行政院國家科學委員會專題研究計畫 成果報告

腎素-血管張力素系統在損害性機械通氣引發大鼠骨骼肌代謝異常所扮演之角色
研究成果報告(精簡版)

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專題研究計畫成果報告
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Acute respiratory failure is a common but significant clinical disorder that has been shown to cost a large amount of medical resources in the intensive care unit, and its mortality has remained substantial despite changes of medical concepts over the past decades and substantial progresses in the modern medical modalities, including mechanical ventilation. As an indispensable modality in managing patients suffering from respiratory failure nowadays, mechanical ventilation has been considered to carry potential risks to exert various deleterious effects on patients with respiratory failure if it is used in an inappropriate way. There have been many speculations and investigations to assess the adverse effects of mechanical ventilation, of which the most striking clinical evidence regards the acute respiratory distress syndrome (ARDS) in a recently published paper by the Acute Respiratory Distress Syndrome Network[1]. Similar to some other previous reports, this clinical trial showed that mechanical ventilation *per se* may be responsible for undesired mortality during the treatment for respiratory failure. A total of 861 patients with acute lung injury or ARDS were randomly assigned to receive ventilation with either a conventional tidal volume (12 ml/kg of predicted body weight) or a lower tidal volume (6 ml/kg) to evaluate their clinical outcomes. The study showed that patients in the lower tidal volume group had a mortality rate that was 22% lower than the conventional tidal volume group. This clinical finding strikingly demonstrated that mechanical ventilation can iatrogenically influence the outcome of critically ill patients treated with mechanical ventilation.

Substantial evidence have also shown that the mortality rate in patients with ARDS remains high with a value of more than 30% and, strikingly, most patients with ARDS die from multiple system organ failure (MSOF) rather than from extreme and refractory hypoxemia[2]. One hypothesis has been proposed and supported by several studies that the use of mechanical ventilation *per se* may be responsible not only for worsening the underlying ALI or ARDS, but may also, by a number of mechanisms, lead to the development of systemic inflammatory response syndrome (SIRS) and MSOF[3,4]. It is therefore believed that studies on the effect of mechanical forces and ventilation on the cells or organs are important for better management of patients requiring ventilatory treatment.

The mechanisms by which mechanical ventilation induces its deleterious effects on the extrapulmonary organs are still incompletely known. In an *in vitro* model, mechanical stretch can induce the release of cytokine IL-8 by alveolar epithelial cells[5]. A new mechanism of injury, “biotrauma”, has been postulated in which the mechanical stress produced by mechanical ventilation leads to up-regulation of pro- inflammatory response as evident by prominent neutrophil infiltrations in the lungs and increased bronchoalveolar lavage (BAL) levels of host inflammatory cells. Mechanical ventilation can lead to release of proinflammatory mediators probably through the following mechanisms[6]. First, stress failure of the plasma membrane with cellular necrosis may cause release of preformed mediators as well as evoke proinflammatory effect by cytosol released from damaged cells. Second, stress failure of the endothelia and epithelial barriers may cause loss of compartmentization and hemorrhage and accumulation of leukocytes in the lungs[7]. Third, overdistension without tissue destruction may occur, evoking mechanotransduction. Fourth, the increased vascular intraluminal pressure and increased shear stress may have effect vasculature independent from stretch and rupture. Previous studies have shown that injurious ventilatory strategies can increase cytokines and *c-fos* mRNA expression in an isolated rat lung model [8]. High tidal volume upregulates intrapulmonary cytokines in an *in vivo* mouse model of ventilator-induced lung injury[9]. There is also evidence that CXCR2 and CXCR2 ligands may play a critical role in the pathogenesis in VILI[10]. Ventilation-induced chemokines and cytokine release has been shown to be associated with activation of NF-κB in the lungs[11]. The increase of cytokines can lead to lung inflammation, and it has been shown that the neutrophil migration into the lung is dependent on stretch-indiced MIP-2 production, and can be blocked by 51% by anti-MIP-2 antibody[12]. Previous reports have also shown that mechanical ventilation can affect local and even systemic cytokine levels in ARDS [13]. In an *in vivo* rabbit model, mechanical ventilation can lead to end-organ apoptosis with biochemical evidence of end-organ dysfunction [14]. In and isolated perfused and ventilated lung model, hyperventilation can induce the release of proinflammatory cytokines, such as TNF-α and IL-6, into the circulation [15].

The possible association between injurious mechanical ventilation and muscle atrophy or altered muscle metabolism has never been reported, despite various studies have been focused on critical illness myopathy (CIM). Of the various complications occurring in critically ill patients receiving mechanical ventilation, myopathies have gained increasing interest during the past years. For the patients, they represent a crucial factor for prolonged intensive care unit treatment and secondary complications[16]. Among the neurological complications occurring in critically ill ICU patients artificially ventilated for more than two weeks, de novo critical illness polyneuropathy and critical illness myopathies are most common[17]. Since the first description of muscular weakness in such patients by Bolton et al in 1984[18], it has become apparent that these symptoms cannot be attributed to immobilization but rather to specific pathological conditions of either the peripheral nerves, the skeletal muscle, or both. Interestingly, the existence of manifest myopathies in these patients has only become apparent in the past few years. Nowadays, these neuromuscular pathologies are clinically well recognized complications of prolonged ICU stays and their prevalence is estimated to be as high as 80% by some authors, with no apparent correlation to underlying condition causing ICU admission[19-21].

Skeletal muscle atrophy is a change that occurs in muscles of adult animals as a result of the conditions of disuse (e.g., immobilization, denervation, muscle unloading), aging, starvation, and a number of diseases states (i.e., cachexia)[22]. Regardless of the inciting event, skeletal muscle atrophy is characterized by a decrease in protein content, fiber diameter, force production, and fatigue resistance[22]. The different types of conditions producing atrophy imply different types of molecular triggers and signaling pathways for muscle wasting. The decision to make or break skeletal muscle is mediated in large part by a network of signal transduction pathways that transmit their activities from extracellular to intracellular factors regulating transcription and post-transcriptional processes. In recent years, several signaling pathways have emerged as key maintenance regulators of muscle mass, including the physphatidylinositol-3-OH kinase (PI(3)K)/Akt pathway, the myostatin pathway and the nuclear factor-κB pathway[23]. The PI(3)K/Akt (protein kinase B) pathway is activated by IGF, the expression of which is induced during muscle differentiation and shown to be essential for myogenesis and maintenance of muscle mass[24]. Activation of PI(3)K recruits and phophrylates the serine.threonine kinase Akt, of which the function in skeletal muscle is essential for both myogenesis and proper maintenance of muscle mass. Investigators appear to be consistent with their thinking that NF-κB activation is relevant in muscle damage and wasting. In vitro differentiation cultures have clearly demonstrated that cytokine and reactive oxygen-induced muscle damage requires the activation of NF-κB[25-27]. *In vivo*, NF-κB activity has been shown to be elevated in skeletal muscles subjected to various forms of injury, including

ischemia/reperfusion[28], freezing[29], disuse[30], and mechanical stress of normal as well as dystrophin-deficient muscles[31].

Myostatin (previously called GDF-8) was originally identified in a screen for novel mammalian members of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors[32]. In adult tissues, myostatin is expressed almost exclusively in skeletal muscle, although clear detectable levels of myostatin RNA are also present in adipose tissue. Mice carrying a deletion of the portion of the gene encoding the C-terminal domain of myostatin were shown to have dramatic and widespread increases in skeletal mass, with individual muscles weighing about twice as much as those of wild-type mice. Analysis of the sections prepared from muscles of homozygous mutant mice showed that these increases in muscle mass result from a combination of increased number of muscle fibers (hyperplasia) and increased fiber size (hypertrophy). Significantly, heterozygous mice are also affected, albeit to a lesser degree; muscles of heterozygous mice weigh approximately 25% more than those of wild-type mice, suggesting that the effect of myostatin is dose dependent. The phenotype of mice lacking myostatin suggested that myostatin normally functions as a negative regulator of muscle growth, and it was on this basis that myostatin was given its name[32]. Systemic administration of myostatin leads to muscle wasting in mice[33], and treatment of cultures muscle cells with recombinant myostatin has resulted in loss of protein and reduced protein synthesis rates[34]. Moreover, myostatin expression is increased in some types of muscle atrophy[35]. Human immunodeficiency virus-infected men have shown higher levels of serum myostatin[36].

The association between inflammatory process and muscle mass regulation has been reported in several studies. In conditions such as pneumonia, the expression of myostatin has been shown to be increased[37], with decreased protein accretion. Proinflammatory cytokines directly regulate the expression of muscle regulatory factor MyoD[25]. MyoD plays a role in skeletal muscle regeneration[38], and decreased expression of MyoD in response to pathogen exposure would likely lead to decreased ability to synthesize new muscle proteins.

The renin-angiotensin system (RAS) has been suggested to play an important role in the development as well as the progression and evolution of the acute lung injury [39-41]. In our previous studies[41] (also: Jerng JS et al. Submitted), we found that the renin-angiotensin system is actively involved in the development of ventilator-induced lung injury (Figure 1 and 2). Angiotensin II, the major effector peptide of RAS, is now recognized as a growth factor that regulates cell growth and fibrosis, besides being a physiological mediator restoring circulatory integrity. Recent data also support the hypothesis that RAS is key mediator of inflammation [42]. Angiotensin II increases vascular permeability that initiates the inflammatory process. Angiotensin II can also contribute to the recruitment of inflammatory cells into the tissue through the regulation of adhesion molecules and cytokines by resident cells [43]. Moreover, angiotensin II can directly activate infiltrating immunocompetent cells, including chemotaxis, differentiation and proliferation [42]. Recent data also suggest that RAS activation could play a certain role even in immunologically induced inflammation [43]. Transcriptional regulation, predominantly via the nuclear factor-κB and AP-1 activation [44], and second mediator systems, such as endothelin-1, the small G protein (Rho) an redox-pathways are shown to be involved in the molecular mechanism by which angiotensin II exerts those functions. Systemic infusion of angiotensin II into normal rats may activate NF-κB[44]. It was shown that angiotensin II activates NF-κB through both the AT1 and AT2 receptor pathways[45]. Angiotensin II also participates in tissue repair and remodeling, through the regulation of cell growth and matrix synthesis.

Some studies have also associated the renin-angiotensin system to muscle metabolism. Angiotensin II has been shown to directly induce protein degradation in skeletal muscle through an increased activity and expression of ubiquitin-proteasome proteolysic pathway[46], and activation of NF-κB has been shown to be responsible for the increased protein degradation[46]. Angiotensin II has also been shown to induce skeletal muscle wasting through enhanced protein degradation and down-regulate autocrine IGF-1[47]. Angiotensin II and VEGF are shown to be involved in angiogenesis induced by short-term exercise training[48]. In humans, it has been shown that the circulating angiotensin converting enzyme activity is correlated with muscle strength, supporting the role of the RAS in the regulation of human skeletal muscle strength[49]. Angiotensin II has also been shown to be required for optimal overload-induced skeletal muscle hypertrophy, acting at least via the AT1 receptor-dependent pathway[50]. In patients with congestive heart failure, the AT1 receptor for angiotensin II is shown to be expressed in the skeletal muscles, but not the AT2 receptor[51].

We therefore hypothesize that the renin-angiotensin system, in addition to its active role in ventilator-induced lung injury, is actively involved in the development of ventilator-induced alteration of muscle metabolism and possible resultant muscle atrophy. Treatment or prophylactic use of agents that block the action or reduce the production of angiotensin II may attenuate the muscle atrophy induced by injurious mechanical ventilation.

研究目的(Specific Aims):

In this proposed study, we wish to investigate the following hypotheses:

(1) Injurious mechanical ventilation can induce a systemic inflammatory response, which may be indicated by increased circulating levels of proinflammatory cytokines as well as organ-specific evidence of local inflammatory responses.

(2) Injurious ventilation, mainly high-volume ventilation, can induce an altered expression of myostatin and other related factors, and regulate muscle atrophy.

(3) Injurious mechanical ventilation can activate the renin-angiotensin system (RAS) in skeletal muscles of the rats.

(4) Ventilator-induced myostatin expression is associated with an increased systemic activity of the RAS, and blocking the production of angiotensin II can attenuate the expression of myostatin in skeletal muscle.

Materials & Methods

Reagents

Dexamethasone, captopril, PD123319, lipopolysaccharide from Escherichia coli (serotype 055:b5) and all other chemicals, unless indicated, will be purchased from Sigma-Aldrich (St. Louis, MO). Losartan, a specific antagonist of type 1 angiotensin receptor (AT1) for angiotensin II, is a gift from Merck (Merck & Co., Inc., Whitehouse Station, NJ). PD123319, a specific antagonist of type 2 angiotensin receptor (AT2) for angiotensin II, will be purchased from Sigma. Antibodies to rat tumor-necrosis factor-α, type 1 and type 2 angiotensin II receptors, nuclear factor-κB p65, myogenin and MyoD will be purchased from Santa Cruz (Santa Cruz, CA). Antibodies to rat I-κB and phosphorylated I-κB will be purchased from Cell Signaling (Beverly, MA). Antibody to myostatin will be perchased from R&D system (#AF788).

Animal Preparation

Male Sprague-Dawley rats weighing 200-250 g will be cared, handled and maintained in the animal resource facility of the National Taiwan University College of Medicine in accordance with the Institutional Guidelines. The source of the animals is the experimental animal center of the Academia Sinica. The rats will

be fed with rat chow and water ad libitum and housed in standard care facilities for ten days before being used for experiments. All animal experiments will be performed according to the guidelines of the institution at a specialized lab for small animals in the experimental animal center of National Taiwan University College of Medicine.

Generally, the animals will be randomly assigned to ventilated groups and non-ventilated groups, with details described below. For the animals assigned to the MV groups, they will be generally anesthetized by intraperitoneal injection of urethane (1.3 g/kg) shortly before being applied with mechanical ventilation. Tracheostomy will then be performed, followed by arterial and venous catheterizations. The tracheostomy tube will then be connected to a volume-controlled ventilator for small animals (New England Medical Instruments, Inc., Medway, MA) and ventilated according to the study design protocol and the general descriptions of the manufacturer.

Mechanical Ventilation Protocol

The animals will be randomly assigned to the following three experimental groups: 1) non-ventilated control group; 2) mechanical ventilation with high tidal volumes (40 ml/kg tidal volume, zero PEEP, 20 breaths/min), with room air; 3) mechanical ventilation with low tidal volumes (7 ml/kg tidal volume, zero PEEP, 100 breaths/min), with room air. For the animals of the control group, only urethane will be injected intraperitoneally, and will be observed closely for physiologic changes for 4 hours. For the low-tidal volume and high-tidal volume treatment groups, mechanical ventilation will be applied for 4 hours for each animal, and the peak airway pressure, blood pressure and signs of spontaneous respiration will be monitored throughout the course of mechanical ventilation. Blood samples, 0.1 ml each time, will be obtained from the left femoral artery via the cannula immediately before starting ventilation and every 60 minutes after starting ventilation. Arterial blood gas analysis will be performed on site immediately after blood sampling using a portable analyzer (i-STAT, Abbott Laboratory, Abbott Park, IL, USA) performed according to the manufacturer's description. Intravenous infusion of normal saline will be used to maintain a MAP of no less than 70 mmHg, while during the experiments, normal saline, 0.5 ml/hr, will be infused via the femoral vein to prevent dehydration. After four hours of ventilation, animals will be given a lethal dose (150 mg/kg) of intravenous pentobarbital for euthanasia. The lungs, liver, diaphragm and gastrocnemius muscle will be then removed en block after the pulmonary vessels are flushed with intracardiac injection of normal saline. The tissues will be frozen in liquid nitrogen immediately after removal and stored at -80˚C for further analysis, while some will be fixed with preservative agent and stored at room temperature for histologic studies. Another group of non-ventilated rats will receive E. coli LPS (Sigma Chemical, St. Louis, MO) instilled transtracheally at a dose of 0.2 mg/100 g of body weight dissolved in 0.5 mL of phosphate- buffered saline. The LPS solution will be dispersed in the trachea just above the level of tracheal bifurcation while the animals are breathing spontaneously.

To study the effect of different levels of positive end-expiratory pressure (PEEP), the rats will also be assigned to receive different PEEP levels (0, 8 and 15 cmH_2O, respectively), set on the ventilator as instructed by the manufacturer. The other settings will be the same as above described.

Treatment with Dexamethasone, Captopril, Losartan or PD123319

To study the effect of simultaneous treatment or prophylactic medications for blocking the action of angiotensin II on the prevention of ventilator-induced muscle atrophy, additional groups of rats will be randomly assigned to receive captopril before receiving mechanical ventilation, or to be treated with either losartan or PD123319 during mechanical ventilation. For animals to receive captopril, 500 mg/l of captopril will be added in the drinking water since for three days before the initiation of mechanical ventilation. For the losartan- or PD123319-treated groups, either losartan (10 mg/kg) or PD123319 (10 mg/kg) will be intravenously administered via a pump simultaneously during the 4-hour course of mechanical ventilation. Dexmethasone, a strong anti-inflammatory glucocorticoids agent, at different dosage of 1, 5, 20 and 100 μg/100g BW, will be administered by intraperitoneal injection for other groups 24 hours prior to being submitted to mechanical ventilation or LPS instillation, in addition to control groups for observation. All pre-medicated animals will be subjected to experiments according to the protocol described above for different ventilatory strategies, or simple observation.

Tissue RNA Extraction

Total cellular RNA from rat muscles obtained four hours after treatment will be isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. Briefly, the tissue will be homogenized with liquid nitrogen, and 1 ml of TRIzol was added. One mililiter of the mixture will be obtained and 200 μl of chloroform will be added. The mixture will be centrifuged 13,000 X g at 4°C for 15 min. The supernatant will be obtained and mixed with 0.5 ml isopropanol, and then centrifuged at 13,000 X g at 4°C for 15 min. The supernatant will be discarded and the sample will be washed with 1 ml of 75% ethanol. The ethanol then will be discarded and the pellet will be dissolved in 20 μl of DEPC-H_2O. The purity and integrity of the RNA samples will be assessed by OD_{260}/OD_{280} spectrophotometric measurements and by agarose gel (1% agarose-formaldehyde gel containing 20 mM morpholinosulfonic acid, 5 mM sodium acetate, and 1 mM EDTA, pH 7.0). According to the literature and experiences, the mRNA amount of myostatin may be low in the rat tissues under current experimental design; therefore we will apply further purification procedures of mRNA from total RNA to obtain high mRNA concentration. The Qiagen mRNA purification kit will be used (Qiagen), and the procedure will be performed according to the instructions by the manufacturer.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR)

One microgram RNA will be subjected to first-strand cDNA synthesis in a 20-μl reaction mixture containing avian myeloblastosis virus reverse transcriptase (10 U), 2 μl of dNTP mixture (2.5 mM concentrations of each dNTP), 0.5 μl of RNAase inhibitor, 1 μl of oligo(dT)12–18 primers (10 μM), and reaction buffer as supplied with the enzyme (50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM spermidine, and 10 mM DDT). The samples will be incubated in a MJ Research PTC-200 thermal cycler (MJ Research) at 42°C for 60 min followed by enzyme denaturation step at 94°C for 2 min. The reverse transcription mixture will be stored at -20°C for use in PCR. All reagents will be obtained from Promega (Southampton, U.K.).

Four microlitters of reverse transcribed products will be used as the template for RT-PCR. The PCR amplification will be performed using rTaq DNA polymerase (Promega) under the following conditions: an initial denaturation at 95°C for 5 min, amplification will be conducted through 30 cycles, with the denaturation for 30s, annealing for 30 s and extension 45 s, final extension is at 72°C for 7 min. Gene-specific oligonucleotide primers of myostatin, myogenin, MyoD, Myf-5 and GAPDH are list in table 1 as described previously[56,57] except for myostatin, which are designed by the authors, with cycling temperature described thereafter. The PCR products will be electrophoresed by 2% agarose gel and stained with ethidium bromide. Bands of each target transcript will be visualized by ultraviolet transillumination and captured using a digital camera. ODs for each band will be quantified by image analysis software AlphaImager System (Alpha Innotech Corporation). The levels of gene expression of each transcript will be normalized to that of the housekeeping gene GAPDH.

Extraction of Cytosolic and Nuclear Protein from Rat Lung Tissue

The rat tissues will be homogenized and washed by cold phosphate buffer saline. For extraction of cytosolic and nuclear proteins, 200 µl 0f cold buffer A (10mM HEPES-KOH, 1.5mM $MgCl_2$, 10mM KCl) containing protease inhibitor 10 µl/l buffer (Sigma Protease Inhibitor, Sigma-Aldrich), will be added and placed on ice for 10 minutes. The mixture will be centrifuged at 8000 X g for 1 minute at 4°C. The supernatant will be collected as cytosolic protein, and the remnant will be resuspended with cold Buffer C (20 mM HEPES, 1.5 mM MgCl2, 420 mM NaCl, 0.2 mM EDTA, and 25% glycerol), and placed on ice for 10, then centrifuged at 13000 X g for 2 minutes at 4°C. The supernatant will be collected as nuclear protein.

Western blotting for proinflammatory cytokines, RAS components, myostatin, MyoD and myogenin in the skeletal muscles

The presence and relative abundance of the pro-inflammatory cytokines, the RAS components, myostatin, MyoD and myogenin will be determined using Western blotting. After the skeletal muscles are removed, they will be homogenized in lysis buffer (50 µM Tris-HCl, 3 mM sucrose, 0.1% Triton X-100, and 1mM protease inhibitor cocktail; Calbiochem-Novabiochem, La Jolla, CA, USA). The supernatant will be removed, and protein content will be estimated using a Micro BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA). Aliquots from muscle homogenates will be diluted in reducing sample buffer (0.5 M Tris-Cl, 2% β-ercaptoethanol, 87% glycerol, 10% SDS, and 1% bromophenol blue). Protein (40 (g/well) will be loaded in 6% polyacrylamide gels. Proteins will be separated by electrophoresis, transferred to nitrocellulose membranes (Bio-Rad, Mississauga, Ontario, Canada), and then blocked for nonspecific binding in a 7% skimmed milk solution. Membranes will be incubated with primary rabbit antibodies for 2 hours at a 1:500 dilution. Membranes will be washed and incubated for 1 hour with peroxidase-conjugated goat anti-rabbit IgG (Jackson ImunoResearch, Bio/Can Scientific, Mississauga, Ontario, Canada). After repeated washing, membranes will be incubated with enhanced chemiluminescence reagent (Amersham Pharmacia Biotech) and placed in a Fluor-X Max Imager, where the image will be captured, and bands will be analyzed by densitometric analysis. Results on all samples will be normalized to control samples as well as the (-actin densities.

Western blot analysis for NF-κB and I-κB of the rat skeletal muscles

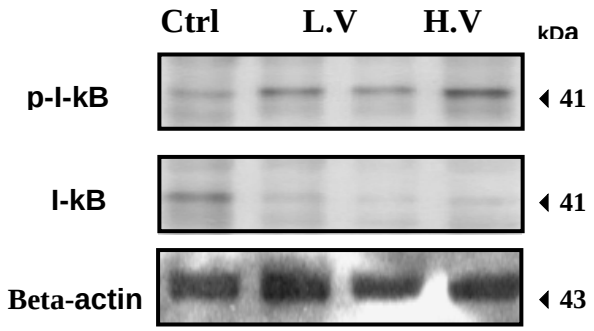
For analysis of the p65 component of NF-(B and I-(B, 20 (g of cytosolic or nuclear protein will be electrophoresed on 10 % SDS-PAGE gel and transferred onto a polyvinylidine difluoride (PVDF) membrane (Amersham, Arlington Heights, IL). Membranes will be blocked for I h in 5 % non-fat dried milk in TBST (0.24 % (w/v) Tris, 0.8 % (w/v) NaCl, 0.05 % (v/v) Tween 20, pH 7.6) and incubated with either monoclonal anti-rat NF-κB p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or monoclonal anti-rat I-κB and anti-phosphorylated I-κB antibodies (Cell Signaling, Beverly, MA) for 1 h. After washing three times in TBST, the blots will be incubated with a peroxidase-conjugated-goat anti-(rabbit IgG) for 1 h and washed extensively three times in TBST for 10 min. Immunocomplexes will be detected with the ECL reagent (Amersham). Analysis of the immunoreactive bands will be performed with AlphaImager 2200 System (Alpha Innotech Corporation).

Emzyme-linked Immunosorbent Assay (ELISA) for TNF-α and MIP-2

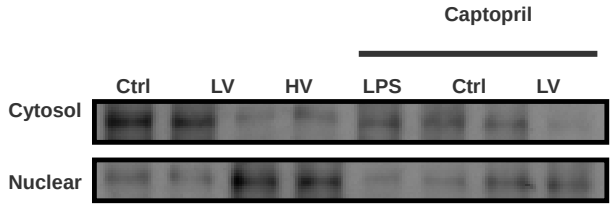
For the tissue TNF-α or MIP-2 assays, 0.5 g of frozen rat lung will be added to 2 ml of cold-acid-ethanol (93% ethanol, 2% concentrated HCl). 85 µg/ml phenylmethylsulfonyl fluoride, and 5 µg/ml pepstatin A, and homogenized 1min with a polytron homogenizer. The samples will be extracted overnight. TNF-α or MIP-2 will be then measured using a rat TNF-α or MIP-2 ELISA kit (Biosource International, Camarillo, CA). Each sample will be run in duplicate according to the manufacturer's instructions. For determination of serum TNF-α or MIP-2 levels, the same procedures will be performed by using the serum as samples, according to the manufacturer's instructions.

Results

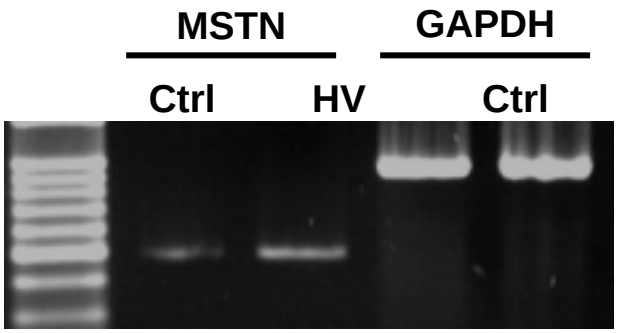
Previously we have shown that mechanical ventilation can induce a proinflammatory response in the rat lung. In the figure below we show that mechanical ventilation with high tidal volume can result in increased level of phosphorylated I-kappaB in the rat lungs.



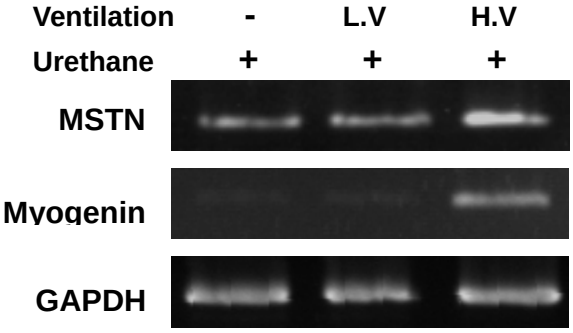
The cytosolic portion of nuclear factor (NF)-kappaB was decreased in the high-volume ventilation group, while there was a concomitant increase of nuclear portion of NF-kappaB, as shown in the figure below. Pretreatment with captopril partially attenuated the response.



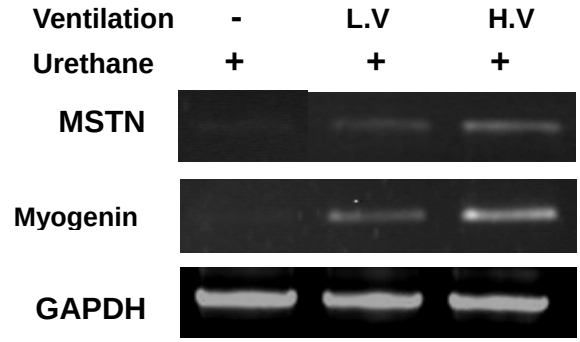
We then assessed the response of rat diaphragmatic muscles to mechanical ventilation with high tidal volume. In the figure below we show that mechanical ventilation increases the expression of myostatin in the rat diaphragm muscle.



To eliminate the influence of muscle relaxant, we used urethane instead for general anesthesia. The figure below shows that high-volume mechanical ventilation increased the expression of myostatin (MSTN) as well as myogenin in the rat diaphragmatic muscles.



High-volume mechanical ventilation also increases the expression of myostatin and myogenin in the rat skeletal muscles, as shown in the figure below.



Discussion

We found that injurious mechanical ventilation influences the expression of genes involving in the metabolism of skeletal muscles of the rat. Since myostatin in a major gene that regulate the muscle mass of animals, our findings might suggest that injurious mechanical ventilation affects the muscle metabolism, which probably will result in muscle wasting of the animals.

Muscle wasting is a prominent feature of patients undergoing prolonged mechanical ventilation. In patients with COPD, systemic muscle wasting is also a prominent feature, which might result in debilitation of the patient, and might contribute to limited life expectancy. In patients with acute respiratory failure, the post-ICU prognosis remained poor, even many patients have been weaned from the ventilators. Mechanical ventilation might result in substantial morbidity, and probably some related to muscle wasting, therefore the relationship between injurious mechanical ventilation and muscle wasting warrants further intensive investigation.

Alveolar overdistension is a miroscopic phenomenon that cannot be excluded by clinical observation only, therefore even using normal settings of MV, there still are possibilities that ventilator-induced lung injury may develop as a result of overdistension of some alveoli. In the preliminary results of our study, injurious mechanical ventilation may result in overexpression of myostatin in the skeletal muscles of rats, suggesting that muscle metabolism might be rapidly affected if the settings of mechanical ventilation becomes injurious, mainly high tidal volume.

The exact mechanism how injurious mechanical ventilation affects muscle metabolism remains unclear. We wish to further investigate the underlying mechanism for this abnormality.

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Table 1. Oligonucleotide sequences used as primers and probes for real-time RT-PCR

Primers	Sequences (5’ to 3’)	Product length	Temperatures (°C)
Myostatin	AGTGGGAAGCACATCAAAAGTT CATTATTTCGTCCAATCCTGGT	494	94-56-72
GAPDH	TGAAGGTCGGTGTCAACGGATTTGGC CATGTAGGCCATGAGGTCCACCAC	983	94-56-72
Myogenin	GACCTGATGGAGCTGTAT AGACAATCTCAGTTGGGC	688	94-60-72
Myf-5	GAGCCAAGAGTAGCAGCCTTCG GTTCTTTCGGGACCAGACAGGG	441	94-60-72
MyoD	TGGCGCGCTGCCTTCTACG ACACGGCCGCACTCTTCCCTG	221	94-60-72