The efficacy of spironolactone in the treatment of acute respiratory distress syndrome-induced rats

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ABSTRACT

Introduction: This study aimed to test the feasibility of spironolactone treatment in comparison with a surfactant in the early stage of acute respiratory distress syndrome (ARDS) in rats, as assessed by the acute lung injury (ALI) score, blood gas, brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Methods: A total of 40 rats were randomly allocated into one of five groups (n is eight). The baseline group (Group B) was subjected to neither tracheotomy nor ARDS induction, while the sham group (Group N) was subjected to tracheotomy upon ARDS induction by acid aspiration. The other three groups were administered either a single dose of spironolactone (100 mg/kg, Group Sp) or surfactant (100 mg/kg, Group S), or were untreated (Group A). Blood samples were collected from the femoral artery for blood gases, BNP and NT-proBNP measurements.

Results: ARDS induction decreased the blood PO_2/FiO_2 ratio and increased the BNP and NT-proBNP levels (p is less than 0.001). Compared with the ARDS-untreated group, spironolactone treatment was more effective at reducing the elevated BNP (72 percent versus 37 percent) and NT-proBNP (53 percent versus 23 percent) levels and ALI score (28 percent versus 7 percent) than surfactant treatment. Moreover, the blood PO_2/FiO_2 ratio was negatively correlated with the BNP (r is −0.79), NT-proBNP (r is −0.85) and ALI scores (r is −0.85).

Conclusion: Spironolactone is an effective form of treatment for ARDS at an early stage, as reflected by an increased blood O_2/FiO_2 ratio, decreased BNP and NT-proBNP levels, and ALI score.

Keywords: acute lung injury, acute respiratory distress syndrome, blood gas, brain natriuretic peptide, spironolactone, surfactant

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INTRODUCTION

Direct or indirect injury to the lungs causes acute respiratory distress syndrome (ARDS), which is also known as noncardiogenic pulmonary oedema. It is associated with hypoxia and oedema due to increased permeability of the pulmonary capillary endothelia.[1] The common characteristics of ARDS include type II alveolar cell hyperplasia, interstitial fibrosis and metaplasia. Gastric acid aspiration can cause severe acute lung injury (ALI) with characteristics similar to those of ARDS.[2] Surfactants decrease alveolar surface tension and protect pulmonary mechanics and fluid balance.[3] Aldosterone is partially responsible for increases in the extracellular matrix turnover, as observed in fibrosis of the cardiac, kidney and lung tissues,[4,5] and exerts its effects primarily on lung epithelium. Spironolactone is an anti-aldosteronic substance that has the ability to ameliorate pulmonary fibrosis.[6-8] Elevations in the brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in patients with a pulmonary disease could be related to hypoxia and sympathetic activation despite having been secreted from cardiac tissue.[9]

It was hypothesised that spironolactone would be effective in the treatment of ARDS by correcting lung diffusion abnormalities. The primary aim of this study was to compare the therapeutic efficacy of spironolactone with a surfactant in the early stage of ARDS induced by acid respiration in rats. The secondary objective was to evaluate the correlation between blood gases, BNP and NT-proBNP for monitoring ARDS development and its responsiveness to treatment.

METHODS

A total of 40 male Sprague Dawley rats weighing 270–280 g were obtained from the Medical Experiments and Research Centre with the approval of the Ethics Board on Experimental Animal Use. The rats were disease-free and had not been used in any other experiment or

exposed to any drug prior to the current experiment, and they were on a standard diet. The rats were randomly allocated into five groups of eight rats each. There were two control groups: the baseline group (Group B), where the rats were not subjected to tracheotomy or ARDS induction, and the sham group (Group N), where the rats were subjected to tracheotomy but not to ARDS induction. The sham group was designed to evaluate the effects of tracheotomy on the markers. The other 24 rats were assigned to the untreated group (Group A), with either oesophageal sprinolactone administration (Group Sp) or intratracheal surfactant administration (Group S) following ARDS induction.

All procedures were performed under general anaesthesia following an eight-hour starvation period. Rats in all the groups were injected with 50 mg/kg thiopental intraperitoneally for immobilisation, in order to insert the femoral artery catheter while allowing them to breathe spontaneously. A 24G intravenous polyethylene cannula was placed inside the femoral vein after the saphenous nerve was carefully isolated and secured with 3/0 non-traumatic silk suture thread to the proximal and distal segments of the artery. Ringer lactate solution was administered as maintenance fluid.

After the injection of 50 mg/kg thiopental intraperitoneally, the cervical regions of the rats in Group N were shaved and a vertical incision was made on the midline, 1 cm above the carina, and using a 14G intravenous cannula, a tracheotomy cannula was put in place. Anaesthesia was maintained through the inhalation of 35% oxygen and 3% Sevorane (Sevoflurane, 250 ml, Abbott, London, England). Respiration was assisted through the use of a volume-controlled mechanical ventilator (Servo 900 D, Siemens, Solna, Sweden). The mechanical respirator was set at 7 ml/kg for the tidal volume (Vt) and a 60-per-minute frequency of breathing (f). The peak inspiratory pressure was 20 cm H_2O when the inspiration/expiration ratio was set at 1:2.

After tracheotomy, ARDS induction in Group A rats was performed through the administration of 0.1 mol/l hydrochloric acid (HC1) (pH 1.25) into the lungs at a dose of 0.4 ml/kg. The acid was instilled drop-by-drop intratracheally; half of the acid was administered while the animal lay in the left-lateral position and the remaining half when the animal was in the right-lateral position. Rats in Group Sp were injected with a single dose of spironolactone (100 mg/kg Aldactone, Pfizer, New York, NY, USA) via an oesophageal tube in the early stage of the lung injury. Rats in Group S were administered 100 mg/kg surfactant through the trachea (Survanta intratracheal suspension, [25 mg phospholipids and 9 mg sodium chloride/ml, 8 ml], Abbott, Chicago, IL, USA). In addition, one group of rats was not treated (Group A).

Femoral artery catheterisation was performed to measure the arterial blood gas levels and to draw blood samples in all groups. After a stabilisation period of 30 minutes in Group B and six hours in the other groups, blood samples for arterial blood gas, BNP (AssayMax Rat BNP-32 ELISA Kit, Assaypro, St Charles, MO, USA) and NT-proBNP (p-BNP Fragment EIA, Biomedica, Wien, Austria) were taken from the femoral arteries of the rats. The rats were then sacrificed at the end of the procedure by administering tiopental. The lungs were excised and fixed in 10% formaldehyde. The sections (5–7 μ) were stained with haematoxylin-eosin and examined under a light microscope (Olympus EX50, Olympus Optical Company, Tokyo, Japan) under 200 times magnification. ALI was evaluated by the sum of the presence of alveolar congestion, haemorrhage, infiltration of neutrophils to or their aggregation in the alveolus or vessel walls, and thickening of the alveolar wall/formation of hyaline membrane. Minimal or negligible, mild, moderate, severe or maximal damage were scored from 0 to 4, respectively.

The data was analysed using the one-way ANOVA test in the Statistical Package for the Social Sciences version 15.0 (SPSS Inc, Chicago, IL, USA). The groups were compared using Duncan's multiple range and Mann-Whitney-U tests for continuous and discrete data. The Pearson's correlation test was also used to determine the correlations between the response variables. Statistical significance was declared at $p < 0.05$.

RESULTS

The sham tracheotomy operation did not affect the blood PO_2/FiO_2 ratio, BNP and NT-proBNP levels, as well as the ALI score, when compared with the basal group (Table I). ARDS induction decreased the PO_2/FiO_2 ratio by 62% and increased the BNP and NT-proBNP levels and ALI score by 253%, 131% and 142%, respectively. Compared with the ARDS-untreated group, intraperitoneal spironolactone administration was more effective at decreasing the elevated BNP (by 72% vs. 37%) and NT-proBNP levels (by 53% vs. 23%) and ALI score (by 28% vs. 7.0%) than intratracheal surfactant administration. The BNP value in Group Sp was lower than that in Group S ($p < 0.001$). The BNP and NT-proBNP level reached their basal levels with intraperitoneal spironolactone administration but not with intratracheal surfactant administration ($p < 0.001$). Moreover, both drugs equally increased the blood $PO_2/$

Table I. BNP and NT-proBNP levels, PO_2/FiO_2 ratio and ALI score in ARDS and upon treatment with a single dose of surfactant or spironalactone.

	Mean value ± SD (range)				
	Group B	Group N	Group A	Group Sp	Group S
PO_2/FiO_2 ratio	538 ± 45 (487–600)	520 ± 41 (460–587)	202 ± 23* (195–212)	519 ± 35 (482–550)	491 ± 37 (470–520)
BNP (ng/ml)	0.09 ± 0.01 (0.08–014)	0.17 ± 0.02 (0.15–0.20)	0.60 ± 0.07† (0.32–0.85)	0.17 ± 0.02 (0.15–0.20)	0.38 ± 0.02¶† (0.37–0.40)
NT-proBNP (fmol/ml)	292 ± 25 (231–346)	309 ± 35 (255–367)	714 ± 69† (489–600)	338 ± 29 (305–379)	552 ± 39† (500–605)
ALI score	1.38 ± 0.1 (1–2)	1.50 ± 0.1 (1–2)	3.63 ± 0.6[a] (3–4)	2.62 ± 0.5[a] (2–3)	3.38 ± 0.4[a] (3–4)

* Significant differences with the other groups ($p < 0.001$). † Significant differences with Groups B, N and Sp. ($p < 0.001$). ¶ Significant differences with Group A ($p < 0.005$). [a] Significant differences with Groups B and N ($p < 0.001$).
Group B: not subjected to tracheotomy surgery and ARDS induction; Group N: underwent tracheotomy surgery without ARDS induction; Group A: untreated rats after ARDS induction; Group Sp: treated with spironolactone after ARDS induction; Group S: treated with surfactant after ARDS induction; BNP: brain natriuretic peptide; NT-proBNP: N-terminal pro-brain natriuretic peptide; ALI: acute lung injury; ARDS: acute respiratory distress; SD: standard deviation

FiO_2 ratio (by 150%) upon its depression due to ARDS. The ALI score of the ARDS group increased significantly in comparison to the normal group ($p < 0.001$). However, both treatments failed to lower the ALI score.

The response variables were autocorrelated. There was a negative correlation between the blood PO_2/FiO_2 ratio and BNP ($r = -0.79$) and NT-proBNP ($r = -0.85$) levels as well as the ALI score ($r = -0.85$) ($p < 0.001$ for all). However, the correlation between the BNP level and ALI score was weaker ($r = -0.56$, $p < 0.001$).

The histological appearance of the lungs of a rat from Groups B, N, A, Sp and S are shown in Figs. 1–5, respectively. Severe alveolar wall destruction and congestion, haemorrhage, infiltration of neutrophils to or their aggregation in the alveolus and alveolar fluid were observed in ARDS-induced and untreated rats (Fig. 3). Spironolactone treatment was more effective than surfactant treatment in reducing congestion, haemorrhage and alveolar fluid (Figs. 4 & 5).

DISCUSSION

The treatment efficacy of ARDS induced by HCl aspiration with spironolactone and surfactant was compared with respect to arterial blood gas and lung histology, and the significance of alterations to the BNP and NT-proBNP levels during the induction and post-treatment of ARDS was substantiated. In the intensive care unit, respiratory failure often results from ARDS, and patients exhibit intense inflammatory responses such as alveolar flooding and collapse, reduced lung compliance, increased effort in breathing and impaired gas exchange capability in association with the dysfunction of the lung parenchyma.[10,11]

The BNP and NT-proBNP levels were found to be higher than normal in patients with pulmonary disease and dyspnoea, and we believe that this could be related to an increase in the release of natriuretic peptides in response to hypoxia and sympathetic activation.[12] A cut-off BNP level < 100 pg/ml has been reported in acute dyspnoeic patients.[13,14] A BNP cut-off level of 100 pg/ml was thus selected for clinical use in this study (sensitivity 90%, specificity 76%). The prevalence of heart failure in this study population was 47%. The BNP levels were significantly higher (mean 675 pg/ml) among patients with a final diagnosis of heart failure, but were lower (mean BNP 346 pg/ml) among patients with dyspnoea and those with no left ventricular dysfunction or heart failure (mean BNP 110pg/ml).[15] A NT-proBNP level < 300 pg/ml may be sufficient to exclude heart disease.[16] A NT-proBNP level < 450 pg/ml had a sensitivity of 98% and a specificity of 76%.[15] Cut-off values for BNP and NT-proBNP in ARDS can be determined without further studies.

The ARDS treatment is basically symptomatic or supportive, and includes the utilisation of mechanical ventilation with low tidal volumes,[17] positive end expiratory pressure to open the collapsed alveoli,[18] oxygen supplementation and maintenance of the functions of other organs. In this study, ARDS induction remarkably depressed the PO_2/FiO_2 ratio and elevated the blood BNP level and ALI score. Due to the high mortality rate, research has focused on finding effective therapies. In ARDS or ALI patients, increased alveolar permeability is partially due to inactivation of the alveolar surfactant, which is caused by a leakage of plasma proteins into the airspaces in the lungs and a degradation of the alveolar surfactant, which is caused by the actions of proteolytic enzymes or reactive oxygen molecules.[19] Increasing the effective surfactant concentration in the lung augments both quantitative and functional surfactant

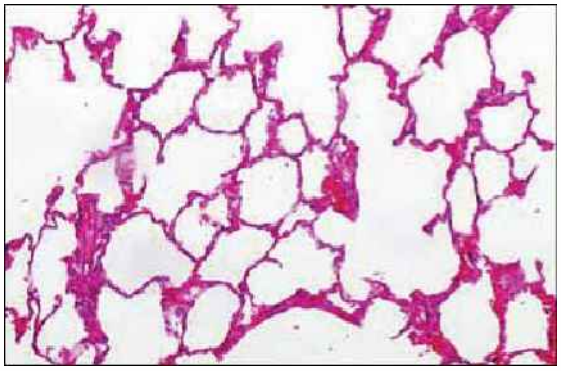

Fig. 1 Photomicrograph shows the lung of a rat not subjected to tracheotomy and acute respiratory distress syndrome induction (Haematoxylin & eosin, × 200).

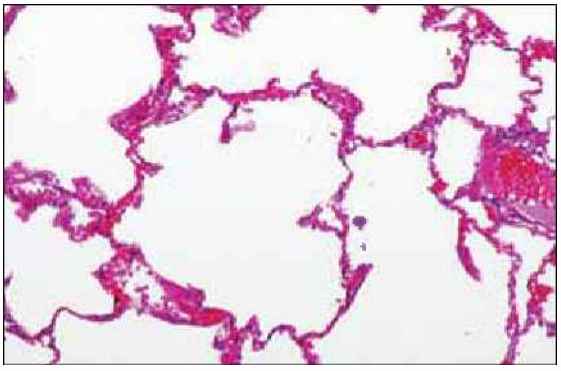

Fig. 2 Photomicrograph shows the lung of a rat that underwent a sham operation (Haematoxylin & eosin, × 200).

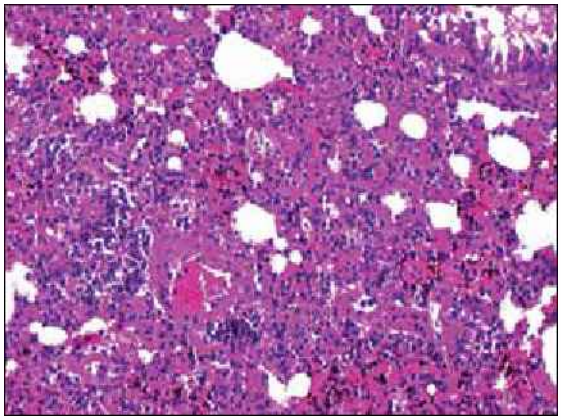

Fig. 3 Photomicrograph shows the lung of an acute respiratory distress syndrome-induced and untreated rat. (Haematoxylin & eosin, × 200).

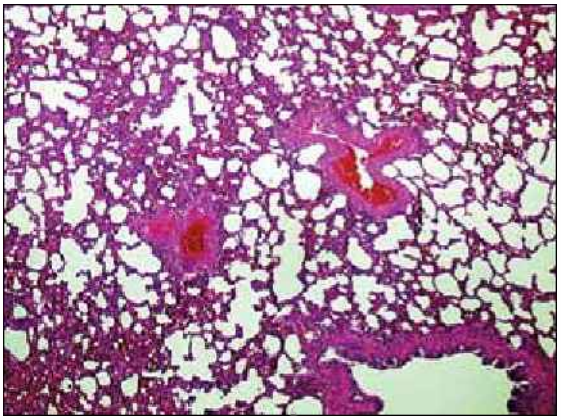

Fig. 4 Photomicrograph shows the lung of a rat treated with intratracheal surfactant after acute respiratory distress syndrome induction (Haematoxylin & eosin, × 200).

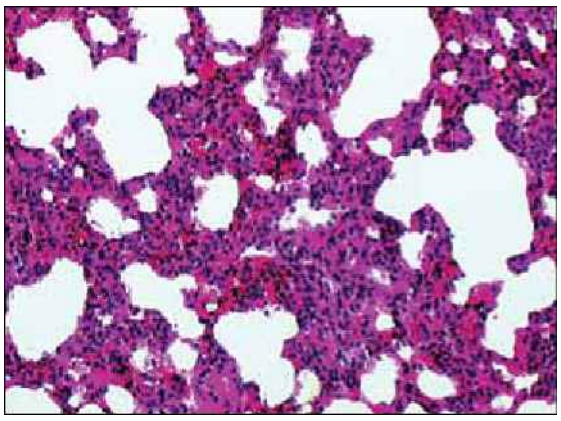

Fig. 5 Photomicrograph shows the lung of a rat treated with oesophageal spironolactone (100 mg/kg) after acute respiratory distress syndrome induction (Haematoxylin & eosin, × 200).

deficiencies, as is reflected by increased oxygenation.[20] Thus, the administration of an exogenous pulmonary surfactant is an adjunctive therapy and benefits adult patients with ARDS.[21-23] The surfactant decreases alveolar surface tension, thereby preventing alveolar collapse and enabling efficient gas exchange at low transpulmonary pressures.[24] Moreover, surfactants play a role in enhancing the immune system, as reflected by suppressed cytokine expression and pro-inflammatory cytokines.[7] Intratracheal surfactant administration in our study restored the blood PO_2/FiO_2 ratio and tended to suppress the blood BNP and NT-proBNP levels, but failed to ameliorate the ALI score.

In ARDS or ALI patients, inflammation causes endothelial dysfunction, fluid extravasation from the capillaries and impaired drainage of fluid from the lungs. This pulmonary oedema increases the thickness of the alveolo-capillary space, thus increasing the distance for the oxygen to diffuse into the blood.[1,2] This impairs gas exchange, leading to hypoxia, an increase in the lung connective tissue, including its matrix, and eventually induces fibrosis of the airspace. Aldosterone is related to extracellular matrix turnover increase, which is associated with cardiac, and possibly lung fibrosis.[4,5,25] Lung cells have aldosterone receptors, and under physiological conditions, aldosterone participates in active sodium transport across the alveolar-capillary membrane.[7,26] It has been shown that aldosterone aids in maintaining the fluid-free lumen of the lung,[27] and is present at

high concentrations in pulmonary fibrosis.[8] In practice, following entubation, fluid aspiration within 12 hours improves the prognosis of ARDS. Aldosterone thus promotes interstitial fibrosis, possibly through local dehydration. Spironolactone administration has been shown to reduce pulmonary fibrosis,[27,28] as well as pulmonary congestion and oedema,[29] by correcting the gas diffusion capacity[30] and renal mineral status.[25] We observed an increase in PO_2/FiO_2 and a reduction in the BNP and NT-proBNP levels and ALI score with spironolactone treatment to a greater extent than with surfactant treatment.

In conclusion, the blood PO_2/FiO_2 ratio was reduced, whereas the BNP and NT-proBNP levels and ALI score were increased due to ARDS. Oesoephageal spironolactone administration increased the blood PO_2/FiO_2 ratio as well as decreased the BNP and NT-proBNP levels and ALI score to a greater extent than intratracheal surfactant administration, thus indicating its efficacy in the treatment of ARDS at an early stage. In addition, the blood PO_2/FiO_2 ratio as well as BNP and NT-proBNP levels can be considered as markers during the treatment and follow-up of patients with ARDS.

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