ORIGINAL ARTICLE

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Evaluation of the cardiac morphological alterations secondary to the pulmonary emphysema: experimental study in rats

Avaliação das alterações morfológicas cardíacas secundárias ao enfisema pulmonar: estudo experimental em ratos

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Abstract:

Objective: **The purpose of this study is to evaluate the occurrence and repercussions of chemically induced pulmonary emphysema and the morphological alterations present in rats' hearts of post-induction and to follow the progression afterwards.**

Method: **Seventy five rats divided into two groups: papain (N=50) and control (N=25), were submitted to intratracheal spraying of papain and saline solution, respectively, and were evaluated. The animals were sacrificed 30, 60, 90, 120 or 180 days post-spraying. Arterial blood gases and cardiac and pulmonary morphometrical analysis were performed.**

Results: **Papain spraying produced alveolar tissue destruction similar to the morphological alterations observed in pulmonary emphysema. The papain group presented mean alveolar diameter higher than controls in all periods evaluated ($p<0.05$). Right ventricle wall thickness and interventricular septum did not show significant macroscopic alterations until six months after emphysema induction. The right ventricle area presented enlargement 120 days after induction of pulmonary emphysema, with mean area higher than controls at 120 and 180 days ($p=0.001$). The left ventricle presented significant cavity area decrease 90 days after induction of the pulmonary emphysema, followed by slight wall thickening.**

Conclusions: **The adopted experimental model was efficient to morphologically induce pulmonary emphysema. The presence of pulmonary emphysema did not provoke morphological changes in the right ventricle wall and interventricular septum. The alveolar destruction induced left ventricular hypertrophy and enlargement of the right ventricle.**

Descriptors: **Pulmonary emphysema, chemically induced. Pulmonary emphysema, physiopathology. Papain, pharmacology. Disease models, animal. Heart ventricle, anatomy & histology.**

Work performed in the Thoracic Surgery Research Laboratory of the Medical School, São Paulo University

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Resumo

Objetivo: **Este trabalho tem como objetivo avaliar a ocorrência e as repercussões de enfisema pulmonar quimicamente induzido e as alterações morfológicas presentes em corações de ratos após sua indução, acompanhando sua progressão ao longo do tempo.**

Método: **Foram avaliados 75 ratos divididos em dois grupos, papaína (N=50) e controle (N=25), submetidos à instilação intratraqueal de papaína e solução fisiológica, respectivamente. Os animais foram sacrificados 30, 60, 90, 120 ou 180 dias após a instilação. Foi realizada gasometria do sangue arterial, análise morfométrica dos pulmões e coração.**

Resultados: **A instilação de papaína produziu destruição do tecido alveolar, mimetizando alterações morfológicas encontradas no enfisema pulmonar, com diâmetro alveolar médio maior no grupo papaína em relação ao controle em todos os momentos avaliados (p<0,05). A espessura da parede do ventrículo direito e septo interventricular não apresentaram alterações macroscópicas significativas no período de até seis meses após a indução do enfisema. A cavidade do ventrículo direito apresentou dilatação, a partir de 120 dias após indução do enfisema, com área média superior aos respectivos controles aos 120 e 180 dias (p=0,001). O ventrículo esquerdo apresentou significativa redução da área de sua cavidade, 90 dias após a indução do enfisema pulmonar, acompanhada de discreto espessamento de sua parede.**

Conclusões: **O modelo experimental empregado foi eficiente para induzir morfologicamente o enfisema pulmonar. A presença de enfisema pulmonar não provocou alterações morfológicas na parede do ventrículo direito e septo interventricular. A destruição alveolar induziu hipertrofia do ventrículo esquerdo e dilatação do ventrículo direito.**

Descritores: **Enfisema pulmonar, induzido quimicamente. Enfisema pulmonar, fisiopatologia. Papaína, farmacologia. Modelos animais de doenças. Ventrículo cardíaco, anatomia & histologia.**

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the main causes of death and disease throughout the world, with more than 16 millions of individuals affected in the United States alone [1]. In Brazil it is responsibly for around 30 thousand deaths per year and is the fifth most common cause of death. It is estimated that there are three millions of sufferers of the disease in Brazil today. As opposed to other chronic diseases whose prevalence is declining, the progress of COPD is of great concern with figures increasing worldwide [2]. It is forecasted that by 2020 it will be the third most common cause of death, only surpassed by ischemic heart disease and cerebral vascular disease [2]. In Brazil, according to the data of DATASUS, the government statistics institution, 280 thousand Brazilians with COPD are hospitalized annually [3].

The denomination COPD includes a series of diseases of different etiologies which have in common the presence of an obstruction to the air flow that is not-totally reversible. Limitation to the air flow is generally progressive and associated to an abnormal inflammatory response of the lungs to particles and/or irritating gases. Among patients with COPD, approximately 20% present with pulmonary emphysema, whilst the other 80% suffer from chronic bronchitis or a combination of these two ailments [3].

Given its high prevalence, COPD requires special attention as a concern of public health, becoming a subject of much scientific investigation, particularly pulmonary emphysema because of its controversial physiopathology. Thus, experimental models that result in both morphological and physiological alterations, mimicking the conditions found in the human lung of this type of patient, are extremely important.

Apart from the already well known repercussions that occur in the lungs of patients with pulmonary emphysema, several organs and structures suffer the impact of these alterations, including the diaphragm, the heart and the carotid body.

Despite of extensive publications on pulmonary emphysema, there are few articles evaluating the effects, in particular over the long term, of experimentally induced emphysema on the heart.

Thus, this study has as its objective to evaluate the morphological alterations seen in rats' hearts suffering from chemically induced pulmonary emphysema, accompanying its progression over time.

METHOD

This work was developed in the Thoracic Surgery research laboratory of the Medical School of the University of São Paulo after approval from the Scientific Ethics Committee of the Heart Institute and the Ethics Committee for the Analysis of Research Projects of the Clinical Directorate of the Hospital das Clinicas of the Medical School of the University of São Paulo.

All the animals received care according to the norms established in the "*Guide for the Care and Use of Laboratory Animals*" (*Institute of Laboratory Animal Resources, National Academy of Sciences*, Washington, D.C., 1996) and following the ethical principles of animal experimentation in the Brazilian code on experimentation.

In this study the model employed was pulmonary emphysema induced by intratracheal spraying of papain [4].

Seventy-five healthy adult male Wistar rats that weighed between 200 and 300 g (mean 275.3 ± 16.9 g), were randomly allocated to two experimental groups:

- Papain Group (n=50) – animals submitted to intratracheal spraying of physiological solution containing papain.
- Control Group (n=25) – animals submitted to intratracheal spraying of 0.9% isotonic physiological solution.

The animals were kept for a period of between 30 to 180 days, with 10 animals of the Papain Group and five of the Control Group being sacrificed at 30-day intervals after the spraying of the solution (30, 60, 90, 120 and 180 days). Thus, five subgroups of the Papain Group (P30, P60, P90, P120 and P180) and five subgroups of the Control Group (C30, C60, C90, C120 and C180) were formed.

All the animals were submitted to anesthesia using 2% isoflurane under 100% O_2 utilizing a gas sprayer. After orotracheal intubation, the rats were connected to a small animal ventilator at a air current of 10 mL/kg and respiratory rate of 80 cycles per minute (Figure 1).

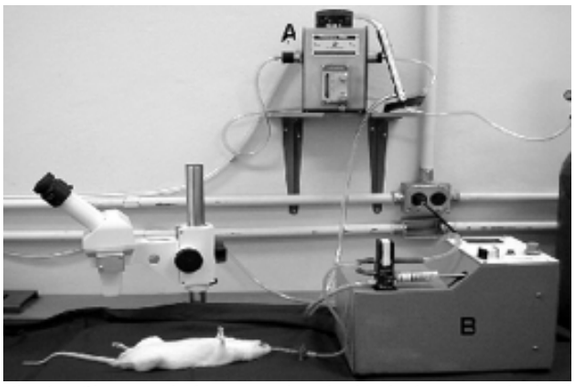

Fig. 1 – Photograph demonstrating the intubed rat, anesthetized and under mechanical ventilation ready for the spraying of papain solution. A – Gas sprayer. B – Small animal ventilator

The animals of the Papain Group were submitted to intratracheal spraying of 6 mg/kg of papain dissolved in 1 mL of 0.9% physiological solution slowly diffused over two minutes by means of an orotracheal catheter. The same technique was employed for the animals in the Control Group except only physiological solution without papain was instilled.

Consequently the animals were removed from the ventilator, maintained under observation until spontaneous respiration was reestablished and extubated and later taken to the animal house.

After the established intervals (30, 60, 90, 120 and 180 days) a blood sample was drawn directly from the abdominal artery using a syringe with heparin to measure the blood gases, the animals were sacrificed by bleeding and the heart and lungs were removed by median sternotomy.

The lungs were fixed under pressure for 48 hours and subsequently three samples from each lung were collected from the central and peripheral portions. These sections were submitted to the routine histological techniques and stained with hematoxylin-eosin.

Aiming at investigating the morphology of the samples, 20 non-successive microscopic fields of each slide (randomly chosen and coded) were examined with the help of a reticulated eyepiece coupled to an optic microscope with a magnification of 400 x.

The pulmonary emphysema was evaluated by histopathologic analysis (presence of alveolar destruction) and by measurement of the mean alveolar diameter that consists of the determination of the number of times that alveolar wall structures intercept a set of coherent straight lines [5].

After fixing the hearts by immersion in a 10% buffered formaldehyde solution, cross-sectioning was performed of the ventricles midway between the apex and the coronary groove of the heart.

Using a digital camera, the hearts were photographed and the digitalized images of the cross-sections of the ventricles were measured using the Image Tool software developed by Department of Dental Diagnostic Science of the University of Texas Health Science Center, San Antonio, USA (Figure 2).

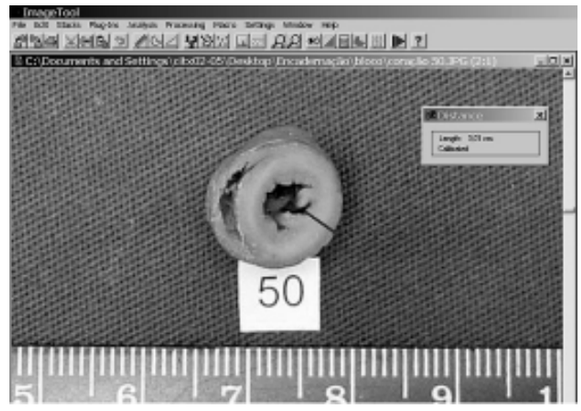

Fig. 2 – Representation of the measurement of the thickness of the left ventricular wall utilizing the Image Tool software

The thickness of the free wall of the right and left ventricles and the interventricular septum were measured, as were the areas of the right and left ventricles.

To test the hypothesis, Analysis of Variance of two factors was used: Papain and Control Groups, intratracheal post-papain spraying intervals (30, 60, 90, 120 and 180 days). The differences among the groups were compared using

the Bonferroni t-test. The level of significance for analysis was set at 5% (p-value > 0.05).

RESULTS

In the Papain Group there were morphologic alterations characteristic of emphysematous lesions with a pattern of panacinar emphysema, that is, involvement of actins with a diffuse widening from the hilar region to the periphery of the lungs. There was destruction of the septa and an increase in the alveolar spaces, with formation of structures at the ends of this rupture known as "drum sticks" (Figure 3).

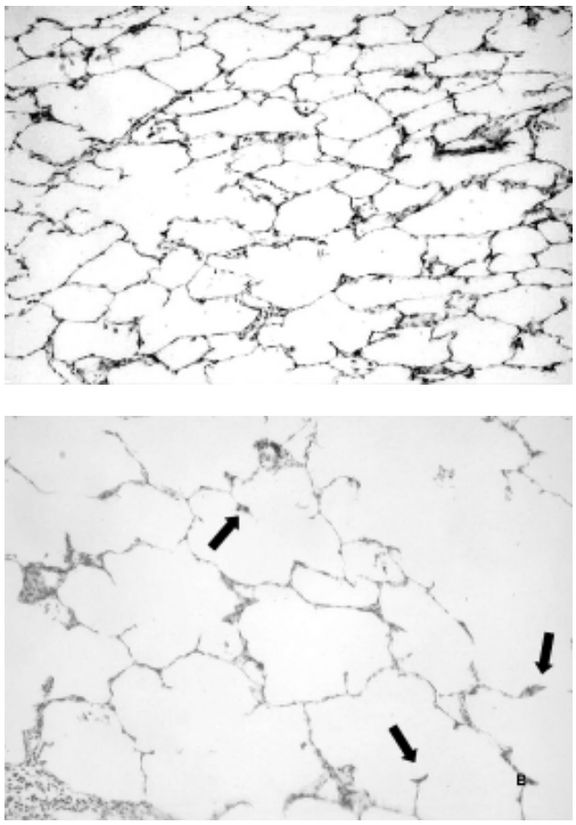

Fig. 3 – Photomicrography of the pulmonary parenchyma of rats submitted to the spraying of physiologic solution (A) or papain (B). Arrows show the formation of "drum sticks" HE staining Magnification 100 x

The increase in the mean alveolar diameter and consequent reduction of the alveolar surface were observed in both lungs of the animals submitted to papain spraying (Figure 4).

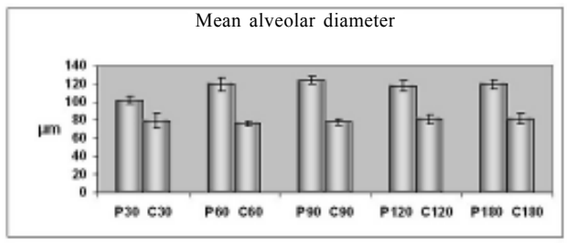

Fig. 4 – Mean alveolar diameter (in µm) in the Papain and Control Groups at different time intervals

The Papain Group presented with mean alveolar diameters greater than the Control Group (p-value <0.05). The mean alveolar diameter in the Control Group ranged between 77.78 and 81.26 ì m, without significant differences among the different intervals assessed. The Papain Group presented a mean alveolar diameter which varied between 101.71 and 124.02 ì m at the different intervals. Except for the P30 subgroup, the mean alveolar diameter in the Papain Group was constant during the evaluation period.

From 90 days after the induction of pulmonary emphysema, there was an increase in the PCO_2 in the Papain Group when compared to the Control Group (p-value < 0.05). The other blood gasometric parameters presented slight alterations, though withoutshowing statistical significance.

The thickness of the right ventricular free wall was similar in the Papain and control Groups (p-value = 0.9735), varying from 1.35 and 1.87 and between 1.39 and 1.69 in the two groups, respectively. No statistically significant differences were observed at the different intervals (p-value = 0.0555) and thus there was no evidence of cor pulmonale (Figure 5).

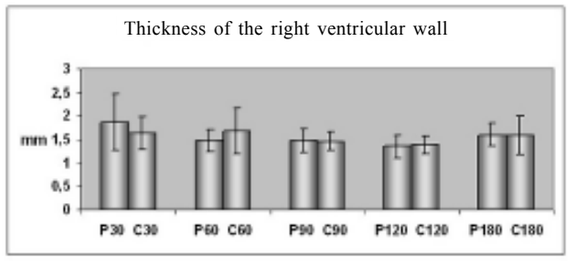

Fig. 5 – Mean thickness of the right ventricular wall (in mm) in the Papain and Control Groups at different time intervals

The septal thickness varied from 3.68 to 3.98 mm in the Papain Group and from 3.43 to 3.82 mm in the Control Group. So, no statistical significant differences were seen in the thickness of the interventricular septa between the Papain and Control Groups (p-value = 0.0737) and at the different time intervals (p-value = 0.9944). The thickness of the

interventricular septa of the hearts from the Papain Group increased with time from the 90th day post-spraying, even though no statistically significant differences were observed.

The thickness of the left ventricle wall showed an increase in the hearts submitted to intratracheal spraying of Papain when compared with the Control Group (p-value = 0.0497). Thus, similar to the interventricular septum, the thickness of the free wall of the Papain Group increased over time from the 90th post-spraying day although no significant differences were seen. The thickness of the left ventricle free wall of the Papain Group varied from 4.29 to 4.93 mm whilst in the Control Group the thickness ranged from 4.28 to 4.63 mm.

As can be seen in Figure 6, in the Papain Group, the mean area of the left ventricle cavity presented with a decrease from the 90th day which was not observed in the Control Group. In some animals, the reduction of the left ventricle cavity was very significant (Figure 7).

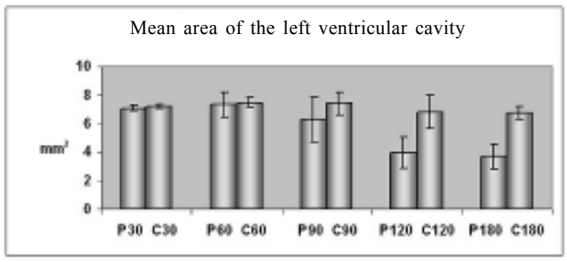

Fig. 6 – Mean area of the left ventricular cavity (in mm2) in the Papain and Control Groups at different time intervals

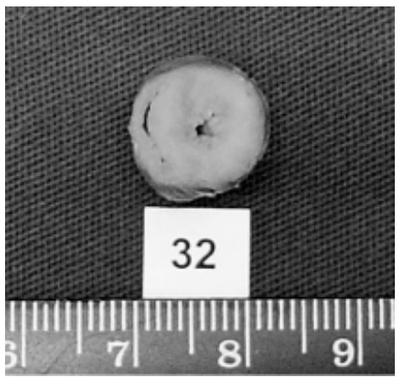

Fig. 7 – Photograph of a rat's heart submitted to intratracheal spraying of papain (P180 Group) demonstrating significant hypertrophy of the left ventricular wall and a reduction of the cavity lumen

The mean area of the right ventricle cavity was similar in both the Papain and Control Groups at the different time intervals, except for the P120 and P180 subgroups which presented with larger mean areas compared to their respective controls (p-value = 0.001) – Figure 8.

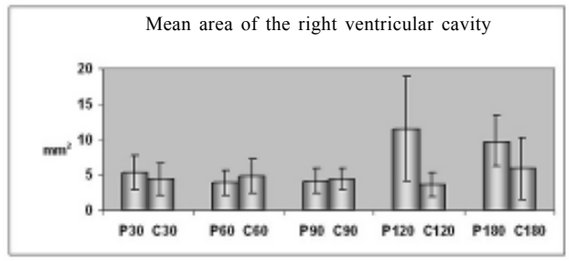

Fig. 8 – Mean area of the right ventricular cavity (in mm2) in the Papain and Control Groups at different time intervals

COMMENTS

An investigation into the mechanisms of in vivo destruction of the pulmonary parenchyma by the administration of proteolytic enzymes such as papain that cause pulmonary emphysema and COPD, has been one of the areas related to pulmonary diseases most intensely researched over the last forty years [6].

Papain is recognized for its potent elastolytic activity, degrading not only the elastin, but also the collagen of the tissues [6].

The histopathologic evaluation of the lungs of the animals submitted to papain administration in this study demonstrated diffuse alveolar destruction, characterizing panacinar-type emphysema, a pattern also reported by other investigators [4,7].

The calculation of the mean alveolar diameter employed in this study is a classically used method to measure pulmonary emphysema. It is well known that, in healthy rats without pulmonary diseases, the mean alveolar diameter is about 70 μm [5]. In this study, the animals of the Papain Group presented with mean alveolar diameters of between 101.71 and 124.02 μm, statistically different to the Control Group. Other authors also found similar values to our findings [4,8,9]. These data demonstrate that, in the lungs of animals submitted to the action of papain, there was a significant increase in the alveolar spaces consequent to the rupture of interalveolar septa.

The analysis of the alveolar diameter in our experiment shows that the P30 subgroup presented with a mean alveolar diameter smaller than the other subgroups exposed to the action of papain, and that these subgroups did not exhibit

differences between each other. The destruction of the pulmonary parenchyma starts with the aggression of the papain, progressing until it stabilizes in the period between 30 and 60 days after exposure, when a significant reduction in the internal alveolar surface is observed. These data corroborate the claim of Fusco et al. [4] that emphysema stabilizes at about 40 days. A similar pattern was observed in hamsters after the spraying of papain or pancreatic elastase verifying the significant progression of emphysema between 1 and 2 months after exposure, followed by stabilization of the histologic injury [10]. Other authors have stated that the injury stabilizes in about the third month after the initial exposure to papain [8,9]. MARTORANA et al. [7] observed that the alterations in the pulmonary structure seen after the administration of papain are progressive over a few weeks and after stabilize, with no differences observed in dogs between 3 and 6 months after the induction of the emphysema. In a work performed by JOHANSON et al. [11] the rats were submitted to a single administration of papain and sacrificed at different time intervals which varied from hours to 3 months after exposure. They verified with this experiment that the major expression of the alterations (alveolar hemorrhage, polymorphonuclear infiltration, inflammation, dilation of the centrilobular air spaces) is observed between 2 and 4 weeks and after there is no regression or progression of the injury.

One of the most common complications reported in patients suffering from COPD is cor pulmonale, characterized by dilation and hypertrophy of the right ventricle and potentially secondary insufficiency and primary hypertension [12].

Although cor pulmonale is frequently observed in patients, we did not evidence signs of its presence in our animals as was initially expected. Several mechanisms have been considered as pathogenic factors of cor pulmonale in pulmonary emphysema, including hypoxia, reduction of the pulmonary vascular territory and increase of the pulmonary arterial pressure and increase in the viscosity of the blood caused by polycytemia secondary to hypoxia [13].

In the study performed by ICOCHEA et al. [13], hypoxia was identified as one of the factors responsible for the development of cor pulmonale in hamsters with experimentally induced pulmonary emphysema, as therapy using oxygen produced an improvement in the right ventricular function. Hypertrophy of the right ventricle can be considered indicative of pulmonary hypertension, which is related to the degree of alveolar hypoxia [14]. Alveolar hypoxia is a powerful constrictor of the pulmonary vessels and there is a proportional inverse relationship between the pulmonary arterial pressure and the PaO_2 in patients with cor pulmonale [14].

Perhaps the absence of significant gasometric blood alterations, remembering that hypoxic vasoconstriction is one of the determinants of pulmonary hypertension, might explain why no alterations were seen in the right ventricles of the animals in this current study over a period of 180 days.

Another aspect to be considered is that the degree of alveolar destruction, caused by the method of pulmonary emphysema induction that we employed, may not have been sufficient to reproduce the morphophysiological conditions necessary for the development of cor pulmonale. Nevertheless, some authors state that there is no relationship between the presence of right ventricular hypertrophy and the percentage of the lung destroyed by emphysema [14-16].

Additionally, it is possible that cor pulmonale develops at a later stage, requiring a longer period of observation. However, according to some authors, the first signs of right ventricular hypertrophy are already seen at three months after the induction of emphysema, with evident hypertrophy at six months [8].

We observed in our study an increase in the area of the right ventricular cavity, from about 120 days after the spraying of papain, associated with hypertrophy of the left ventricle.

Several authors have stated that the presence of left ventricular hypertrophy in patients with pulmonary emphysema is a controversial subject [12] and many attribute it to systemic arterial hypertension [14,17], even without clinical detection. Thus, one hypothesis that we put forth to explain the presence of hypertrophy of the left ventricle in our animals was the possibility that papain introduced systemic arterial hypertension, as hypertrophy is generally associated to a pressure overload. We did not find any published articles that associate the drug to systemic arterial hypertension. However, there are some authors that affirm that papain has an action on the calcium channels, a fact which requires further investigation [18].

According to ISHIKAWA et al. [16], one of the most embarrassing problems faced by clinicians and pathologists is the supposed existence of biventricular hypertrophy in obstructive pulmonary disease.

MURPHY et al. [19] claimed that the incidence of left ventricular hypertrophy in patients with pulmonary emphysema varies in different publications, affecting between 24 and 86% of patients. Although some postmortem studies [17,20] have reported left ventricular hypertrophy in patients with COPD, works performed using non-invasive methods and catheterization demonstrated normal sizes and functioning in these patients [21]. Left ventricular hypertrophy in isolation, without right hypertrophy, was also found in some postmortem cases [17]. SUTINEN et al. [22], found left ventricular hypertrophy in many corpses with pulmonary emphysema, however without showing

statistical significance.

In conclusion, we agree with the authors who state that the association between pulmonary emphysema and left ventricular hypertrophy is an undecided question [17], with obscure causes [19], requiring further investigations.

CONCLUSIONS

In face of what was found, we conclude that the model employed for the induction of pulmonary emphysema was efficient, producing alveolar tissue destruction and mimicking the morphological alterations found in pulmonary emphysema, which are established in up to 60 days after the initial aggression without further progression.

The presence of pulmonary emphysema did not cause morphological alterations in the right ventricular wall or interventricular septum, while the alveolar destruction induced hypertrophy of the left ventricle and dilation of the right ventricle.

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