ORIGINAL ARTICLES

Lung–lung interaction in isolated perfused unilateral hyperventilated rat lungs

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The technique of conducting high tidal volume (TV) ventilation-induced lung inflammation including remote organs is still open to discussion, and our aim is to investigate this issue in isolated ventilated rat lungs perfused with salt solution. Selective right lung (RL) hyperventilation (TV of 15 mL/kg with air containing 5% CO_2 on zero or 2.5 cm H_20 end expiratory pressure (ZEEP or PEEP) in addition to left lung (LL) on 2.5 cm H_20 continuous positive airway pressure (CPAP) for 60 min, was realized after 30 min both lungs ventilation by occluding the left main bronchus, and it was allocated to the following 5 groups: groups 1 and 2 underwent hyperventilation under ZEEP, groups 3 and 4 underwent hyper ventilation under PEEP with recirculation or nonrecirculation (R-ZEEP or NR-ZEEP and R-PEEP or NR-PEEP), and group 5 served as the control group. Recirculation means the same perfusate recirculates the system throughout the procedure. The wet/dry ratio and protein content of bronchoalveolar lavage fluid (Prot-BALF), cytokine messenger RNAs (mRNAs), localization of tumor necrosis factor-α (TNF-α) by immunofluorescence double staining, and TNF-α concentration in the perfusate and BALF in each lung were measured and compared between groups by Kruskal-Wallis test. Lung injury (increased wet/dry ratio, Prot-BALF, and TNF-α on endothelial and epithelial cells) was shown in the hyperventilated RLs with ZEEP compared with their corresponding CPAP LLs. PEEP prevented these injuries. Lung injury was also demonstrated in the recirculated LL compared with the nonrecirculated LL (Prot-BALF, TNF-α and interleukin-1β (IL-1β) mRNAs: the LL of the R-ZEEP is greater than the LL of NR-ZEEP by $P < 0.01$). Unilateral hyperventilated lungs with ZEEP induced TNF-α, increased permeability, and injured the control lung via perfusion. (Translational Research 2010;155:228–237)

Abbreviations: BAL = bronchoalveolar lavage; BALF = BAL fluid; CPAP = continuous positive airway pressure; FITC = fluorescein-5-isothiocyanate; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IL-1β = interleukin-1β; LA = left atrium; LL = left lung; MODS = multiple organ dysfunction syndrome; mRNA = messenger RNA; NR-PEEP = nonrecirculation with PEEP; NR-ZEEP = nonrecirculation with PEEP; PA = pulmonary artery; PEEP = positive end-expiratory pressure;

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R-PEEP = recirculation with PEEP; RL = right lung; RT-PCR = reverse transcriptase polymerase chain reaction; R-ZEEP = recirculation with ZEEP; TNF-α = tumor necrosis factor-α; TV = tidal volume; VILI = ventilator induced lung injury; ZEEP = zero end expiratory pressure

AT A GLANCE COMMENTARY

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Background

High tidal volume ventilation results in ventilator-induced lung injury (VILI); however, the pathophysiology and the remote organ damage by VILI is still open to discussion. Our isolated perfused and preferentially ventilated lung model is unique because it contains an internal control that can be used as a typical remote organ distant from the hyperventilated lung.

Translational Significance

Our experimental results showed a conclusion about the possible involvement of inflammatory cytokines and their injuring effect on remote organ by VILI. Moreover, we found a harmful effect of sustained inflation of lung without respiratory movement. These findings will contribute to the improvement of respiratory management.

The high mortality rate of acute respiratory distress syndrome is caused not by exacerbation of respiratory failure but mainly by multiple organ dysfunction syndrome (MODS),[1] and the lower tidal volume (TV) ventilation decreases the mortality rate compared with the conventional TV ventilation.[2] This finding suggests that the conventional TV ventilation would sometimes cause MODS; in other words, it will induce lung injury and remote organ damage. Prior to clinical randomized studies, a few prime works[3-5] revealed that the high TV ventilation (hyperventilation) induces lung damage and remote organ damage. The causes of lung damage were suggested to be the activation of cytokine genes, and the induction of tumor necrosis factor-α (TNF-α) messenger RNA (mRNA) was verified in an isolated nonperfused hyperventilated lung model.[6] However, experiments by different investigators under the same conditions and using the same animal models showed the contradictory results.[7] Experimental and clinical works have suggested the involvement of inflammatory cytokines in lung injury caused by high TV ventilation; however, definite conclusions have not yet been reached.[8,9]

We have developed a unilateral lung injury model using isolated perfused ventilated rat lungs, in which 1 lung could be exposed to ischemia and/or hyperventilation, and simultaneously the other control lung could maintain normal perfusion and/or ventilation (ie, 1 lung can be used as an internal control and the other lung can be used as an experimental lung).[10-13] Such a model is suitable for exploring gene expression because any experimental burden would easily activate cytokine genes, and careful evaluation would be required to reach a conclusion. Also, using our model, one can use the control lung as a typical remote organ distant from the injured lung, and we have already proved that the ischemia reperfusion lung injures the control lung via circulation with TNF-α and superoxide anions liberated from the injured lung.[13,14] We are adopting this model to the unilateral hyperventilation model, in which 1 lung is exposed to hyperventilation (high TV ventilation) and the other lung is maintained at continuous positive airway pressure (CPAP) with both lungs perfused.

We hypothesize that unilateral hyperventilated lungs induce inflammatory cytokine mRNAs and liberate inflammatory cytokines, which injures the hyperventilated lungs as well as the control lung via perfusion.

METHODS

This experiment was approved by The Institutional Animal Use Committee of Tokyo Medical and Dental University.

Isolated perfused rat lung preparation. As the procedures have been reported previously,[10,11] only an outline is described here. The rats (male Sprague-Dawley rats weighing 261 $\pm$ 17 g, mean $\pm$ standard deviation [SD]) were mechanically ventilated at 70 breaths/min using a humidified gas (air containing 5% CO_2), and after cannulating to pulmonary artery (PA) and left atrium (LA), Krebs-Henseleit solution containing bovine serum albumin (low-endotoxin grade; Sigma Chemical Co., St. Louis, Mo) at 4 g/dL was perfused with a flow rate of 0.04 mL/g body weight per minute. The heart and lungs were freed and transferred to a humidified constant-temperature chamber; positive end-expiratory pressure (2.5 cm H_20 PEEP) was applied and TV was adjusted to 10 mL/kg. Pulmonary perfusion and LA pressures were measured at the main PA and at the tip of the LA cannula; airway pressure was measured at the orifice of tracheal cannula.

Selective ventilation and perfusion of each lung (Fig 1). The left and right main bronchi, as well as the PAs, were isolated from the surrounding tissues to enable a soft clip to pass around each of the main bronchi