HISTOARCHITECTURAL CHANGES OF RABBIT LUNG PARENCHYMA IN THE COURSE OF EXPERIMENTAL BAROTRAUMA EVALUATED BY QUANTITATIVE METHOD

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Received: June 25, 2011 Accepted: February 10, 2011

Abstract

The aim of the study was to estimate pulmonary inflation in rabbits after experimental pulmonary barotrauma (PB) using the previously described original method for quantitative description of pulmonary histostructure. Rabbits allotted into control (P) and experimental (E) groups were used. The experimental group animals were subjected to PB. Taking into consideration post-traumatic management, group E was divided into two subgroups. Subgroup D animals were sacrificed immediately after the injury while DO subgroups – 3 weeks after the injury. The number of alveoli and other pathologically closed air spaces were counted during histological examinations of frontal cross-sections of the lungs. In D and DO subgroups, pulmonary inflation increased significantly in relation to group P and in DO group the changes were more visible. It has been concluded that PB evokes an increase in air spaces in the lungs and this change, when untreated, intensifies with time.

Key words: rabbits, pulmonary barotrauma, morphology, quantitative methods, morphometry.

Pulmonary barotrauma (PB) is a life threatening complication, which is connected with exposing a man to pressure changes of external environment or/and disproportion of respiratory tract capacity to the volume of breathing factor. PB is connected with a man’s stay under the conditions of both increased (divers, hyperbaric treatment) and decreased (aviation) pressure. In slightly different mechanism, it may occur due to the action of air shock wave (explosions) whereas in clinical practice it is most frequently found during the treatment with aided or substitutive respiration, particularly in hospital intensive care units and neonatal departments (2, 4, 8, 12, 14). The air contained in the lungs and in other gas spaces of the organism increases its volume in the course of turning from higher to lower pressure, resulting in the sudden dilation of histoarchitectonic structures of the organ. On this route, it comes to injuries in divers or airmen. In intensive care units and neonatal departments, the mechanism of pulmonary damage is associated with ventilating patients with constant volume of respiratory mixture, with simultaneously decreasing (inflammatory process, oedema, hyaline membrane syndrome, atelectasis due to other reasons) lung capacity (3, 6, 8, 13, 15). According to Rouby et al. (12), 86% of patients with PB showed dilation of air spaces, mainly in the form of bronchial and vesicular tree distension, and also formation of subpleural and/or intraseptal pseudocysts. These authors think that the observed changes are specific for PB. An increase in normal and pathologic air spaces of the lungs in PB was also observed by other authors (1, 3, 7, 9, 11, 13). Evaluation of rabbits’ pulmonary inflation after experimental barotrauma was performed with the application of own half-quantitative method of slide analysis.

Material and Methods

The experiment was carried out on German Lop rabbits of both sexes, aged 28-34 weeks and body mass of 2.24-5.25 g. Before the experiment, the animals were subjected to X-ray examination of chest in order to exclude animals with pathological changes in their lungs. The rabbits were divided into two groups; a control (P) and experimental one (E). Both groups of the animals had identical breeding conditions. Two subgroups were separated from the control group. The first one (P) comprised 12 animals on which no procedures were performed. The second, procedure control (PZ), consisted of seven animals on which all procedures – except PB – were performed as in group E.
The rabbits of experimental group were subjected to pulmonary barotrauma. This group was divided into two subgroups according to the time of observation:

- in subgroup D – 19 animals – barotrauma was performed and the rabbits were sacrificed immediately after the procedure
- in subgroup DO – 13 animals – after the sustained injury observation was carried out for 3 weeks.

The investigations were carried out in the pressure chamber where an intubated rabbit was placed. The air pressure in the chamber was raised at the rate of 1 ata per minute up to the value of 3 ata. At the peak of inspiration, their respiratory tract was blocked and pressure in the chamber was lowered during 1 min to atmospheric pressure, imitating the mechanism of pulmonary barotrauma in divers. In the course of autopsy, after cutting the integuments of neck, the trachea was dissected free and then double ligated in order to keep the lungs expanded during fixing them. The lungs were fixed in containers (appropriate for their dimensions) with 10% neutralised formalin for 14 d at 0-4°C. The slides were made with paraffin method and stained with haematoxylin and eosin. The slides for further examinations were selected in the course of routine microscopic assessment from sections comprising cross-section of the whole lung. The specimens selected for the examinations comprised of proper cross-sections of the fixed fully aerated lungs with visible: bronchial tree, pulmonary hilus, and parenchyma, with no pathological changes in parenchyma (inflammatory process, fibrosis, cysts etc.).

The examinations were carried out under light microscope, at magnification of 40x. In each cross-section of the lung, two fields of vision were selected for the count from the zones: subpleural, central and parahilar in the upper part of the lung, middle part, and basilar part (further on called “lobes”). About 20 reference alveoli were selected in each of these zones. In the field of vision one closed air space identified as alveolus or emphysematous bulla was selected randomly.

The method of histoquantitative estimation of pulmonary parenchyma was previously described (17, 18). The numbers of interalveolar septa adjacent to the randomly selected reference alveolus were counted. Reference alveoli, in relation to which the above-mentioned index was counted, were selected in microscopic specimens at magnification of 200x. The count method is presented in Figs 1, 2, and 3. Statistical analysis was performed using multivariate analysis of variance in modification for non-parametric data (ANOVA according to Friedman and ANOVA according to Kruskall-Wallis with tests of median).

Fig. 1. Histological structure of cross-section of rabbit lungs. (20x)

Fig. 2. Enlarged and simplified diagram of lung framework. (200x)

Fig. 3. Refilled diagram of lung framework. The numbers determine the number of nodes for each pulmonary alveolus (numbers of adjoining alveolus).
Results

The statistical analysis demonstrated significant differences of means between joint groups P and PZ and joint groups E and EO (P<0.001) (Fig. 4). The differences between group E and EO and group P and PZ compared in pairs proved to be statistically insignificant (P>0.05).

The counted number of interalveolar septa coming away from the wall of the selected air space did not change even when taking into consideration the division into “lobes” (Fig. 5) and zones (Fig. 6) of the lung. No statistically significant differences were observed in mean number of septa falling to the alveolus between the left and right lung and in the superior, middle, and inferior “lobe”, both in experimental and comparative groups.

Fig. 4. Differences of means between joint groups. The numbers on the y-axis determine the number of nodes for each pulmonary alveolus (numbers of adjoining alveolus).

Fig. 5. Differences of means between joint groups considering the division into “lobes”. The numbers on the y-axis determine the number of nodes for each pulmonary alveolus (numbers of adjoining alveolus). The letters under determine hypothetical upper lobes (g), middle (s), and bottom (d) of the lung.
Discussion

The experimental model used in own examinations made it possible to investigate the changes of pulmonary inflation evoked by PB and to analyse their intensification. The results of this analysis revealed that PB caused a considerable increase in pulmonary aeration (distension). When disruption of alveolar walls occurs, their regeneration is no longer possible, leading to formation of bigger or smaller emphysematous bullae as permanent consequences of past PB. This phenomenon explains among others the distinct increase in pulmonary parenchyma inflation in experimental groups compared with control groups, irrespectively of the applied treatment. In our studies, a dilation of air spaces of vesicular tree was found in all animals subjected to PB, which was manifested by a decrease in the number of interalveolar septa coming away from the wall of the selected air space. Excessive aeration of pulmonary parenchyma increased in time. The obtained results of the quantitative examinations of the lungs after PB correspond with the data published before. In these studies we have demonstrated that after PB, the inflation of pulmonary parenchyma increases in a statistically significant manner and when not treated with recompression-decompression, it intensifies. It confirms the results of clinical examinations by Thorsen et al. (16). They evaluated breathing capacity of the lungs in 24 divers practising deep diving. After 4 years, they carried out renewed examinations of the same divers and found a decrease in breathing capacity of the lungs (traits of emphysema-distension). These authors emphasised that the results of the examinations confirmed earlier observed dependences between breathing capacity of the lungs and number of dives. The screening examinations carried out on professional divers demonstrated an impairment of lung capacity increasing together with the increase of the number of dives (16), which should be connected with asymptomatic episodes of PB. The described above method of quantitative analysis of lung histoarchitecture should be easily adopted for automatised image analysis systems (17) and applied in other investigative purposes in lung pathology. The applied morphometrical method allowed evaluating the changes of pulmonary inflation after PB, which evoked the extension of air spaces of the lungs. This change, when untreated, intensified together with experiment duration.

References


