Surfactant protein A is decreased in the lung of rats with hepatopulmonary syndrome[1]

Lucas Souto Nacif[I], Wellington Andraus[II], Márcia Saldanha Kubrusly[III], Flávia Saldanha Kubrusly[IV], Vera Cristina Bugelli Cainelli Gebara[IV], Andrea Ishizawa[V], Luiz Augusto Carneiro D'Albuquerque[VI]

[I]Fellow Master degree, Postgraduate Program in Medicine (Surgical Gastroenterology), School of Medicine, University of Sao Paulo (USP), Brazil. Acquisition of data, surgical procedures, statistical analysis, manuscript preparation.
[II]Assistant Professor, Coordinator, Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, School of Medicine, Medical Investigation Laboratory (LIM 37), USP, Sao Paulo-SP, Brazil. Surgical procedures, manuscript writing, critical revision.
[III]Fellow PhD degree, Postgraduate Program in Medicine (Surgical Gastroenterology), Biochemistry, Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, School of Medicine, Medical Investigation Laboratory (LIM 37), USP, Sao Paulo-SP, Brazil. Statistical analysis, manuscript preparation.
[IV]Fellow PhD degree, Postgraduate Program in Biological Sciences (Biology Genetics) from the University of São Paulo, Biochemistry, Biotechnology Center, Butantan Institute, Sao Paulo-SP, Brazil. Designed the indirect ELISA for evaluation of SP-A concentration, critical revision.
[V]Graduate student, Biochemistry, Biotechnology Center, Butantan Institute, Sao Paulo-SP, Brazil. Designed the indirect ELISA for evaluation of SP-A concentration.
[VI]Full Professor, Chairman, Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, School of Medicine, Medical Investigation Laboratory (LIM 37), USP, Sao Paulo-SP, Brazil. Conception and design of the study, manuscript writing, critical revision.

ABSTRACT

PURPOSE: To evaluate surfactant protein A levels in an hepatopulmonary syndrome rat model. To date, there have been no studies aimed at evaluating surfactant levels in the setting of cirrhosis or hepatopulmonary syndrome.

METHODS: A total of 35 rats were divided into control, sham, and experimental HPS groups. We evaluated surfactant protein A levels in rats and the experimental model designed to induce hepatopulmonary syndrome was common bile duct ligation. Statistical analysis was performed using GraphPad Prism Software®. Differences were considered statistically significant when $p < 0.05$.

RESULTS: Lung homogenate of surfactant protein A levels were lower in the experimental hepatopulmonary syndrome and sham groups in comparison to the control group ($p < 0.05$). Serum SP-A levels were the same in experimental hepatopulmonary syndrome and control groups but decreased in the sham group compared with the experimental groups ($p < 0.05$). Myeloperoxidase activity was higher in the experimental hepatopulmonary syndrome group than the other two groups ($p < 0.05$).

CONCLUSION: Surfactant protein A is present in experimental hepatopulmonary syndrome and leads to an imbalance between serum and pulmonary levels due to systemic inflammatory response.

Key words: Hepatopulmonary Syndrome. Pulmonary Surfactant-Associated Protein A. Models, Animal. Rats.

Introduction

Hepatopulmonary syndrome (HPS) is formed by the clinical triad of chronic liver disease, intrapulmonary vascular dilatation, and hypoxemia. This condition is present in 4% to 32% of patients with cirrhosis. The pathogenesis of this condition is not well defined, but it is speculated that a combination of factors, such as an imbalance in the response of vascular endothelin receptors, pulmonary microvascular remodeling, and genetic predisposition, leads to intrapulmonary vascular dilatation and bacterial translocation[1-4]. The pathophysiology of hypoxemia in this syndrome is multifactorial, however, predominant mechanisms include pulmonary shunts, impairment of the ventilation-perfusion ratio, low diffusion, diffusion-perfusion mismatch, or changes in the alveolar-capillary oxygenation gradient[5,6].

Hypoxemia in patients with this syndrome initially responds to low-flow supplemental oxygen, however oxygen supplementation requirements usually increase over time. Mild disease improvement has been reported with several treatments, such as sympathomimetics, almitrine bismesylate, estrogen blockers, indomethacin, octreotide, methylene blue, acetyl salicylic acid, and an inhibitor N-nitro-L-arginine methyl ester (L-NAME). Currently, no pharmacological intervention can readily improve arterial oxygenation and alter the course of HPS. Thus, liver transplantation is the only effective therapeutic option for the resolution of HPS[1,2,6].

The pathophysiological features of experimental HPS induced by common bile duct ligation (CBDL) includes alterations in the pulmonary microvasculature, including vasodilation, intravascular monocyte accumulation, and angiogenesis[2-4,7]. Some authors have shown that an increase in the expression of inducible nitric oxide synthase (iNOS) in the lungs of CBDL animals can also contribute to local nitric oxide production during the progression of HPS[2-4,8].

Pulmonary surfactant, which is secreted by alveolar type II pneumocytes, reduces surface tension at the air/liquid interface of the alveoli and stabilizes alveoli at low lung volumes[9-11]. Endogenous surfactant is a mixture of phospholipids (90%) and specific proteins (10%) such as SP-A (surfactant protein A), SP-B, SP-C and SP-D. SP-A, however, it is the most abundant protein and represents approximately about 5–6% of the dry weight[12,13]. The function of pulmonary surfactant is to increase lung compliance, reduce surface tension, and prevent atelectasis and lung collapse[14]. The absence or reduction of surfactant is associated with neonatal respiratory distress syndrome and congenital surfactant deficiency[15,16]. Conversely, there is an increase in pulmonary alveolar proteinosis, which is related to significant respiratory dysfunction. Exogenous surfactant preparations were initially developed to treat neonatal respiratory distress syndrome. Surfactant supplementation with SP-A and SP-D has been used as a therapy for lung disease in infancy and the inflammatory and immune protection benefits of this treatment have been proven[15-17].

The pathophysiology of HPS is not completely understood and we hypothesized that arterial blood gas changes in the setting of systemic inflammation and pulmonary disease (HPS) may alter surfactant production. The aim of this study was to evaluate the presence or absence of SP-A using a HPS rat model.

Methods

This project was approved by the Ethics Committee for the Guidelines of Animal Experimentation of the University of Sao Paulo, School of Medicine. The study was designed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Council for international Organization of Medical Sciences (CIOMS).

Thirty five male Wistar rats (200–250g, LIM 37/USP, Sao Paulo, Brazil) were housed at 19°C ± 3°C with a 12-h (06:00–18:00 hours) artificial light cycle. Two or three animals from the same treatment group were housed per cage. The animals had free access to tap water and standard food during the entire experiment. Food intake was not measured.

Study design

The animals underwent a common bile duct ligation (CBDL group n=16) as previously described[18,19]. The sham group (n=8) underwent laparotomy and mobilization of the common bile duct. The control group (n=5) only underwent the experimental analysis for data collection. All procedures began with the intraperitoneal anesthesia using 5% ketamine hydrochloride 30 mg/kg (Ketalar®, Cristália). Animals were kept warm with a 45W, 127V halogen bulb and body temperature was monitored using a rectal digital thermometer (YSI 4000A Precision Thermometer, USA) and maintained between 35°C and 37°C.

Arterial and venous blood analysis

After 28 days, animals were anesthetized and underwent a laparotomy. We collected blood samples using abdominal aorta (arterial) and superior vena cava (venous) punctures. Then, the animals were sacrificed.

Arterial blood gas analysis (oxygen saturation and PaO$_2$) was performed with an ABL 800 radiometer (Radiometer Copenhagen, Denmark) and results of oxygen saturation are expressed in % and PaO$_2$ in mmHg. The following laboratory (venous) biochemical parameters were measured: aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TB), direct bilirubin (DB), gamma glutamyl transferase (GGT), and alkaline phosphatase (AP). The quantification of AST, ALT, BT, GGT, and AP was performed using an ultraviolet optimized method (Cobas Mira, Roche) according to the International Federation of Clinical Chemistry. Results are expressed in units per liter (U/L).

Inclusion criteria

For the evaluation of surfactant protein A, we adopted the following inclusion criteria: an increase in GGT (>30 U/L), TB (>5.0 U/L), and DB (>4.0 U/L), changes in arterial blood gas by true measurement of PO$_2$ <80 mmHg and decreased O$_2$ saturation <90%.

Evaluation of surfactant protein A (SP-A) in rats

Initially, for establishing and detecting SP-A in rats, we purified lung porcine SP-A and made a standard curve to identify the best dilution of polyclonal rabbit antiserum against porcine-SP-A to detect SP-A at an optical density (OD) above 0.5. Different concentrations of SP-A versus dilutions of the polyclonal rabbit antiserum against porcine-SP-A (primary antibody) and dilution fixed anti-rabbit* IgG conjugated with peroxidase (Sigma® Chemical Co.) (secondary antibody). (*Product No. A-0545 Lot 074H4829 Anti-Rabbit IgG (whole molecule) Peroxidase Conjugate Antibody Developed in Goat Affinity Isolated Antigen Specific Antibody Adsorbed with Human IgG)[20].

We ultimately chose a dilution 1:1600 of polyclonal rabbit antiserum against porcine-SP-A to detect μg/50μL 0.0625 or 1.25 mg/mL. Then we investigated SP-A in control rat serum and lung homogenates using polyclonal rabbit antiserum against porcine-SP-A (indirect ELISA). The dilution selected was 1:5. Finally, the determination of SP-A in rat serum and lung homogenates of control, sham, and cirrhosis (CBDL) groups is shown in Table 1. The SP-A concentration is expressed in μg/ml[10,12].

Myeloperoxidase activity

Lung myeloperoxidase (MPO) activity was used as an indicator of neutrophils in the lung[21]. Samples were homogenized

TABLE 1 – SP-A serum and lung homogenate of control, sham and rats with experimental HPS (CBDL).

	Serum			Lung Homogenate		
	control	sham	CBDL	control	sham	CBDL
OD	0.136	0.061	0.134	0.430	0.245	0.281
Ug/ml	1.380	0.276	1.000	3.974	1.239	2.075
%	14	3	10	40	12	21

Note: surfactant protein A (SP-A); optical density (OD); Hepatopulmonary syndrome (HPS); common bile duct ligation (CDBL).

in PBS containing 0.5% hexadecyl and 5 mM EDTA, pH 6.0, sonicated and then centrifuged at 3000xg for 30 min. The supernatant was measured spectrophotometrically for MPO activity based on optical density (460 nm) changes due to the decomposition of H$_2$O$_2$ in the presence of *o*-dianisidine. Results are expressed as OD at 460 nm.

Statistical analysis

Statistical analysis was performed using GraphPad Prism Software®. Differences were considered significant with the p-value was <0.05. Data are presented as the mean ± standard deviation (SD) for continuous variables. Comparisons between groups were made using one way analysis of variance Kruskal-Wallis test (nonparametric ANOVA) and the post-hoc Dunn-Bonferroni test was used to perform multiple comparisons.

Results

To establish HPS, 20 rats were used in the CBDL group (three were excluded because HPS was not established and one died during anesthesia). For the sham group, 10 rats were used (one died during anesthesia and one died during the second post-operative period). The control group consisted of five rats. Macroscopic findings 28 days after surgical bile duct ligation showed evidence of cirrhosis in all experimental rats (CBDL group) including cirrhotic aspects of the liver, splenomegaly and impairment of lung tissue.

In the laboratory analysis, we observed a significant elevation of liver enzymes (AST and ALT), total and direct bilirubin, and GGT in the CBDL group in comparison with the control and sham groups. Only the AP value showed no significant differences as shown in Table 2. Assessment of the arterial blood gases in the CBDL group showed lower levels of PO$_2$ and O$_2$ saturation than other groups (Table 2).

TABLE 2 - Findings of the laboratory biochemical and arterial blood gas analyses.

Parameters	Control group (n=5)		Sham group (n=8)		CBDL group (n=16)		p*
AST(U/L)	181.6	(±52.88)	114.8	(±46.35)	439.2	(±177.3)	<0.0001
ALT(U/L)	59.80	(±09.88)	43.88	(±15.08)	592.1	(±406.1)	<0.0001
TB (U/L)	00.10	(±00.00)	0.33	(±0.15)	06.32	(±1.20)	<0.0001
DB (U/L)	00.00	(±00,00)	0.18	(±0.08)	04.65	(±1.21)	<0.0001
AP (U/L)	368.2	(±34.97)	330.8	(±211.7)	592.1	(±406.1)	0.3780
GGT (U/L)	00.00	(±00.00)	4.62	(±2.38)	61.06	(±29.72)	<0.0001
PA O$_2$ (mmHg)	79.74	(±04.03)	90.28	(±9.11)	72.74	(±17.33)	0.0014
Sat O$_2$ (%)	88.44	(±01.37)	97.33	(±1.16)	80.68	(±16.96)	0.0011

Note: *p value, comparison between control group vs sham group vs CBDL group. The mean and standard deviation or number is presented. CBDL, common bile duct ligation; AST, aspartate transaminase; ALT, alanine transaminase; TB, total bilirubin; BD, direct bilirubin; GGT, gamma glutamyl transferase; AP, alkaline phosphatase; PaO$_2$, partial pressure of oxygen; Sat O$_2$, oxygen saturation; and N/A, not applicable.

Levels of surfactant protein A (SP-A) were significantly decreased in the lung homogenate in sham and CBDL groups compared with the control group (Figure 1A). The serum SP-A levels in the sham group were significantly lower than the control group, however, levels measured in the CBDL group did not differ from the control group (Figure 1B). Myeloperoxidase (MPO) activity was significant higher in the CBDL group than the other groups, however control and sham groups showed no significant differences (Figure 1C).

Macroscopic features of lungs and livers from the CBDL group showed evidence of damage, which was not present in the control group. A histological analysis was conducted in only a few samples and the results were not analyzed statistically. In the CBDL group, lung tissue showed alveolar edema (Figure 2A) and the liver showed fibrosis and inflammatory cells (Figure 2B).
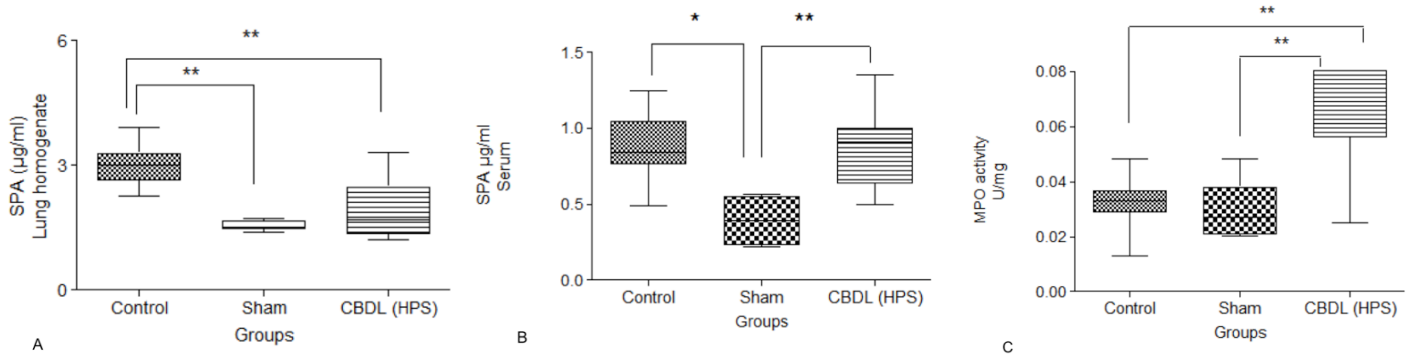

FIGURE 1 – Comparative SP- A lung homogenate, SP-A serum and myeloperoxidase (MPO) activity in groups. Note: p value (**p <0.01 and p <0.05) comparison between control group *vs* sham group *vs* CBDL group. CBDL, common bile duct ligation; MPO, myeloperoxidase; SP-A, surfactant protein A; and N/A not applicable.
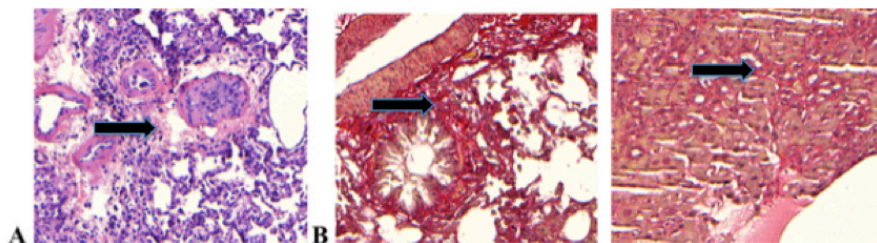

FIGURE 2 - Histological findings in the lungs and liver of rats in CBDL group demonstrating HPS. NOTE: A - Lung tissue stained with Verhoeff. B- Hepatic tissue stained with Picrosirius. Magnification, 200x. Arrows pointing out edema and inflammatory cell infiltrate.

Discussion

This is the first study to evaluate surfactant protein A (SP-A) levels in hepatopulmonary syndrome in a rat model. Interestingly, it was observed that lung surfactant was consumed in not only experimental HPS but also in a systemic inflammatory process (surgical trauma). Moreover, an imbalance between the level of surfactant protein A in lung homogenate and serum was also observed in the setting of experimental HPS.

As demonstrated by some authors previously, the induction of biliary cirrhosis by common bile duct ligation (CBDL) in rats is an established experimental model for HPS[18,19]. Our study is in accordance with the literature and HPS was established in this study in more than 85% of rats using a previously described model.

SP-A and SP-D are members of a family of innate immune molecules called collectins. The most well defined function of the collectins is the ability to opsonize pathogens, including bacteria and viruses, regulate a variety of immune cell functions, and facilitate phagocytosis by innate immune cells, such as macrophages and monocytes[13,22]. SP-A and SP-D have direct bactericidal activities in addition to their well-described opsonic activity and ability to regulate inflammatory mediator production[23].

Myeloperoxidase (MPO) levels were significantly increased in the CBDL group as compared with other groups. MPO activity in cirrhosis models with hepatopulmonary syndrome (HPS) was explored by other authors and it has been proposed that MPO may be involved in the regulation of iNOS expression[21]. Additionally, previous studies have shown that SP-A stimulates the chemotactic activity of alveolar neutrophils[11] and myeloperoxidase activity is increased in hepatopulmonary syndrome in rats[26].

The presence of SP-A in serum was reported in pulmonary fibrosis[24]. In fact, the present study found surfactant protein A (SP-A) in the blood in all groups of animals. It was found that surgical trauma (sham group) resulted in a decrease in serum and pulmonary (lung homogenate) SP-A, which did not normalize after 28 days. In contrast, in the experimental HPS group (CBDL group), pulmonary SP-A was also consumed but an imbalance was observed between serum and pulmonary levels.

The alveolar cell in the lung produces surfactant (SP-A) and HPS starts with pulmonary inflammation that progress to vascular shunts or microvascular lung changes with decrease in arterial blood-gas with $PO_2 < 80$ mmHg and decreased O_2 saturation $< 90\%$. We speculate that this alteration may explain the consumed pulmonary SP-A in experimental HPS (CBDL group), while serum level remains higher. As cited by Zhang and Fallon[8], the main features in experimental HPS are pulmonary microvascular dilation and angiogenesis that lead to abnormal pulmonary gas exchange[8]. In acute pancreatitis, the expression of SP-A in rat lung was reported to be reduced[25]. Based on the findings of this study, the hypothesis that SP-A may play a role in systemic inflammation is strengthened.

The above explanation may be simple, but the experimental HPS group also underwent laparotomy and the serum SP-A was not consumed. It was previously demonstrated that SP-A increases in the blood circulation of patients with acute lung injury/acute respiratory distress syndrome[15-17].

Lung SP-A levels first underwent alteration most likely due to systemic inflammation and surgical trauma (Sham group). However, the later development of pulmonary inflammation or microvascular changes would impair the balance between the body compartments, and as expected, SP-A remained higher in the blood.

Conclusion

Surfactant protein A is present in experimental Hepatopulmonary Syndrome and leads to an imbalance between serum and pulmonary levels due to a systemic inflammatory response.

References

1. Palma DT, Fallon MB. The hepatopulmonary syndrome. J Hepatol. 2006 Oct;45(4):617-25. PMID: 16899322.
2. Fallon MB. Mechanisms of pulmonary vascular complications of liver disease: hepatopulmonary syndrome. J Clin Gastroenterol. 2005 Apr;39(4 Suppl 2):S138-42. PMID: 15758649.
3. Luo B, Tang L, Wang Z, Zhang J, Ling Y, Feng W, Sun JZ, Stockard CR, Frost AR, Chen YF, Grizzle WE, Fallon MB. Cholangiocyte endothelin 1 and transforming growth factor beta1 production in rat experimental hepatopulmonary syndrome. Gastroenterology. 2005 Aug;129(2):682-95. PMID: 16083721.
4. Zhang J, Ling Y, Tang L, Luo B, Pollock DM, Fallon MB. Attenuation of experimental hepatopulmonary syndrome in endothelin B receptor-deficient rats. Am J Physiol Gastrointest Liver Physiol. 2009 Apr;296(4):G704-8. doi: 10.1152/ajpgi.90627.2008.
5. Vercelino R, Tieppo J, Dias AS, Marroni CA, Garcia E, Meurer L, Picada JN, Marroni NP. N-acetylcysteine effects on genotoxic and oxidative stress parameters in cirrhotic rats with hepatopulmonary syndrome. Basic Clin Pharmacol Toxicol. 2008 Apr;102(4):370-6. doi: 10.1111/j.1742-7843.2007.00181.x.
6. Lima BL, Franca AV, Pazin-Filho A, Araujo WM, Martinez JA, Maciel BC, Simoes MV, Terra-Filho J, Martinelli AL. Frequency, clinical characteristics, and respiratory parameters of hepatopulmonary syndrome. Mayo Clin Proc. 2004 Jan;79(1):42-8. PMID: 14708947.
7. Zhang J, Luo B, Tang L, Wang Y, Stockard CR, Kadish I, Van Groen T, Grizzle WE, Ponnazhagan S, Fallon MB. Pulmonary angiogenesis in a rat model of hepatopulmonary syndrome. Gastroenterology. 2009 Mar;136(3):1070-80. doi: 10.1053/j.gastro.2008.12.001.
8. Zhang J, Fallon MB. Hepatopulmonary syndrome: update on pathogenesis and clinical features. Nat Rev Gastroenterol Hepatol. 2012 Sep;9(9):539-49. doi: 10.1038/nrgastro.2012.123.

9. Bailey TC, Maruscak AA, Petersen A, White S, Lewis JF, Veldhuizen RA. Physiological effects of oxidized exogenous surfactant in vivo: effects of high tidal volume and surfactant protein A. Am J Physiol Lung Cell Mol Physiol. 2006 Oct;291(4):L703-9. PMID: 16632516.

10. Dias S, dos Santos F, Sakauchi D, Iourtov D, Raw I, Kubrusly F. The Porcine Pulmonary Surfactant Protein A (pSP-A) Immunogenicity Evaluation in the Murine Model. Pharmacology & Pharmacy. 2010;1:27-32.

11. Kresch MJ, Block M, Karim MR, Zhu L, Hussain N, Thrall RS, Sha'afi RI. Surfactant protein A stimulates release of neutrophil chemotactic factors by alveolar type II pneumocytes. Lung. 2010 Dec;188(6):491-7. doi: 10.1007/s00408-010-9243-6.

12. Kubrusly FS, Iourtov D, Leme E, Raw I. Pulmonary surfactant protein A isolation as a by-product of porcine pulmonary surfactant production. Biotechnol Appl Biochem. 2004 Oct;40(Pt 2):173-9. PMID: 14709163.

13. Precioso AR, Sakae PP, Mascaretti RS, Kubrusly FS, Gebara VC, Iourtov D, Rebello CM, Vaz FA, Raw I. Analysis of the immunogenicity and stability of a porcine pulmonary surfactant preparation administered in rabbits. Clinics (Sao Paulo). 2006 Apr;61(2):153-60. PMID: 16680333.

14. Wright JR. The "wisdom" of lung surfactant: balancing host defense and surface tension-reducing functions. Am J Physiol Lung Cell Mol Physiol. 2006 Nov;291(5):L847-50. PMID: 16861381.

15. Dushianthan A, Cusack R, Goss V, Postle AD, Grocott MP. Clinical review: exogenous surfactant therapy for acute lung injury/acute respiratory distress syndrome - where do we go from here? Crit Care. 2012 Nov 22;16(6):238. PMID: 23171712.

16. Bae YM, Bae CW, Oh MH, Lee SH, Woo KM, Jung KB. Effect of exogenous surfactant therapy on levels of pulmonary surfactant proteins A and D in preterm infants with respiratory distress syndrome. J Perinat Med. 2009;37(5):561-4. doi: 10.1515/JPM.2009.100.

17. Bersani I, Speer CP, Kunzmann S. Surfactant proteins A and D in pulmonary diseases of preterm infants. Expert Rev Anti Infect Ther. 2012 May;10(5):573-84. doi: 10.1586/eri.12.34.

18. Fallon MB, Abrams GA, McGrath JW, Hou Z, Luo B. Common bile duct ligation in the rat: a model of intrapulmonary vasodilatation and hepatopulmonary syndrome. Am J Physiol. 1997 Apr;272(4 Pt 1):G779-84. PMID: 9142908.

19. Kountouras J, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br J Exp Pathol. 1984 Jun;65(3):305-11. PMID: 6743531.

20. Kubrusly FS, Sakauchi D, Dias SC, Iourtov D, Gebara VCBC, Horton DSPQ, Piazza RMF, Precioso AR, Mascaretti RS, Rebello CM, et al. Polyclonal Rabbit Antiserum Against Porcine-SP-A Able to Detect Human SP-A Using Immunochemical Methods. In: ERLICH II 2nd World Conference on Magic Bullets Nürberg: October 3-5 2008; Germany; 2008: 279.

21. Wang Y, Wang W, Zhang Y, Zhao X, Yang D. Clinical significance of a myeloperoxidase gene polymorphism and inducible nitric oxide synthase expression in cirrhotic patients with hepatopulmonary syndrome. J Huazhong Univ Sci Technolog Med Sci. 2010 Aug;30(4):437-42. doi: 10.1007/s11596-010-0445-1.

22. Floros J, Wang G, Mikerov AN. Genetic complexity of the human innate host defense molecules, surfactant protein A1 (SP-A1) and SP-A2--impact on function. Crit Rev Eukaryot Gene Expr. 2009;19(2):125-37. PMID: 19392648.

23. Wu YZ, Medjane S, Chabot S, Kubrusly FS, Raw I, Chignard M, Touqui L. Surfactant protein-A and phosphatidylglycerol suppress type IIA phospholipase A2 synthesis via nuclear factor-kappaB. Am J Respir Crit Care Med. 2003 Sep 15;168(6):692-9. PMID: 12882758.

24. Mazur W, Toljamo T, Ohlmeier S, Vuopala K, Nieminen P, Kobayashi H, Kinnula VL. Elevation of surfactant protein A in plasma and sputum in cigarette smokers. Eur Respir J. 2011 Aug;38(2):277-84. doi: 10.1183/09031936.00110510.

25. Zhang XM, Chen HL, Wang ZH. The expression and function of pulmonary surfactant protein A in severe acute pancreatitis in rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2008 Jul;20(7):390-2. PMID: 18611334.

26. Nacif LS, Andraus W, Kubrusly MS, Molan N, Chaib E, D'Albuquerque LA. Myeloperoxidase activity is increased in hepatopulmonary syndrome in rats. Arq Bras Cir Dig. 2013 Nov-Dec;26(4):293-5. PMID: 24510037.

Correspondence:
Wellington Andraus
Divisão de Transplantes de Fígado e Órgãos do Aparelho Digestivo
Departamento de Gastroenterologia, USP
Rua Dr. Enéas de Carvalho Aguiar, 255/9º andar, sala 9113/9114
05403-900 São Paulo – SP Brasil
Tel.: (55 11)2661-3323
Fax: (55 11)2661-9008
wellington@usp.br