reduced. The effectiveness of MSCs in minimising the manifestation of VILI was in agreement with findings observed in bleomycin or LPS-induced lung injury [10, 11].

Data from bleomycin or LPS in ALI models also suggest that the protective effect of MSCs may be similar, regardless of the route of injection [10, 11, 14]. Our group is the first to show evidence, in a single study, that either intravenous or

intratracheal administration of MSCs may result in the same potential benefits in the reduction of VILI. We observed that MSCs injected *via* the intra-alveolar route were recovered in the BALF, highlighting that this injection method would maximise cell delivery in the lung and directly reach the injury site. When MSCs were administered *via* the systemic route, however, they became localised in the alveolar region, confirming the finding that MSCs arrive first at the pulmonary microcirculation. Their retention in the lung may be facilitated by their large size with respect to the capillary diameter, and by the expression of adhesion molecules, such as VCAM-1 and P-selectin [25]. However, the fact that systemic application of MSCs can be as effective as local application in protecting against lung injury might have some practical clinical relevance as intravenous administration is much simpler than intratracheal administration in patients, even those who are intubated and mechanically ventilated.

Since several studies have shown that pulmonary engraftment of MSCs in the lungs does not occur very easily, the ability of MSCs to reduce lung injury in different ALI models is currently attributed to a paracrine effect [26, 27]. Irrespective of the route of delivery, MSCs may reduce lung injury and promote repair by interacting with injured resident alveolar

FIGURE 6. a–d) Representative images of vascular cell adhesion protein (VCAM)-1 immunohistochemical staining. In rats subjected to high-volume ventilation pre-treated with mesenchymal stem cells (MSC) either intravenously (VEN; c) or intratracheally (TRA; d), VCAM-1 expression greatly decreased compared with over-ventilated rats (b). e) Data are presented as mean ± SEM. #: $p < 0.0001$.

epithelial or lung endothelial cells, or by modulating the responses of neutrophils, monocytes, activated macrophages, lymphocytes with the release of several secreted factors such as angiopoietin (Ang)-1, prostaglandin E_2 (PGE_2), IL-1RA, transforming growth factor- β (TGF- β). Furthermore, MSCs may secrete growth factors, such as vascular endothelial growth factor (VEGF), KGF and HGF, which have cytoprotective

and reparative properties [5, 26, 27]. In our acute study, we observed increased expression of IL-1RA mRNA in lung tissue when over-ventilated animals were pre-treated with MSCs, irrespective of the administration route, while no major changes in the mRNA expression of KGF or HGF were detected among all the groups after 3 h of injurious ventilation. In an LPS-induced ALI model, a significant decrease in pro-inflammatory

cytokines (tumour necrosis factor (TNF)- α , MIP-2) and an increase in anti-inflammatory cytokines, including IL-10 and IL-13, were observed when MSCs were given *via* the intra-tracheal route [11]. Similar results were achieved when the culture medium of MSCs was administered for treatment of LPS-induced ALI in an *ex vivo* perfused human lung [14]. Further evidence that paracrine factors released by MSCs or the conditioning medium play a beneficial role in the reversal of ALI comes from studies on perinatal hyperoxic models of lung injury [28].

Our study was conceived to test the hypothesis that MSCs may reduce VILI. Although the experimental design was close to that of conventional studies using rodent VILI models and MSCs infusion, the present work shows some limitations because not all the possible ranges of variables were investigated. To induce VILI we did not investigate different ventilator settings (amplitude of tidal volume, effect of positive end-expiratory pressure (PEEP) or pressure- rather than volume-controlled ventilation). A previous study, however, showed that these variables can modulate the magnitude of VILI but not its mechanisms and consequences (lung membrane leakage and inflammation) [29]. A further limitation, in common with most research studies on treatment with MSCs is about the timing of MSCs instillation with respect to the start of the injurious stimulus, in our study, mechanical ventilation. Whether the protective effects of MSCs could depend on these parameters remains to be elucidated. The effects could be different, in fact, if MSCs were applied as a preventive treatment and a different concentration with respect those ones we used, or once lung injury has been well established. Finally, we focused on the early effects of VILI (3 h). Extending the duration of the experiments would allow assessing the study in more details, specifically the expression and role played by different biomarkers in the mid- and long-term. Accordingly, the potential therapeutic effect of MSC in chronic models of VILI requires further investigation. In particular, future experiments assessing the effect of MSCs on already well established VILI are of major interest to better approach the clinical problem. Regardless of its limitations, this is the first study, to our knowledge, that provides proof for the concept that both local and systemic pre-treatment with MSCs could play a role in protecting the lungs from the injuries caused by mechanical ventilation of high amplitude.

MSCs are emerging as a potential therapeutic tool in patients. In fact, a considerable number of clinical trials using this type of cells have recently been completed, or are ongoing, for various diseases, including acute myocardial infarction, ischaemic heart failure, renal failure, multiple sclerosis, aseptic necrosis of the hip, inflammatory bowel disease, osteogenesis imperfecta and also respiratory diseases such as idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease [5]. To date, most of the research in MSCs has been carried out with cells obtained from the bone marrow. It is expected, however, that MSCs obtained from more accessible niches, for instance umbilical cord blood and, particularly, adipose tissue, could facilitate both the heterologous and autologous infusion of MSCs. Although MSCs from different niches do show some differences, their main biological features are similar. In fact, some studies have shown that MSCs derived from adipose tissue and from bone marrow perform similarly, for instance

for ischemic stroke therapy [30]. Accordingly, both types of adult stem cells are currently used in clinical trials [31]. It is therefore likely that future research could prove that MSCs obtained from sites other than bone marrow could also be helpful in alleviating VILI and, more generally, ALI/ARDS.

SUPPORT STATEMENT

This research was supported in part by the Ministerio de Ciencia e Innovación (SAF2008-02991 and PI081908). L. Chimenti is the recipient of a European Respiratory Society and Marie Curie Joint Research Fellowship (MC 1636-2010). The research leading to these results has received funding from the European Respiratory Society and the European Community's Seventh Framework Programme FP7/2007-2013 – Marie Curie Actions under grant agreement RESPIRE, PCOFUND-GA-2008-229571.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

The authors wish to thank I. Almendros, R. Nieto and M.A. Rodríguez (Unit of Biophysics and Bioengineering, Faculty of Medicine, University of Barcelona, Barcelona, Spain) for their valuable technical help. The mesenchymal stem cells employed in this work were kindly provided by the Tulane Center for Gene Therapy, New Orleans, LA, USA.

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