

Angiotensin-converting enzyme inhibitor captopril attenuates ventilator-induced lung injury in rats

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Jiang J-S, Wang L-F, Chou H-C, Chen C-M. Angiotensin-converting enzyme inhibitor captopril attenuates ventilator-induced lung injury in rats. *J Appl Physiol* 102: 2098–2103, 2007. First published February 22, 2007; doi:10.1152/jappphysiol.00514.2006.—We hypothesized that lung inflammation and parenchymal apoptosis in ventilator-induced lung injury (VILI) are related to ANG II and assessed the ability of the angiotensin-converting enzyme inhibitor captopril to attenuate VILI in rats. Adult male Sprague-Dawley rats were randomized to receive two ventilation strategies for 2 h: 1) tidal volume of 40 ml/kg, respiratory rate of 25 breaths/min, and inspiratory O₂ fraction of 0.21 [high-volume, 0 positive end-expiratory pressure (HVZP) group] and 2) injection of captopril (100 mg/kg ip) 30 min before HVZP ventilation (HVZP + CAP group). Another group, which did not receive ventilation, served as the control. Mean arterial pressure was significantly lower in the HVZP + CAP group than in the HVZP group at 2 h of ventilation. Total protein levels were significantly higher in bronchoalveolar lavage fluid (BALF) recovered from HVZP-ventilated rats than from controls. BALF macrophage inflammatory protein-2 and lung ANG II were significantly higher in the HVZP group than in the control and HVZP + CAP groups. Lung ANG II levels correlated positively with BALF protein and macrophage inflammatory protein-2. The number of apoptotic airway and alveolar wall cells was significantly higher in the HVZP and HVZP + CAP groups than in the control group and significantly lower in the HVZP + CAP group than in the HVZP group. These results suggest that the efficiency of captopril to attenuate VILI is related to reduction of inflammatory cytokines and inhibition of apoptosis and indicate that VILI is partly mediated by the local angiotensin system.

apoptosis; bronchoalveolar lavage; macrophage inflammatory protein

MECHANICAL VENTILATION has been used to support acutely ill patients for several decades. Despite the lifesaving potential of this support, it has several potential disadvantages and complications (30). Mechanical ventilation with high tidal volumes causes lung hemorrhage and edema and activates inflammatory pathways. This process is referred to as ventilator-induced lung injury (VILI) (5, 21). Research has revealed that the manifestations of VILI were physiologically and histopathologically indistinguishable from acute lung injury. The spectrum of VILI includes disruption of endothelial and epithelial cells, increases in endothelial and epithelial permeability, and increases in pulmonary inflammatory mediators (5, 21, 28, 32). The mechanisms underlying VILI have not been fully elucidated (27). Full understanding of the mechanisms that mediate lung injury may permit possible strategies to prevent VILI early in the course of illness.

Apoptosis, a process of programmed cell death, is important in the development and remodeling of tissues during normal repair processes (25). It is triggered by the activation of an internally encoded suicide program as a result of extrinsic or intrinsic signals (34). Apoptosis has been connected to the pathogenesis of disease in many organs, including the lung (7, 14). The initiation and mechanisms of the pulmonary cell death process in VILI remain unclear. ANG II can be generated locally in the lung tissue and may have autocrine and paracrine actions at the cellular level (6). This local renin-angiotensin system regulates apoptosis of alveolar epithelial cells (6, 34) and mediates acid- and coronavirus-induced lung injury in mice (11, 12). However, little is known about the role of ANG II in the pathogenesis of VILI *in vivo*. Captopril is a common angiotensin-converting enzyme (ACE) inhibitor for the treatment of hypertension, heart failure, and other cardiovascular and renal diseases (16). ACE converts the inactive peptide ANG I to the active vasoconstrictor ANG II while inactivating the vasodilator bradykinin. We hypothesized that lung parenchymal apoptosis, leukocyte infiltration into air space, and inflammatory cytokine increase are related to ANG II in VILI and that these deleterious effects can be attenuated with the ACE inhibitor captopril.

METHODS

Animal preparation. The experimental protocol was approved by the Institutional Animal Use Committee at Taipei Medical University. Adult male Sprague-Dawley rats (250–300 g body wt) were maintained on a 12:12-h light-dark cycle with free access to food and water. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott, North Chicago, IL), and a polyethylene (PE-50, Becton Dickinson, Sparks, MD) catheter containing heparinized, isotonic saline was placed in one carotid artery to sample blood for gas analysis. Blood gas tensions and mean arterial pressure were measured with a blood gas analyzer (model 1620, Instrumentation Laboratories, Lexington, MA) and an intravascular blood pressure transducer (model BP-100, iWorx/CB Sciences, Dover, NH), respectively. A tracheostomy was performed, and a 14-gauge plastic cannula was inserted into the trachea. The high-volume zero-positive end-expiratory pressure (HVZP) group was ventilated for 2 h at a tidal volume of 40 ml/kg, zero positive end-expiratory pressure, respiratory rate of 25 breaths/min, and inspiratory O₂ fraction of 0.21 via a volume-cycled small animal ventilator (model SAR-830/AP, CWE, Ardmore, PA). The HVZP + CAP group was injected intraperitoneally with 1 ml of the ACE inhibitor captopril (100 mg/kg; Sigma, St. Louis, MO) 30 min before HVZP ventilation. The dose of captopril was based on the work of Gavin et al. (8). Another group, which did not

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receive ventilation, served as the control. Control and HVZP groups were injected with an equal volume of 0.9% NaCl. The rats were randomized to receive one of these two ventilation strategies. All animals were kept in the supine position for the duration of the experiment, and arterial blood gases and mean arterial pressures were measured at the beginning of the experiment and every hour after randomization.

Bronchoalveolar lavage. After 2 h of ventilation, the chest was opened and the lungs were removed intact from the animal; the tracheostomy tube remained in place. The lungs were instilled with 7 ml of 0.9% saline at 4°C; the saline solution was washed into and out of the lungs three times and then recovered. This washing procedure was repeated twice more for each animal, and the three washes were finally pooled and the total volume was recorded. There were no differences in the total volume of saline infused or recovered after the lavage procedure between the three experimental groups. An aliquot of the bronchoalveolar lavage fluid (BALF) from each animal was used to measure the total protein content, with bovine serum albumin as the standard and macrophage inflammatory protein-2 (MIP-2) using an ELISA kit (R & D Systems, Minneapolis, MN); values are expressed as milligrams per kilogram of body weight and picograms per milliliter, respectively.

Measurement of ANG II in lung tissue. Lung tissue was homogenized in lysis buffer and centrifuged at speeds recommended by the manufacturer. The supernatant solution was used for the measurement of ANG II levels with an ELISA kit (SPI-BIO, Massy Cedex, France). Protein content was measured by the method of Lowry et al. (15).

Histological examination. Immediately after the bronchoalveolar lavage was finished, the right lung was fixed by instillation of a 10% formaldehyde solution at 20 cmH₂O. Specimens were embedded in paraffin, stained with hematoxylin and eosin, and examined by a pathologist who was blinded to the protocol and experimental groups. Lung injury was scored according to the following four criteria: 1) alveolar congestion, 2) hemorrhage, 3) infiltration of neutrophils in the air space or vessel wall, and 4) thickness of the alveolar wall (17). Each item was graded according to a five-point scale as follows: 0 for minimal (little) damage, 1 for mild damage, 2 for moderate damage, 3 for severe damage, and 4 for maximal damage.

In situ detection of apoptotic epithelial cells. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) was carried out to detect apoptotic DNA damage using the Apo-BrdU-IHC In Situ DNA fragmentation assay kit (BioVision, Mountain View, CA) and confirmed by immunohistochemistry for caspase-3 (Cell Signaling Technology, Beverly, MA). Briefly, after fixation with 4% buffered formalin, the lung sections were deparaffinized and hydrated. Then the TUNEL method was applied to label the 5' end of double-stranded DNA fragments with bromodeoxyuridine triphosphate. Methyl green solution was used for counterstaining. Positive staining was indicated as brown and background staining as blue-green. For semiquantitative measurement of apoptosis, the number of TUNEL-positive and -negative cells in the airway and alveolar wall were counted. A pathologist who was blinded to the treatment group systematically and randomly counted the cells until reaching a total number of ~200 airway and 2,000 alveolar wall cells; values are expressed as percentage of apoptotic cells.

Statistical analysis. The lung injury score data are given as the median (range); other data are means \pm SE. Statistically significant differences were analyzed by ANOVA followed by Scheffé's post hoc analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Effects on gas exchange and mean arterial pressure. The arterial blood gas tensions and mean arterial pressures were comparable among the three study groups before mechanical ventilation (Table 1). Mean pH was higher and mean PCO₂ was lower in the HVZP group throughout the study period. Mean

Table 1. Arterial blood gases and mean pressures at baseline and during ventilation

| | pH | PaO ₂ , Torr | PaCO ₂ , Torr | MAP, mmHg |
|----------|-----------------|-------------------------|--------------------------|-------------|
| Control | | | | |
| 0 h | 7.32 \pm 0.03 | 94 \pm 2 | 41 \pm 2 | 119 \pm 4 |
| HVZP | | | | |
| 0 h | 7.31 \pm 0.04 | 91 \pm 3 | 43 \pm 2 | 122 \pm 4 |
| 1 h | 7.58 \pm 0.03 | 86 \pm 3 | 20 \pm 1 | 110 \pm 5 |
| 2 h | 7.53 \pm 0.02 | 81 \pm 3 | 22 \pm 2 | 98 \pm 4 |
| HVZP+CAP | | | | |
| 0 h | 7.32 \pm 0.02 | 92 \pm 2 | 41 \pm 1 | 120 \pm 4 |
| 1 h | 7.49 \pm 0.03 | 90 \pm 2 | 21 \pm 2 | 105 \pm 3 |
| 2 h | 7.48 \pm 0.02 | 85 \pm 2 | 20 \pm 2 | 88 \pm 2* |

Values are means \pm SE ($n = 6$). HVZP, 2 h of ventilation at tidal volume of 40 ml/kg, respiratory rate of 25 breaths/min, and inspiratory O₂ fraction of 0.21 [high-volume 0-positive end-expiratory pressure (HVZP)]; HVZP + CAP, captopril (100 mg/kg ip) injected 30 min before HVZP; PaO₂ and PaCO₂, arterial PO₂ and PCO₂; MAP, mean arterial pressure. * $P < 0.05$ vs. HVZP at equivalent time of ventilation.

arterial pressure was significantly lower in the HVZP + CAP group than in the HVZP group at 2 h of ventilation.

Lavage protein and MIP-2. Total protein contents recovered from BALF were significantly higher in rats ventilated with HVZP protocols than in control animals, and the values were comparable between HVZP and HVZP + CAP groups (Fig. 1A). MIP-2 concentrations in BALF increased after HVZP ventilation, and the values were approximately three-fold higher in the HVZP group than in the control group (Fig. 1B). MIP-2 was significantly lower in the HVZP + CAP group than in the HVZP group.

Lung ANG II concentration. Lung ANG II concentration was significantly higher in the HVZP group than in the control and HVZP + CAP groups (Fig. 2). Lung ANG II levels were comparable in the control and HVZP + CAP groups. Lung ANG II levels correlated positively with total protein and MIP-2 in BALF in all study animals ($r = 0.608$, $P < 0.01$ and $r = 0.712$, $P < 0.001$, respectively).

Histology. After 2 h of ventilation, the lung injury score was significantly higher in the HVZP and HVZP + CAP groups than in the control group (Table 2) and significantly lower in the HVZP + CAP group than in the HVZP group. The lung injury was characterized by patchy areas of hemorrhage and inflammatory cell infiltration (Fig. 3). These findings were consistent with the extent of alveolar damage in acute lung injury. No major histological abnormalities were present in the control animals. The TUNEL assay showed a greater number of apoptotic airway and alveolar wall cells in ventilated rats (Fig. 4); semiquantitative counting of TUNEL-positive and -negative cells in the airway and alveolar wall confirmed the ability of captopril to reduce the number of apoptotic cells (Fig. 5). Immunohistochemistry for caspase-3 further confirmed these findings (Fig. 6).

DISCUSSION

Our in vivo model showed that mechanical ventilation at a high tidal volume increased total protein and MIP-2 in the BALF and increased the lung injury score and the number of apoptotic airway and alveolar wall cells. These phenomena are consistent with the alterations of VILI. The main finding of this study is that the development of VILI was associated with an

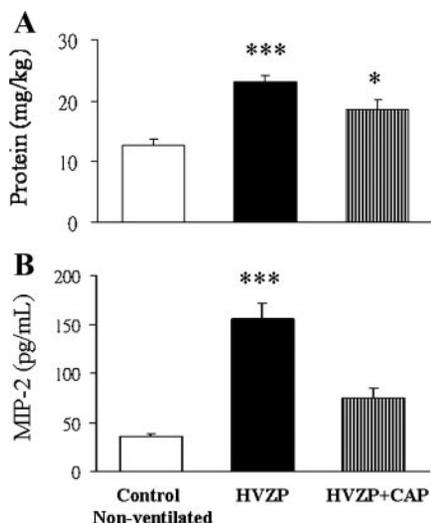


Fig. 1. Total protein and macrophage inflammatory protein (MIP)-2 in bronchoalveolar lavage fluid (BALF) in control rats ($n = 7$), rats subjected to 2 h of ventilation at tidal volume of 40 ml/kg, respiratory rate of 25 breaths/min, and inspiratory O_2 fraction of 0.21 [high-volume 0-positive end-expiratory pressure ventilation (HVZP), $n = 7$], and rats injected with captopril (100 mg/kg ip) 30 min before HVZP ventilation (HVZP + CAP, $n = 7$). A: total protein contents recovered from BALF were significantly higher in HVZP and HVZP + CAP than in control. $*P < 0.05$; $***P < 0.001$ vs. control. B: MIP-2 concentrations were significantly higher in HVZP than in control and HVZP + CAP. $***P < 0.001$.

increase of lung ANG II levels, and the deleterious effects were attenuated by an ACE inhibitor. These results are consistent with previous data indicating that high-volume ventilation may injure the lungs and suggest that the local tissue angiotensin mediates these harmful events in VILI.

Our finding of the highest BALF MIP-2 levels in rats subjected to HVZP ventilation suggests that mechanical ventilation can have a major influence on the inflammatory environment of normal lungs and may initiate or augment lung injury. Lung ANG II and BALF MIP-2 levels were increased in the ventilated groups, and there was a linear relation between lung ANG II and BALF MIP-2 levels ($r = 0.712$, $P < 0.001$). These data imply that ANG II is involved in pulmonary inflammatory cytokine release and neutrophil recruitment into alveolar spaces in VILI. These results were consistent with the observations of Nabah et al. (19), who found that intraperitoneal administration of ANG II induces neutrophil accumulation and release of MIP-2 in peritoneal exudate fluid in rats.

Pulmonary edema is a prominent feature of VILI in small animal models (5). The high protein content of the edema fluid

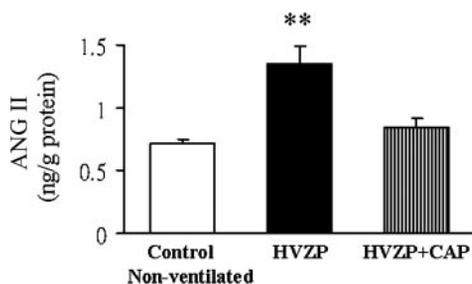


Fig. 2. Lung ANG II in control, HVZP, and HVZP + CAP groups. Lung ANG II concentrations were significantly higher in HVZP than in control and HVZP + CAP. $**P < 0.01$.

Table 2. Lung injury scores

| Treatment | Alveolar Congestion | Hemorrhage | Neutrophil Infiltration | Alveolar Wall Thickness | Lung Injury Score |
|-----------|---------------------|------------|-------------------------|-------------------------|-------------------|
| Control | 0 (0-1) | 1 (0-3) | 2 (1-3) | 0 (0-1) | 2 (1-8) |
| HVZP | 3 (3-3) | 3 (2-3) | 3 (3-3) | 0 (0-2) | 9 (8-11)† |
| HVZP+CAP | 1 (1-2) | 1 (1-2) | 3 (2-3) | 1 (1-2) | 6 (5-8)*‡ |

Values are medians, with range in parentheses ($n = 7$). $*P < 0.05$; $†P < 0.001$ vs. control; $‡P < 0.01$ vs. HVZP.

suggests that it is due to increased permeability and implicates changes in the epithelial and microvascular endothelial barriers. In this study, we found that 2 h of injurious mechanical ventilation led to severe pulmonary edema as assessed by the high concentrations of protein in BALF compared with that in

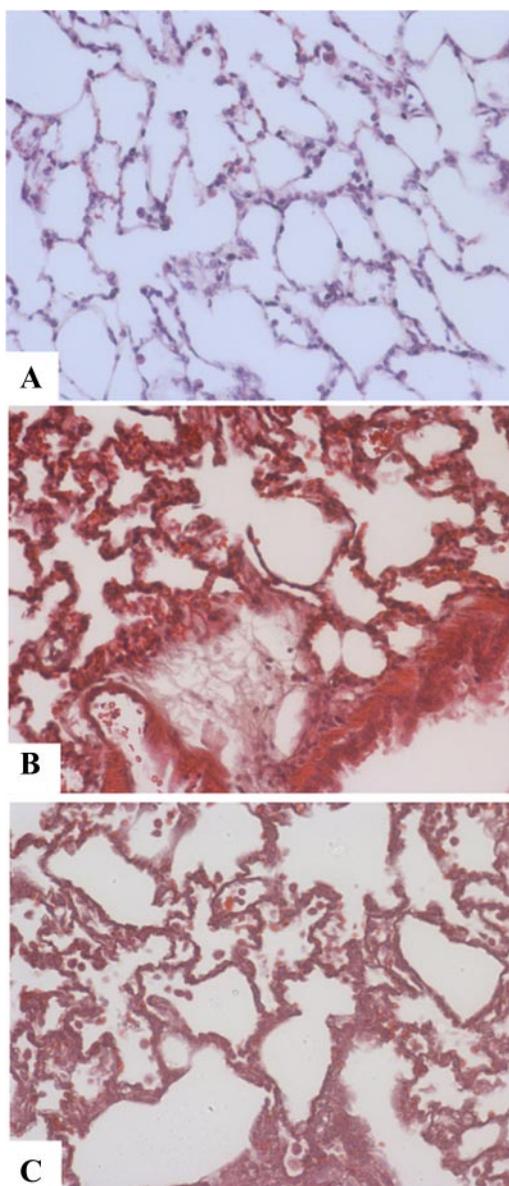


Fig. 3. Representative lung tissue photomicrographs. Magnification $\times 200$. A: no major histological abnormalities in control group. B: patchy areas of hemorrhage, thickened alveolar walls, and inflammatory cell infiltration in HVZP group. C: less hemorrhage and mild inflammatory cell infiltration in HVZP + CAP group.

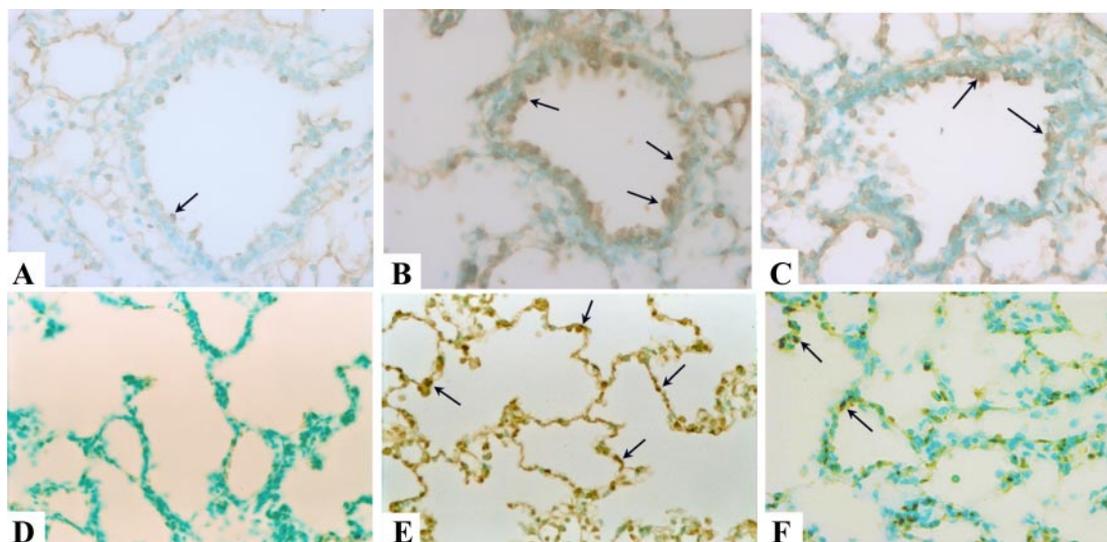


Fig. 4. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling of airway and alveolar wall. Magnification $\times 400$. A, B, and C: airway from lungs of control, HVZP, and HVZP + CAP rats, respectively. D, E, and F: alveolar region from lungs of control, HVZP, and HVZP + CAP rats, respectively. Positive staining is indicated by brown (arrow); background staining is blue-green. Note reduced staining in C and F.

control rats. Bronchoalveolar lavage levels of cytokines have been shown to be key mediators of injurious ventilation (3, 31). Blocking of inflammatory cytokines has been found to attenuate the severity of VILI in animals (10). MIP-2 is a rodent homolog of human IL-8, and both are important mediators of neutrophil recruitment and activation. The lung lavage level of MIP-2 was examined in this study, inasmuch as MIP-2 is known to be released during VILI (22, 35). We found increased BALF MIP-2 after mechanical ventilation, and the highest level was seen in the HVZP group. These findings are consistent with the observations of Ricard et al. (24), who found that BALF protein concentrations were markedly higher in high-volume-ventilated intact rats than in low-volume-ventilated

animals and that BALF MIP-2 concentrations were similar in these two groups. These results suggest that the mechanism of pulmonary edema may differ from the mechanism of cytokine release and that cytokines might not be necessary for initiation of pulmonary edema.

Apoptosis has been connected to the pathogenesis of lung diseases (7). The initiation and mechanisms of pulmonary cell death processes in VILI remain unclear. The renin-angiotensin system plays an important role in the regulation of blood pressure, fluid, and electrolyte homeostasis (23). ANG II is released from its precursor angiotensinogen by enzymatic processing with renin and then by ACE. ANG II, the principal biologically active peptide, causes arteriolar vasoconstriction and stimulates aldosterone secretion. Although angiotensinogen is mainly synthesized in the liver and secreted into the circulating blood, angiotensin formation has also been shown to occur in diverse tissues other than the liver (2). ANG II can be generated locally in the lung tissue and may have autocrine and paracrine actions at the cellular level (6). Wang et al. (33) reported that ANG II induces apoptosis in rat alveolar epithelial cells *in vitro* and that these effects are blocked by an ANG II receptor antagonist. Using a cyclic mechanical stretch model, Hammerschmidt et al. (9) found that captopril prevents alveolar type II cell apoptosis induced by high-amplitude mechanical stretching.

Acute administration of a single dose of captopril (100 or 50 mg/kg) significantly decreased mean arterial pressure in initially normotensive rats and mildly sodium-depleted normal humans, respectively (1, 8). A significant reduction of ANG II in lung tissue after captopril administration demonstrates that ACE was efficiently inhibited in our procedure. In this study, the reduction of blood pressure in the HVZP + CAP group might produce lactic acid and, hence, counteract hypocapnic alkalosis (4). Hypercapnic acidosis reduces lung microvascular permeability induced by ischemia-reperfusion or high airway pressure (18, 26). Hypocapnia may directly injure the lung and augment acute lung injury following ischemia-reperfusion (13). A dose-response relation was demonstrated for the dele-

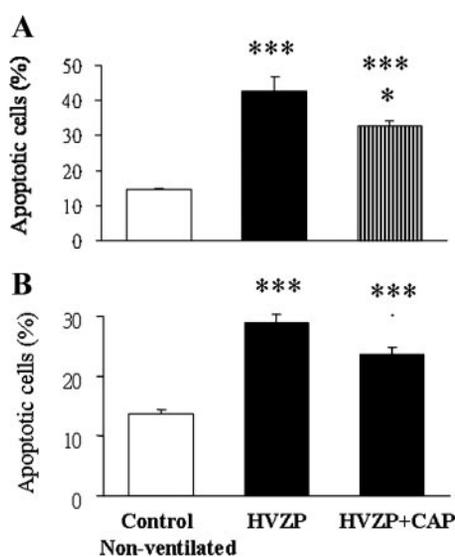


Fig. 5. Semiquantitative measurement of apoptosis. Number of TUNEL-positive and -negative cells were counted in airway (A) and alveolar wall (B) from lungs of control, HVZP, and HVZP + CAP rats. Number of apoptotic airway and alveolar wall cells was significantly greater in HVZP and HVZP + CAP than in control and significantly reduced in HVZP + CAP compared with HVZP. * $P < 0.05$ vs. HVZP. *** $P < 0.001$ vs. control.

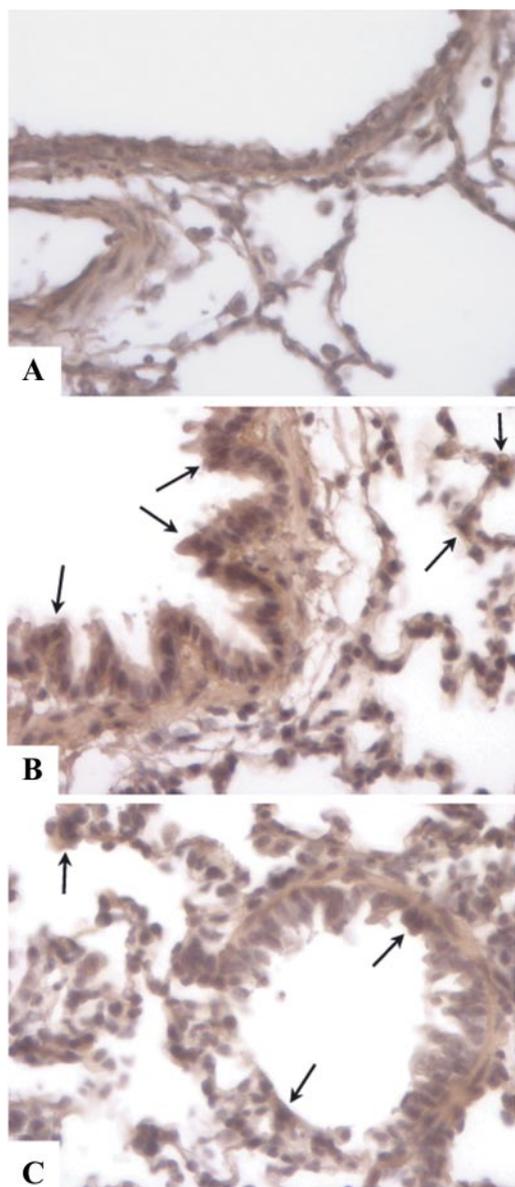


Fig. 6. Immunohistochemistry for caspase-3 in pulmonary epithelial cells of control (A), HVZP (B), and HVZP + CAP (C) rats. Magnification $\times 400$. Arrows indicate positive staining.

terious effects of hypocapnic alkalosis, with the degree of injury proportional to the severity of the hypocapnic alkalosis (13). Nin et al. (20) suggested that an inflammatory, rather than a hemodynamic, mechanism is involved in the changes induced by high-tidal-volume ventilation. These results suggest that our data are consistent with the possibility that captopril attenuates VILI through a mechanism dependent partly on its ability to decrease local angiotensin.

In conclusion, we have demonstrated that high-tidal-volume ventilation increased total protein and MIP-2 in the BALF and increased the number of apoptotic airway and alveolar wall cells and the level of ANG II in lung tissue and that the deleterious effects were attenuated by captopril. These results imply that ANG II is involved in the pathogenesis of VILI and suggest that the protective mechanism of captopril to attenuate VILI is related to reduction of inflammatory cytokine and inhibition of apoptosis.

GRANTS

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