# The Therapeutic Effects of Methylprednisolone and Surfactant in Acute Respiratory Distress Syndrome in a Rat Model

## Metilprednizolon ve Sürfaktanın Akut Solunum Sıkıntısı Sendromunda Terapötik Etkileri

Nazım DOĞAN, MD,[a]
Şeyda AYKAN, MD,[a]
Canan ATALAY, MD,[a]
Cemal GÜNDOĞDU, MD,[b]
M. Sait KELEŞ, MD[c]

Departments of
[a]Anesthesiology and Reanimation,
[b]Pathology,
[c]Biochemistry,
Atatürk University Faculty of Medicine, Erzurum

Geliş Tarihi/*Received:* 29.11.2009
Kabul Tarihi/*Accepted:* 21.06.2010

*This study is presented as poster in Turkish Anesthesiology and Reanimation Congrees (TARK), 28 October -1 November 2009, Antalya, Turkey.*

Yazışma Adresi/*Correspondence:*
Canan ATALAY, MD
Atatürk University Faculty of Medicine, Department of Anesthesiology and Reanimation, Erzurum, TÜRKİYE/TURKEY
cananatalay@hotmail.com

**ABSTRACT Objective:** To compare the efficacy of methylprednisolone and surfactant in early stages of acute lung injury in rats and to determine whether acute respiratory distress syndrome (ARDS) development and its responsiveness to medical treatment could be assessed by brain natriuretic peptide (BNP) and pro-N type BNP. **Material and Methods:** Fourty rats were randomly allocated into one of five groups, with 8 replicates. Rats in the baseline group (group B) were not subjected to either tracheotomy or ARDS induction, whereas rats in sham group (group N) were subjected only to tracheotomy. After tracheotomy and induction of ARDS by acid aspiration, remaining rats (n= 24) were treated with either a single dose of methylprednisolone (20 mg/kg, group M) or surfactant (100 mg/kg, group S) or left untreated (group A). Six hours later, arterial blood samples were collected for blood gases, BNP, and Pro N-Type BNP measurements. **Results:** Sham tracheotomy did not affect $PaO_2/FiO_2$ ratio, BNP and pro-N type BNP levels, or the acute lung injury (ALI) score. ARDS induction decreased $PaO_2/FiO_2$ ratio by 62% and increased BNP and pro-N type BNP levels, as well as ALI score by 3.5, 2.3, and 2.4-folds, respectively. $PaO_2/FiO_2$ in group A was significantly lower than the controls ($p< 0.001$). We noted an increase in $PaO_2/FiO_2$ with methylprednisolone and surfactant treatment ($p< 0.001$). Both methylprednisolone and surfactant treatments increased $PaO_2/FiO_2$ ratio and decreased BNP and pro-N type BNP levels. There was an increase in BNP in the ARDS group ($p< 0.001$). Compared to the ARDS group, significant reductions were observed in the methylprednisolone and surfactant groups ($p< 0.001$, $p< 0.05$). BNP value in group M was lower than the group S ($p< 0.05$). Pro N-Type BNP increased in rats of the ARDS group ($p< 0.001$). ALI score of the ARDS group increased significantly in comparison to the normal group ($p< 0.001$). However, both treatment modalities failed to reduce Pro N-Type BNP and ALI score. Blood $PaO_2/FiO_2$ ratio showed negative correlations with BNP ($r= -0.78$, $p< 0.001$) and pro-N type BNP ($r= -0.81$, $p< 0.001$) levels. **Conclusion:** $PaO2/FiO_2$ ratio increased whereas BNP level decreased following intraperitoneal methylprednisolone and intratracheal surfactant administration in ARDS-induced rats. $PaO_2/FiO_2$ ratio and BNP may be considered as markers during treatment and follow-up of patients with ARDS.

**Key Words:** Respiratory distress syndrome, adult; brain natriuretic peptide-45; methylprednisolone; pulmonary surfactant.

**ÖZET Amaç:** Sıçanlarda akut akciğer hasarının erken aşamalarında metilprednizolon ve sürfaktanın etkinliğini karşılaştırmak ve akut solunum sıkıntısı sendromu (ARDS) gelişimi ile tıbbi tedaviye cevabının beyin natriüretik peptid (BNP) ve pro-N tipi BNP ile değerlendirilip değerlendirilemeyeceğini belirlemek. **Gereç ve Yöntemler:** Kırk sıçan rastgele beş gruba ayrıldı. Bazal gruptaki (Grup B) sıçanlara ne trakeotomi ne de ARDS indüksiyonu uygulandı, oysa sham grubundaki (Grup N) sıçanlara trakeotomi yapıldı. Asit aspirasyonu ile ARDS indüksiyonunun ardından geri kalan sıçanlara (n= 24) ya tek doz metilprednizolon (20 mg/kg, Grup M) veya sürfaktan (100 mg/kg, Grup S) verildi veya hiçbir tedavi verilmedi (Grup A). Altı saat sonra arteryel kan gazları, BNP ve pro-N tipi BNP ölçümleri için femoral arter kan örnekleri alındı. **Bulgular:** Sham trakeotomi operasyonu $PaO_2/FiO_2$ oranını, BNP ve pro-N tipi BNP düzeylerini ve akut akciğer hasarı (ALI) skorunu etkilemedi. ARDS indüksiyonu $PO_2/FiO_2$ oranını %62 oranında azalttı ve BNP ve pro-N tipi BNP düzeylerini arttırdı ve ALI puanını sırasıyla 3.5, 2.3 ve 2.4 kat artırdı. Grup A'da $PaO_2/FiO_2$ kontrollere göre anlamlı derecede daha düşüktü ($p< 0.001$). Metilprednizolon ve sürfaktan tedavisi ile $PaO_2/FiO_2$'de de bir artış saptadık ($p< 0.001$). ARDS grubunda BNP'de artış vardı ($p< 0.001$). ARDS grubu ile karşılaştırıldığında, metilprednizolon ve sürfaktan gruplarında anlamlı düşüş gözlendi (sırasıyla $p< 0.001$, $p< 0.05$). Grup M'de BNP değeri Grup S'den anlamlı şekilde düşüktü ($p< 0.05$). Pro-N tipi BNP ARDS grubu sıçanlarda arttı ($p <0.001$). ARDS grubunun ALI skoru normal gruba göre anlamlı şekilde arttı ($p <0.001$). Ancak, her iki tedavi pro-N tipi BNP ve ALI skorunu azaltmada başarısız oldu. Kan $PaO_2/FiO_2$ oranı, BNP ($r= -0,78$, $p< 0.001$) ve pro-N tipi BNP ($r= -0,81$, $p< 0.001$) düzeyleri ile negatif korelasyon gösterdi. **Sonuç:** ARDS indüklenmiş sıçanlarda intraperitoneal metilprednizolon ve intratrakeal sürfaktan uygulamasını takiben BNP düzeyi azalırken $PaO_2/FiO_2$ oranı artmıştır. $PaO_2/FiO_2$ oranı ve BNP ARDS'li hastaların tedavisinin izlenmesi için marker olarak kullanılabilir.

**Anahtar Kelimeler:** Solunum sıkıntısı sendromu, yetişkin; beyin natriüretik peptid-45; metilprednizolon; pulmoner sürfaktan

doi:10.5336/medsci.2009-16315



**Turkiye Klinikleri J Med Sci 2011;31(3): 679-85**

Acute respiratory distress syndrome (ARDS) is a functional disorder of the pulmonary capillary endothelia and alveolar epithelia resulting from direct or indirect insult to the lungs.[1,2] Gastric acid aspiration can cause severe acute lung injury (ALI) with characteristics similar to that of ARDS.[3] Increased capillary permeability resulting from decreased blood oxygen saturation leads to a vascular leakage (exudative phase) during the pathophysiological sequelae of the human ARDS. This effluence becomes apparent as a growing perivascular and intraalveolar edema (E) with infiltration of noncellular blood factors, like fibrin, into the lung alveoli.[4,5] These detrimental alterations lead to an increase in inhibition of surfactant activity.[4]

The major causes of mortality in patients who survive acute lung injury/ARDS (ALI/ARDS) develop due to the extensive tissue remodelling and fibrosis.[5,6] Glucocorticoids inhibit both acute, and particularly, chronic inflammatory processes. Inflammation can be inhibited regardless of the etiology. Methylprednisolone can prevent development of pulmonary injury when given before or at the beginning of the inflammation.[7] Surfactant is a substance that decreases alveolar surface tension and protects pulmonary mechanics and fluid balance. Surfactant plays a modulating role in the immune system via inhibiting the cytokine expression and suppressing the releases of pro-inflammatory cytokines, tumor necrosis factor-alpha (TNFα), and interleukin-6 (IL-6).[8] Brain natriuretic peptide (BNP) and N-terminal fragment brain natriuretic peptide (Pro N-type BNP) are secreted from the heart.[9] It may be elevated in ARDS patients, which can be used to differentiate the pulmonary etiology from the cardiac etiology.[10,11]

In the present study, we examined changes in histology and arterial blood gases in response to intraperitoneal methylprednisolone or intratracheal surfactant administrations in early stages of acute lung injury induced by acid aspiration in rats. We also aimed to determine whether the development of ARDS and response to treatment can be monitored by BNP and pro-N type BNP.

## MATERIAL AND METHODS

### RATS

Forty male Sprague Dawley rats weighing 270-280 g were obtained from the Medical Experiments and Research Center of Atatürk University. The experimental procedures mentioned below were approved by the Ethics Board on Experimental Animal Use. Rats were disease-free and not used in any experiment before or exposed to any drug prior to our experiment. They were on a standard diet.

### EXPERIMENTAL GROUPS

Rats were allocated randomly into five groups with eight replicates: Baseline group (Group B), not subjected to tracheotomy and ARDS induction; sham or normal group (Group N), subjected to tracheotomy; and other 24 rats were subjected to tracheotomy and ARDS induction. They were then further divided into three subgroups to be not treated (Group A) and treated with methylprednisolone (Group M) or surfactant (Group S).

### SURGICAL INTERVENTIONS, ARDS INDUCTION, AND TREATMENTS

After eight hours of feed deprivation, all procedures were performed under general anesthesia. Rats in all groups were injected with 0.06 mg atropine sulphate (0.25 mg/ml, Galen İlac San, Türkiye) and 50 mg/kg thiopental intraperitoneally for immobilization for femoral artery catheterization while breathing spontaneously. A 24G intravenous polyethylene cannula was placed inside the femoral vein after isolating it carefully from the saphenous nerve and the cannula secured with a 3/0 non-traumatic silk suture thread on proximal and distal segments of the artery. Ringer lactate solution was given as the maintenance fluid.

After anesthesia induction, injection of 50 mg/kg thiopental intraperitoneally, cervical regions of the rats in Group N were shaved. A vertical incision was made at the midline, 1 cm above the carina, and using a 14G i.v. cannula, a tracheotomy cannula was placed. Anesthesia was maintained by inhalation of 35% oxygen and 3% sevorane (Sevof-

lurane, 250 ml, Abbott, U.S.A). Respiration was assisted by volume-controlled mechanical ventilator (Servo 900 D, Siemens, Sweden). The mechanical ventilator was set at 7 ml/kg for the tidal volume (Vt) and 60/min frequency of breathing (f). Peak inspiratory pressure was 20 cm $H_2O$ while the inspiration/expiration ratio was set at 1:2.

After tracheotomy, ARDS induction was performed via administration of 0.1 mol/l HCl (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 given while the animal lied at left-lateral position and the remaining half when the animal was at right-lateral position.[3]

Upon ARDS induction, rats in Group M were injected with a single dose of intraperitoneal methylprednisolone (20 mg/kg; Prednol-L, 20 mg lyophilized methylprednisolone vial, 2 ml distilled water, Mustafa Nevzat İlaç San, Türkiye) at the early stage of lung injury. Rats in Group S were administered 100 mg/kg surfactant through trachea (Survanta, beractant, intratracheal suspension, 25 mg phospholipids and 9 mg sodium chloride/ml, 8 ml, Abbott, U.S.A). On the other hand, a group of rats were not treated (Group A).

### SAMPLE COLLECTION

Femoral artery catheterization was performed to measure arterial blood gas levels and draw blood samples in all groups. After a stabilization period of 30 minutes in Group B and six hours in other groups, blood samples for arterial blood gas, BNP (Assay Pro, Assay Max Rat BNP-32 ELISA Kit, Lot# 0112824, USA) and Pro N-type BNP (Biomedica, BNP Fragment EIA, Lot# L74BM, USA) were obtained from the femoral arteries of the rats. Rats were sacrificed at the end of the procedure with tiopenthal.

Five rats died during femoral arterial catheterization, while placing tracheotomy cannula, or maintaining mechanic ventilation.

### HISTOPATHOLOGY

Lungs were excised and fixed in 10% formaldehyde. Five-seven micron-thick sections were stained with hematoxylin-eosin and examined under light microscope (Olympus EX50, X40-X400, Japan) under X200 magnification. Acute lung injury (ALI) was evaluated by the sum of presence of alveolar congestion, hemorrhage, infiltration of neutrophils to or their aggregation in the alveolus or vessel walls, and thickening of the alveolar wall/formation of hyaline membrane. Scores were given as 0, 1, 2, 3 or 4 for minimal (negligible), mild, moderate, severe, or maximal damage, respectively.

### STATISTICAL ANALYSIS

Kruskal-Wallis test was used to compare continuous variables and the Chi-square for categorical variables. Binary comparisons were performed using Dunnett's post hoc analysis. A p-value less than 0.05 was considered as statistically significant.

## RESULTS

Sham tracheotomy operation had no effect on $PaO_2/FiO_2$ ratio, BNP and pro-N type BNP levels, or ALI score when compared with basal group (Table 1). ARDS induction dramatically decreased $PaO_2/FiO_2$ ratio (by 62%), which was elevated at a similar magnitude by methylprednisolone (by 127%) and surfactant (by 143%) treatments. $PaO_2/FiO_2$ in Group A was significantly lower than the controls ($p< 0.001$). We noted an increase in $PO_2/FiO_2$ with methylprednisolone and surfactant treatment ($p< 0.001$). Both methylprednisolone and surfactant treatments increased $PaO_2/FiO_2$ ratio and decreased BNP and pro-N type BNP levels. Both methylprednisolone and surfactant treatments decreased BNP level at 60 and 39%, respectively, compared with Group A. There was an increase in BNP in ARDS group ($p< 0.001$). Compared to the ARDS group, significant reductions were observed in the methylprednisolone and surfactant groups ($p< 0.001$, $p< 0.05$). BNP value in Group M was lower than Group S ($p< 0.05$). Pro N-Type BNP was higher in rats of the ARDS group ($p< 0.001$). ALI score of the ARDS group was higher significantly when compared to the normal group ($p< 0.001$). However, both treatments failed to reduce Pro N-Type BNP and ALI score. Blood $PaO_2/FiO_2$ ratio was negatively correlated with BNP ($r= -0.78$, $p< 0.001$) and pro-N type BNP ($r= -0.81$, $p< 0.001$) levels.

**TABLE 1:** Brain natriuretic peptide (BNP) and pro-N type BNP levels, $PO_2/FiO_2$ ratio, and acute lung injury (ALI) score in acute respiratory distress syndrome (ARDS) and upon its treatment with a single dose of intraperitoneal methylprednisolone (20 mg/kg) or intratracheal surfactant (100 mg/kg).

| | Group B | Group N | Group A | Group M | Group S |
|---|---|---|---|---|---|
| $PaO_2:FiO_2$ | 538 ± 45 | 520 ± 41 | 202 ± 23 | 459 ± 35 | 491 ± 37 |
| (Min-max) | (487-600) | (460-587) | (195-212) | (432-487) | (470-520) |
| Median | 528 | 518 | 202* | 461 | 482 |
| BNP, ng/ml | 0.09 ± 0.01 | 0.17 ± 0.02 | 0.60 ± 0.07 | 0.25 ± 0.02 | 0.38 ± 0.03 |
| (Min-max) | (0.08-0.14) | (0.10-0.15) | (0.42-0.85) | (0.23-0.30) | (0.30-0.40) |
| Median | 0.10 | 0.14 | 0.63[ab] | 0.24[+] | 0.38[+b] |
| Pro-N BNP fmol/ml | 292 ± 25 | 309 ± 35 | 714 ± 69 | 560 ± 39 | 552 ± 39 |
| (Min-max) | (231-346) | (255-367) | (687-742) | (475-600) | (500-605) |
| Median | 292 | 302 | 719[+] | 518[+] | 562[+] |
| ALI score | 1.38 ± 0.1 | 1.50 ± 0.1 | 3.63 ± 0.6 | 3.13 ± 0.4 | 3.38 ± 0.4 |
| (Min-max) | (1-2) | (1-2) | (3-4) | (3-4) | (3-4) |
| Median | 1 | 1.5 | 4+ | 3[+] | 3[+] |

Group B= group of rats not subjected to tracheotomy surgery and ARDS induction; N= group of rats undergone tracheotomy surgery without ARDS induction; A= group of untreated rats after ARDS induction; M= group of rats treated with methylprednisolone after ARDS induction; S= group of rats treated with surfactant after ARDS induction. Values are mean ± SD. *significant differences from other groups (p< 0.001). [+]significant differences from Group B and Group N. (P< 0.001). a significant differences from Group B, Group N and Group M (P< 0.001). [b]significant differences from Group M (P< 0.05).

Histological appearances of the lungs of rats in groups B, N, A, M, and S are shown in Figures 1-5, respectively.

## DISCUSSION

In this study, an ARDS model was developed by HCl aspiration in rats and then treatment efficacies of methylprednisolone and surfactant were compared with respect to arterial blood gas and lung histology. Moreover, significance of alterations in BNP and pro-N-type-BNP during induction and treatment of ARDS was questioned. BNP and pro-N-type-BNP are powerful prognostic markers in patients with cardiac disease[11] and BNP has been demonstrated to predict outcome in acute respiratory distress syndrome. Patients with acute respiratory distress syndrome (ARDS) suffer from other illnesses such as cardiac and pulmonary derangement associated with right ventricular strain and noncardiogenic pulmonary edema. These can alter concentrations of cardiac natriuretic peptides.[10]

Corticosteroids are shown to act as immunemodulators and anti-inflammatory agents in the treatment of ARDS, but there is no consistent evidence about their optimal dose, timing, and duration.[12] Recently, Meduri et al.[13] found that the early use of low-dose prolonged methylprednisolone in patients with severe ALI/ARDS significantly relieved the systemic inflammatory response and improved pulmonary and extrapulmonary organ function. Wang et al.[5] reported that low-dose dexamethasone could reduce pulmonary inflammation and fibrosis after LPS-induced ALI in rats. Steinberg et al.[14] reported that methylprednisolone could be harmful to the patient if started 2 weeks after ARDS had developed. Steroids enhance pulmonary functions and improve survival via suppressing the cellular and biochemical markers of

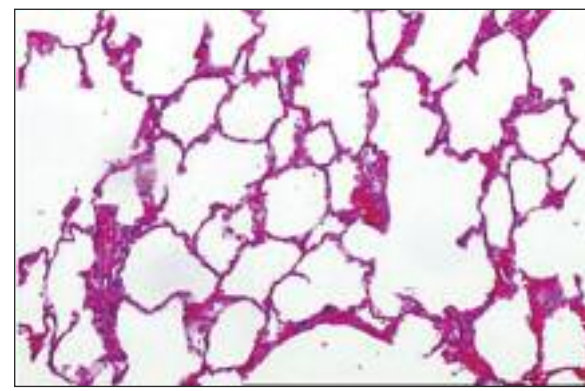

**FIGURE 1:** Histopathological appearance of lung of a rat not subjected to tracheotomy and ARDS induction (LM, H&E, x200).

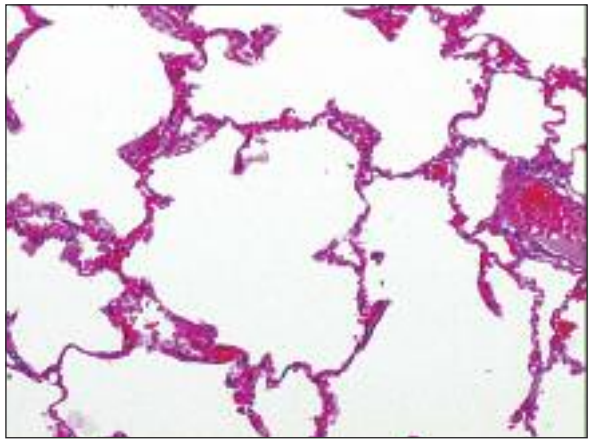

**FIGURE 2:** Histopathological appearance of lung of a rat undergone tracheotomy (LM, H&E, x200).

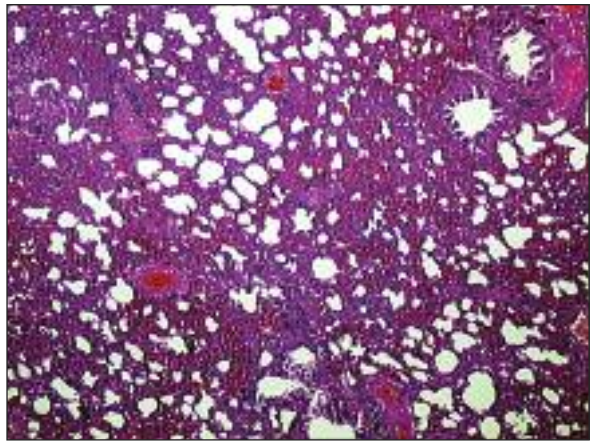

**FIGURE 3:** Histopathological appearance of lung of an ARDS-induced rat (LM, H&E, x200).

inflammation.[15] Meduri et al.[13] reported reductions in lung injury and multiple organ dysfunction scores when methylprednisolone was given for 28 days. Similarly, Annane et al.[16] showed that mortality in ARDS reduced with steroid treatment in early stage. Zhou et al.[17] reported that low-dose hydrocortizone treatment had the most remarkable effects of improving the biological indexes of lung injury, inflammatory mediators and pathological changes in rats. A meta-analysis showed that high dose corticosteroids for a short time was not suggested in the treatment of early-stage ARDS.[18] In fact, prolonged glucocorticoid treatment was shown to result in significant improvement as well as survival rate.[19] In the present experiment, a single dose of methylprednisolone administration intraperitoneally in early stage of ARDS resulted in arterial blood gas oxygenation and reductions in alveolar congestion, hemorrhage, neutrophil infiltration and formation of hyaline membranes. However, effects of repeated corticosteroid administration remain to be investigated.

Surfactant used in this study was extracted from bovine lung and administered directly to the lungs. Hafner et al. reported a dose-dependent increase in reduced $PaO_2$ by protein-containing surfactants.[20] Playfor and Nootigattu showed that exogenous surfactant treatment improved oxygenation and decreased mortality.[21] Halliday reported that natural surfactants were beneficial when admi-

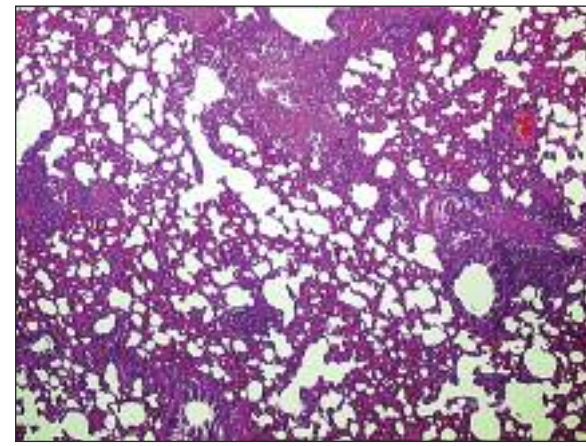

**FIGURE 4:** Histopathological appearance of lung of an ARDS-induced rat treated with intraperitoneal methylprednisolone (LM, H&E, x200).

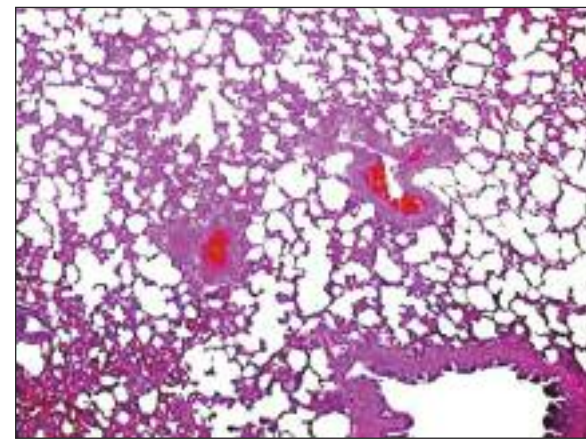

**FIGURE 5:** Histopathological appearance of lung of an ARDS-induced rat treated with intratracheal surfactant. (LM, H&E, x200).

nistered to the lungs by means of a rapid bolus, whereas their delivery by nebulization was ineffective.[22] Hafner and Germann showed that the variation of lavage volume and repetition of lung lavage as well as use of surfactants in their rat model demonstrated good reproducibility of different severity grades and states of the acute lung injury.[23] We found in the present study that surfactant given intratracheally in early-stage primary ARDS improved $PaO_2/FiO_2$ ratio. Exogenous surfactant has been shown to improve surfactant functions and survival in term infants and children with ALI and ARDS, however did not have a significant effect in adult ARDS patients.[24] Taut et al. gave normal intensive care treatment and 50 mg/kg intratracheal rsp-c surfactant to 266 ARDS patients and concluded that rsp-c surfactant improved oxygenation and decreased mortality, irrespective of the predisposing factor.[25] We used a different protein-containing surfactant intratracheally and it was in the same group with rsp-c, and blood gas analysis showed that oxygenation was improved in rats. In our study, surfactant was as effective as methylprednisolone.

The present results raise the question whether BNP and Pro N-Type BNP could be used as indicators in primary ARDS, because we observed that both were elevated in ARDS and reduced with treatment, and negatively correlated with the $PaO_2/FiO_2$ ratio. In general, BNP is less than 25 pg/ml and Pro-N-Type BNP is less than 70 pg/ml in 90% of healthy people. In acute dyspneic patients, BNP less than 100 pg/ml and Pro-N-Type BNP less than 300 pg/ml may be sufficient to exclude heart disease.[26] Karmpaliotis et al. reported that BNP in ALI/ARDS patients was 325 pg/ml, whereas it was 1260 pg/ml in patients with cardiogenic pulmonary edema, suggesting that cardiogenic pulmonary edema and ARDS could be differentiated by BNP values.[26] In this experiment, both BNP and Pro N-Type BNP levels increased with ARDS induction and BNP level decreased with treatment. Their increases were associated with reduction in $PaO_2/FiO_2$ ratio in ARDS, suggesting that BNP release was triggered by hypoxia and sympathetic activation.[27] BNP and ProN-Type BNP were found higher than normal in patients with a pulmonary disease and dyspnea and the authors believed this could be related to an increase in the release of natriuretic peptides in response to hypoxia and sympathetic activation.[28] Cut-off values for BNP and Pro N-Type BNP in ARDS can be determined with further studies.

## CONCLUSION

The results of the present study show that intraperitoneal methylprednisolone and intratracheal surfactant administrations increased $PaO_2/FiO_2$ ratio and decreased BNP level. Both $PaO_2/FiO_2$ and BNP may be considered as markers during treatment and follow-up of patients with ARDS. Cut-off values for BNP and Pro N-Type BNP in ARDS can be determined with further studies.

## REFERENCES


1. Özdemir Kumbasar Ö. [Pulmonary edema and ards]. Turkiye Klinikleri J Surg Med Sci 2006;2(11):79-82.
2. Wiener-Kronish JP, Gropper MA, Matthay MA. The adult respiratory distress syndrome: definition and prognosis, pathogenesis and treatment. Br J Anaesth 1990;65(1):107-29.
3. Safdar Z, Yiming M, Grunig G, Bhattacharya J. Inhibition of acid-induced lung injury by hyperosmolar sucrose in rats. Am J Respir Crit Care Med 2005;172(8):1002-7.
4. Thiel M, Zourelidis C, Peter K. [The role of polymorphonuclear neutrophilic leukocytes in the pathogenesis of acute respiratory distress syndrome (ARDS)]. Anaesthesist 1996;45(2):113-30.
5. Wang XQ, Zhou X, Zhou Y, Rong L, Gao L, Xu W. Low-dose dexamethasone alleviates lipopolysaccharide-induced acute lung injury in rats and upregulates pulmonary glucocorticoid receptors. Respirology 2008;13(6):772-80.
6. Foner BJ, Norwood SH, Taylor RW. The acute respiratory distress syndrome. In: Civetta JM, Taylor RW, Kirby ER, eds. Critical Care. 3rd ed. Philadelphia: Lippincott-Raven; 1997. p.1825-39.
7. Meduri GU, Belenchia JM, Estes RJ, Wunderink RG, el Torky M, Leeper KV Jr. Fibroproliferative phase of ARDS. Clinical findings and effects of corticosteroids. Chest 1991;100(4):943-52.
8. Fanaroff AA, Martin RJ. The respiratory distress syndrome and its management. Neonata-Perinatal Medicine. Diseases of the Fetus and Infant. 5th ed. St Louis, Missouri: Mosby Year Book; 1992. p.810-20.
9. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003;362(9380):316-22.
10. Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC. Prognostic value of plasma N-terminal probrain natriuretic peptide levels in the acute respiratory distress syndrome. Crit Care Med 2008;36(8):2322-7.

11. Berdal JE, Stavem K, Omland T, Hall C, Smith-Erichssen N. Prognostic merit of N-terminal-proBNP and N-terminal-proANP in mechanically ventilated critically ill patients. Acta Anaesthesiol Scand 2008;52(9):1265-72.
12. Peter JV, John P, Graham PL, Moran JL, George IA, Bersten A. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. BMJ 2008;336(7651):1006-9.
13. Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest 2007;131 (4):954-63.
14. Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, et al.; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med 2006;354(16): 1671-84.
15. Reade MC, Milbrandt EB. Is there evidence to support a phase II trial of inhaled corticosteroids in the treatment of incipient and persistent ARDS? Crit Care Resusc 2007;9(3): 276-85.
16. Annane D, Sébille V, Bellissant E; Ger-Inf-05 Study Group. Effect of low doses of corticosteroids in septic shock patients with or without early acute respiratory distress syndrome. Crit Care Med 2006;34(1):22-30.
17. Zhou T, Fan XM, Wang YQ, Qi YJ, Chen H, Qian SY. [Effects of different doses of hydrocortisone on acute lung injury in rats with early septic shock induced by Escherichia coli]. Zhonghua Er Ke Za Zhi 2004;42(9):644-8.
18. Deal EN, Hollands JM, Schramm GE, Micek ST. Role of corticosteroids in the management of acute respiratory distress syndrome. Clin Ther 2008;30(5):787-99.
19. Meduri GU, Marik PE, Chrousos GP, Pastores SM, Arlt W, Beishuizen A, et al. Steroid treatment in ARDS: a critical appraisal of the ARDS network trial and the recent literature. Intensive Care Med 2008;34(1):61-9.
20. Häfner D, Germann PG, Hauschke D. Comparison of rSP-C surfactant with natural and synthetic surfactants after late treatment in a rat model of the acute respiratory distress syndrome. Br J Pharmacol 1998;124(6):1083-90.
21. Playfor SD, Nootigattu VK. Exogenous surfactant in paediatric Acute Lung Injury and Acute Respiratory Distress Syndrome. Curr Drug Saf 2006;1(2):159-68.
22. Halliday HL. Surfactants: past, present and future. J Perinatol 2008;28(Suppl 1):S47-56.
23. Häfner D, Germann PG. A rat model of acute respiratory distress syndrome (ARDS) Part 2, influence of lavage volume, lavage repetition, and therapeutic treatment with rSP-C surfactant. J Pharmacol Toxicol Methods 1999;41(2-3):97-106.
24. Willson DF, Chess PR, Notter RH. Surfactant for pediatric acute lung injury. Pediatr Clin North Am 2008;55(3):545-75, ix.
25. Taut FJ, Rippin G, Schenk P, Findlay G, Wurst W, Häfner D, et al. A Search for subgroups of patients with ARDS who may benefit from surfactant replacement therapy: a pooled analysis of five studies with recombinant surfactant protein-C surfactant (Venticute). Chest 2008;134(4):724-32.
26. Karmpaliotis D, Kirtane AJ, Ruisi CP, Polonsky T, Malhotra A, Talmor D, et al. Diagnostic and prognostic utility of brain natriuretic Peptide in subjects admitted to the ICU with hypoxic respiratory failure due to noncardiogenic and cardiogenic pulmonary edema. Chest 2007;131(4):964-71.
27. Worster A, Balion CM, Hill SA, Santaguida P, Ismaila A, McKelvie R, et al. Diagnostic accuracy of BNP and NT-proBNP in patients presenting to acute care settings with dyspnea: a systematic review. Clin Biochem 2008;41(4-5):250-9.
28. Maeder M, Ammann P, Rickli H, Diethelm M. Elevation of B-type natriuretic peptide levels in acute respiratory distress syndrome. Swiss Med Wkly 2003;133(37-38):515-8.