行政院國家科學委員會補助專題研究計畫成果報告

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※ 利用微陣列基因晶片分析呼吸器誘發肺損傷相關基 ※
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計畫編號：NSC　90－2314－B－002－236
執行期間：90 年 08 月 01 日至 91 年 07 月 31 日

計畫主持人：王 鶴 健
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計畫參與人員：

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一、中文摘要

關鍵詞：微陣列基因晶片，呼吸器誘發肺損傷，基因分析

儘管重症加護醫學的進步，但是急性肺損傷的的病人死亡率依然很高。這類病人死亡的原因大多為多重器官衰竭。最近的研究顯示急性肺損傷的病人接受人工呼吸器的治療，呼吸器誘發的肺損傷對病人的預後扮演重要的負面角色。人工呼吸如何造成病人進一步肺損傷的原因仍不清楚，但是有證據顯示肺泡的過度充氣及塌陷的肺泡因重覆張開、閉和所產生的剪力，可以導致肺損傷和發炎反應。由於呼吸器誘發的肺損傷，其分子機轉並不清楚。因此我們以大鼠之動物模式，利用微陣列基因晶片，來分析肺臟對呼吸器誘發肺損傷的基因表現。利用正常或內毒素誘發肺損傷之大鼠，接受不同呼吸器設定(正常潮氣容積，高潮氣容積±呼吸末期正壓)，於不同的時間收集其肺組織，進行廣泛基因分析。結果經由比較正常和呼吸器誘發肺損傷大鼠基因表達的差異，我們初步發現經由呼吸器誘發肺損傷的相關特定基因，包括細胞生長和細胞週期之調控基因群，與細胞生長有關之細胞激素和發炎有關之介質，蛋白酶和細胞黏附因子等基因群。更進一步的分析個別基因在呼吸器誘發肺損傷上所扮演的角色，有助於加速對呼吸器誘發肺損傷的預防和治療策略的發展。

Abstract

Keywords：Microarray, ventilator-induced lung injury, global gene analysis

Despite advances in critical care, the mortality rate in patients with acute lung injury remains high. Furthermore, most patients who die do so from multiple-system organ failure. It has been postulated that ventilator-induced lung injury (VILI) plays a key role in determining the negative clinical outcome of patients exposed to mechanical ventilation. How mechanical ventilation exerts its detrimental effect is as yet unknown, but it appears that overdistension of lung units or shear forces generated during repetitive opening and closing of atelectatic lung units exacerbates, or even initiates, significant lung injury and inflammation. The molecular mechanisms of VILI are poorly understood. We used microarrays to analyze the gene expression programs that underlie lung injury in response to mechanical ventilation. Sprague-Dawley rats were randomly assigned to receive ventilation with either a high tidal volume (20 ml/Kg) or a normal tidal volume (10 ml/Kg) with or without PEEP (10 cmH_2O). The same ventilator setting will be also applied to Sprague-Dawley rats with LPS-induced lung injury. Different time points of rat lung tissue were obtained for global analysis of gene expression. Changes in gene expression were monitored. Analysis of the time course of induction of these genes identify sequential genetic programs that characterize VILI. The results showed that VILI associated genes could be categorized into different gene groups according to related gene functions: cell growth and cell cycle

regulator, cytokine growth factor and inflammatory mediator, proteinase and adhesion molecule and miscellaneous genes. Further studies focusing on the verification of the relationship of the specific genes on VILI should accelerate the development of more effective strategies for intervention at various stages in the development of VILI of the lung.

二、研究計畫之背景及目的

Despite advances in critical care, the mortality rate in patients with acute respiratory distress syndrome (ARDS) remains high at values exceeding 30%. Furthermore, most patients who die do so from multiple-system organ failure (MSOF) rather than from hypoxia (1), an observation that has puzzled both clinicians and scientists interested in acute lung injury (ALI). Despite the life-saving potential of this assistance, mechanical ventilation per se may be responsible not only for worsening the underlying ALI but, by a number of mechanism, may also lead to the development of a systemic inflammatory response syndrome (SIRS) and MSOF (1). The postulate is that overdistension of lung units and/or shear forces generated during repetitive opening and collapse of atelectatic regions of the lung exacerbate, or even initiate, significant lung injury and inflammation (1-3), with or without concomitant gross structural disruption. Ventilator-induced lung injury (VILI) is thus the result of a complex interplay among various mechanical forces acting on lung structures during mechanical ventilation. The weight of evidence obtained from experimental animal studies, correlative human studies addressing the detrimental effects of different ventilatory strategies quite convincingly points to the role of this entity in determining clinically significant outcomes in ventilated patients (5-8). However, mechanical ventilation is clearly an indispensable tool for patients who require ventilator support; nonetheless, if we are to succeed in eliminating its iatrogenic consequences, we must understand the biomolecular and cellular effects of exposing the lung to the forces generated by mechanical ventilation. Previous studies of participation of inflammatory cytokines and gene expression in the course of VILI have provide some clues to pathophysiology (9-11), but have generally been limited to analysis of one or a few genes, and therefore have provided little information about the coordinated pattern of gene expression involved in this response.

Microarray is a modified Northern blot, where one analyzes the relative expression level of a gene by determining the amount of messenger RNA (mRNA) that is present. Unlike a conventional Northern blot where one can analyze one, two, or even 10 to 20 mRNAs, a microarray allows the simultaneous analysis of the expression levels of hundreds, thousands, or even tens of thousands of genes in a single experiment (12). This capability, coupled with sophisticated computer analysis tools (bioinformatics) will improve our understanding of pulmonary biology and pulmonary diseases. Experiments concerning global analysis of gene expression and cluster analysis of genes in various diseases have been reported recently (13).

To understand the molecular basis

for VILI, in more detail, we have set up a rat model of VILI. A murine microaaray genechip has also been developed in our laboratory. We analyzed changes in gene expression in response to VILI by using this oligonucleotide microarrays that permit a simultaneous and quantitative measurement of the expression of thousands of genes.

三、結果與討論

Analysis of the time course of induction (or suppression) of genes (more than 2 folds) associated with VILI by microarray revealed 132 genes. To further narrow down the genes to be induced or suppressed more than 3 folds, 21 genes were obtained. These 21 genes could be categorized into different groups of genes according to related gene functions:

(1) Cell growth and cell cycle regulator: eukaryotic translation initiation factor 2 alpha kinase 3, cycline-dependent kinase 4, inhibitor of DNA binding 3- domain negative helix-loop-helix protein, Transforming growth factor beta receptor II, eukaryotic translation initiation factor IV gamm3, neuropilin II, CD7 antigen (P41), cyclin-dependent kinase 2.

(2) Cytokine growth factor and inflammation mediator: prostagladin-endoperoxide synthase I, tachykinin, interleukin 19, metrix metalloproteinase 12, hepatocyte growth factor, insulin-like growth factor-binding protein 4, hepatocyte growth factor receptor, endothelin 1, CD 44 antigen.

(3) Proteinase and adhesion molecule: platelet/endothelial cell adhesion molecule, tissue inhibitor of metalloproteinase 1.

(4) Miscellaneous gene: dickkopf (Xenopus laevis) homolog 3, collagen type 1 alpha 1, CD36 antigen, thrombospondin 3, collagen type 4 alpha 3.

Most of the genes were upregulated by VILI, only genes such as insulin-like growth factor-binding protein 4, hepatocyte growth factor receptor, CD 7 antigen, endothelin1, cyclin-dependent kinase 2 were down regulated by VILI. Through this study, a comprehensive profile of the pulmonary response to VILI was generated with this arrays containing probe sets for approximately 6000 murine genes and expressed sequence tags (ESTs). Changes in gene expression were monitored after different time point & ventilator settings in VILI. Analysis of the time course of induction of these genes identifies sequential genetic programs that characterize VILI. Further studies focusing on the verification of the relationship of the specific genes on VILI should accelerate the development of more effective strategies for intervention at various stages in the development of VILI of the lung.

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