Cytokine productions and gene expressions caused by mechanical stretching of normal human pulmonary artery endothelial cells

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Keywords: mechanical stretching, VALI, cytokine production, gene expression

1 Introduction

Mechanical ventilators are indispensable for patients with many types of respiratory failure. However, excessive mechanical ventilation causes some complications, because it is not completely physiological. One of the serious complications is ventilator-associated lung injury (VALI). It is generally agreed that VALI induces multiple organ failure which leads to the death of a patient [1], [2]. For the understanding of the molecular mechanism of VALI, we measured cytokine productions and gene expressions caused by in vitro excessive mechanical stretching of normal human pulmonary artery endothelial cells (HPAEC). Mechanical stretching was applied to the cells cultured on a flexible silicoelastic membrane, using an FX-4000T™ Flexercell® Tension Plus™ System.

2 Method and Results

2.1 Materials and Methods
HPAEC passaged 7-8 times were seeded on six-well flexible silicoelastic membrane culture plates (BioFlex® Culture Plates-Collagen Type I). Outer area of the membrane was coated by PDMS so that the cells would adhere to only inner area where uniform strain was imposed on them. The membrane was stretched by an FX-4000T™ Flexercell® Tension Plus™ System and a circular-shaped loading post [3]. Stretching had square waveforms of 15 cycles/min and the ratio of stretching to relaxing was 1:2. Elongations of 20% were applied to the cells cultured on the membrane for durations of 1h, 3h, 6h and 12h. Simultaneously, as the control, the other group of the cells was cultured on the membrane without stretching. Cytokine productions (IL-6, IL-8) were measured by the ELISA method (BIOSOURCE). The same experiment was repeated five times to make statistical comparisons of the cytokine productions using Kruskal-Wallis and Scheffe tests ($P < 0.05$). Gene expressions were measured by the GeneChip microarray analysis method (Affymetrix).

2.2 Results

Cytokine productions. No significant changes were observed in the IL-8 production, regardless of stretching. The IL-6 production was greater in the cells stretched for 0h, 1h and 3h than in the cells stretched for 6h and 12h after culturing started (Fig.1).

Gene expressions. Stretching increased the amounts of mRNA of IL-6, IL-6 signal transducer (Fig.2), IL-6 (interferon, beta 2, Fig.3) and IL-6 receptor (Fig.4). The amounts of mRNA of IL-6 and IL-6 signal transducer reached a peak at early stages of stretching, while the amount of mRNA of IL-6 receptor was increased even 12 hours after culturing started.
3 Discussion

Cyclic stretching increased the IL-6 production and the mRNA expressions related to IL-6 production. The time courses of the mRNA expressions were different. Examination of the relationship between the mRNA expressions will show pathways of signal transmission from stretching to inflammation.

References

