Enliven Archive
Intensifying Thoughts

Research Article www.enlivenarchive.org Enliven: Journal of Anesthesiology and Critical Care Medicine

ISSN:2374-4448

Magnesium on Blockage of Nuclear Factor-κB Activation at Ventilator-Induced Lung Injury: Random Trial

Regina Grigolli Cesar, MD, PhD[1*], Werther Brunow de Carvalho, MD[2,3]

[1]*Santa Casa De Misericórdia De São Paulo, School of Medicine*

[2]*Full Professor, Intensive Care/Neonatology, Children's Institute, University of São Paulo*

[3]*Faculty of Medicine, Clinics Hospital*

[*]**Corresponding author**: Regina Grigolli Cesar, Santa Casa De Misericórdia De São Paulo, School of Medicine, Brazil, Tel: 55(11) 98101-1617; E-mail: reginautiped@uol.com.br

Received Date: 30th June 2015
Accepted Date: 29th July 2015
Published Date: 04th August 2015

Citation: Ubale P, Mhamane R, Raju S, Pallavi G, Sharma P, et al. (2015) Anaesthetic Management of a Child with Laryngeal Papillomas. Enliven: J Anesthesiol Crit Care Med 2(7): 019.

Abstract

Rationale

The mechanical ventilation may aggravate the severe acute lung injury and trigger a systemic inflammatory response. The pathophysiology evolves Biotrauma, meaning that the over distension and the cyclical opening/collapse of structures on the lung during ventilation with high PIP associated with low PEEP, results in excessive activation of nuclear factor-{kappa}Beta (NF-κB), a regulator of the inflammatory response, that exacerbates the local response and becomes it systemic. The magnesium, a modulator of signaling pathways of the inflammatory cascade, deserves investigation in inhibiting the NF-κB activation.

Objectives

Investigate experimentally the effect of hypermagnesemia to inhibit the activation and migration of NF-κB from cytoplasm to nucleus in cells of lungs injured by mechanical ventilation.

Methods

Two experimental groups of 15 Sprague-Dawley rats each, one receiving 1.25 mL/kg/h of a 10% $MgSO_4$ solution, other receiving equal volume of saline, both under mechanical ventilation with VT of 40 mL/kg, PEEP of 3 cmH_2O, PIP of 35 cm H_2O, RR of 40 cycles per minute. Lungs were excised after 50 minutes of experimental interventions and prepared for further immunohistochemical analysis.

Measurements and Main Results

ANOVA shown no differences when compared the NF-κB activation values in the cytoplasm (712.44 ± 253.86) and nucleus (974.27 ± 344.59) of cells in $MgSO_4$ group with the respective values in cytoplasm (653.99 ± 272.98) and nucleus (952.29 ± 346.68) of cells in Saline group ($F_{[1, 28]} = 0.90$, $p > .05$).

Conclusions

There was no inhibitory effect of magnesium on the activation of NF-kB nor on its expression through migration to the nucleus.

Keywords: Lung injury; Acute; Nuclear factor-{kappa}Beta; Animal experimentation; Rats; Sprague-Dawley

Abbreviations

PIP: Peak Inspiratory Pressure; PEEP: Positive End-Expiratory Pressure; NF-κB: Nuclear Factor-{kappa}Beta; V_T: Tidal volume; RR: Respiratory Rate; CPM: Cycles per Minute; ANOVA: Analysis of Variance; VILI: Ventilator Induced Lung Injury; ARDS: Acute Respiratory Distress Syndrome; IL: Interleukin; mRNA: Messenger RNA; HMGB1: Inflammatory Factor High Mobility Group Box 1; Mg^{2+}: Magnesium ion; $[Mg^{2+}]$: Concentration of Magnesium Ion; $MgSO_4$: Magnesium Sulphate; NMDA: N-Methyl-D-Aspartate (NMDA); PI3K/Akt: Phosphoinositide 3-Kinase/Akt; h: hour; kg: Kilogram; LSAB: Streptavidina-Biotina-Peroxidase (LSAB); I.V.: Intravenous; L: Litre; mL: Milliliter; mm: Milimeter; $PaCO_2$: Partial pressure of carbon dioxide in arterial blood; PaO_2: Partial pressure of oxygen in arterial blood; SaO_2: Oxygen saturation in arterial blood; δ Glass' Effect Size; ρ: Intraclass correlation; Δ: Critical Effect Size; mmHg: Millimeters of Mercury; mEq/L: Milliequivalents per Liter

Introduction

The mortality rate associated with severe acute lung injury exceeds 50%, and this is relate to the multiple organ failure than to hypoxia by respiratory failure [1-5]. Mechanical ventilation can aggravate [6] or even be among the causes of acute lung injury [7], and trigger a systemic inflammatory response.

Ventilator Induced Lung Injury (VILI) do not differ in essence of Acute Respiratory Distress Syndrome (ARDS) [8-10], with edema containing red cells, neutrophils, serum proteins and surfactant, pointing to the increase of permeability of alveolar-capillary barrier [11-18], which pathophysiology involves "biotrauma" [4,19-24]. High pressures during mechanical ventilation potentially stresses the pulmonary architecture, leading to (by over-distension) a loss of integrity of plasmatic membrane in alveolar cells with decompartmentalization into the alveolar space and release of intracellular cytokines to the interstitium and systemic circulation [25]. The cyclical opening/collapse of pulmonary structures release (by mechanical transduction) cytokines and chemokines that induce an excessive activation of nuclear factor-{kappa}Beta (NF-κB) [4,16,26-37].

Some strategies aiming to block the NF-κB activation are based on inhibition of its migration for the nucleus [38-41]. Angiotensin-converting enzyme-2 [42] and the activator protein (AP)-1 [43], e.g., have been tested in the lipopolysaccharide–induced lesion model of the pulmonary microvascular endothelial cells, but not in the VILI model. Since Trendelenburg (1912) accepted, and Rosello and Pla (1936) demonstrated, the effect of magnesium as a potential bronchodilator, its low oral intake has been related to airway hyper-reactivity, and oral supplementation apparently helps to control asthma in pediatric patients [44]. Magnesium has a potential effect in inhibiting inflammation [45].

Alveolar macrophages of hypomagnesemic rats show high serum levels of IL 1-b e IL 6, as well as induction of mRNA for IL 1-β and TNF-α [46], possibly explaining the bronchial hyperreactivity [47]. Moreover, hypomagnesemia is frequently observed in patients with higher risk to develop ARDS [48,49]. Magnesium sulphate ($MgSO_4$), useful in acute exacerbation of asthma at high doses [50], inhibits (in vitro) the secretion of the macrophage secretion of the inflammatory factor high mobility group box 1 (HMGB1) during experimentally induced sepsis [51], and can attenuates pulmonary oxygen toxicity [52]. The magnesium-related mechanism of inhibition of the NF-κB activation and its signaling pathway apparently involves the calcium channels regulation [53-55], especially the L-type calcium channels [56,57] and N-methyl-D-aspartate (NMDA) antagonist [57], with anti-inflammatory effect mediated by increase of the phosphoinositide 3-kinase/Akt (PI3K/Akt) activity [58].

This study aimed to investigate experimentally the effect of hypermagnesemia to inhibit the activation of NF-kB in lungs injured by mechanical ventilation.

Methods and Materials

This study was approved by the Review Board for human and animal studies of the Santa Casa de São Paulo School of Medicine.

Trial Design

Two experimental groups, composite by Sprague-Dawley rats randomly selected to receive high dose of magnesium sulfate ($MgSO_4$ Group), or equal volume of saline solution (Saline Group: controls), both under mechanical ventilation with harmful parameters. Their lungs were compared for the cytoplasm and nuclear values of activated NF-κB (obtained by immunohistochemical analysis) after exposition to the experimental condition ($MgSO_4$ or Saline).

Histopathology and Immunohistochemistry

The histopathologycal changes in lungs, useful for determination of the success in obtain VILI, are reversible, so its need be fixed during the experimental conditions that induce lesions [13], before euthanasia. After excised, the lungs may be fixed and conserved in 10% formalin for 48h, embedded in paraffin, and routinely processed. The lungs were stained with hematoxylin and eosin for routine histological analysis [59].

The expression of transcription nuclear factor-{kappa}Beta (NF-κB) p65 [21] was analyzed by Complex Streptavidina-Biotina-peroxidase (LSAB) Method. The coloration guarantee the identification on nucleus (NF-κB actived) and on cytoplasm.

The extension of the lesion and the intensity of the immunohistochemical expression were determinated by Stereologic Method [60].

Data for normal lungs (non-ventilated) of Sprague-Dawley rats were previously determinated, and revealed that usually exist a small Density (cells or points of fluid/mm^2) of red cells, neutrophils and linfocites in the parenchyma, and perivascular fluid (Table 2).

All the procedures involved in histopathology and immunohistochemistry from the fixation of excised lungs, were performed by a pathologist expert in lung analysis from Department of Pathology of Santa Casa de Misericórdia de São Paulo.

Table 2. Means and standard deviations (s) for inflammatory cells, hemorrhage and interstitial edema, expressed in terms of Density (points of lesion/μm^2), used to verify the efficacy to obtain lung injury in ventilated rats of experimental groups ($MgSO_4$ and Saline) when compared to non-ventilated rats (normal lungs).

Density*	$MgSO_4$ group	Control group	Non-ventilated
Inflammatory cells	1468.50 ± 93.54	1262.02 ± 129.37	799.99 ± 157.38
Red cells	2843.82 ± 152.85	2655.51 ± 200.67	1649.97 ± 285.32
Interstitial edema	13752.00 ± 3541.06	23006.49 ± 2264.14	9209.43 ± 1741.90

* = cells or points of edema/mm2

The values are presented as mean ± SEM

The inflammatory cells are neutrophils and linfocites.

Sample Size Determination and Statistical Analysis

No background information was found to estimate the standard deviation in the Sprague-Dawley population for values of the independent and dependent variables considered in this study: so, the Glass' Effect Size (δ) remains 0.5 [61] for all outcomes. A high (0.9) intraclass correlation (ρ) is expected for data that are obtained of each subject is your own control. Indeed, this study involves two independent samples, with each group treated as a single sample in a pre-post design [61]. Thus, Critical Effect Size (Δ) is 0.75, and at least 11 (10+1) cases per group are necessary for a significance level of 5% for one-tailed parametric tests, and guarantee 90% power for Paired *t*-Test and Two-factor Analysis of Variance with repeated measures on one factor [61,62] and comparisons among treatment means by Fisher-*lsd* [62].

Subjects

Thirty four male Sprague-Dawley rats were necessary to compound the two experimental groups with a equal minimal size of 11 subjects, considering that the ventilated rats were randomly exposed to one of the two experimental conditions and that 4 rats data were discharged: 2 rats that died during induction of pulmonary lesions and 2 lungs not good for analysis. At end, did remain for analysis data of 15 rats in "$MgSO_4$ Group" and 15 rats in "Saline Group".

For allocation, the author used a list of random numbers: even numbers: infusion of 10% $MgSO_4$ solution; odd numbers: infusion of saline solution.

Materials

JELCO® catheter (16 gauge); Siemmens-900® ventilator; ampoules of 10% magnesium sulfate solution, thiopental sodium, saline solution, 10% formalin solution, provided by the Central Laboratory of Santa Casa de Misericórdia de São Paulo; *kit* NF-κB p50, United States Biological.

Procedures

– Jejum for 6h before anesthesia. The RR was registered at rest. Then, anesthesia with thiopental sodium (50 mg/kg) by via intra-peritoneal injection;

– Tracheal intubation for mechanical ventilation, remained at physiological parameters (V_T: 5 mL/kg; FiO_2: 21%; PEEP: 5 mmHg; RR: 40 cpm) during the surgical procedures: right jugular vein dissection and cannulation for intravenous infusions (I.V.); dissection of right carotid artery for laboratory tests;

– Obtaining of blood sample (2 mL) for baseline values of [Mg^{2+}], $PaCO_2$, PaO_2 and SaO_2;

– Then, aiming to reach hypermagnesemia, magnesium was infused in a high dose (1,25 mL/kg/h of a 10% $MgSO_4$ solution) for rats numbered with even numbers, and an equivalent volume of saline for the other rats;

– At the start of continuous I.V. infusion, the mechanical ventilator was adjusted to harmful values: V_T = 40 mL/kg; PEEP = 3 cm H_2O, PIP = 35 cm H_2O; RR = 40 cpm, remained until the end of infusion, *i.e.*, 50 minutes.

– After the end of the infusion, new blood samples were obtained for serum [Mg^{2+}], $PaCO_2$, PaO_2 and SaO_2 determinations.

– Then, infusion in right inner jugular vein of 20 mL of saline, followed by 20 mL of 10% formalin solution for the microscopy fixation of the lungs, that were then excised *en bloc*;

– At last, euthanasia with I.V. infusion of thiopental sodium, 50 mg/kg in *bolus*.

Conclusion

It is concluded that, for the subjects of this study, there were no inhibitory effect of high dose of magnesium on the activation of NF-κB neither on its expression through migration to the nucleus. In respect to its role on the systemic inflammatory response and on failure of multiple organs, both would not have been avoided in these rats. About local (pulmonary) inflammatory response, seems that magnesium was the responsible by avoiding interstitial edema.

Results and Discussion

Efficacy in Control of Potentially Intervenient Variables

A *post hoc* analysis of variance for multiple comparisons between $MgSO_4$ Group (N = 15) and Saline Group (N = 15) means (Table 1) revealed (considering each subject as your own control) that these two groups were similar for these means when they were compared at baseline and at post-ventilation states of $PaCO_2$ ($F_{[1, 28]} = 3.06$, $p > .05$).

After mechanical ventilation, both the groups showed higher PaO2 means ($F_{[1, 28]} = 6.10$, $p < .05$), without difference between them.

The $MgSO_4$ Group had higher SaO_2 mean after ventilation ($F_{[1, 28]} = 5.14$, $p < .05$) (Table 1).

When compared with normal lungs (N = 15) predetermined means (Table 2), both ventilated groups had higher means of inflammatory infiltrate (neutrophils and linfocites) ($F_{[2, 42]} = 6.53$, $p > .05$) and of red cells ($F_{[2, 42]} = 7.95$, $p > .05$), without differences between the groups, but the MgSO4 Group did not develop interstitial edema ($F_{[1, 42]} = 1.40$, $p > .05$).

Table 1. Gasometric parameters and serum concentration of magnesium ([Mg2+]), respectively used to verify the acid-base homeostasis and the efficacy to obtain hypermagnesemia, registered at pre (baseline) and post interventions (sulphate magnesium infusion and mechanical ventilation), for MgSO4 group (N = 15) and Saline group (N = 15).

	MgSO4 group	MgSO4 group	Saline group	Saline group
	pre	post	pre	post
PaCO2 (mmHg)	45.98 ± 3.56	39.67 ± 2.95	39.64 ± 3.53	44.88 ± 4.80
PaO2 (mmHg)	99.99 ± 6.32	144.40 ± 21.18	82.90 ± 8.69	103.18 ± 21.30
SaO2 (%)	0.967 ± 0.004	0.979 ± 0.003	0.909 ± 0.025	0.873 ± 0.057
[Mg2+] mEq/L	1.9 ± 0.1	11.1 ± 2.2	2.1 ± 0.1	1.8 ± 0.1

The values are presented as mean ± SEM

Paired *t*-test of comparisons between the serum [Mg^{2+}] at base-line and after infusions (Table 1), reveals post-infusion hypermagnesemia in $MgSO_4$ Group ($t = 4.130$, $df = 14$, $p < .05$), and consume of magnesium in the controls (Saline Group) ($t = 5.691$, $df = 14$, $p < .05$).

Hypermagnesemia and the Blockage of NF-κB Activation

The effectiveness of inhibiting NF-κB activation and expression in lungs harmed by mechanical ventilation was analyzed through the comparison of the proportion of activated NF-κB in the cytoplasm and in the nucleus of the lungs of the subjects of experimental groups, reflecting the migration to nucleus for its expression.

Analysis of variance (two-way, repeated measures on one factor) reveal that activated NF-κB density means were higher in nucleus (974.27 ± 344.59) than in the cytoplasm (712.44 ± 253.86) in $MgSO_4$ Group lung cells, and also higher in nucleus (952.29 ± 346.68) than in the cytoplasm (653.99 ± 272.98) in Saline Group lung cells, without difference between groups (Table 3 and Table 4, Figure 1 and Figure 2).

Table 3. Summary table of analysis of variance, for comparisons among NF-κB activation Density at cytoplasm and nucleus of pulmonary cells for the ventilated rats ($MgSO_4$ and Saline groups).

Source of variation	SS	DF	MS	F
Citoplasm versus nucleus	1176552.28	1	1176552.28	99.87*
Between groups	24258.00	1	24258.00	0.13
Within groups	5338531.27	28	190661.83	
Cytoplasm versus nucleus versus groups	4987.37	1	4987.37	0.42
Subjects versus cytoplasm versus nucleus	329850.27	28	11780.37	
Total	6874179.19	59		

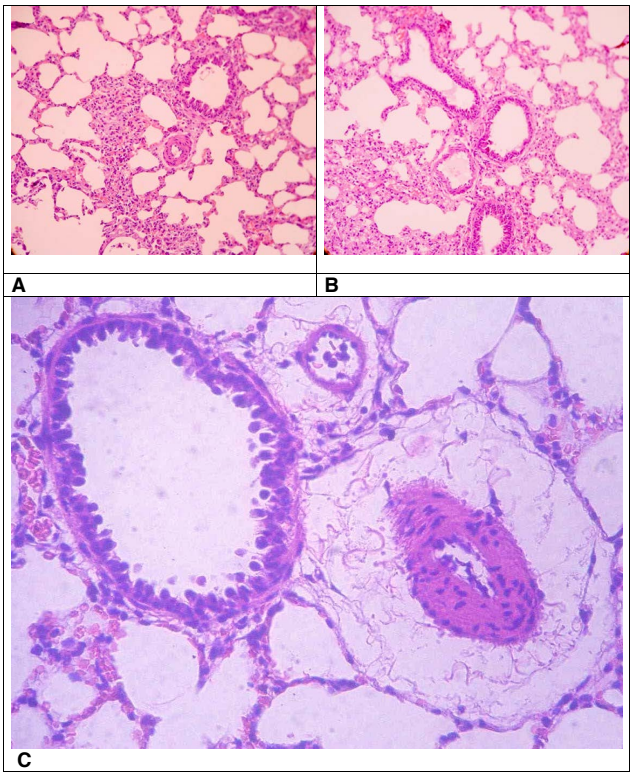
* = significance ($p < 0.05$), F0,95[1, 28] = 4.20

Abbreviations: SS = sum of squares; df = degrees of freedom; MS = mean squares

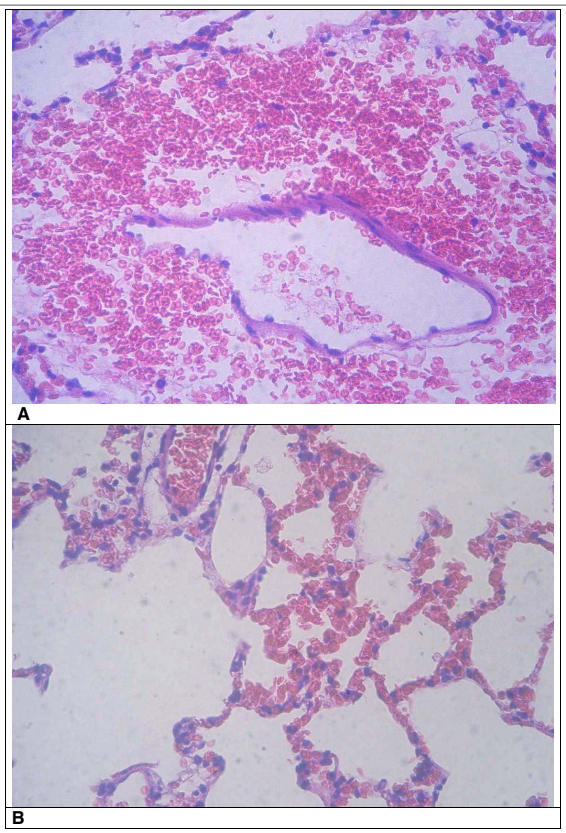
Table 4. F values for comparisons by Fisher-lsd.

Comparações	*F*
Saline group: cytoplasm versus nucleus	56.65*
MgSO4 group: cytoplasm versus nucleus	43.65*
Cytoplasm: Saline group versus MgSO4 group	0.31
Nucleus: Saline group versus MgSO4 group	2.17

* = significance ($p < 0.05$), F0,95[1, 28] = 4.20



Hematoxylinandeosinstain. 100x. A: Lungofthe non-ventilatedrat n. 6; B: Lungoftherat n. 15 (MgSO4group), without perivascular edema; C: Perivascular/intersitialedema, rat n. 7 (Saline Group).



Hematoxylinandeosinstain. 100x. A: Lungofthe non-ventilatedrat n. 6; B: Lungoftherat n. 15 (MgSO4group), without perivascular edema; C: Perivascular/intersitialedema, rat n. 7 (Saline Group).

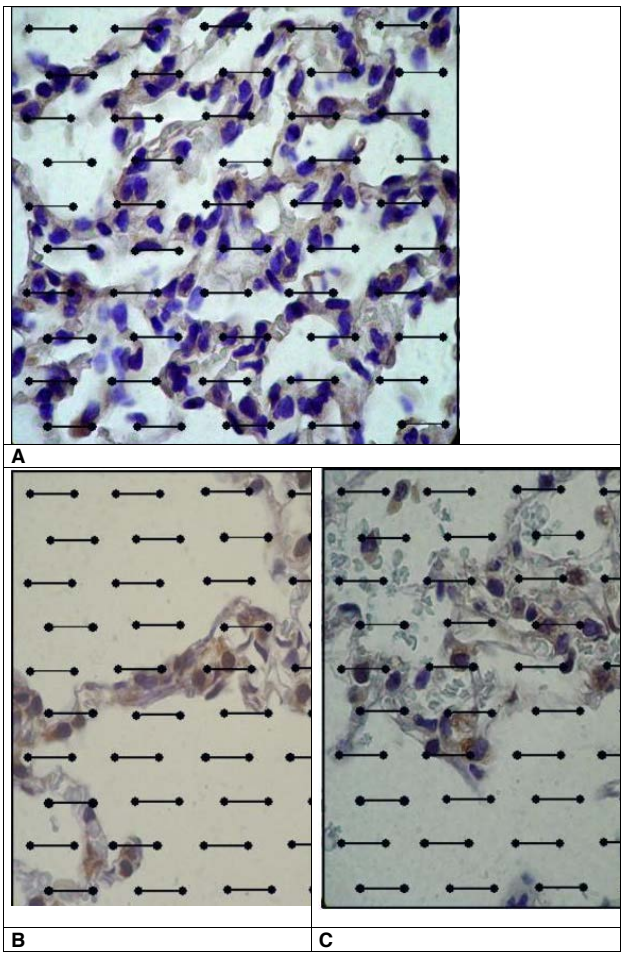


Figure 4. It isconsidered positive expressionoftheantigen nuclear transcriptionfactor- {kappa} B (NF-κB) p65when it isobservedcytoplasmicand nuclear staining in brownliningcellsoftheairways (internal positive control), pneumocytesand alveolar macrophages. A: Non-ventilatedrat n. 8; B: rat n. 6 of Saline Group; B: rat n. 11 ofMgSO4group.

Discussion

Although any significant difference among subjects of a same breed (Sprague-Dawley rats) was not expected before the experimental treatments, a *post hoc* analysis revealed that the $MgSO_4$ and Saline (control) groups, so that each subject, were comparable at the base line state. Moreover, in comparison with control group, data of experimental group revealed that the intravenous $MgSO_4$ infusion posology was enough to induce hypermagnesemia.

The histopathological analysis showed that there were success in the induction of lung damage with the mechanical ventilation parameters adopted in both groups, so that the effect of independent variable, probably, may not be suffered meddling of spurious variability: The isolation of the hypermagnesemia effect on the NF-κB activation was, seemingly, guaranteed.

The results obtained reveal that the NF-κB activation occurred during the induction of lung injury in both control and $MgSO_4$ groups in a same extension, leading to conclusion that, in these Sprague-Dawley rats, $MgSO_4$ did not have effect on this activation. However, two findings deserved attention: the consumption of Mg^{2+} during lung damage induction; in the group that received $MgSO_4$, the lungs showed less perivascular edema. The consumption of Mg^{2+} is not surprising, to the extent that is frequently observed in critically ill patients, and that Dedhia and Banks [48] and Bohmer [49] already had related hypomagnesemia as a higher risk of ARDS development. Maybe this consumption is related to the pathophysiologic mechanisms of acute lung injury, deserving future investigation.

About the perivascular edema, the lungs of the rats that received $MgSO_4$ showed, at mean, the same proportion of perivascular fluid as the non-ventilated subjects; those that received isotonic saline solution and developed hypomagnesemia post-VPM evolved with pulmonary edema. These results suggest a protective effect of magnesium on the formation of lung edema during pulmonary ventilation with potentially harmful parameters.

Whereas that in the VILI the edema imply fluidic overflow for the interstitial space, with alveolar flood partially due to high pressures over lung structures during alveolar recruitment [23,24], this finding deserves experimental investigation, particularly because the magnesium, that has a confirmed effect as an coadjuvant on control of acute asthma (edema has a relevant role in Asthma), may be involved in the tissular fluid homeostasis.

Acknowledgments

The authors thank to:

Fabíola Del Carlo Bernardi, MD, PhD, Pathologist, by histopathological and immunohistochemical analysis;

Hugo Hideo Kunni, MD, who intermediated the Sprague-Dawley rats acquisition at the Laboratory of Animal Research of University of Campinas (UNICAMP), São Paulo, Brazil, and its transportation to the Unity of Experimental Surgical Technique (UTECE) of Santa Casa de São Paulo School of Medicine (FCMSCSP), São Paulo, Brazil.

References

1. Montgomery AB, Stager MA, Carrico CJ, Hudson LD (1985) Causes of mortality in patients with adult respiratory distress syndrome. Am Rev Respir Dis 132: 485-489.
2. Ferring M, Vincent JL (1997) Is outcome from ARDS related to severity of respiratory failure? Eur Respir J 10: 1297-1300.
3. Hudson LD (1999) Progress in understanding ventilator-induced lung injury. JAMA 282: 77-78.
4. Dos Santos CC, Slutsky AS (2000) Invited review: Mechanisms of ventilator-induced lung injury: A perspective. Cellular responses to mechanical stress. J Appl Physiol 89: 1645-1655.
5. Lee WL, Detsky AS, Stewart TE (2000) Lung-protective mechanical ventilation strategies in ARDS. Intensive Care Med 26: 1151-1155.
6. Webb HH, Tierney DF (1974) Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures; protection by positive end-expiratory pressure. Am Rev Respir Dis 110: 556-565.
7. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, et al. (2013) European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants - 2013 update. Neonatology 103: 353- 368.
8. Tsuno K, Miura K, Takeya M, Kolobow T, Morioka T (1991) Histopathologic pulmonary changes from mechanical ventilation at high peak airway pressures. Am Rev Respir Dis 143: 1115-1120.
9. Dreyfuss D, Saumon G (1998) Ventilator-induced lung injury: lessons from experimental studies. Am J Respir Crit Care Med 157: 294-323.
10. Martin GS (2001) Update on Acute Lung Injury: Mechanisms of Action. 30th International Educational and Scientific Symposium of the Society of Critical Care Medicine - 2001. Crit Care Med 29: 225-226.
11. Baughman RP, Gunther KL, Rashkin MC, Keeton DA, Pattishall EN (1996) Changes in the inflammatory response of the lung during acute respiratory distress syndrome: prognostic indicators. Am J Respir Crit Care Med 154: 76-81.
12. Nève V, de la Roque ED, Leclerc F, Leteurtre S, Dorkenoo A, et al. (2000) Ventilator-induced overdistention in children. Dynamic versus low-flow inflation volume-pressure curves. Am J Respir Crit Care Med 162: 139-147.
13. Vlahakis NE, Hubmayr RD (2000) Cellular responses to mechanical stress. Invicted Review: Plasma membrane stress failure in alveolar epithelial cells. J Appl Physiol 89: 2490-2496.
14. Ricard JD, Dreyfuss D, Saumon G (2002) Ventilator-induced lung injury. Curr Opin Crit Care 8: 12-20.
15. Kao SJ, Su CF, Liu DD, Chen HI (2007) Endotoxin-induced acute lung injury and organ dysfunction are attenuated by pentobarbital anaesthesia. Clin Exp Pharmacol Physiol 34: 480-487.
16. Chiang CH, Pai HI, Liu SL (2008) Ventilator-induced lung injury (VILI) promotes ischemia/reperfusion lung injury (I/R) and NF-kappaB antibody attenuates both injuries. Resuscitation 79: 147-154.
17. Henes J, Schmit MA, Morote-Garcia JC, Mirakaj V, Köhler D, et al. (2009) Inflammation-associated repression of vasodilator-stimulated phosphoprotein (VASP) reduces alveolar-capillary barrier function during acute lung injury. FASEB J 23: 4244-4255.
18. Calverley P, Miserocchi G, Maestu LP, Aliverti A, De Backer W, et al. (2010) The changing face of respiratory physiology: 20 years of progress within the ERS. Eur Respir J 35: 945-948.
19. Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, et al. (1999) Effect of mechanical ventilation on inflamatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. JAMA 282: 54-61.
20. Allen GB, Pavone LA, DiRocco JD, Bates JHT, Nieman GF (2005) Pulmonary impedance and alveolar instability during injurious ventilation in rats. J Appl Physiol 99: 723-730.
21. Zhang XT, Liu J, Yu X, Ning Q, Luo XP (2009) Lipopolysaccharide and hyperoxia induce nuclear factor-kappa B expression in human embryo lung fibroblasts in vitro. Zhongguo Dang Dai Er Ke Za Zhi 23: 4244-4255.
22. Hubmayr RD (2005) Ventilator-induced lung injury without biotrauma? J Appl Physiol 99: 384-385.
23. Bernard GR, Artigas A, Brighan KL, Carlet J, Falke K, et al. (1994) Definitions, mechanisms, relevant outcomes, and clinical trial coordination. The American-European Consensus in ARDS. Am J Respir Crit Care Med 149: 818-824.
24. Artigas A, Bernard GR, Carlet J, Dreyfuss D, Gattinoni L, et al. (1998) Ventilatory, pharmacologic, supportive therapy, study design strategies, and issues related to recovery and remodeling. The American-European Consensus Conference on ARDS, Part 2. Am J Respir Crit Care Med 157: 1332-1347.
25. Halbertsma FJJ, Vaneker M, Scheffer GJ, van der Hoeven JG (2005) Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. The Netherlands Journal of Medicine 63: 382-392.
26. Held H-D, Boettcher S, Hamann L, Uhlig S (2001) Ventilation induced chemokine and cytokine release is associated with activation of nuclear factor-kB and is blocked by steroids. Am J Respir Crit Care Med 163: 711-716.

27. Poynter ME, Cloots R, van Woerkom T, Butnor KJ, Vacek P (2004). NF-κB activation in airways modulates allergic inflammation but not hyperresponsiveness. J Immunol 173: 7003-7009.

28. Cheng DS, Han W, Chen SM, Sherrill TP, Chont M, et al. (2007) Airway epithelium controls lung inflammation and injury through the NF-kB pathway. J Immunol 178: 6504-6513.

29. Alcorn JF, Ckless K, Brown AL, Guala AS, Kolls JK, et al. (2010) Strain-dependent activation of NF-kappaB in the airway epithelium and its role in allergic airway inflammation. Am J Physiol Lung Cell Mol Physiol 298: L57-66.

30. Desmet C, Gosset P, Pajak B, Cataldo D, Bentires-Alj M, et al. (2004) Selective blockade of NF-κB activity in airway immune cells inhibits the effector phase of experimental asthma. J Immunol 173: 5766-5775.

31. Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr (1999) Control of inducible chemoresistance enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-[kappa]B. Nat Med 5: 412-417.

32. Sun Z, Anderson R (2002) NF-[kappa]B activation and inhibition: a review. Shock 18: 99-106.

33. Lentsch AB, Pathrose P, Kader S, Kuboki S, Collins MH, et al. (2007) The Ron Receptor Tyrosine Kinase regulates acute lung injury and suppresses nuclear factor [kappa]B activation. Shock 27: 274-280.

34. Sheller JR, Polosukhin VV, Mitchell D, Cheng DS, Peebles RS, et al. (2009) Nuclear factor kappa B induction in airway epithelium increases lung inflammation in allergen-challenged mice. Exp Lung Res 35: 883-895.

35. Pantano C, Ather JL, Alcorn JF, Poynter ME, Brown AL, et al. (2008) Nuclear factor-κB activation in airway epithelium induces inflammation and hyperresponsiveness. Am J Respir Crit Care Med 177: 959-969.

36. Sadikot RT, Han W, Everhart MB, Zoia O, Peebles RS, et al. (2003) Selective IκB kinase expression in airway epithelium generates neutrophilic lung inflammation. J Immunol 170: 1091-1098.

37. Everhart MB, Han W, Sherrill TP, Arutiunov M, Polosukhin VV, et al. (2006) Duration and intensity of NF-κB activity determine the severity of endotoxin-induced acute lung injury. J Immunol 176: 4995-5005.

38. Granja T, Köhler D, Mirakaj V, Nelson E, König K, et al. (2014) Crucial role of Plexin C1 for pulmonary inflammation and survival during lung injury. Mucosal Immunol 7: 879-891.

39. Taggart CC, Cryan SA, Weldon S, Gibbons A, Greene CM, et al. (2005) Secretory leucoprotease inhibitor binds to NF-κB binding sites in monocytes and inhibits p65 binding. J Exp Med 202: 1659-1668.

40. von Bismarck P, Klemm K, García Wistädt CF, Winoto-Morbach S, Schültze S, et al. (2009) Selective NF-kappaB inhibition, but not dexamethasone, decreases acute lung injury in a newborn piglet airway inflammation model. Pulm Pharmacol Ther 22: 297-304.

41. MacIntyre NR (2000) A view to the future of treatments for acute lung injury. 96th International Conference of the American Thoracic Society.

42. Li Y, Cao Y, Zeng Z, Liang M, Xue Y, et al. (2015) Angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas axis prevents lipopolysaccharide–induced apoptosis of pulmonary microvascular endothelial cells by inhibiting JNK/NF–κB pathways. Sci Rep 5: 8209.

43. Liu G, Ye X, Miller E, Liu SF (2014) NF-κB-to-AP-1 Switch: A mechanism regulating transition from endothelial barrier injury to repair in endotoxemic mice. Sci Rep 4: 5543.

44. Gontijo-Amaral C, Ribeiro MA, Gontijo LS, Condino-Neto A, Ribeiro JD (2007) Oral magnesium supplementation in asthmatic children: a double-blind randomized placebo-controlled trial. Eur J Clin Nutr 61: 54-60.

45. Dibaba DT, Xun P, He K (2014) Dietary magnesium intake is inversely associated with serum C-reactive protein levels: meta-analysis and systematic review. Eur J Clin Nutr 68: 510-516.

46. Nakagawa M, Oono H, Nishio A (2001) Enhanced production of IL-1β and IL-6 following endotoxin challenge in rats with dietary magnesium deficiency. J Vet Med Sci 63: 467-469.

47. Hoang BX, Levine SA, Graeme Shaw D, Pham P, Hoang C (2006) Bronchial epilepsy or broncho-pulmonary hyper-excitability as a model of asthma pathogenesis. Med Hypotheses 67: 1042-1051.

48. Dedhia HV, Banks DE (1994) Pulmonary response to hyperoxia: Effects of magnesium. Environ Health Perspect 102: 101-105.

49. Bohmer T (1995) Magnesium in lung diseases. Tidsskr Nor Laegeforen 115: 827-828.

50. Gao F, Ding B, Zhou L, Gao X, Guo H, et al. (2013) Magnesium sulfate provides neuroprotection in lipopolysaccharide-activated primary microglia by inhibiting NF-κB pathway. J Surg Res 184: 944-950.

51. Liu Z, Zhang J, Huang X, Huang L, Li S, et al. (2013) Magnesium sulfate inhibits the secretion of high mobility group box 1 from lipopolysaccharide-activated RAW264.7 macrophages in vitro. J Surg Res 179: 189-195.

52. Flink EB, Dedhia HV, Dinsmore J, Doshi HM, Banks D, et al. (1992) High-dose magnesium sulfate attenuates pulmonary oxygen toxicity. Crit Care Med 20: 1692-1698.

53. Lindeman KS, Hirschman CA, Freed AN (1989) Effect of magnesium sulfate on bronchoconstriction in the lung periphery. J Appl Physiol 66: 2527-2532.

54. Bloch H, Silverman R, Mancherje N, Grant S, Jagminas L, et al. (1995) Intravenous magnesium sulfate as an adjunct in the treatment of acute asthma. Chest 107: 1576-1581.

55. Tolsa JF, Gao Y, Raj JU (1999) Developmental change in magnesium sulfate-induced relaxation of rabbit pulmonary arteries. J Appl Physiol 87: 1589-1594.

56. Kao MC, Jan WC, Tsai PS, Wang TY, Huang CJ (2011) Magnesium sulfate mitigates lung injury induced by bilateral lower limb ischemia-reperfusion in rats. J Surg Res 171: e97-106.

57. Lee CY, Jan WC, Tsai PS, Huang CJ (2011) Magnesium sulfate mitigates acute lung injury in endotoxemia rats. J Trauma 70: 1177-1185.

58. Su NY, Peng TC, Tsai PS, Huang CJ (2013) Phosphoinositide 3-kinase/ Akt pathway is involved in mediating the anti-inflammation effects of magnesium sulfate. J Surg Res 185: 726-732.

59. Luna LG (1968) Armed Forces Institute of Pathology (AFIP) Manual of Hystologic Staining Methods. Blakiston Division/ McGraw-Hill, New York, NY, USA.

60. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, MØller A, et al. (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96: 379-394.

61. Kraemer HC, Thiemann S (1987) Equality of means: z- and t-Tests, balanced ANOVA. How many Subjects? SAGE Publications, Newbury Park, Ca, USA.

62. Winner BJ, Brown DR, Michels KM (1991) Two factor experiments with repeated measures on one factor. Statistical Principles in Experimental Design. (3rd edtn), McGrall-Hill, New York, NY, USA.