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Rapamycin reduces high-amplitude, mechanical stretch-induced apoptosis in pulmonary microvascular endothelial cells

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ABSTRACT

Alveolar epithelial and endothelial cell death caused by mechanical over-distension is likely to contribute to ventilator-induced lung injury (VILI). Consequently, the search for cytoprotective interventions is of interest. We investigated the effect of the mTOR inhibitor rapamycin on human pulmonary microvascular endothelial cell (HMVEC-L) viability in a model of high-amplitude mechanical stretch. Cyclic mechanical stretch (30% increase in membrane surface area, cycling frequency 40/min), employed for 24 h induced apoptosis in HMVEC-L. This effect was reduced by rapamycin treatment. Focusing on possible mechanisms of action we demonstrated that the stretch-induced reduction in the anti-apoptotic messenger pAkt could be restored by rapamycin treatment. Furthermore, we observed rapamycin-induced modifications in the HMVEC-L actin cytoskeleton architecture and global cellular f-actin content which functionally resulted in an increased global cellular mechanical stability – as indicated by an increased HMVEC-L osmomechanical resistance – thereby possibly desensitizing HMVEC-L to mechanical stimulation.
According to the data from the present study, rapamycin represents a promising cytoprotective agent under mechanically challenging conditions such as mechanical ventilation.

Introduction

The use of low tidal volume ventilation improves mortality and morbidity in acute lung injury/acute respiratory distress syndrome (ALI/ARDS) (The Acute Respiratory Distress Syndrome Network, 2000). This concept of protective ventilation recognizes that high tidal volume ventilation may induce or aggravate lung injury. Indeed, alveolar overdistension is regarded as a crucial mechanism in ventilator-induced lung injury (VILI) (1999).

In alveolar type II (AT II) cells, high-amplitude mechanical stretch induces necrosis (Tschumperlin and Margulies, 1998; Tschumperlin et al., 2000) and apoptosis (Edwards et al., 1999; Hammerschmidt et al., 2004). Previously, we found that AT II cell apoptosis was induced by high-amplitude cyclic deformation whereas low amplitude did not affect cell viability as compared to static cells (Hammerschmidt et al., 2004). High-amplitude stretch-induced apoptosis has been shown to be abrogated by angiotensin-converting enzyme inhibition (Hammerschmidt et al., 2004), by PI3K-stimulating agents (Hammerschmidt et al., 2007), and by nitric oxide (Edwards et al., 2000; Hammerschmidt et al., 2004).

In addition to alveolar epithelial cells, the alveolocapillary barrier is also made up of endothelial cells. Here, sharing a common basal membrane, the two types of cells are coupled mechanically (West, 2003) and thus experience a similar degree of cyclic mechanical stretch during ventilation. The effect of cyclic overdistension on pulmonary microvascular endothelial cell viability has not been extensively studied yet. Therefore, the aim of this study was to investigate high-amplitude stretch-induced apoptosis in HMVEC-L.

Due to the inhomogeneous nature of most lung injuries, ventilation with low tidal volume in injured lungs may still cause alveolar overdistension because of the derecruitment of alveolar units and an increased risk of overdistending the remaining open lung units. Therefore, interventions that reduce the susceptibility of the lung to mechanical stress may be beneficial.

Rapamycin, a specific inhibitor of the serine/threonine kinase mTOR, is a promising candidate for modulating mechanical stretch-induced apoptosis. This assumption is based on two experimental findings: a) rapamycin has been characterized as an apoptosis-modulating agent in models of malignant cells, here exhibiting both pro-apoptotic (Shi et al., 1995; Muthukkumar et al., 1995; Hosoi et al., 1999) and anti-apoptotic (Asnaghi et al., 2004) effects; and b) rapamycin was shown to affect the cellular actin-cytoskeletal architecture (Gomez-Cambronero, 2003; Qian et al., 2004, 2005), which in turn is a major determinant of the global cellular mechanical state. As cellular reactions toward mechanical stimulation – for example, apoptosis following mechanical stretch – are governed by

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ATII cell, alveolar type II cell; FCS, fetal calf serum; HMVEC-L, human microvascular endothelial cells of the lung; LDH, lactic acid dehydrogenase; mTOR, mammalian target of rapamycin; PBS, phosphate buffered saline; PI, propidium iodide; VILI, ventilator-induced lung injury.

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the mechanical properties of the cell itself (Ingber, 2006), this might offer a means by which rapamycin can modulate apoptosis.

This study tests two hypotheses: (i) Rapamycin modulates the extent of mechanical stretch-induced apoptosis in HMVEC-L. (ii) Rapamycin alters the basal mechanical state of HMVEC-L by modifying the HMVEC-L cytoskeletal architecture.

To this end, the effect of rapamycin on mechanical stretch-induced apoptosis was characterized by using an established model of applying mechanical stretch to simulate cyclic overdistension of the lung. Actin cytoskeletal architecture was analyzed by confocal microscopy and filamentous (f-)actin quantification. Cellular mechanical state was evaluated by determining cellular osmomechanical resistance.

Materials and methods

Drugs

Rapamycin (Rapa), latrunculin B (Lat), and wortmannin (WM) were purchased from Sigma-Aldrich (Deisenhofen, Germany).

Cell culture and HMVEC-L preparation for experimental use

HMVEC-L were purchased from Cambrex (Verviers, Belgium). Cells were maintained in Endothelial Basal Medium-2 (EBM®-2) supplemented by EGM-2 MV SingleQuots (both Cambrex, Verviers, Belgium) at 37 °C, 5% CO_2 in a humidified atmosphere. Cells were propagated to passages 9 or 10 for experimental use.

For use in the cyclic stretch experiments HMVEC-L (2.5×10^5 cells/well) were plated on the central area of fibronectin-coated silicon membranes (BioFlex®-6-well-culture plates, Flexcell International, Hillsborough, NC, USA). Previously plates were additionally coated with human fibronectin (100 μg/ml; Roche Diagnostics, Penzberg, Germany) for at least 3 h at 4 °C. Experiments were started at 22 h of cellular adherence.

Cyclic stretch

BioFlex®-wells were subjected to cyclic sinusoidal stretch employing a FX 3000T™ Flexercell Tension Plus™ system (Flexcell International, Hillsborough, NC, USA) or were left unstretched within the same incubator as static controls for the entire time of the experiment. We used a pattern of cyclic stretch characterized by an amplitude of 30% change in surface area of the silicon membranes at a frequency of 40/min. This stretching pattern was adopted from previous studies investigating rat alveolar type II cells (Hammerschmidt et al., 2004, 2005, 2007).

After the stretching period, cells were harvested and analyzed for cellular viability or Akt activation, respectively.

Detection of cellular viability (apoptosis/necrosis)

Cellular viability was detected using a TACS™ Annexin V-FITC Apoptosis Detection Kit (R&D Systems, Wiesbaden-Nordenstadt, Germany) as previously described (Hammerschmidt et al., 2004).

Staining behavior was analyzed by flow cytometry (5000 events/condition) (Coulter® Epics® XL™; Beckman Coulter, Krefeld, Germany). Cells negative for both annexin V and PI staining (A−/PI−) were regarded as viable, cells staining positive for annexin V without PI uptake (A+/PI−) as apoptotic, and cells with structural damage to the plasma membrane as indicated by PI uptake (PI+) as necrotic (regardless of their annexin V staining behavior).

Analysis of Akt activation (Akt phosphorylation)

Akt activation was analyzed using a PathScan® Phospho-Akt1 (Ser473) Sandwich ELISA Kit (Cell Signaling Technology, Danvers, MA, USA) according to the manufacturer's instructions.

ELISAs were analyzed in a spectrophotometric reader (Tecan Spectra Classic; Tecan, Crailsheim, Germany). OD450 readings were normalized to total protein concentration, which was determined in

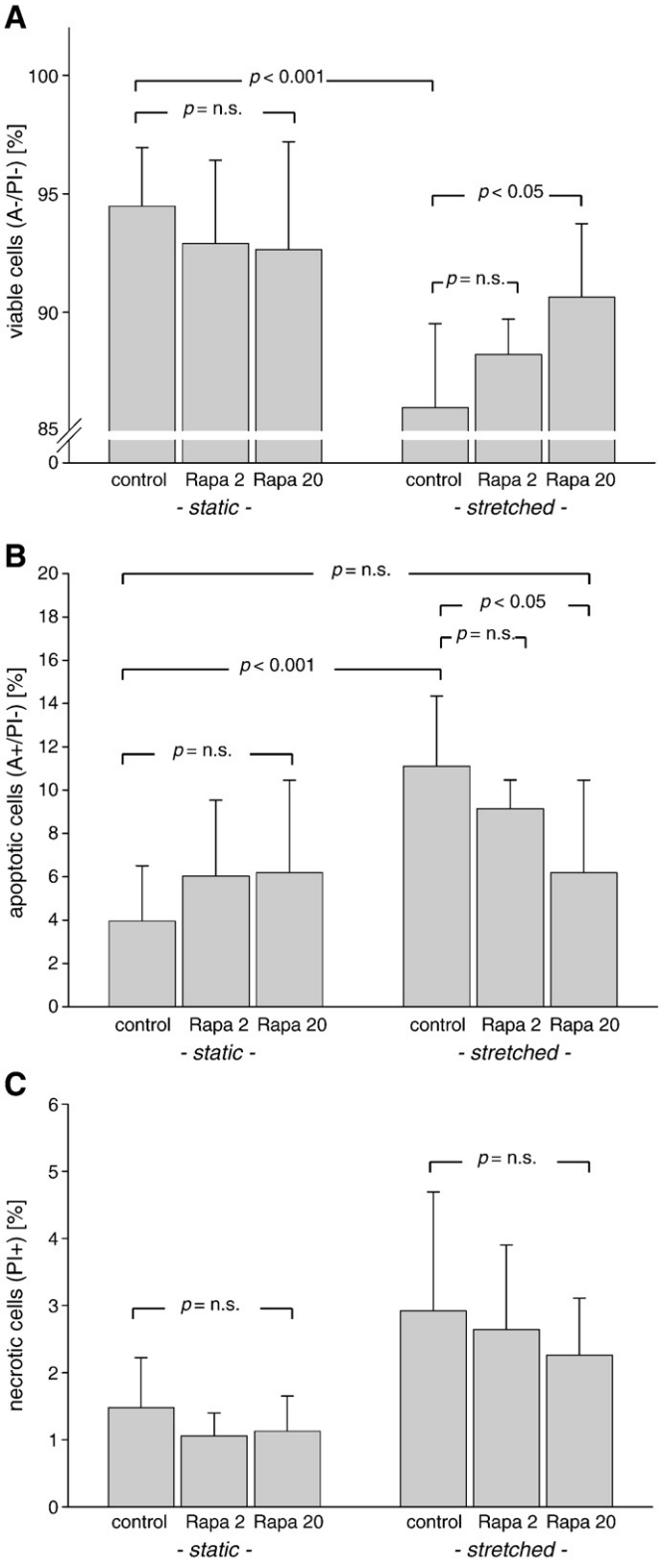

Fig. 1. The effect of high-amplitude stretch and rapamycin treatment on cell viability. Panels A–C depict results from flow cytometric analysis of cell viability. Each condition represents $n = 9$ cell isolations with triplicate measurements. Rapamycin (Rapa; 2 ng/ml or 20 ng/ml, respectively) was added 6 h before experiments were started. Values are mean ± SD.