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Intranasal instillation of distilled water, hypertonic saline and sodium bicarbonate promotes redox imbalance and acute lung inflammation in adult mice

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ABSTRACT

Bronchial obstruction, caused by retained secretions, is often treated by the administration of mucoactive agents including distilled water, saline, hypertonic saline, and sodium bicarbonate. However, the inflammatory effect of these solutions on the lungs remains unclear. This study evaluated the instillation effects of different solutions on oxidative stress and lung inflammatory response in C57BL/6 mice. Fifty C57BL/6 mice were divided into 5 groups: control (CG); distilled water (DWG), hypertonic saline (HSG), saline (SG) and sodium bicarbonate (SBG). CG was exposed to ambient air while DWG, HSG, SG and SBG had 50 µl of respective solutions administered intranasally for 5 consecutive days. Twenty-four hours after the last intranasal instillation, all animals were euthanized for subsequent analysis. All solutions promoted increased recruitment of inflammatory cells to the lung compared to controls. Superoxide dismutase activity was lower in HSG compared to all other groups; catalase activity was reduced in SG, while it increased in SBG and DWG compared to CG. Finally, there was an increase in the inflammatory markers TNF-α, CCL2 and IFN-γ in DWG compared to CG, SG and HSG. In conclusions, the intranasal instillation of different solutions promotes redox imbalance and inflammation on lungs of adult mice.

1. Introduction

Respiratory failure is one of the most frequent causes of admission to the intensive care unit. In this context, invasive mechanical ventilation is used in order to prevent the worsening of lung injury and to minimize the mortality rate in patients with respiratory failure (Caparros, 2014). Although it presents an important therapeutic role, the insertion of an artificial airway such as endotracheal, nasotracheal or tracheostomy, can inhibit mucociliary clearance and promote the retention of secretions in the airways. This can lead to increased oxygen needs, worsening ventilation and a potential increase in morbidity and mortality (Paratz and Stockton, 2009; Icard and Rubio, 2017). Thus, the management of airway secretions in mechanically ventilated individuals is fundamental for successful ventilatory weaning. Aspiration is one of the most commonly performed procedures in intensive care units, with the purpose of removing secretions from the respiratory tract, maintaining airway permeability and preventing complications (Paratz and Stockton, 2009).

Treatment of bronchial obstruction caused by retained secretions is accomplished by administration of mucoactive agents including distilled water, saline, hypertonic saline, and sodium bicarbonate (Icard and Rubio, 2017). In the mid-1960s, the first studies were carried out with the application of ultrasonic nebulization of saline solution and distilled water associated with respiratory therapy (Paez and Miller, 1971). Distilled water, isotonic saline and hypertonic saline are widely used in medicine to increase the expectoration of secretions that can

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lead to upper airway obstruction (Campos et al., 2015). In addition, saline instillation combined with artificial ventilatory support was associated with a lower incidence of ventilator-associated pneumonia (Favretto et al., 2012).

It is well described in the literature that the inhalation of distilled water does not appear to act directly on the smooth muscles of the airways (Mochizuki et al., 1999). However, studies suggest that inhalation of distilled water may induce rapid ionic or osmolar changes in airway fluids. This in turn leads to the activation of inflammatory cells, or stimulates sensory nerve endings (Hook and Siraganian, 1981; Bienenstock et al., 1988), resulting in reduction of forced expiratory volume in one second in asthmatic patients (Mochizuki et al., 1999). In addition, the use of sodium bicarbonate as a mucoactive agent has not been well studied, however, a few studies suggest that bicarbonate sequesters calcium ions into mucin granules, allowing expansion and improving mucociliary clearance (Chen et al., 2010; Cooper et al., 2013; Icard and Rubio, 2017).

The use of these mucoactive agents is related to the recruitment of inflammatory cells into the air space resulting in the production of inflammatory cytokines and reactive oxygen species (ROS) (Mochizuki et al., 1999). The production of reactive species at high concentrations is related to redox imbalance, which is characterized by a transient or chronic increase in reactive species levels. This leads to a breakdown of the balance between oxidants and antioxidants and can cause damage to cellular biomolecules, resulting in tissue damage (Holguin, 2013; Lushchak, 2014). In this context, this study aims to analyze the effects of intranasal instillation of isotonic and hypertonic saline, distilled water and sodium bicarbonate solution on lung inflammatory response and redox imbalance in adult mice.

2. Material and methods

2.1. Animals

Fifty male C57BL/6 mice aged between 10 and 12 weeks were obtained from the Experimental Nutrition Laboratory of Federal University of Ouro Preto (UFOP). The animals were conditioned under controlled parameters of temperature, humidity, and luminosity (24 °C, 50 ± 10%, and 12 h light/dark respectively), and had access to balanced standard feed and water ad libitum. The experimental procedures followed the ethical principles established by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (CEUA-UFOP) and was approved with the protocol number 2016/67.

2.2. Experimental design

The animals were randomly divided into 5 groups (n = 10): control (CG) exposed to ambient air, and experimental groups exposed to distilled water (DWG), saline (0.9% sodium chloride) (SG), hypertonic saline (20.0% sodium chloride) (HSG) and sodium bicarbonate solution (8.4% sodium bicarbonate) (SBG) by intranasal instillation. Prior to each administration, the animals were anesthetized one by one in chambers containing isoflurane and received the respective solution while unconscious. Twice a day (6 a.m./6 p.m.) for five consecutive days, 12.5 µL of solution were administered into each nostril, totaling 25 µL per exposure. Twenty-four hours after the last administration, the animals were euthanized by overdose of ketamine (130 mg/kg) and xylazine (0.3 mg/kg).

2.3. Collection and analysis of bronchoalveolar lavage fluid

Immediately after euthanasia, the thorax of each animal was opened. The right lung was clamped, the trachea cannulated and the left lung washed three times with 500 µL of saline solution (0.9%) for collection of BALF samples. The samples were kept on ice until the end of the experiment to avoid cell lysis. A Neubauer chamber was used to determine the number of leukocytes in BALF. For the differential cell count, 250 µL samples were centrifuged in Cyto-centrifuge (INBRAS health equipment, São Paulo, BR) and stained with a rapid panoptic kit (Newprov, Pinhais, Paraná). A total of 100 cells per slide were counted, which were differentiated into lymphocytes, macrophages, neutrophils and eosinophils, according to their standard morphological criteria (de Souza et al., 2018; Ramos et al., 2018).

2.4. Lung collection

After BALF collection, the right ventricle was perfused with saline solution (0.9%), the right lung was clamped, and the left lung was instilled via the trachea with 4% buffered formalin (pH 7.2) at a pressure of 25 cmH_2O per 2 min. The left lung was then removed and immersed in fixative solution for 48 h. The material was then processed and stained with hematoxylin and eosin (HE) to perform morphometric analyzes. The right lung was removed, placed in a polypropylene tube homogenized in phosphate buffer (pH = 7.8), and centrifuged at 13,000 rpm for 10 min, the supernatant collected and stored in a freezer (−80 °C) (Soares et al., 2016).

2.5. Biomarkers of oxidative damage and antioxidant defense

The assay for lipid peroxidation analysis was performed according to the method described by Buege and Aust (Buege and Aust, 1978).. This method is based on the ability of thiobarbituric acid to bind to oxidized lipids. For the determination of carbonylated protein, our protocol was adapted from the method described by Reznick and Packer (Reznick and Packer, 1994). Superoxide dismutase (SOD) activity was measured according to the method based on the ability of SOD to inhibit auto-oxidation of pyrogallol, this method was described by Marklund and Marklund (Marklund and Marklund, 1974). The activity of Catalase (CAT) was measured according to the method described by Aebi (Aebi, 1984) from the decrease of hydrogen peroxide at an absorbance of 240 nm. The concentration of glutathione was adapted from commercial kit Sigma # CS0260, which uses a kinetic method to measure total glutathione levels (GSH + GSSG) in biological samples by the reduction of 5,5′-dithio-bis-(2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid (Griffith, 1980). Protein analysis was performed on the lung homogenate supernatant according to the method described by Bradford (Bradford, 1976).

2.6. Immunoenzymatic assay

Tissue homogenate was used for the analysis of monocyte chemoattractant protein 1 (CCL2), tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ). Assays were performed on 96-well plates, and 100 µl of monoclonal antibody against the peptides (or proteins), reconstituted in phosphate buffered saline (PBS), were added. After a 12 h incubation period at room temperature, the blockade was carried out with solution containing PBS and 1% fetal bovine serum for two hours. Samples were added in a volume of 25 µl per well. Subsequently, secondary antibodies diluted in PBS and 1% fetal bovine serum were added. The staining intensity was read in an ELISA reader at 450 nm. Inflammatory markers in the samples were quantified based on the optical density obtained with the standard curve of known concentrations of peptides (Ramos et al., 2018).

2.7. Stereological analyzes of lung parenchyma

Stereological and morphometric analysis of the pulmonary parenchyma were performed on the H&E stained slides. The slides were photographed in the Multiuser Laboratory of the Nucleus of Biological Sciences Research (NUPEB) of UFOP, using a light microscope equipped with Leica BM5000 digital camera (Leica DFC 300 FX) coupled with image capturing software, Leica Application Suite, in microscopic

objective of 40 × . The alveolar septal volume density (Vv) analysis was performed in a test system composed of 16 points and a known test area. The test system was coupled to the monitor and 20 random fields of the photographs were analyzed in a 40x magnification according to the method described by Campos et al (Campos et al., 2017).

2.8. Statistical analysis

Data are expressed as mean values ± standard errors of the means. Evaluation of data normality was performed using the Kolmogorov-Smirnov test. One-way ANOVA followed by Tukey's *post-hoc* test was used for parametric data. The Kruskal-Wallis test followed by Dunn's *post-hoc* test was used for nonparametric data. The difference was considered significant when $p < 0.05$. All analyses were performed using GraphPad Prism software version 5.00 for Windows 7 (GraphPad Software, San Diego, CA).

3. Results

3.1. Cell recruitment to Bronchoalveolar lavage fluid

To evaluate the presence of inflammatory cells in the lung parenchyma, total and differential cell counts were performed. After five days, the intranasal administration of distilled water, saline, hypertonic saline and sodium bicarbonate solution led to a greater recruitment of total inflammatory cells to the lung compared to controls ($p < 0.0001$). However, the cell profile within the groups varied, and the animals of DWG and SBG showed higher macrophage counts compared to the CG ($p < 0.0001$). Lymphocyte analysis revealed higher counts in groups exposed to saline, hypertonic saline and sodium bicarbonate solution compared to controls $p < 0.0001$). SG and HSG presented higher neutrophil counts in the bronchoalveolar lavage compared to CG ($p < 0.0001$). In addition, there was an increase of neutrophils in HSG compared to DWG and SBG ($p < 0.05$) (Table 1).

3.2. Redox imbalance in pulmonary parenchyma

Protein oxidation levels were higher in the hypertonic saline and sodium bicarbonate groups compared to controls ($p < 0.05$). Superoxide dismutase activity was lower in HSG when compared to all other experimental groups ($p < 0.05$). Catalase activity was higher in DWG and SBG and lower in SG when compared to the CG ($p < 0.0001$). The ratio of reduced glutathione to oxidized glutathione was lower in DWG and HSG compared to the CG ($p < 0.0001$) (Table 2).

3.3. Levels of inflammatory markers in the pulmonary parenchyma

The inflammatory markers CCL2, TNF-α, and IFN-γ were analyzed for inflammatory status of the lungs (pg/mL). TNF-α levels were higher in the DWG (779.1 ± 49.76) compared to CG (559.5 ± 13.83), SG (536.1 ± 7.08), HSG (547.9 ± 18.53) and SBG (653.6 ± 19.05). In addition, the levels of TNF-α were higher in SBG compared to SG and HSG ($p < 0.0001$). CCL2 levels were higher in the DWG (3073 ± 171.5) and SBG (2691 ± 106.3) compared to CG (2261 ± 63.67) and SG (2168 ± 80.49) ($p < 0.0001$). IFN-γ levels were higher in DWG (760.5 ± 5.91) compared to CG (646.7 ± 17.34) and SG (611.7 ± 13.41) ($p < 0.05$) (Fig. 1).

3.4. Stereological of the pulmonary parenchyma

Stereological analyzes of the pulmonary parenchyma showed no differences in airspace volume density (Vv [a]) and volume density of alveolar septa (Vv [sa]) (Fig. 2).

4. Discussion

The purpose of this study was to evaluate the instillation effects of different solutions on oxidative stress and lung inflammatory response in C57BL/6 mice. We found that the administration of distilled water, isotonic saline, hypertonic saline and sodium bicarbonate solutions promoted the recruitment of inflammatory cells to the lungs. In addition, intranasal instillation of distilled water, sodium bicarbonate and hypertonic saline led to an increase in inflammatory markers and pulmonary oxidative stress.

The mechanism of action of most mucoactive agents are well known. It is reported that isotonic saline improves mucociliary clearance mainly by inducing a productive cough with higher expectoration. Hypertonic saline exert its effects by increasing mucociliary clearance through stimulation of ciliary beat frequency and thinning of mucus (Ural et al., 2009). Studies have shown that nebulization with hypertonic saline is involved in the improvement of pulmonary function and respiratory symptoms in fibrocystic patients (Reeves et al., 2011). Distilled water is involved in the stimulation of bronchial hyperreactivity, which could induce ionic and osmotic changes in respiratory secretions related to the activation of inflammatory cells, such as neutrophils and alveolar macrophages (Chen et al., 2010; Cooper et al., 2013; Icard and Rubio, 2017). However, in vivo studies are needed to observe the effects triggered by administration of sodium bicarbonate on the respiratory system.

Bronchoalveolar lavage is a simple technique capable of revealing variations in the type and number of nucleated immune cells and the presence of soluble acellular components in the lower respiratory tract (Gharsalli et al., 2018). In this study, the administration of distilled water, saline, hypertonic saline and sodium bicarbonate led to the recruitment of inflammatory cells into the lungs. Furthermore, the cell profile between groups was different. For example, the instillation of distilled water led to the recruitment of macrophages, while isotonic saline and hypertonic saline recruited neutrophils and lymphocytes. Finally, the administration of sodium bicarbonate promoted the

Table 1
Effects of intranasal instillation of different solutions on recruitment of cells to BALF.

	CG	DWG	SG	HSG	SBG
Leukocytes ($x10^3$/mL)	85.0 ± 7.49	137.1 ± 2,85[a]	138.0 ± 6.11[a]	165.0 ± 4.53[a,b,c]	158.9 ± 4.54[a]
Macrophages ($x10^3$/mL)	83.17 ± 7.03	125.2 ± 3.22[a]	107.1 ± 6.14	100.4 ± 7.34	148.3 ± 4.32[a,c,d]
Lymphocytes ($x10^3$/mL)	1.39 ± 0.20	3.95 ± 0.84	4.8 ± 0.57[a]	7.84 ± 1.49[a]	5.32 ± 0.77[a]
Neutrophils ($x10^3$/mL)	0.44 ± 0.14	7.55 ± 1.29	26.07 ± 2.90[a]	56.57 ± 8.95[a,b,e]	5.11 ± 0.50
Eosinophils ($x10^3$/mL)	0.0	0.4 ± 0.25	0.0	0.16 ± 0.16	0.17 ± 0.17

Control (CG); distilled water (DWG), saline (SG), hypertonic solution (HSG) and sodium bicarbonate (SBG) groups. The letter (a) represents a significant difference between groups when compared to CG. The letter (b) represents a significant difference compared to DWG. The letter (c) represents a significant difference compared to SG. The letter (d) represents a significant difference compared to HSG. Data were expressed as mean ± standard error of the mean and were analyzed by one-way ANOVA followed by Tukey post-test, n = 10 animals per group ($p < 0.05$).

Table 2
Biomarkers of oxidative stress on lung parenchyma.

	GC	DWG	SG	HSG	SBG
SOD (U/mg PTN)	39.55 ± 3.27	32.92 ± 2.03	34.61 ± 2.68	20.53 ± 0.74[a,b,c,e]	38.46 ± 4.65
CAT (U/mg PTN)	1.65 ± 0.10	2.34 ± 0.17[a]	1.20 ± 0,02[a,b,e]	1.30 ± 0,11[b,e]	2.68 ± 0.13[a]
GSH/GSSG ratio	3.33 ± 0.23	2.42 ± 0.09[a,c]	3.89 ± 0.25	2.52 ± 0.12[a,c]	2.61 ± 0.07[c]
Protein carbonyl (nmol/mg PTN)	25.00 (20.71; 26.46)	34.78 (27.42; 35.14)	36.12 (26.99; 46.83)	47.06 (32.06; 76.16)[a]	44.59 (31.46; 52.90)[a]

Control (CG); distilled water (DWG), saline (SG), hypertonic solution (HSG) and sodium bicarbonate (SBG). The letter (a) represents a significant difference between groups when compared to CG. The letter (b) represents a significant difference compared to DWG. The letter (c) represents a significant difference compared to SG. The letter (d) represents a significant difference compared to HSG. The letter (e) represents a significant difference compared to SBG. SOD, CAT and protein carbonyl data were expressed as mean ± standard error of the mean and were analyzed by one-way ANOVA followed by Tukey post-test, n = 10 animals per group. GSH/GSSG ratio data were expressed in median, minimum and maximum value and were analyzed by Kruskal-Wallis followed by Dunn's post-test, n = 10 animals per group ($p < 0.05$).

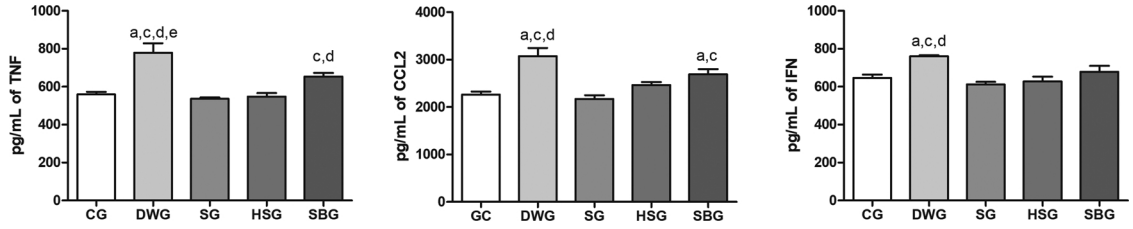

Fig. 1. Biomarkers of inflammation on lung parenchyma. (A) TNF-α (pg/mL) (B) CCL2 (pg/mL) (C) IFN-γ (pg/mL). The letter (a) represents a significant difference between groups when compared to CG. The letter (b) represents a significant difference compared to DWG. The letter (c) represents a significant difference compared to SG. The letter (d) represents a significant difference compared to HSG. The letter (e) represents a significant difference compared to SBG. Data were expressed as mean ± standard error of the mean and were analyzed by one-way ANOVA followed by Tukey post-test, n = 10 animals per group.

recruitment of macrophages and lymphocytes. Studies evaluating the effects of these solutions on the inflammatory response are scarce. However, a recent study published by our research group demonstrated that ultrasonic nebulization with distilled water and saline for a long period resulted in recruitment of macrophages and neutrophils (with distilled water) and lymphocyte (with saline) (Campos et al., 2015). The results of this study, taken in conjunction with our previous study on long term exposure, suggest that all mucoactive solutions are capable of generating an influx of cells into the lungs, yet the response triggered by short-term exposure differs from long-term exposure.

The airways respond to inflammatory stimuli, such as cystic fibrosis, chronic obstructive pulmonary disease and mechanical ventilation, by promoting goblet cell hyperplasia and hypersecretion of mucus. In this sense, the use of mucoactive agents increases the mucus expectoration capacity and/or decreases hypersecretion (Rubin, 2002; Icard and Rubio, 2017). However, to our knowledge there are no studies evaluating the short-term exposure of these substances in our experimental model. Therefore, in this study, we analyzed whether mucoactive agents generate an inflammatory response in the lungs of mice after a short term of 5 days; and determined which markers were involved (CCL2, IFN-γ, or TNF-α).

Cytokines and chemokines are produced at the site of inflammation by different cell types and act to reduce the action of noxious stimuli (Aghasafari et al., 2019). The chemokine CCL2 is a trophic molecule and mediator of extravasation of monocytes to regulate the inflammatory process (Navratilova, 2006). Our results demonstrate an increase of CCL2 in the groups exposed to distilled water and sodium bicarbonate. A previous study by Rancan et al. demonstrated an increase in CCL2 in animals with lung injury (Rancan et al., 2017). In addition, a study published by our research group showed an increase of CCL2 in animals submitted to mechanical ventilation (de Souza et al., 2018). Furthermore, the increase of CCL2 in the DWG and SBG was associated with an increase of macrophages in the air space, indicating that this cell type is involved in the immune response triggered by the administration of distilled water and sodium bicarbonate.

In addition to increased CCL2, an increase in TNF-α was observed in the distilled water and sodium bicarbonate groups. Macrophage recruitment to the site of inflammation is involved with increased production of this cytokine, since macrophages produce TNF-α and activate the signaling pathways involved with the inflammatory response (Jung et al., 2019). Wang et al. observed an increase of TNF-α, in a model of lung injury induced by mechanical ventilation (Wang et al., 2018). Finally, in the group exposed to distilled water, an increase of IFN-γ was observed. Interferon-gamma is a cytokine produced by a subpopulation of T cells and is involved in the activation of macrophages at the site of inflammation (Davis et al., 2000). In summary, intranasal administration of distilled water and sodium bicarbonate solution leads to an inflammatory response mediated primarily by macrophages. Therefore, it is possible, based on this and previous studies, that the use of distilled water and sodium bicarbonate may induce lung injury through the recruitment of macrophages and increase in CCL2, TNF-α and IFN-γ.

Multiple immune cells occupy the respiratory mucosa in healthy animals and humans, however when lung injury occurs, there may be recruitment and activation of leukocytes in the respiratory mucosa (Chen et al., 2018). In this study, we observed an increase in the number of inflammatory cells, cytokines and chemokines in the groups submitted to intranasal instillation. It is known that macrophages and neutrophils directly produce reactive oxygen and nitrogen species, which may induce oxidative stress. To counterbalance the reactive species produced by inflammatory cells, the lungs mount an antioxidant response, that includes the enzymes superoxide dismutase, catalase and glutathione peroxidase (Pham-Huy et al., 2008). In our study, in order to evaluate the effects caused by intranasal instillation of distilled water, saline, hypertonic saline and sodium bicarbonate, we analyzed oxidized protein levels and activity of antioxidant enzymes. The detection of carbonylation of proteins is a widely used method for the analysis of damage induced by oxidative stress (Ramos et al., 2018). Our results demonstrate that exposure to hypertonic saline and sodium bicarbonate solution presented higher levels of carbonylated protein.

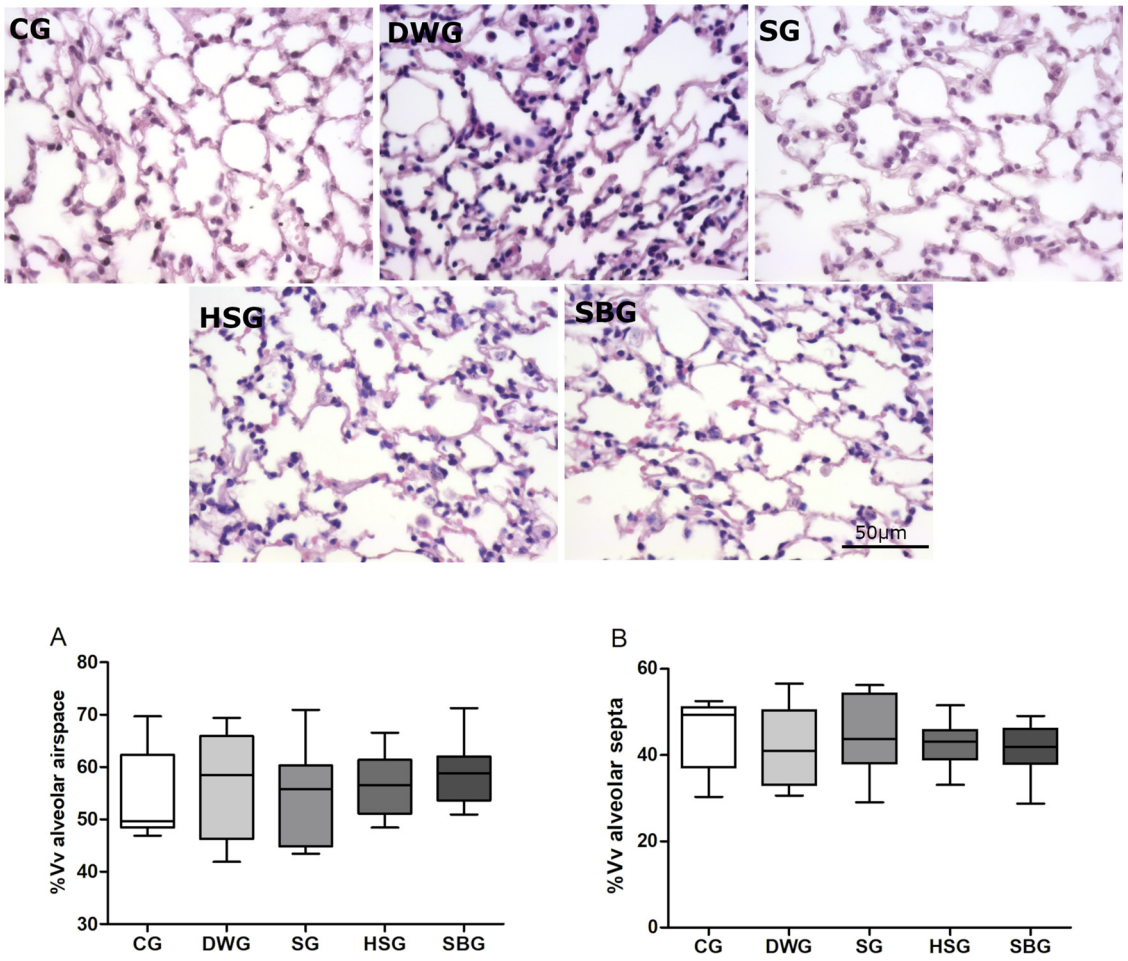

Fig. 2. Stereological analyses of lung sections. Photomicrographs of lung sections stained with hematoxylin and eosin. Bar = 50 µm, 400× magnification. (A) Volume density of alveolar septa. (B) Volume density of alveolar airspace. For A and B data were expressed in median, minimum and maximum value and were analyzed by Kruskal-Wallis followed by Dunn's post-test, n = 10 animals per group.

Murta et al. observed similar results in a formaldehyde exposure model (Murta et al., 2016) and Xie et al. observed increased oxidative damage in rabbits nebulized with saline (Xie et al., 2017).

In addition to the higher levels of carbonylated protein, intranasal instillation of hypertonic saline showed a decrease in superoxide dismutase, catalase activity and a lower ratio between reduced glutathione and oxidized glutathione. Furthermore, hypertonic saline was associated with an increase in neutrophil counts. Superoxide dismutase is the main enzyme defense in the lungs and acts by removing the superoxide radical in hydrogen peroxide, which is a substrate for catalase and glutathione peroxidase (Campos et al., 2013). Murta et al (Murta et al., 2016) observed similarly high levels of carbonylated protein in rats nebulized with different concentrations of formaldehyde. In addition, they demonstrated a decrease in alveolar septa and an increased alveolar area in animals nebulized with 5% formaldehyde. It is possible that the effects of administered hypertonic saline are mediated by the recruitment of neutrophils into the lungs and the production of reactive species. Our results combined with those by Murta et al (Murta et al., 2016) suggest that hypertonic saline has the potential to promote airway lesion both through an increased inflammatory response and redox imbalance.

The groups exposed to distilled water and sodium bicarbonate had an increase in catalase activity and a decrease in the ratio of reduced glutathione and oxidized glutathione, indirectly demonstrating an increase in the activity of glutathione peroxidase. Catalase and glutathione peroxidase are two important antioxidant enzymes and are responsible for the oxidative balance in the lungs, regulating oxidative stress (Birben et al., 2012). The results of our study resemble those published by Ramos et al. (Ramos et al., 2018), who reported a significant increase in catalase activity in the group exposed to cigarette smoke for a short period compared to controls. Campos et al (Campos et al., 2015) demonstrated, in a long-term nebulization model with distilled water and saline, a decrease in catalase activity in the distilled water group compared to controls. These studies suggest that the antioxidant mechanisms occurring with short-term exposure differ from the mechanisms associated with long-term exposure. In this context it is possible that the response triggered by the administration of distilled water and sodium bicarbonate solution is related to the recruitment of macrophages to the lungs, and these lead to oxidative stress.

We also examined the pulmonary parenchyma to evaluate for structural changes. Oxidation of membrane proteins, lipids and inflammatory cells, such as macrophages and neutrophils, are involved in the remodeling of pulmonary histoarchitecture (de Souza et al., 2018). Campos et al. (Campos et al., 2015) observed in animals nebulized with distilled water for six months, an increase in the volume density of the alveolar septa. In this study, although the results showed cellular recruitment and oxidative damage, we did not find alterations in pulmonary histoarchitecture. We believe that the short period of exposure to the different solutions was not enough to promote structural alterations.

The management of airway secretions to treat various lung diseases is a common practice (Ehrmann et al., 2013). In mechanically ventilated patients, this practice is fundamental for successful weaning. Mucoactive agents are used in the management of airway secretions,

although their use is based on expert recommendations, not on validated studies (Icard and Rubio, 2017). In this study, we demonstrate that despite the clinical relevance, the administration of hypertonic saline is capable of generating pulmonary oxidative stress.

5. Limitations

In our study, animals from the control group did not receive anesthesia with isoflurane. However, we believe that the duration of exposure to isoflurane in the experimental groups was not sufficient enough to cause an inflammatory response in the lungs and therefore has little impact on the results of this study. Future studies might examine the relationship of duration of exposure to isoflurane and lung inflammation in this experimental model.

6. Conclusions

In summary, the administration of distilled water and sodium bicarbonate, for a short period, leads to the influx of inflammatory cells into the lungs, primarily macrophages, which increased inflammatory response and oxidative stress in adult mice.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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