The effects of enalapril and losartan on mechanical ventilation–induced sympathoadrenal activation and oxidative stress in rats

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ABSTRACT

Background: Mechanical ventilation (MV) is a method of maintaining appropriate gas exchange in patients who are unable to sustain adequate alveolar ventilation. While life-saving in the short-term, prolonged MV leads to altered cardiovascular responses and enhanced lung injury, but the exact mechanism is unknown. Therefore, we investigated the involvement of the sympathoadrenergic and renin–angiotensin system in MV-induced altered cardiovascular responses.

Methods: Sprague–Dawley rats were divided into six groups: (1) spontaneous breathing (SB); (2) SB + enalapril (100 μg/kg intravenous infusion); (3) SB + losartan (100 μg/kg infusion); (4) 12 h of MV; (5) MV + enalapril; and (6) MV + losartan. After the animals were sacrificed, blood and tissue samples were collected. Tyrosine hydroxylase, dopamine beta hydroxylase, and neuropeptide Y were measured in adrenal medulla and hypothalamus, whereas AT1 was measured in lung tissues by Western blot. Norepinephrine enzyme-linked immunosorbent assay and total antioxidant capacity were assayed in plasma.

Results: Our findings indicated that MV increases the sympathetic activation markers in adrenal medulla and hypothalamus. Moreover, oxidative stress was increased in lung and brain tissues. Treatment with enalapril or losartan reduced the lipid peroxidation in lung and brain tissues, while preserving the tissue glutathione content and plasma antioxidant capacity.

Conclusions: These data demonstrate that the inhibition of the renin–angiotensin system by enalapril or losartan may reduce the MV-induced increase in sympathetic activity markers and oxidative stress, and thus, may have a beneficial effect as adjuvant therapy.

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1. Introduction

Mechanical ventilation (MV) is a method to mechanically assist or replace spontaneous breathing (SB). Although it is often a lifesaving intervention in critically ill patients, it carries many potential complications including pneumothorax, acute lung injury, and ventilator-associated pneumonia. Other complications include diaphragm atrophy, decreased cardiac output, and oxygen toxicity [1]. The inflammatory response in the lungs due to MV may lead to distal organ dysfunction.

Recent research indicates a cross talk between lungs and other organs, including brain [2]. The hypothalamic sympathoadrenal medullary axis leads to marked activation of the adrenal medulla and sympathetic ganglia characterized by elevated activity of the catecholamine biosynthesizing enzymes such as tyrosine hydroxylase (TH) and dopamine beta hydroxylase (DBH), resulting in a rise in circulating epinephrine and norepinephrine (NE). TH is the rate-limiting step in catecholamine biosynthesis as it catalyzes the hydroxylation of tyrosine to dopamine, whereas DBH catalyzes the conversion of dopamine to NE. In addition to catecholamines, neuropeptide Y (NPY) is synthesized in the adrenal medulla and is co-released with epinephrine and NE. The previously mentioned factors, TH, DBH, and NPY are considered as biomarkers of sympathetic nervous system activity [3].

Sympathoactivation contributes to systemic stress and cardiovascular complications. The patients who have prolonged MV display blood pressure alterations and abnormal autonomic responses [4], which may be because of MV-associated activation of the hypothalamic-pituitary-adrenal axis or hypothalamic sympathoadrenal medullary axis. Whether MV activates these axes is unknown.

The present study was aimed to test the hypothesis that a 12 h exposure to MV results in increased activation of the hypothalamic sympathoadrenal medullary axis besides increasing oxidative stress in plasma and other organs. Thus, we measured TH, DBH, and NPY protein expression in the adrenal medulla and hypothalamus, lung angiotensin II type 1 (AT1) receptor protein levels, oxidative stress in lung and brain, and plasma NE. In addition, the effects of enalapril-atenolol converting enzyme inhibitor (ACE) and losartan-AT1 receptor blocker on MV-induced changes were evaluated.

2. Materials and methods

2.1. Animals

Adult female Sprague–Dawley rats were obtained from Charles River Labs and were aged 4–6 mo and ~ 300 g at the time of sacrifice. All animals were housed at the University of Florida Animal Care Services Center according to the guidelines set forth by the Institutional Animal Care and Use Committee. Animals were maintained on a 12-h light–dark cycle and provided food (AIN93 diet) and water ad libitum throughout the experimental protocol. Animals were divided into six groups (10 animals/group) as follows: (1) 12 h of SB; (2) SB group treated with the ACE inhibitor enalapril; (3) SB group treated with the AT1 antagonist losartan; (4) 12 h of MV; (5) 12 h of MV with the ACE inhibitor enalapril; and (6) 12 h of MV with the AT1 antagonist losartan.

Losartan group received an intraperitoneal priming dose (30 mg/kg) followed by intravenous infusion (100 μg/kg/min, infusion rate 0.30 mL/h), and enalapril group received enalapril (40 mg/kg) followed by an intravenous infusion (100 μg/kg/min, infusion rate 0.30 mL/h) while during the 12 h experimental period.

2.2. Experimental protocol

Animals were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally). After reaching a surgical plane of anesthesia, tracheostomy was performed on the animals using aseptic techniques and mechanically ventilated with a controlled pressure-driven ventilator (Servoventilator 300; Siemens; Bridgewater, NJ) for 12 h with the following settings: upper airway pressure limit, 20 cm H2O; pressure control level above positive end-expiratory pressure 4–6 cm H2O; respiratory rate, 80 beats/min; and positive end-expiratory pressure 1.0 cm H2O. In general, we estimate that these ventilator settings result in a tidal volume of ~1 mL/100 g of body weight. All surgical procedures were performed as previously described in detail [15]. Briefly, cannulas were inserted into the carotid artery to permit continuous measurement of blood pressure and the collection of periodic arterial blood samples during MV. Blood samples were analyzed for pH, pO2, and pCO2 using an electronic blood gas analyzer (GEM Premier 3000; Instrumentation Laboratory; Lexington, MA). If necessary, adjustments were made to the ventilator to ensure that the arterial blood gas and pH measures were within the desired physiological ranges. PaO2 was maintained at 70 mm Hg throughout the experiment by adjustments in FiO2 (22%–25% oxygen). Cannulas were inserted into the jugular vein for the constant infusion of sodium pentobarbital (~10 mg/kg/h). Body temperature was maintained between 36° C and 37° C by using a recirculating heating blanket. Continuous care during the MV protocol included lubricating the eyes, expressing the bladder, removing airway mucus, rotating the animal, and passive limb movement. After 12 h of MV, the animals were immediately killed, and blood and tissue samples were collected.

2.3. Enzyme-linked immunosorbent assay measurements of plasma NE

Blood samples were taken via cardiac puncture at the time of sacrifice and centrifuged at 5000 rpm for 10 min at 4° C. The samples were stored at −80° C until further analysis by an enzyme-linked immunosorbent assay kit (Rocky Mountain Diagnostics, Inc Colorado Springs, CO) following the instructions of the manufacturer.

2.4. Total antioxidant capacity in the plasma

Total antioxidant capacity in the plasma was evaluated by Oxiselect kit (Biocell Laboratories, San Diego, CA) according to the manufacturer’s instructions.
Plasma total antioxidant capacity (tAOC) expressed as copper reducing equivalent (CRE) and NE concentrations in SB, enalapril, losartan, and MV groups at 12 h. Each group consists of six to eight rats. *P < 0.05 versus SB and +P < 0.05 versus MV. Values are presented as mean ± standard error of mean.

<table>
<thead>
<tr>
<th>Plasma concentration</th>
<th>SB</th>
<th>SBE</th>
<th>SBL</th>
<th>MV</th>
<th>MVE</th>
<th>MVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>tAOC (CRE mmol/mL)</td>
<td>218.10 ± 12.8</td>
<td>225.60 ± 19.8</td>
<td>193.00 ± 12.4</td>
<td>125.90 ± 18.6</td>
<td>196.90 ± 21.5</td>
<td></td>
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<tr>
<td>NE (ng/mL)</td>
<td>2.32 ± 0.9</td>
<td>2.34 ± 0.6</td>
<td>2.48 ± 0.9</td>
<td>1.86 ± 0.9</td>
<td>2.95 ± 0.9</td>
<td>3.00 ± 1.1</td>
</tr>
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</table>

MVE = MV with the ACE inhibitor enalapril; MVL = MV with the AT1 antagonist losartan; SBE = SB group treated with the ACE inhibitor enalapril; SBL = SB group treated with the AT1 antagonist losartan.

2.5. Tissue preparation

After completion of the experimental protocol, animals were overanesthetized with pentobarbital (120 mg/kg intraperitoneally) and the adrenal medulla, hypothalamus, and lung rapidly removed, immediately frozen in liquid nitrogen and stored at -80°C until subsequent analyses. Before homogenization, adrenals were decapsulated and the medullae were separated from the cortex.

2.6. Western blot analysis

Adrenal medulla, hypothalamus, and lung were homogenized and assayed for protein levels of the catecholamine biosynthetic enzymes, TH and DjH, along with NPY using antibodies directed to TH (1/8000; Pel-Freez Biologicals, Rogers, AR), DjH (1/2000; Novus Biologicals, Littleton, CO), NPY (1/500; Santa Cruz Biotechnology, Santa Cruz, CA), and AT1(1/2000; Abcam, Cambridge, MA). All secondary antibodies were used in a concentration of 1/2000. An equal amount of protein for each adrenal medulla homogenate (2 µg protein for TH, 10 µg for DjH, and 35 µg for NPY), hypothalamus homogenate (4 µg protein for TH, 0.2 µg protein for DjH, and 40 µg for NPY), and lung (40 µg protein for AT1) was applied to the gels.

2.7. Malondialdehyde (MDA) and glutathione (GSH) assays

Tissue samples were homogenized with potassium phosphate buffer (pH 6.0) and ice-cold trichloroacetic acid (1 g tissue plus 10 mL 10% trichloroacetic acid) in an ultrasonic tissue homogenizer. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously [5]. Lipid peroxidation is expressed in terms of MDA equivalents using an extinction coefficient of 1.56 × 10^5 /M/cm and the results are expressed as nmol MDA/g tissue.

GSH measurements were performed using a modification of the Ellman procedure [6]. Briefly, after centrifugation at 2000g for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/L Na2HPO4 2H2O solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate) was added, and the absorbance at 412 nm was measured immediately after mixing. The results are expressed in µmol GSH/g tissue.

2.8. Statistics

Data are presented as mean ± standard error of mean. Comparisons between groups were done by analysis of variance followed by Tukey multiple comparison test or Student t-test using a GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Significance was established at P < 0.05.

3. Results

MV caused a decrease in the total antioxidant capacity of the plasma in MV group. Enalapril and losartan treatments significantly restored the plasma antioxidant capacity (Table).

Moreover, the plasma NE levels had a tendency to decrease in the MV group; however, this decrease was not significant because of the large variation between samples. Treatment with enalapril or losartan prevented this decrease (Table).

MV caused an increase in sympathetic activation markers, that is, TH, DjH, and NPY in adrenal medulla, therapeutic intervention with either enalapril or losartan reversed these MV-induced changes (Fig. 1). In contrast, TH remained unchanged in hypothalamus, whereas DBH and NPY were elevated with MV. In the hypothalamus, enalapril failed to inhibit the increases in DjH and NPY while losartan had efficacy (Fig. 1).

AT1 protein was assessed in the lungs. AT1 receptor protein was elevated with MV, and although there was no change with drug treatment in the absence of MV, enalapril and losartan reversed the MV-induced increase (Fig. 2).

MV caused an increase in both lung and brain tissues lipid peroxidation as evidenced by the increase in the malondialdehyde content, that is, thiobarbituric acid reactive substance. Also there was a concomitant decrease in GSH content in these tissues. Treatment with either enalapril or losartan prevented the decrease in GSH and reduced the lipid peroxidation (Fig. 3).

4. Discussion

MV not only causes an injury in the lung, but also may lead to oxidative stress and injury in distant organs [7]. It is known that there are a multiple pathways enabling cross talk between lung and other organs including the brain. An insult to the lung may alter physiological factors and may lead to an imbalance in the brain. Because the integrity of the brain function mainly depends on O2 and glucose, a peripheral homeostatic imbalance likely triggers an inflammatory response through cytokines and other mediators [2,8].

Previous reports have shown that MV increases the oxidative stress and causes ventilation-induced acute lung injury and secondary injuries in other organs such as...
diaphragm, heart, or kidney [7,9,10]. It has been also shown that MV leads to a decrease in tissue GSH levels [11]. In the present study, we have consistently observed that MV caused a decrease in GSH levels in lung and brain, and a concomitant increase in lipid peroxidation as evidenced by the increase in the malondialdehyde content, a thiobarbituric acid reactive substance. Moreover, plasma antioxidant capacity was decreased probably because of the extensive oxidative stress in the body. GSH is one of the principle endogenous antioxidants in all tissues, and the oxidative injury is a consequence of GSH depletion [12]. Therefore, antioxidants and GSH analogs have been widely studied to prevent or reduce oxidative injury in various models.

In the present study, both losartan and enalapril were effective in reducing lipid peroxidation and partially in preserving the GSH content in lung. On the other hand, in the brain, losartan demonstrated similar results, but not enalapril. Although both losartan and enalapril do not have a sulphydryl moiety have previously been shown to exert antioxidant effects in various studies [13,14]. Therefore, our finding that losartan and enalapril reduce oxidative stress and increase antioxidant capacity was consistent with these previous reports [15]. However, the failure of enalapril to protect brain tissue could be a result of its inability to cross the blood brain barrier. For instance, it has been claimed that losartan effects are limited to the periphery except for the cases with brain injury [16]. On the other hand, there are several studies that support the central effects of losartan. Kucuk et al. [17] showed that losartan treatment attenuated hypertension-induced blood–brain barrier permeability. Moreover, functional evidence has been shown that systemic administration of losartan crosses blood–brain barrier in the rat and attenuates the pressor response to electrical stimulation of subfornical organ [18].

Fig. 1 – Western blot analysis of the protein levels of TH, DßH, and NPY in the adrenal medulla and hypothalamus tissues of the saline or enalapril (E) or losartan (L)-treated SB or MV groups. Each group consists of n = 6–8 rats. *: P < 0.05 versus SB and +: P < 0.05 versus MV.
MV caused an increase in sympathetic activation markers, that is, TH, DβH, and NPY in adrenal medulla. Although TH remained unchanged in hypothalamus, DβH and NPY were elevated. Both drugs (enalapril and losartan) reversed these MV-induced changes in the adrenal medulla. However, enalapril failed to inhibit the increase in DβH and NPY while losartan had some efficacy. This increase in the protein levels of the biosynthetic enzymes (TH and DβH) was probably because of a compensatory mechanism to restore NE plasma levels; we observed that plasma NE levels tended to decrease at 12 h after MV.

The alteration of plasma epinephrine and NE levels because of MV was previously reported by several researchers. Aneman et al. [19] showed NE was inconsistent or unstable in patients. Monteverde [20] showed that the NE requirement was increased in pediatric patients in prolonged MV and Barbieri et al. [21] showed that the plasma epinephrine and NE increased at the first hour of MV in patients, and this increase was restored to baseline levels by the termination of MV. Moreover, Wieske et al. [22] have proposed that the autonomic dysfunction caused abnormal heart rates in intensive care unit patients.

Activation of angiotensin system contributes to several pathophysiological effects in the cardiovascular system via the activation of sympathetic nervous system [23]. Previous studies have reported an increase in lung or bronchoalveolar fluid angiotensin II levels at 2 or 4 h of MV [24–27]. However, lung ACE levels were found to be unchanged [25] or increased in bronchoalveolar fluid [26]. Moreover, captopril was effective in reducing the MV-induced lung injury and elevated ACE activity, and blockade of bradykinin receptors did not attenuate the effect of captopril. Moreover, the serum ACE levels were unchanged, although angiotensin II levels were increased in the bronchoalveolar fluid. Thus, indicating the role of a non-ACE pathway for the production of angiotensin II [26].

A recent study has also shown that angiotensin 1–7 analogs protected the lung against acute lung injury, thereby increasing the ratio of ACE2/ACE activity and reducing the

![Figure 2](image1.png)

**Fig. 2** — Western blot analysis of the protein levels of AT1 receptor in the lung tissues of the saline or enalapril (E)-or losartan (L)-treated SB or MV groups. Each group consists of n = 6–8 rats. **: P < 0.01 versus SB and : P < 0.05 versus MV.

![Figure 3](image2.png)

**Fig. 3** — Malondialdehyde and GSH levels in lung (A and C) and brain (B and D) tissues of the saline or enalapril (E) or losartan (L)-treated groups. Each group consists of n = 6–8 rats. : P < 0.05 versus SB and : P < 0.05 versus MV.
bioavailability of angiotensin II and AT1 receptor actions [28]. Increased angiotensin or AT1 levels have been correlated with the severity of the injury. Both receptor types AT1 and AT2 in the lungs were also shown to increase after MV [25,27]. Similar to treatment with ACE inhibitors, treatment with an angiotensin receptor blocker, losartan, has been shown to reduce the lung edema, inflammation, and injury score and AT1 receptor messenger RNA because of MV [25,27].

In the present study, we did not measure the ACE activity but we found that lung AT1 receptor protein was increased in the lung at 12 h of MV, and both enalapril and losartan treatments were effective in preventing this increase. As we previously discussed, both treatments also reduced the oxidative stress in the lungs and sympathetic nervous system activity markers in the adrenal medulla. On the other hand, it would have been valuable to evaluate the AT1 receptor protein in the aorta and to evaluate the sympathetic nervous system activity in different brain regions other than hypothalamus.

5. Conclusions

In summary, these data, for the first time, demonstrate that inhibition of renin–angiotensin system by enalapril or losartan may reduce the MV-induced increase in sympathetic activity markers and oxidative stress, and thus, may have a beneficial effect as adjuvant therapy.

On the other hand, in humans and a number of species, including the hamster, quantitatively important chymase-independent Ang II formation from Ang I occurs in the heart, arteries, and kidney. However, chymase differs in rats and rabbits and is not active in the conversion of Ang I to Ang II, but is involved in Ang II degradation. Consequently, one would anticipate that blockade of the system at the ACE step in rats would be equivalent to inhibition of the Ang II receptor in humans. On the other hand, AT1 receptor blockers may be more effective than ACE inhibitors in humans [29].

Although the preliminary data of this study are important in terms of showing the involvement of the interaction of
renin–angiotensin system with the MV-induced sympathetic activation (Fig. 4), further studies are needed to elucidate the detailed mechanisms and implement it into the clinical setting.

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Author contributions: H.Z.T. collected the data, performed statistical analysis, and prepared the manuscript. O.S.K. adapted the animal model, performed the surgery, and collected the data. Y.S., K.L., and N.K. collected the data and performed analysis. K.J.S., M.P.W., A.J.S., and E.E.T performed the surgery, follow up the animals, and collected the data. S.P., P.J.S., and N.T. were responsible for experimental design and prepared the manuscript.

Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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