Morphological evaluation of the lungs in rats with experimentally induced renal failure

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Abstract

The study aimed at morphological evaluation of changes in the lungs of rats during experimentally induced uremia. The studies were performed in three (3) groups of rats: C - a control group (15 animals), SO - 15 rats, submitted to sham operation, U - an uremic group - 21 rats with experimental renal failure. After 1, 2 and 4 weeks from the surgery, the collected lungs were fixed in Bouin's fluid and in 2.5% purified glutaraldehyde for electron microscopy. Paraffin specimens were cut into 5 μm slices and stained by H+E, by Azan's method and with silver, according to Grimelius. Ultra thin sections contrasted with uranyl acetate and lead citrate. Blood serum urea and creatinine levels were determined. In the uremic rats, increased concentrations of serum creatinine and urea were observed.

Chronic renal failure affected the progress and the degree of development of the changes in the lungs, the final effect of which was fibrosis. In microscopic pictures of the lungs from the uremic rats, numerous changes were observed, characteristic for chronic oedematous conditions of different intensification.

Key words: chronic renal failure, lung, morphology.

Introduction

The final stage of chronic renal failure (CRF) is chronic uremia, characterised by severe metabolic disorders, inducing clinical symptoms in the majority of organs [1, 2].

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Oedema with pleural exudate is the most frequently observed pulmonary complication, fairly often asymptomatic in its course. Pulmonary oedema results from an increased hydrostatic blood pressure in capillary vessels, the pressure rise being in proportion to fluid overload, as well as to an increased permeability of pulmonary capillaries [3, 4]. Mechanic and haemodynamic changes in the lungs may occur without any basic clinical syndromes, leading to serious pulmonary function disturbances, which may further result in injuries of the walls of the perialveolar capillaries, reducing the efficiency of gas exchange [4].

In order to increase the knowledge on the pathogenesis of respiratory disorders in (CRF), as well as with regards to the lack of an unequivocal evaluation of causes of the changes, observed in the respiratory tract, it seemed fairly interesting to attempt a thorough study of the dynamics of morphology in changes of the lung in experimentally induced renal failure.

The goal of the performed studies was morphological evaluation of changes in the lungs in the course of experimentally induced uremia.

Materials and Methods

The study was carried out on 51 young male Wistar rats, 200-250 g body weight (mean 220±10g) at the beginning of the experiment. The animals had a free access to drinking water and standard granulated diet. The experimental uremia was produced in 21 rats. The control animals underwent a sham operation, i.e. decapsulation and removal of the adherent fat (15 rats). The other control group did not undergo any surgical procedure (15 animals). Experimental uremia was induced, using the method, described by Ormrod and Miller [6], and Azzadin [5]. The rats were anesthetized with pentobarbital, administered intraperitoneally in a dose of 50 mg/kg. Nephrectomy of the right kidney was performed and 70% of the left kidney cortex was removed, leaving the renal medulla intact.

Figure 1. Lung of a control animal. H+E stain, mag. 200x.

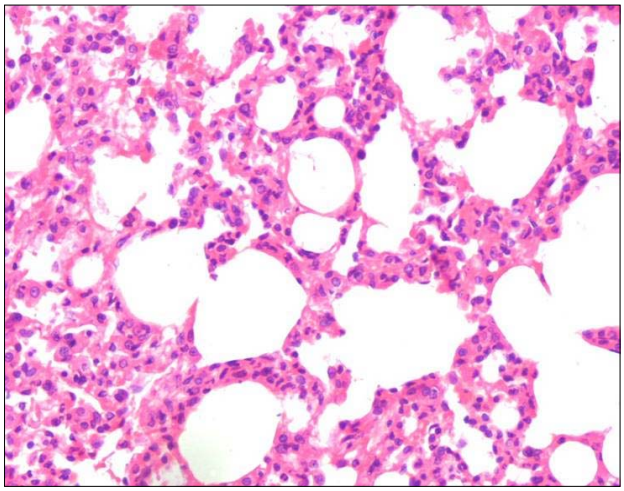

Figure 2. A lung fragment of a rat with uremia after 1 week of the experiment. Advanced atelectasis and oedema. Azan stain, mag. 250x.

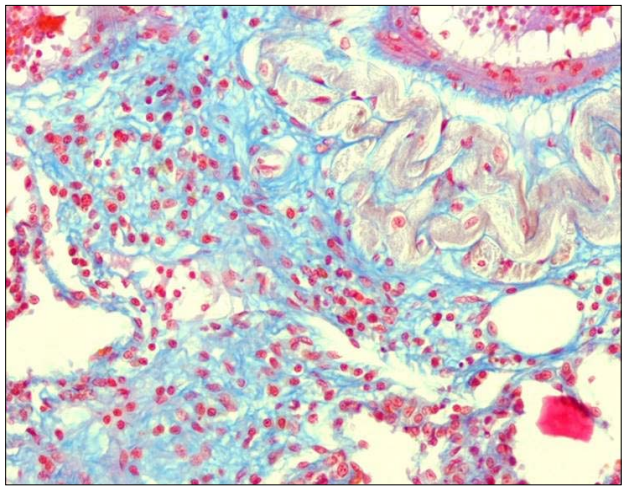

Figure 3. Lung specimens of rats with uremia after 2 and 4 weeks of the experiment. Lung fibrosis, obliterative bronchiolitis - connective tissue cells within the bronchial fibrosis, metaplasia of bronchial epithelium. H+E stain, mag. 250x.

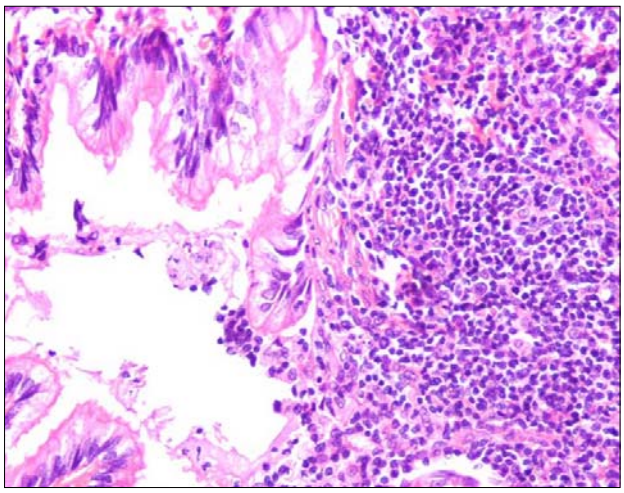

Figure 4. Degenerative changes of epithelial and endothelial cells. Micropinocytosis (representative of the group rats with uremia after 4 weeks of the experiment), mag. 7 000x.

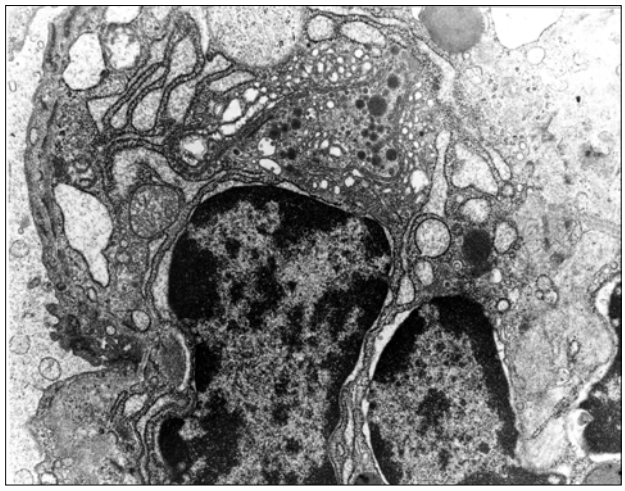

Table 1. Serum concentrations of creatinine and urea in control and uremic rats (mg/dl).

	control	1 week	2 weeks	4 weeks	* p-value
Creatinine	0.52 ± 0.052	0.63 ± 0.092	0.94 ± 0.058	0.72 ± 0.12	<0.05
Urea	35.33 ± 5.98	52.91 ± 16.39	95.10 ± 11.90	85.58 ± 9.766	<0.05

After 1, 2 and 4 weeks from the surgery, the rats were anaesthetised with pentobarbital and blood was collected from their hearts. Then, the animals were sacrificed by decapitation and the lung was fixed in Bouin's fluid for 24 h in temperature of +4^{0}C and embedded in paraffin in a routine way. For electron microscopy, the specimens were embedded in 2.5% purified glutaraldehyde. The specimens were cut in 5µm slices and stained by hematoxylin-eosin, by Azan's method and with silver, according to Grimelius.

Ultra thin sections, contrasted with uranyl acetate and lead citrate and were examined in an OPTON 900 PC electron microscope.

Blood serum urea and creatinine levels were determined.

Statistical analysis was performed by the Shapiro-Wilk's test. The analysis was performed, using the SAS ATAT software package. A probability level $p<0.05$ was considered significant.

Results

No significant differences were found between the control groups of rats and, therefore, only the results referring to the animals subjected to a sham operation are discussed.

Increased concentrations of urea and creatinine are one of the indicators of renal insufficiency.

Table 1. Serum concentrations of creatinine and urea in the control and the uremic rats (mg/dl).

A statistical increase of serum creatinine and urea concentrations was demonstrated in all the groups of animals with experimental renal insufficiency, when compared with respective values in the control groups (Table 1).

Morphological pictures of the lungs from the control animals were normal. In turn, in the group of uremic rats, changes, atelectatic in character, and erythrorrhagia were found in their lungs, evaluated after 1, 2 and 4 weeks from the surgery. Collapses of capillary lumen and obliterations of interalveolear septa were observed (Fig. 1). In the preparations from the 14th and the 28th day, the fluid, which normally occurs in the pulmonary alveoli, was also observed in lumen of the upper airways, where it accumulated on the epithelial surface. Also, young connective tissue cells occurred in the pictures, distorting the lung structures - the pictures corresponding to pulmonary fibrosis (Fig. 2). In that mass of fibrotic pulmonary tissue, various sections of the respiratory tract with obliterative bronchiolitis were found (Fig. 3).

Using an electron microscope, moderate atrophy of type II pneumocytes and sectional smoothing of microvilli on the free surface of those cells were seen. Necrotic changes of cells were also observed. In the endothelial, as well as in the epithelial cells of the respiratory tract, translucent sites were found in cytoplasm (Fig. 4).

Discussion

Effects of uremia on the respiratory tract are still little known. It has been found that, in the course of renal failure, changes of different character may occur in the lungs. Disturbed pulmonary function and weaker respiration control are among the uremic consequences in the respiratory tract [2, 3, 4].

In the performed studies, the morphological pictures of the lungs from the uremic rats corresponded to changes characteristic for massive atelactasis, chronic oedematous conditions and bronchiolitis, as well as to pulmonary fibrosis. In the course of renal failure, a progression of pulmonary changes was noted, regarding their degree and dynamics.

Pathomorphological changes, observed in uremia, may - via different mechanisms - lead to surfactant function disturbances or to injury of the alveolo-capillary barrier, reducing the carbon monoxide diffusion capacity, what may result in pulmonary oedema [4]. In the course of renal insufficiency, chronic pulmonary interstitial oedema is often observed [3, 7, 8]. In uremia, atrophy of both bronchial and alveolar epithelium (especially of type II pneumocytes) is found. This process may, however, be accompanied by regeneration attempts of the epithelium.

The advanced progression of pulmonary changes, as observed during experimental chronic uremia, indicates the existence and a continuous effect of factors, damaging the pulmonary tissue. So far, no particular factor has been found, which could be responsible for the pulmonary changes, observed in uremia.

Summing up, it can be stated that experimental CRF causes changes in the lungs, which are characteristic for the syndrome of uremic lung. The degree of progression of the pulmonary changes may be related to the time period of renal insufficiency.

References

1. Adachi Y, Sasagawa I, Tateno T, Tomaru T, Kubota Y, Nakada T. Testicular histology in experimental uremic rats. Arch Androl, 1998; 41: 51-55.

2. Levillain O, Marescau B, Possemiers I, Al Banchaabouchi M, De Deyn PP. Influence of 72% injury in one kidney on several organs involved in quanidino compound metabolism: a time course study. Pflügers Arch, 2001; 442: 558-69.

3. Słomian M, Mosiewicz J, Myśliński W. Lung function in chronic uremia. Ann Univ Mariae Curie Sklodowska, 2000; 55: 148-53.

4. Kalender B, Erk M, Pekpak M, Apaydin S, Ataman R, Serdengecti K, Sariyar M, Erek E. The effect of renal transplantation on pulmonary function. Nephron, 2002; 90: 72-77.

5. Azzadin A, Wollny T, Pawlak R, Małyszko J, Małyszko JS, Myśliwiec M, Buczko W. L-carnitine effects on anemia in uremic rats treated with erythropoetin. Nephron, 1999; 83: 370-71.

6. Ormord D, Miller T. Experimental uremia. Nephron, 1980; 26: 249-254.

7. Lee HY, Stretton TB, Barnes AM. The lung in renal failure. Thorax, 1975; 30: 46-53.

8. Bush A, Gabriel R. Pulmonary function in chronic renal failure: Effects of dialysis and transplantation. Thorax, 1991; 46: 424-28.