Airway and lung parenchyma morphology during the respiratory cycle

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Summary. Objective: Describe the morphological changes that take place in the lung parenchyma and in the airways during the respiratory cycle with a view to establishing a relationship between them. Subjects: Adult Wistar rats. Interventions: The lungs were fixed at seven different points in the respiratory cycle: Inflation, 10 and 20 cm transpulmonary pressure, total lung capacity. Deflation, 20, 15, 10 and 0 cm transpulmonary pressure. Measurements: The lungs were processed for morphometric study and bronchial and parenchymal variables, such as lung volume, number of alveoli, anatomic dead space, bronchial lumen surface and bronchial wall surface were quantified. The results were compared by analysis of variance (ANOVA) or the Kruskal-Wallis and Mann-Whitney’s U tests. Results: The lung volume, the number of alveoli and the anatomic dead space increased with the increase of the transpulmonary pressure and decreased with the decrease of it, the obtained values in deflation being higher than those in inflation (p<0.05). The bronchial lumen and the bronchial wall surfaces generally showed higher values in inflation than in deflation (p<0.05). Conclusions: The anatomic dead space was altered as a consequence of the variations in airway diameter and length. Lung parenchyma tension may have been of influence in the variations of the bronchial wall.

Key words: Alveolar recruitment, Anatomic dead space, Lung hysteresis, Rat

Introduction

During the respiratory cycle the lung undergoes structural changes both at parenchymal and airway levels. Various functional and morphological studies have proved that the number of peripheral airspaces (PAS) increase with lung volume increase on inflation (Mead et al., 1957; Frazer et al., 1985; Lum and Mitzner, 1987). This process is referred to as alveolar recruitment. It is caused by the opening of PAS, which remain closed at low transpulmonary pressure (Ptp) (Lum and Mitzner, 1987). The opposite occurs on deflation: a decrease takes place in the number of PAS, which is known as alveolar de-recruitment (Escolar et al., 2002). With respect to the airway, it has been proved that anatomic dead space (ADS) increases during inflation and decreases during deflation (Froeb and Mead, 1968; Escolar et al., 2003b). These changes in ADS are caused by some modifications both in bronchial diameter and bronchial length: bronchial diameter increases at the beginning of inflation (Marshall, 1962; Hughes et al., 1972), whereas at the end of the process the bronchi are lengthened (Chelucci et al., 1999).

These changes taking place in the lung parenchyma during the respiratory process may be related to the changes that occur in the airways (Froeb and Mead, 1968; Hughes et al., 1972). However, respiratory cycle patterns depict lung parenchyma behavior as being unrelated to airway behavior. A morphological model of the respiratory cycle of the lung is presented below, with a description of the parenchyma and the airways. It is suggested that the changes in the airways are related to the lung parenchyma variations.

Materials and methods

Seventy-five healthy 5-month-old male Wistar rats were used. The animals were supplied by Iffa Credo laboratories. Experimental protocol was approved by the Ethics Committee for Animal Experiments (University of Zaragoza). For handling purposes, all of them were anesthetized with pentothal (0.1 mg/g of body weight). The experiment was organized in three protocols:

1) The purpose was to describe the pressure-volume curve in the rat. Ten rats were used. An air-filled syringe...
was installed in a perfusion pump and connected to the rat trachea; the system had a manometer.

2) The aim was to establish guidelines that would enable the lung to be fixed through the pulmonary artery while maintaining it full of air at constant Ptp. Thirty animals were used; a flexible cannula through which the fixing solution was perfused was introduced in each of them from the inferior vena cava to the atrium. The most common problems were pneumothorax and the positioning of the cannula in a hepatic vein. In order to avoid this, when the cannula was located behind the liver, the trunk of the animal was hyperextended. Ptp was maintained constant by means of a water column that ended in a recipient, with an orifice in the upper part that was connected to the trachea.

3) The purpose was to describe the lung morphometrically during a respiratory cycle. Thirty-five animals were organized in seven groups, five animals in each group, according to the point in the respiratory cycle at which they were fixed; the first, second and third groups were fixed respectively at 10, 20 and 27 cm water Ptp in inflation. The fourth to seventh groups were fixed at 20, 15, 10 and 0 cm Ptp in deflation. All the animals were processed for histological study.

Fixation was performed with formalin at 10% for 48 hours. The lungs were post-fixed by immersion in the same fixing substance for 15 days. The fixing substance was perfused at a pressure of 30 cm of water

Histological processing

After the postfixation period the heart was separated from the lungs and the pulmonary volume was measured. Next the lungs were cut transversally into 0.5 cm-thick sections. Three slices per lung were chosen at random. They were dehydrated and placed in paraffin. 7 µm cuts were made and stained with methylene blue and hematoxylin eosin.

Morphometric study

The following variables were studied:

Lung volume (Pv): after the lungs were fixed the trachea was ligated and the lungs were immersed in a saline solution. The volume of displaced liquid was measured. It is expressed in cm³.

For capture, an Olympus BX 50 microscope, a Sony XC57CE video camera, a Power Macintosh 7200 90 computer, a Grabber digitalization card and Grabber capture program were used. The images were captured in 256 gray tones at a size of 551 400 pixels. A Power Macintosh 7200 90 computer, the program NIH image 1.60b7 and another program developed by ourselves (Escolar et al., 2002) were used for the processing and the quantification of the images.

The following protocol was used for the quantification of the lung parenchyma variables: seven fields were captured at random in the cuts stained with methylene blue, at x100 and in 256 gray tones (Fig. 1); 1,470 fields were studied; the images were binarized (Fig. 2); the following variables were measured in the resultant image (Escolar et al., 2002).

Alveolar chord (Ac): distance between two walls of a single alveolus (Fig. 2). The result is expressed in µm.

Alveolar wall thickness: expressed in µm (Fig. 2).

Lung parenchyma surface: applies to the gray color surface in figure 2. The results of this variable are not presented.

PAS surface: section area of an alveolus (Fig. 2). It is expressed in µm².

Alveolar volume: calculated considering that the alveolar chord is the mean diameter of an alveolus; the sphere volume formula was applied. The results of this variable

Fig. 1. Histological cut stained with methylene blue and captured in 256 gray tones (x 100).

Fig. 2. Figure 1 in which the threshold option was applied. White: air; gray: tissue. Black line surrounding the tissue: internal alveolar perimeter. Ac: Alveolar chord. Wc: alveolar wall thickness.
are not presented.

*Tissue volume*: the anatomic dead space was subtracted from the lung volume and the result was related to the lung parenchyma surface and the anatomic dead space. The results of this variable are not presented.

*Alveolar air volume*: the anatomic dead space and the tissue volume were subtracted from the lung volume. The results are expressed in cm³.

*Number of PAS in each lung*: the alveolar air volume was divided by the alveolar volume.

*Internal alveolar perimeter*: calculated directly by the computer. It is represented by the black line surrounding the tissue in figure 2. The results of this variable are not presented.

*Internal alveolar surface (IAS)*: calculated from the internal alveolar perimeter and the lung volume. The results are expressed in cm².

The bronchial variables were quantified from the cuts stained with hematoxylin eosin and methylene blue (Escolar et al., 2003b):

*Anatomic dead space*: the fields were captured x20 in the cuts stained with methylene blue. The area occupied by the airway was selected interactively from the cuts; this value was converted into percentage form and the proportion of lung volume occupied by the airway was obtained from the overall lung volume. This is expressed in cm³.

The following variables were quantified from the cuts stained with hematoxylin eosin. All the bronchi located in the x40, x100 and x200 fields were captured (Fig. 3A) (Escolar et al., 2003b). Bronchi that failed to fulfill the criteria of Bai and cols (Bai et al., 1994) were discarded.

*Internal bronchial area (Ai)*: bronchial luminal area (Fig. 3). It is expressed in µm².

*Total wall area (WAt)*: bronchial wall area. It is expressed in µm².

*Inner wall area (WAi)*: bronchial mucosa surface. It was quantified interactively (Fig. 3B) and is expressed in µm².

Fig. 3. A. Bronchial cut stained with hematoxylin eosin and captured at x200. B: black color: mucosal surface (internal bronchial area). C. black: adventitiial surface (Outer wall area).

Fig. 4. Results of the variables: lung volume, alveolar air volume, distal air space number and internal alveolar surface. The mean and one standard deviation are presented. Continuous lines and black circles represent inflation values; dotted lines and spotted rhombus, deflation values. +: p<0.05 with respect to the 20 cmI group. #: p<0.05 with respect to the 20 cmD group. ·p: <0.05 with respect to the 15 cmD group. +: D p<0.5 with respect to the 10 cmD group.
Outer wall area (W Ao): bronchial adventitia surface. It was quantified interactively (fig. 3C) and is expressed in µm².

Statistical study

The values measured and the dispersion values were calculated. The results were compared by analysis of variance (ANOVA) when close to normal distribution. Otherwise (Kurtosis and Skewness rates), they were compared using the Kruskal-Wallis and Mann-Whitney’s U tests. The mean results are presented 1 standard deviation. Results were considered statistically significant when p<0.05

Results

The results, which are presented in figures 4 to 7, enable us to point out the following: Lung parenchyma variables, (Fig. 4): the higher the Ptp, the larger the Pv, the alveolar air volume, the number of PAS, and the IAS and vice versa. These values fell when the Ptp was decreased. Given the same Ptp, the values obtained in inflation were lower than those obtained in deflation. The alveolar chord (Fig. 5), the PAS surface and the wall thickness decreased as a result of the increase in Ptp and rose when Ptp was decreased. The values obtained from these two variables remained higher in inflation than in deflation.

Bronchial variables: ADS increased on inflation and decreased on deflation (Fig. 6). The values for A₁, WA₁, WAᵢ and WAₒ (Fig. 7) were generally higher in inflation.

Fig. 5. Results of the variables: alveolar chord, PAS surface and alveolar wall thickness. The mean and one standard deviation are presented. Continuous lines and black circles represent inflation values; dotted lines and spotted rhombus, deflation values: +: p<0.05 with respect to the 20 cmI group. *: p<0.05 with respect to the 28 cm group. #: p<0.05 with respect to the 20 cmD group. h p: <.05 with respect to the 15 cmD group. D: p<0.05 with respect to the 10 cmD group.

Fig. 6. Results of the anatomic dead space variable. The mean and one standard deviation are presented. Continuous lines and black circles represent inflation values; dotted lines and spotted rhombus, deflation values. +: p<0.05 with respect to the 20 cmI group. *: p<0.05 with respect to the 28 cm group. #: p<0.05 with respect to the 20 cmD group. h p: <.05 with respect to the 15 cmD group. D: p<0.05 with respect to the 10 cmD group.
than in deflation; in these four variables a peak of maximums was reached at 10 cm. Ptp in deflation; in the case of variables A_i and WA_i a higher value was reached than in inflation at the same Ptp.

Discussion

Our results coincide with the hypothesis proposed, as during the respiratory process the structure of both the bronchial and parenchymal pulmonary components was altered and, given the same Ptp, the lung architecture displays differences between inflation and deflation. Mead et al. (1957) described the P-V curve and related it with alveolar recruitment. This hypothesis states that during inflation the lung volume increases because the PAS are open and during expiration the lung volume decreases because they are closed (Frazer et al., 1985). In our opinion the increase in the variables Pv, alveolar air volume, number of PAS and IAS (Fig. 4) that takes place during inflation and the further decrease of these variables during deflation lead us to conclude that the lungs increase in volume due to an increase in alveolar air volume, giving rise to alveolar recruitment and an increase of the gas exchange surface. It has been suggested that the increase in the number of PAS that takes place during inflation is due to the detachment of the alveolar walls, which unfold, sharing the ductus and resulting in smaller PAS (Fig. 4) (Lum and Mitzner, 1987). We relate this to the discrete decrease/increase in alveolar size and the thinning/thickening of the alveolar walls that takes place during inflation/deflation.

The differences between inspiration and expiration are related to lung hysteresis. The lung is an imperfect elastic, so during breathing it dispels energy. This property of the lung is called hysteresis (Brusasco and Pellegrino, 1995). Lung hysteresis can be quantified as it applies to the surface that exits between the two parts of the P-V curve (Bachofen and Hildebrandt, 1971). The strain that takes place in the lung parenchyma during breathing can be of influence on the airway (Froeb and Mead, 1968; Hughes et al., 1972) so that parenchymal hysteresis is related to bronchial hysteresis, both of which are part of lung hysteresis (Froeb and Mead, 1968). It has been described that the ADS increases in inflation, decreases during expiration and displays hysteresis (Froeb and Mead, 1968). This coincides with the results obtained in the ADS variable (Fig. 6). The discrete increase in A_i obtained by raising the Ptp from 10 to 20 cm. (Fig. 7) coincides with a previous study performed on rat lungs (Escolar et al., 2003a,b). However when the Ptp was increased from 20 to 27 cm a slight decrease occurred in A_i. It has been reported that the greatest increase in airway diameter takes place at low pressures: between 5 and 12 cm. of
H₂O, according to Hughes et al. (1972) and under 10 cm according to Marshall (1962). It has been proposed that the bronchus length increases with high lung volumes and its caliber can decrease (Chelucci et al., 1999). In order to explain this behaviour two proposals have been considered: one makes the bronchial surfactant responsible, which would avoid a significant increase of the bronchial size (Gayrard et al., 1969; Peter et al., 1970). The second proposal considers that the bronchus is an elastic tube which when it increases in length decreases in thickness (Radford, 1963).

It has been described that in deflation the bronchial wall area is larger than during inflation (Hughes et al., 1972; Escolar et al., 2003a,b) and that during deflation when the pulmonary volume decreases from 100% to 25% of total lung capacity the surface of the bronchial wall increased (James et al., 1988). These results do not coincide with ours, probably because in our experiment the lungs studied were fixed “in situ”, unlike in previous studies. When the lung is distended out of the chest the forces that are opposed to distension are tissue forces and those related to surface tension (Bachofen et al., 1987). In our case, the thoracic wall is also in opposition to the Ptp. Following the functional interpretation of the descending segment of the P-V curve (Frazer et al., 1985), we propose that in the first period of deflation the Ptp decreases and the entire lung, parenchyma and airway retract. When the alveoli decrease in size (Frazer et al., 1985) the surface tension increases; the smaller the alveolar size (Frazer et al., 1985), the greater the surface tension. In this sense it has been suggested that with lower Ptp the surface tension forces are predominant (Bachofen et al., 1987). In the second phase of deflation the surface tension increases and a massive closing of the alveolus comes about (Frazer et al., 1985). This can bring about an increase in lung parenchyma tension, which will be transferred to the peribronchial area, which in turn would explain the increase in airway variables at 10 cm Ptp in deflation. Therefore, the reverse process to that which occurs in inspiration takes place: shortening the length of the bronchus causes the wall thickness to increase (Radford, 1963).

With regard to airflow obstruction following deep inspiration in asthmatics, Burns and Gibson (2002) suggest that following deep inspiration the intrapulmonary forces give rise to traction of the peribronchial area, causing negative pressure on the airway wall, which could lead to liquid extravasation or to the ingurgitation of the lymphatic or venous plexus. In the case of healthy individuals these changes are minor. However, in people suffering from asthma there is more tissue in the bronchial wall and the possible liquid extravasation would be more substantial, meaning that it would take longer for the liquid to be reabsorbed from the bronchial wall. The adventitial and mucosal changes were similar, which suggests that both variables take part in the wall thickness changes to the same extent.

For us there are two kinds of fundamental forces that may have influence on the pulmonary morphology: Those derived from the Ptp, as we have seen in this experiment, and the other derived from the pulmonary artery pressure. We have only modified the Ptp, maintaining a constant arterial pressure. (Conhaim et al., 1989; Conhaim and Rodenkirch, 1996). Although the measurements were taken with static pressure in our experiment, we believe that our respiratory cycle model is closer to the “in vivo” situation than the previous models in which the lungs were cut out from the chest during fixation. We conclude this study by proposing that the tension forces generated in the lung parenchyma during breathing can be of influence in structural changes in the bronchial tree.

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