Respiratory Physiology & Neurobiology 188 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

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

Antioxidant effect of human adult adipose-derived stromal stem cells in alveolar epithelial cells undergoing stretch

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ARTICLE INFO

Article history:
Accepted 8 April 2013

Keywords:
Oxidative stress
Human adipose-derived stromal stem cells
Acute lung injury
Cyclic stretch

ABSTRACT

Introduction: Alveolar epithelial cells undergo stretching during mechanical ventilation. Stretch can modify the oxidative balance in the alveolar epithelium. The aim of the present study was to evaluate the antioxidant role of human adult adipose tissue-derived stromal cells (hADSCs) when human alveolar epithelial cells were subjected to injurious cyclic overstretching.
Methods: A549 cells were subjected to biaxial stretch (0–15% change in surface area for 24 h, 0.2 Hz) with and without hADSCs. At the end of the experiments, oxidative stress was measured as superoxide generation using positive nuclear dihydroethidium (DHE) staining, superoxide dismutase (SOD) activity in cell lysates, 8-isoprostane concentrations in supernatant, and 3-nitrotyrosine by indirect immunofluorescence in fixed cells.
Results: Cyclically stretching of AECs induced a significant decrease in SOD activity, and an increase in 8-isoprostane concentrations, DHE staining and 3-nitrotyrosine staining compared with non-stretched cells. Treatment with hADSCs significantly attenuated stretch-induced changes in SOD activity, 8-isoprostane concentrations, DHE and 3-nitrotyrosine staining.
Conclusion: These data suggest that hADSCs have an anti-oxidative effect in human alveolar epithelial cells undergoing cyclic stretch.

1. Introduction

Mechanical ventilation is a life sustaining therapy for patients with acute respiratory failure and severe acute lung injury (ALI) in the intensive care unit. But mechanical ventilation by itself may cause lung injury through cyclic tissue stretching, a processes known as ventilator-induced lung injury (VILI) (Dreyfuss and Saumon, 1998; Tremblay et al., 1999; Gordo Vidal et al., 2007). It is a serious undesirable complication of ventilator therapy and an important cause of morbidity and mortality in patients (Rubenfeld et al., 2005).

One of the main mechanisms involved in the pathogenesis of VILI is through an increase in oxidative stress (Reddy et al., 2007; Syrkina et al., 2008; Marín-Corral et al., 2010), resulting from an imbalance between the production of reactive oxygen species (ROS) and their elimination. One theory to account for this is through the repetitive mechanical stress induced by the ventilator. Indeed, (AECs) undergoing cyclic stretch show increased oxidative stress (Vlahakis et al., 1999; Chapman et al., 2005; Jafari et al., 2004).

Increased ROS formation affects normal cell function by oxidation of relevant biomolecules such as proteins, lipids and DNA (Comhair and Erzurum, 2002). Superoxide reacts with NO yielding the potent oxidant peroxynitrite, which induces protein and lipid oxidation, and protein nitration.

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http://dx.doi.org/10.1016/j.resp.2013.04.007

Despite a wide body of research there is not as yet an effective therapy for ALI (Adhikari et al., 2004; Christofidou-Solomidou and Muzykantov, 2006). New cell-based therapies, particularly the administration of human adult mesenchymal stem cells (hMSC) are a promising alternative for the treatment of lung diseases. hMSCs are pluripotent adult stem cells found mainly in the bone marrow with the capability of differentiating into multiple cell types (Prockop, 1997; Krause et al., 2001). There are several animal studies showing the anti-inflammatory effects of bone marrow-derived hMSC in different models of ALI (Rojas et al., 2005; Gupta et al., 2007; Ortiz et al., 2003).

hMSC can also be derived from adipose tissue (hADSC). These mesenchymal cells, readily obtained from subcutaneous adipose tissue, display multi-lineage development plasticity and share similarities with bone marrow-derived hMSCs in their surface markers and gene profiling (Zuk et al., 2001, 2002). Because of the relative ease in which they are isolated and purified, there is an increasing interest for their use in novel cell therapies. Specifically, some studies have found that autologous transplantation of hADSC ameliorates pulmonary emphysema by selectively inducing growth factors expression (Shigemura et al., 2006). We hypothesized that hADSCs would serve an antioxidant effect in AECs undergoing cyclic stretch.

2. Methods

2.1. Isolation and culture of hADSC

All aspects of our study received ethics's approval through our institution's Ethics Committee. Adipose tissue biopsies from adult healthy donors and subcutaneous adipose tissue samples (10–50 g, each) were collected from three adult patients (age, 20–60 years) undergoing plastic surgery for routine procedures. Adipose tissue was washed at least 3 times in sterile phosphate-buffered saline (PBS) (Sigma–Aldrich, St. Louis, MO), treated with an equal volume of collagenase type I suspension and digested at 37 °C for 30 min with 2 mg/ml type I collagenase (Sigma–Aldrich, St. Louis, MO). Enzyme activity was neutralized with Dulbecco's modified Eagle's medium (DMEM) (Lonza Walkersville Inc., Walkersville, MD) supplemented with 10% heated fetal bovine serum (FBS) (Lonza Walkersville Inc., Walkersville, MD) and centrifuged at 1500 rpm for 5 min. The pellet was resuspended in stromal culture medium (DMEM + 10% FBS + 1% penicillin/streptomycin/amphotericin) and seeded in culture plates. Cells were maintained at 37 °C and 5% CO_2. When the monolayer of adherent cells reached confluence, cells were trypsinized (0.05% EDTA (0.02%) (T/E) (Lonza Walkersville Inc., Walkersville, MD), resuspended in stromal culture medium, and subcultured. For experiments, 3rd–5th passages of hADSCs from not pooled samples were used.

2.2. Characterization and differentiation of hADSC

As described in detail in the supplementary online data, adherent hADSC population displayed the properties (immunophenotyping and lineage differentiation) consistent with the requirements for the definition of MSCs (Dominici et al., 2006). Briefly, adherent cells showed a typical fibroblast-like morphology and exhibit cell-surface MSC specific markers including positive-staining for CD73, CD105 and CD90, and negative-staining for CD45 and HLA DR. Moreover, using specific culture medium under *in vitro* conditions, stromal cells were able to differentiate into chondrocytes, osteocytes and adipose cells after 21 days in standard conditions of culture, which displayed their multi-lineage potential.

2.3. AECs culture

A549 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (0.3 μg/ml), penicillin (100 U/ml), streptomycin (200 μg/ml), and 10% fetal bovine serum (FBS; GIBCO, Grand Island, NY). A549 cells were grown on six well culture plates with a flexible base (5 cm^2) made of collagen-I-impregnated silicoelastic membrane (Flexcell International Corporation, Hillsborough, NC). The A549 cells were passaged and seeded onto these plates at density of 5.0×10^5 cells/well and grown to confluence in a humidified 95% air – 5% CO_2 incubator at 37 °C. Cells were then washed with warm phosphate buffered saline (PBS), (Sigma–Aldrich, St. Louis, MO) and a fresh serum-free DMEM medium was added and cells were cultured for 24 h before each experiment.

2.4. AEC stretching model

To mimic lung injury induced by mechanical ventilation we used a conventional model of alveolar cell stretch. The cell-stretching device deformed the cell substrate by applying a vacuum to its underside. A cylindrical loading post was located underneath the central region of the well. When the vacuum was applied the outer annular region of the flexible cell substrate, the central region was equibiaxially and homogeneously stretched (Trepat et al., 2006). The cell-stretching device was calibrated as described in detail in the online Data Supplement. We applied a cyclic cell stretch with a pattern of amplitude and frequency simulating that experienced by the epithelial monolayer in patient lungs subjected to high-volume mechanical ventilation. Quasi-sinusoidal cyclic stretch was applied to achieve a 15% change in the basement membrane surface area and performed in a cyclic manner (0.2 Hz). These surface area changes correspond to 60% of total lung capacity (TLC) (Tschumperlin et al., 2000). Cells were stretched at cell density 2.5×10^5 cell/well on 6-well plates for 24 h at 37 °C in a humidified incubator containing 5% CO_2. Nonstretched cells (static cells) were used as controls. Comparisons were made between stretched cells and control cells cultured on the same plates in the absence of cyclic strain.

2.5. Experimental protocol

Fresh DMEM culture medium was placed in cell wells before starting the experiments. To investigate to antioxidant effect of hADSCs in alveolar epithelial cells subjected to cyclic stretch, four groups of identical cell cultures conditions were prepared ($N = 4$): (1) non-stretched and untreated A549 cells; (2) non-stretched A549 treated with hADSC; (3) stretched and untreated A549 cells; (4) stretched A549 cells treated with hADSC.

In all experiments wells were mounted on the cell-stretching device at $t = 0$ and placed in a 37 °C, 5% CO_2 incubator for 24 h, with or without stretching, depending on the experimental group. In groups 3 and 4, 15% strain at 30 cycles/min, 0.5 Hz was applied for 24 h. In groups 2 and 4, hADSC were added to the culture well (1 hADSC per 3 AECs ratio, 84,000 hADSC per well) at $t = 0$. After 24 h, cell-free supernatants were collected and stored at −80 °C for further analysis. Subsequently, adherent cells were detached with 0.25% trypsin/EDTA (Sigma–Aldrich, St. Louis, MO), lysed at 4 °C with a cell lysis reagent (T-PER, Tissue Protein Extraction Reagent, Thermo, Fisher Scientific, Rockford, IL) and stored at −80 °C for analysis of oxidative markers.