Effects of prone and supine position on oxygenation and inflammatory mediator in a hydrochloric acid-induced lung dysfunction in rats[1]

Efeitos da posição prona e supina na oxigenação e mediador inflamatório na disfunção pulmonar induzida por ácido clorídrico em ratos

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ABSTRACT

Purpose: To compare the effectiveness of mechanical ventilation of supine *versus* prone position in hydrochloric acid (HCl)-induced lung dysfunction. **Methods**: Twenty, adult, male, Wistar-EPM-1 rats were anesthetized and randomly grouped (n=5 animals per group) as follows: CS-MV (mechanical ventilation in supine position); CP-MV (mechanical ventilation in prone position); bilateral instillation of HCl and mechanical ventilation in supine position (HCl+S); and bilateral instillation of HCl and mechanical ventilation in prone position (HCl+P). All groups were ventilated for 180 minutes. The blood partial pressures of oxygen and carbon dioxide were measured in the time points 0 (zero; 10 minutes before lung injury for stabilization), and at the end of times acid injury, 60, 120 and 180 minutes of mechanical ventilation. At the end of experiment the animals were euthanized, and bronchoalveolar lavages (BALs) were taken to determine the contents of total proteins, inflammatory mediators, and lungs wet-to-dry ratios. **Results**: In the HCl+P group the partial pressure of oxygen increased when compared with HCl+S (128.0±2.9 mmHg and 111.0±6.7 mmHg, respectively) within 60 minutes. TNF-α levels in BAL do not differ significantly in the HCl+P group (516.0±5.9 pg/mL), and the HCl+S (513.0±10.6 pg/mL). **Conclusion**: The use of prone position improved oxygenation, but did not reduce TNF-α in BAL upon lung dysfunction induced by HCl.

Key words: Lung. Respiration, Artificial. Oxygenation. Inflammation Mediators. Rats.

RESUMO

Objetivo: Comparar os efeitos da ventilação mecânica em posição prona *versus* supina na disfunção pulmonar induzida por ácido clorídrico (HCl). **Métodos**: Vinte ratos, adultos, Wistar-EPM-1 foram anestesiados e distribuídos aleatoriamente em grupos (n=5 animais por grupo): CS-MV (controle, ventilado mecanicamente em posição supina); CP-MV (controle, ventilado mecanicamente em posição prona); instilação bilateral de HCl e ventilação mecânica em posição supina (HCl+S) ou ventilação em posição prona (HCl+P). Todos os grupos foram submetidos a ventilação mecânica por 180 minutos. As pressões parciais de oxigênio e dióxido de carbono no sangue arterial foram mensuradas nos tempos Injúria ácida (10 minutos após instilação de HCl), e ao final de cada período após lesão por HCl, 60, 120 e 180 minutos sob ventilação mecânica. Ao final do experimento os animais foram eutanasiados, os pulmões retirados para avaliação do peso úmido em relação ao peso seco do pulmão direito e realizamos o lavado broncoalveolar (BAL) para determinação de proteínas totais e o mediador inflamatório TNF-α. **Resultados**: No grupo HCl+P a pressão parcial de oxigênio, no tempo de 60 minutos, aumentou quando comparada com o grupo HCl+S (128.0±2.9 e 111.0±6.7 mmHg, respectivamente). Os níveis de TNF-α no lavado broncoalveolar não diferiram de maneira estatisticamente significante quando comparamos os grupos HCl+S (513.0±10.6 pg/mL) *versus* HCl+P (516.0±5.9 pg/mL). **Conclusão**: O uso da posição prona melhora a oxigenação, mas não reduz os níveis de BAL após disfunção pulmonar induzida por HCl.

Descritores: Pulmão. Respiração Artificial. Oxigenação. Mediadores da Inflamação. Ratos.

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Introduction

Gastric acid aspiration is a common etiology of acute lung injury (ALI) and is initially characterized by a chemical "burn" of the pulmonary epithelium, with a subsequent influx of extracellular fluid into the alveolar space that leads to pulmonary edema. Hence, acid aspiration-content is an important cause of acute respiratory distress syndrome (ARDS)[1].

Acute lung injury (ALI) and ARDS are serious complications in trauma and surgical patients[2,3]. In spite of improvements in critical care and ventilator management, mortality rates have been reported to remain as high as 35% to 40%[4,5].

A variety of ventilatory maneuvers are employed to combat it, such as mechanical ventilation with prone and/or supine position, positive end expiratory pressure (PEEP), alveolar recruitment, high-frequency ventilation and non-ventilatory maneuvers such as the administration of nitric oxide and surfactant, fluids management, corticoids[6-8].

The use of prone ventilation has been shown to improve aeration and decrease alveolar shunt in an animal model[9]. Many clinical and experimental studies have since been carried out, but the mechanism responsible for the improvement remains highly controversial[4,10].

During anesthesia and paralysis, the prone position determines a more homogeneous distribution of the gravitational gradient for alveolar inflation[11].

Following aspiration other symptoms can be observed, with microvascular lung injury, hypoxemia, increase alveolar protein content and inflammatory response, etc.

The purpose of this study was to test the hypothesis whether mechanical ventilation with prone position improves arterial oxygenation and decreases local inflammatory dysfunction on an experimental model of ALI.

Methods

Surgical procedure

All procedures were approved by the Institutional Research Committee at Federal University of São Paulo, in accordance with the National Institutes of Health (NIH) Guidelines Regarding Animal Experimentation and the principles of the Brazilian College on Animal Experimentation.

Twenty (n= 20) male Wistar rats weighing 280–340 g were fasted overnight with free access to water. Animals were group-housed in standard Plexiglas bins (2–3 rats per cage). Rats (n=5 in every group) were anesthetized intraperitoneally (i.p.) with sodium thiopental (40 mg/kg body weight; Thionembutal, ABBOTT, SP, Brazil). Muscle relaxation was maintained with 0.8 mg/kg pancuronium bromide (Pavulon, Organon, SP, Brazil) i.p. Animals were placed in the supine position, and 1% lidocaine was injected subcutaneously at the tracheal region.

After anesthesia, a tracheotomy was performed and a 14G cannula was inserted approximately 1 cm above the carina. A hind paw vein (used for drug infusions and compensation of fluid losses) and the left internal carotid artery (used to obtain arterial blood for gases determination) were exposed and cannulated using a PE-50 tubing (Intramedic Polyethylene Tubing, Becton Dickinson, NJ, USA). Normothermia was maintained with an underlying electric hotplate (37 ± 0.6oC [mean±SD])[1].

Mechanical ventilation (MV)

After reaching a surgical plane of anesthesia and muscle relaxation, the animals were ventilated using a rodent ventilator (683 model, Harvard Apparatus; MA, USA) for 3 h using a tidal volume (V_T) set at approximately 6 mL/kg, a positive end-expiratory pressure (PEEP) of 5 cmH_2O, a fraction of inspired oxygen (FiO_2) of 1.0, a respiratory rate (RR) of 30–50 cycles per minutes and an inspiration/expiration (I/E) ratio of 1:1. A venous catheter was placed in the jugular vein to allow the infusion of isotonic saline solution and sodium pentobarbital whenever needed[1].

The animals were divided into 4 different groups (n=5 in every group). The first group (CS-MV) received only conventional MV in supine position; the second group (CP-MV) received only conventional MV in prone position; the third group (HCl+S) received bilateral instillation of HCl and MV and in supine position; the fourth group (HCl+P) received HCl instillation and MV in the prone position for 180 minutes. The animals were maintained in the supine and/or prone position for 10 minutes, hereby defined as the baseline phase. In order to be included in the remainder of this study, each rat had to exhibit an initial arterial partial pressure of oxygen [PaO_2] ≥ 300 mmHg.

Acid instillation lung injury

After 10 min connected in the ventilator for stabilization, the lung injury was performed using a 0.1 N HCl solution (2 mL/kg) instilled in the endotracheal tube in the supine position with the rat disconnected from the ventilator, the aspiration of HCl solution was performed in an inverted Trendelenburg position. When $PaO_2 \leq 150$ mmHg the protocol was started[1].

Measurements

Arterial blood gases measurement

Arterial blood gases (PaO_2 and arterial partial pressure of carbon dioxide [$PaCO_2$]) were determined in blood samples collected with sterile vented plastic syringes (PICO 70, Radiometer, Copenhagen, Denmark) and measured at the times acid injury, 60, 120 and 180 min using an automatic AVL-Compact3 device (Roche Diagnostic, Mannheim, Germany).

Wet/dry (W/D) weight ratio of lungs

After bronchoalveolar lavage, the right lung was placed in a drying oven for 72 h at 60°C and then re-weighed. The W/D ratios of lungs were then calculated.

Bronchoalveolar lavage (BAL)

At the end of 3 h, the animals were euthanized (1 mL/100 g bw T-61 Euthanasia Solution, Hoechst & Roussel). A sternotomy was performed, and the lungs and appending structures were removed and weighed. The left lung was tied, and the right lung was washed three times with 20 mL/kg cold sterile phosphate-buffered saline (PBS; pH 7.2–7.4). Lavage fluids were kept separate and centrifuged (at 400 x **g** for 10 min) at 4ºC, and the supernatants were frozen immediately on dry ice and stored at -80ºC[1].

Protein leakage

An 1-mL aliquot of BAL was used to measure the total protein content using specific kits (QUANT-iT Protein Assay, Invitrogen, CA, USA) and a standard equipment for readings (QUBIT Fluorometer, Invitrogen, CA, USA).

Cytokine assay

TNF-α concentrations in BAL were determined using a rat-specific, commercially available enzyme immunoassay [ELISA] (R&D Systems, Minneapolis, USA).

TABLE 1 - Effects of PEEP after hydrochloric acid (HCl) on the arterial blood levels of PaO_2 and $PaCO_2$ in the time points Injury, 60, 120 and 180 minutes. Data are shown as mean±SD

	Pa_{O_2} (mmHg)				Pa_{CO_2} (mmHg)			
	Injury	**60 min**	**120 min**	**180 min**	**Injury**	**60 min**	**120 min**	**180 min**
CS-MV	311±3	321±3.6	321±2.5	309±9.1	39±4.1	38±3.2	38±2.9	39±2.2
CP-MV	311±3.4	322±4.8	322±2.6	313±6.7	36±2.5	39±1.7	41±3.4	45±2
HCl+S	87±4.4	111±6.7	146±4.1	232±6.3	63±4.1	63±3.7	50±2.1	62±3.5
HCl+P	87±4.4	128±2.9*	152±2.5$^{\#}$	261±8.6§	67±3.7	56±4.5*	51±3.4$^{\#}$	55±3.5§

No differences were observed in the time Injury in the PaO_2 and $PaCO_2$ levels among all groups.
**P*<0.05 compared with HCl+S group in the PaO_2 and $PaCO_2$ measurements (time 60 min);
#*P*<0.05 compared with HCl+S group in the PaO_2 measurements (time 120 min);
§*P*<0.05 compared with HCl+S group in the PaO_2 and $PaCO_2$ measurements (time 180 min).

Statistical analysis

Data were expressed as means ± standard deviation (SD). Values were compared between groups using an ANOVA test for multiple comparisons, and repeated-measures ANOVA was used to compare measurements among groups along the different time periods. Statistical analyses were performed using a standard computer software package (GraphPad Prisma, GraphPad Software, San Diego, CA, USA). *P*-values less than 0.05 were considered statistically significant.

Results

None of the animals died before the end of the experiment. The groups did not differ significantly in terms of the variables studied during the baseline period, either in terms of gas exchange, nor did the body weights differ at the end of experiment.

Between control groups (CS-MV and CP-MV) no significant differences were found concerning the studied variables. Differences could be observed after induced lung injury in groups HCl+S and HCl+P when compared with their respective controls (Table 1, Figures 1, 2 and 3).

Blood gases analyses

After 60 min of mechanical ventilation the PaO_2 values were higher in the HCl+P group compared with HCl+S group (128.0±2.9 *versus* 110.0±6.7 mmHg, respectively). In the time point 120 min the PaO_2 were higher in the HCl+P (152.0±2.5 mmHg) compared with HCl+S (146.0±4.2 mmHg) (Table 1), and the difference was observed at the time 180 min when comparing HCl+S *versus* HCl+P (P<.001; Table 1). At the times 60, 120 and 180 minutes the PaO_2 differ when compared HCl+S and HCl+P with respectively control group (Table 1). $PaCO_2$ values were lower in the HCl+P group compared to the HCl+S group (Table 1) in the times 60, 120 and 180 min. No significant differences were observed among HCl groups at the acid injury time point.

W/D weight of lungs, total proteins, and TNF-α in the BAL

W/D ratios were higher in the HCl+S group as compared to the HCl+P group (*P*<.05; Figure 1).

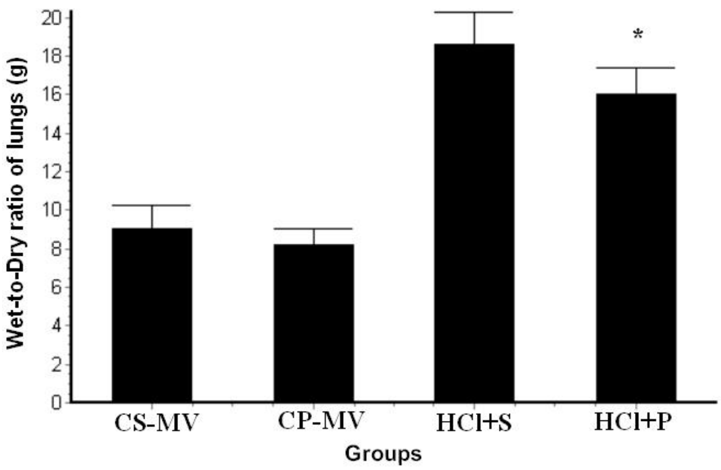

FIGURE 1 - Effect of position on lungs wet to dry ratios from rats, mechanically-ventilated controls (C-MV) and instilled with HCl. **P*<.05 *versus* the HCl+S group. Values are presented as mean ± SD. Values are presented as mean ± SD

The total proteins in BAL were lower in the HCl+P group compared to HCl+S group [38.0±2.9 mg/mL *versus* 47±2.3 mg/mL, respectively; *P*<.001]. In the CS-MV group and in CP-MV, the total protein values in BAL were 26.0±1.6 mg/mL and 23.0±2.3 mg/mL, respectively (Figure 2).

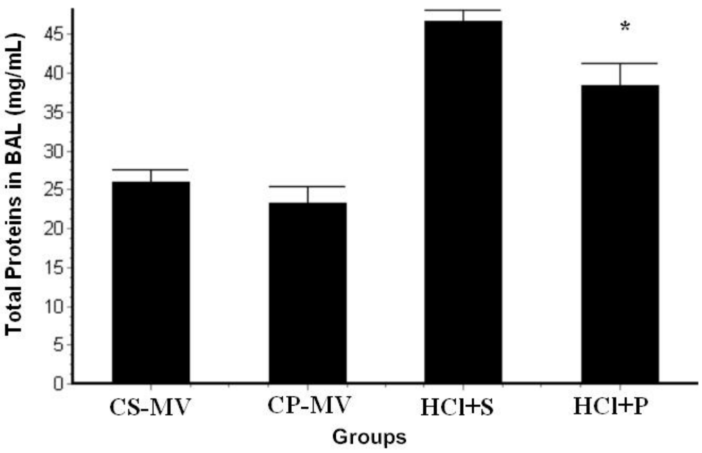

FIGURE 2 - Effect of position on total protein levels in BAL from rats, mechanically-ventilated controls (C-MV) and instilled with HCl. *P<0.05 *versus* the HCl+S group. Values are presented as mean ± SD

The TNF-α concentration in BAL was significantly higher in the group HCl+S and HCl+P when compared with the respective control group (P<.001), but did not differ between HCl-injured groups (Figure 3).

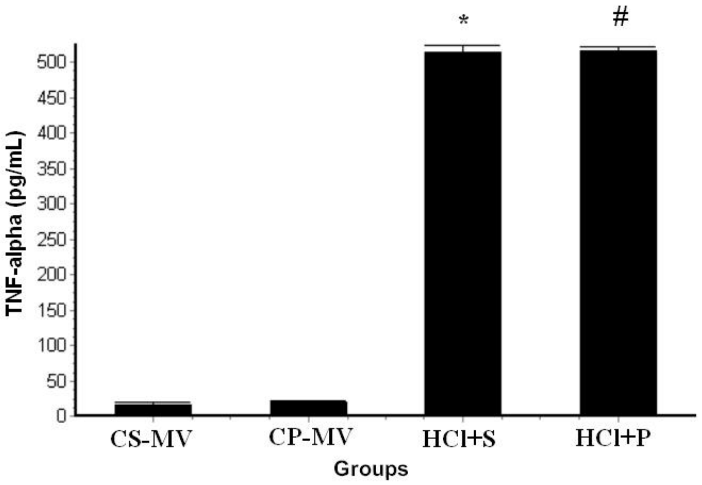

FIGURE 3 - Effect of position on TNF-α levels in BAL from rats, mechanically-ventilated controls (C-MV) and instilled with HCl. *P<0.05 *versus* the CS-MV group, and #P<.05 *versus* the CP-MV group. Values are presented as mean ± SD

Discussion

The present study confirm that prone position promoted the recovery of PaO_2 after HCl instillation. Decreased levels of total proteins in BAL accompained this alleviating effect on lung injury.

Since West's accurate descriptions of the physiology of respiration and the ventilation/perfusion zones[12], position change has been advocated to promote redistribution of gas, recruit alveoli, and redistribute pulmonary blood flow[13].

Prone position was initially introduced in healthy anesthetized and paralyzed subjects for surgical specific reasons. Since then, it has been used during acute respiratory failure to improve gas exchange. The interest on prone position during ALI/ARDS progressively increased, even if the mechanisms leading to a respiratory improvement are not yet completely understood. Our results demonstrated the ameliorating effect on the recovery gas exchange in the group with lung injury and ventilated in the prone position.

Reports of prone positioning to improve oxygenation in patients with ALI/ARDS date back nearly 30 years[14]. Animal studies have demonstrated improved oxygenation and ventilation perfusion ratios, along with restoring aeration, decreasing shunt, and preserving perfusion[1,9,15]. In addition, prone positioning has been shown to eliminate compression of the lungs by the heart[16].

TNF-α is an early-response, pro-inflammatory cytokine that has been shown to be integral in the pathogenesis of other lung injury models that are neutrophil-mediated[17-20]. TNF-α leads to activation of leukocytes and induces the expression of endothelial adhesion molecules that play a major role in the leukocyte-endothelial interactions that lead to neutrophil migration into the alveoli[21]. Our results demonstrated a small reduction in the TNF-α levels in the group HCl+P, but these effects did not differ significantly in comparison with the HCL+S group.

Computerized tomography of the chest shows that, while ARDS injuries are heterogeneous, they primarily affect the dorsal regions of the lungs[22]. Therefore, in the supine position, pulmonary ventilation without PEEP is primarily distributed to the ventral region of the lungs. The ratio of ventilation between ventral and dorsal regions is around 2.5:1. With the addition of PEEP, the distribution of ventilation becomes progressively more homogeneous. It means that ratio of close to 1:1 with PEEP at 20 cmH_2O[23].

Conclusion

The use of the prone position improves arterial oxygenation and reduces the lung edema in a rat model of acid injury.

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