Effects of exercise training on papain-induced pulmonary emphysema in Wistar rats

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Effects of exercise training on papain-induced pulmonary emphysema in Wistar rats. J Appl Physiol 100: 281–285, 2006.—The purpose of the present study was to evaluate the role of exercise training on the development of papain-induced emphysema in rats. Our hypothesis was that the increase in pulmonary tissue stretching associated with exercise could increase the severity of a protease-induced emphysema. Wistar rats were randomly assigned to four groups (n = 10 for each group) that received, respectively, intratracheal infusion of papain (6 mg in 1 ml of 0.9% NaCl) or vehicle and were submitted or not to a protocol of exercise on a treadmill. Rats exercised at 13.3 m/min, 6 days/week, for 9 wk (increasing exercise time, from 10 to 35 min). We measured respiratory system elastance and resistance, the size and weight of the heart, and pulmonary mean linear intercept (Lm). After 9 wk of exercise training, there were no differences in respiratory system resistance and elastance values among the four experimental groups. Volume of the heart was significantly greater in rats submitted to exercise training (P = 0.007) compared with sedentary rats due to increases in volumes of both right and left cardiac chambers. Lm was significantly greater in rats that received papain compared with saline-infused rats (P = 0.025). Surprisingly, this was true, even though there was no significant decrease in elastance, possibly due to connective tissue remodeling. However, Lm was significantly greater in papain + exercise rats compared with rats that received papain and were not submitted to exercise. We conclude that exercise training can increase alveolar damage induced by papain infusion.

CHRONIC OBSTRUCTIVE PULMONARY disease (COPD) is a major cause of respiratory disability and death worldwide (22). Among COPD patients, ~20% present with emphysema, whereas 80% have chronic bronchitis or a combination of emphysema and chronic bronchitis. Emphysema is characterized by permanent destruction of alveolar walls, resulting in air space enlargement (4). The pathophysiology of emphysema is not completely understood.

The most accepted hypothesis concerning emphysema development is the presence of an imbalance between protease and antiprotease activity within the lung tissue, resulting in degradation of elastin (11, 29). However, it has been shown in animal models that it is possible to induce pulmonary emphysema by other mechanisms, such as increased collagenase activity (6) or inhibition of proteoglycan synthesis (30).

Recently, Kononov et al. (13) observed in lungs of rats with elastase-induced emphysema that, during stretching, the newly deposited elastin and collagen fibers undergo larger distortions than in normal tissue. They suggested that mechanical forces during breathing are capable of causing failure of the remodeled extracellular matrix and may contribute to the progression of emphysema.

The role of exercise training in pulmonary rehabilitation of patients with severe COPD has been extensively studied (2). Exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance but has inconsistent effects on measurements of respiratory impairment (1, 5). However, the effects of exercise training on the development of emphysema have not been determined.

Several animal models have been used to study the pathophysiology of pulmonary emphysema. Instillation of proteases with elastolytic activity such as papain and elastase into the lungs of laboratory animals has been extensively used and results in morphological pulmonary changes that are similar to human panlobular emphysema (10, 23).

The purpose of the present study was to evaluate the role of exercise training in the development of papain-induced emphysema in rats. We reasoned that the increase in pulmonary stretching associated with exercise could increase the severity of a protease-induced emphysema.

METHODS

This study was approved by the Institutional Review Board of the School of Medicine of the University of São Paulo. Eight-week-old male Wistar rats weighing 278.2 ± 7.6 g (mean ± SD) were used throughout the study. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” published by the National Institutes of Health (NIH publication 86-23, revised 1985).

Experimenal groups. Rats were randomly separated into four groups (n = 10 for each group): 1) rats that received intratracheal infusion of papain and were submitted to a protocol of exercise on a treadmill [papain-exercise (PE)]; 2) rats that received intratracheal infusion of papain vehicle (NaCl 0.9%; saline) and were submitted to exercise [saline-exercise (SE)]; 3) rats that received intratracheal papain and were not submitted to exercise [papain-sedentary (PS)]; and 4) rats that received intratracheal saline and remained sedentary [saline-sedentary (SS)].

Emphysema induction. The rats were anesthetized with inhalation ether, and a polyethylene cannula was inserted into the trachea. The
tracheal cannula was connected to a rodent ventilator (Harvard 683, Harvard Apparatus, South Natick, MA), and the rats were ventilated with a tidal volume of 10 ml/kg and a respiratory rate of 90 breaths/min and received a mixture of 1% isoflurane (Baxter Healthcare of Porto Rico), diluted in 100% oxygen, at a flow rate of 2 l/min with the use of a gas nebulizer (1224K, Takaoka, Brazil).

Emphysema was induced by a single intratracheal instillation of papain. Six milligrams of papain (10–20 U/mg protein, Sigma Chemical, St. Louis, MO) were diluted in 1 ml of 0.9% NaCl and infused into the tracheal cannula. Control (saline) rats received intratracheal instillation of 1 ml of 0.9% NaCl.

**Exercise training protocol.** Two days after tracheal instillation of either papain or saline, SE and PE groups were subjected to an exercise-training protocol. We used a human motor-driven treadmill (HT 4000, Inbramed), adapted with eight aluminum chambers with Plexiglas covers (10 × 10 × 50 cm each) for training of rats. The treadmill was set at 0° inclination. There was a previous 2 wk of familiarization with the motor-driven treadmill. The rats ran at 13.3 m/min, 6 days/wk, for 9 wk. Rats exercised for 10, 15, 20, 25, 30, 35, and 35 min/day, respectively, on weeks 1–9. All training sessions were carried out between 5:30 and 7:30 PM.

**Measurements of respiratory system mechanics.** Forty-eight hours after the last training session, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip), tracheostomized, and mechanically ventilated. Tracheal pressure (Ptr) was measured with a pressure transducer (DP 45-28-2114, Validyne, Northridge, CA) connected to a side tap in the orotracheal cannula. Airflow (V˙) was measured with a pneumotachograph (Fleisch-4.0, OEM Medical, Richmond, VA) attached to the tracheal cannula and to a differential pressure transducer (Validyne DP 45-16-2114). Ptr and V˙ signs were registered in a Gould RS-3400 recorder (Gould Instruments, Cleveland, OH), sampled at 200 Hz with an analog-to-digital converter (DT 2801 A, Data Translation, Marlboro, MA), and stored in a microcomputer. Lung volume (V) changes were obtained by electronic integration of V˙.

We computed respiratory system resistance (Rrs) and elastance (Ers) by least squares fitting of measured values of Ptr, V, and V˙, over 9–10 respiratory cycles, to the equation of motion of the respiratory system, as follows (19, 20)

\[
\text{Ptr}(t) = \text{Ers} \times \text{V}(t) + \text{Rrs} \times \text{V}(t)
\]

where \(t\) is time. All data were collected and processed by using LABDAT software (RHT-InfoData, Montreal, Quebec, Canada).

**Lung and heart morphological evaluation.** After measurements of respiratory mechanics, the abdominal wall was opened, and the abdominal aorta was cut. The thoracic cavity was then opened, and the lungs and heart were removed.

Left lungs were distended with neutral buffered 10% formaldehyde infused through the main bronchus at 25 cmH2O and fixed for 24 h. After fixation, transversal slices of the lung were embedded in paraffin. Five-micrometer-thick histological sections were obtained and stained with Masson Trichrome.

A Zeiss Axiosplan microscope was connected to a video camera and to a high-resolution video. Using a grid with lines of known length, the average air space distance [mean linear intercept (Lm)] was determined in 30 randomly chosen microscopic fields per lung. The observers that performed the measurements were blinded to the experimental groups of the rats.

Two polyethylene cannulas were inserted into the heart, through the aorta and pulmonary artery, respectively, and neutral buffered 4% paraformaldehyde was infused to fill, respectively, the left and right cardiac ventricles until complete filling of the ventricles was observed. Paraformaldehyde was infused up to a point that reflux of fixative was observed. No increase in pressure or distention of the ventricles was involved in the process. After filling the heart, all arteries and veins were ligated to avoid emptying of the ventricles. All hearts remained in the paraformaldehyde solution for 24 h and were then transferred to 70% ethanol, remaining for another 24 h. The hearts were then weighted with all ventricles filled, then with the left ventricle empty, and then with both left and right ventricles empty, to measure the volume of cardiac ventricles.

**Statistical analysis.** All values are expressed as means ± SE. The statistical significance of differences among values of the weight of the rats was determined by two-way repeated-measures ANOVA. Ers, Rrs, Lm, and weights and volumes of the heart were analyzed with two-way ANOVA followed by Tukey’s test. The statistical software Sigma-Stat 2.0 for Windows (Jandel Scientific, San Rafael, CA) was used.

**RESULTS**

Figure 1 shows body weight values measured during the experimental period. There was a significant increase in the weight of all experimental groups (\(P < 0.001\)). There were no significant differences in weight among the four experimental groups studied.

Figures 2, A and B, shows, respectively, mean (±SE) values of Rrs and Ers obtained in the four groups of rats at the end of the experimental protocol. We did not observe significant differences in values of either Rrs or Ers when the four groups were compared.

Figure 3 shows representative photomicrographs of lungs from rats that received intratracheal instillation of 0.9% NaCl (A) or papain (B). We observed a substantial destruction of alveolar walls, resulting in enlargement of distal airway spaces. Figure 4 shows Lm values measured in the four experimental groups. Animals that received papain instillation showed a significant increase in mean values of Lm (\(P = 0.025\)) compared with rats that received tracheal instillation of vehicle (saline). Mean Lm values of the lungs of rats that received papain and were submitted to the protocol of exercise training were significantly greater than those in both the PS group and the control (SE or SS) groups (\(P < 0.001\)).

Hearts from the rats submitted to exercise training showed an increase in full weight (\(P = 0.007\)) but not in their empty weight (Fig. 5A). The increase in full weight was due to an increase in the volumes of both left and right ventricles (Fig. 5B). We did not observe significant differences between these values when rats submitted to exercise training were compared with rats that remained sedentary.

![Fig. 1. Body weight values obtained during the experimental period in the four groups of rats studied. Time 0, day of tracheal instillation of either papain or saline. Values are means ± SE. There was a significant increase in body weight (\(P < 0.001\)) during the experiments in all groups. There was no significant difference among the groups.](http://jap.physiology.org/Downloaded_from/10.22038/10.22038.1.2017)
DISCUSSION

In the present study, we observed that exercise training starting 2 days after intratracheal papain infusion increases the severity of alveolar damage, worsening emphysema. To our knowledge, there were no previous studies that aimed to determine the effect of exercise after intratracheal infusion of a protease such as papain.

The first reproducible model of emphysema was described in 1965 by Gross et al. by instilling papain (a plant protease) into the lung of rats (7, 9). Together with the observation of Laurell and Eriksson (16) of an association between serum alpha-1 antitrypsin (a protease inhibitor) deficiency and emphysema, the animal model of papain-induced emphysema was the basis of the protease-antiprotease hypothesis of the pathophysiology of emphysema. This experimental model of emphysema has several limitations, since the lung injury is caused by a single massive insult rather than a continuous low-grade inflammation, such as that believed to be present in pulmonary injury due to cigarette smoking (7). In addition, the instillation of a single high dose of a protease into the airways may not reflect the processes that result from the delivery of enzymes in minute amounts by activated inflammatory cells in pulmonary interstitium in human emphysema (18). Interestingly, it has been shown that some of the instilled enzyme appears to be sequestered within pulmonary macrophages and/or complexed with alpha-2 macroglobulin in the lung (25, 27). A slow release of active enzyme from these reservoirs may contribute to

Fig. 2. Respiratory system resistance ($R_{rs}$; A) and respiratory system elastance ($E_{rs}$; B) obtained at the end of the experimental period (9 wk). Values are means ± SE. There were no significant differences in either $R_{rs}$ values or $E_{rs}$ values among the four groups of rats.

Fig. 3. Photomicrographs of lung parenchyma 9 wk after tracheal instillation of 1 ml of either 0.9% NaCl (A) or papain (6 mg/ml; B) (original magnification ×100, Masson Trichrome staining). A: alveolar wall integrity is preserved. B: there is alveolar wall destruction and marked enlargement of distal air spaces.

Fig. 4. Mean linear intercept values measured in the four experimental groups. Values are means ± SE. *Significantly greater than the groups of rats that received intratracheal instillation of saline ($P = 0.025$); **significantly greater than the other three groups ($P < 0.001$).
chronic lung injury. However, as pointed out by Dawkins and Stockley (7), experimental models of emphysema induced by a single instillation of a protease into the airways can be useful for studying events after the insult. In the present study, we aimed just to evaluate the effect of 9 wk of exercise training in the pulmonary response to papain instillation.

In our experimental model, we did not observe a significant influence of papain infusion on Rrs and Ers, although morphometric analysis showed a substantial development of pulmonary emphysema. Many authors have shown that induction of emphysema with either papain or elastase in a variety of animal species produced increases in pulmonary compliance (26). In contrast, Johanson and Pierce (12) studied the time course of a single papain infusion in 2-mo-old rats and observed an increase in lung compliance 2 mo after papain infusion, but these differences were not present anymore when rats were studied at 8 or 18 mo of age. They suggested the presence of lung tissue growth and/or repair with normal elastic properties. These differences in the presence or absence of pulmonary compliance alterations in experimental models of emphysema may be due to differences in experimental protocols, resulting in differences in pulmonary tissue remodeling. Biochemical and histological studies suggest that the amount of connective tissue fibers and/or their organization is an important determinant of mechanical alterations in experimental emphysema (13, 14). Kononov et al. (13) observed in rats that elastase administration induces a significant change in elastin-collagen network, with thickened elastin and collagen fibers. In our study, we observed, in the lungs of rats that received papain, the presence of focal areas of alveolar distortion (Fig. 3), either caused by compression of the dilated air spaces over the surrounding parenchyma or the result of obstructive lesions of the small airways, also observed in papain-treated rats. Because we measured dynamic elastance, our measurements of Ers in emphysematous rats may be affected by the uneven distribution of inspired air. Another possible explanation for the lack of differences in dynamic elastance is that Ers was measured at a higher V in papain-treated rats due to differences in pressure-volume relationships between saline- and papain-treated rats.

The protocol of treadmill exercise training that we used was similar to the protocols used in rats by other research groups (17, 21). We observed an increase in heart ventricle volume, consistent with the volume overload induced by aerobic exercise. We did not observe a significant cardiac hypertrophy, as the weight of empty hearts was not different when sedentary and exercise-trained rats were compared. The volume increase was present in both left and right cardiac ventricles. The increase in the right ventricle was present in rats submitted to exercise training, either with emphysema or not, suggesting that this increase was due to exercise itself and not to cor pulmonale.

The main finding of our study was a worsening of pulmonary emphysema induced by exercise training. Our hypothesis is that the increase in pulmonary distention and/or alveolar stretching, due to the increase in tidal volume, due to exercise training, somehow exacerbated the alveolar alterations induced by papain instillation. There are few studies that provide insights regarding this question. It has been shown that patients with end-stage emphysema who are submitted to V reduction surgery lose lung function over time after surgery at a rate that exceeds that existing preoperatively (8). Because the remaining lung of these patients is stretched to fill the thoracic cavity, it is possible that the increase in mechanical forces on the connective tissue may contribute to the worsening of pulmonary function. West (31) suggested that the distribution of mechanical stresses in the lung is related to the areas of more intense injury in centrilobular emphysema. Kononov et al. (13) developed a technique to measure the stress-strain properties of lung tissue sections and were able to visualize the deformation of the elastin-collagen network labeled with immunofluorescence. During stretching, the newly deposited elastin and collagen fibers presented larger distortions than in normal tissue. In addition, they observed that the threshold for mechanical failure of collagen during stretching is reduced when emphysema tissue is compared with normal tissue. One alternative explanation for the worsening of emphysema induced by exercise was the presence of exercise-induced oxidative stress. It has been demonstrated that strenuous aerobic exercise is associated with oxidative stress and tissue damage (15, 24). However, the protocol we used was not of strenuous exercise, and moderate exercise training is associated with adaptive responses in at least some antioxidant capacities (3, 28).

In conclusion, we observed that, in rats with emphysema induced by papain infusion, the severity of emphysema increased when the rats were submitted to an exercise training protocol. This suggests that the volume changes and mechan-
EXERCISE TRAINING AND PAPAIN-INDUCED EMPHYSEMA

...ical forces related to the pulmonary response to exercise play a role in the alveolar damage induced by papain, but the precise mechanism of this effect remains to be established.

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