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Irradiation as a hazard for mucociliary clearance

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Abstract

OBJECTIVES: In this paper we study effects of irradiation to pulmonary tissue on a micro and ultrastructural level to get insights into the dynamics of morphological changes and associated post-radiative physiological conditions.
METHODS: Animal and human pulmonary tissue with and without radiation damage was subject to light, transmission, scanning and polarization microscopy and morphometric evaluation.
RESULTS: The present investigations on the influence of irradiation on experimental and human lung tissue demonstrate that complex changes are induced in the cells which are essential for mucociliary clearance. These changes are a shortage of alveolar macrophages, cell apoptosis, proliferation of collagen ligament in the barrier of gaseous exchange, retraction of endothelial lining of capillaries and significant broadening of the gaseous exchange barrier, resulting in serious damage for the O_2 and CO_2 exchange.
CONCLUSIONS: These changes at microscopic, cellular, and ciliary level trigger conditions for various diseases of the respiratory system, which is further assessed by a simultaneous computer aided estimation of ciliary function. With the concurrent world-wide increase of respiratory diseases, these findings are important knowledge for the clinical practice.

ORIGINAL ARTICLE

INTRODUCTION

Mucociliary clearance of the respiratory tract, the primary defense of the organism in its contact with the environment, is, among other effects, affected by inhaled aerosols of heavy metals occurring in the air and by radiotherapy of cancer diseases, which may lead to radiation-induced complications of healthy tissue. There are two primary contacts of the human body with the environment: the skin and the lung. The total surface area of respiratory system is approximately 40 times higher than surface area of the skin (total alveoli surface area is about 75 m^2 (Knowles and Boucher

2002, Ross and Pawlina 2006), and skin surface area is 1.5 to 2 m^2 (Bouslimani *et al.* 2015)). A decrease of respirable alveoli area represents a serious health issue (Foltinová *et al.* 2002). Approximately 150 m^3 of air pass daily through the nasal cavities and this inhaled air goes into the lungs at sufficient pressure, volume, moisture, temperature and cleanliness (Kilic *et al.* 2015). Respiration under physiological condition of rest uses only about 5% of total lung capacity, whereas much higher portion of lung capacity is used under load at high physiological activity. Effects of pollutants and heavy metals on lung and other tissues (e.g., placenta & umbilical cord blood) have been studied for example in (Foltin *et al.* 2011; Foltinová *et al.* 2002; Foltin 2016). Effects of drug and vaccine inhalation and smoking were reviewed for example in (Haghi *et al.* 2014; Gizurarson 2015). This paper focuses on radiation-induced changes to lung tissue.

A stimulus, harmless for healthy organisms, may irritate a diseased organism and may trigger chain reactions affecting cells, tissue, and organs. Radiation – a key part of modern cancer treatment concepts – is such a stimulus which can seriously affect the microscopic structure of cells in tissues, especially in lung. The lung tissue is known to be prone to radiation-induced changes which influence its microscopic structure and function. Therapeutic and diagnostic technology is continuously improving to reduce impacts of radiation usage. However, more work is needed to advance our understanding of post-radiative consequences, to stimulate further studies on the tolerance of normal tissue to radiation (highlighted also by Emami *et al.* 1991, updated by Emami 2013 after factoring-in progress during the last decade).

Radiation induced changes on lungs and other organs (Brennan *et al.* 1998; Coggle *et al.* 1986; Cotron *et al.* 1994; Foltinová *et al.* 2000; Lingos *et al.* 1991; Ohyama & Yamada 1997; Osterreicher *et al.* 2000; Mulwany 2002) are known to lead to development of late fibrosis (Intengan *et al.* 2001; Kantak *et al.* 1993; Kraus *et al.* 1991; Kurishita *et al.* 1991; Osterreicher *et al.* 2000; Rosielko *et al.* 1993; Yong & Heath 2000) that reduces the respiratory surface of the lungs. However, the actual knowledge about changes at the structural level of the barrier of gaseous exchange, which together with alveolar macrophages forms the base of the second phase of mucociliary clearance, is incomplete (Abratt & Willcox 1994; Boucher 1994; Brennan *et al.* 1998; Finkelstein *et al.* 1994; Foltinová *et al.* 2002; Gartner & Hiatt 2001; Intengan & Schiffrin 2001; Junqueira *et al.* 1992). Insight into the background of the dynamics of these morphological changes is possible only at the cellular level (Baum *et al.* 1988; Foltinová *et al.* 2002).

We are presenting, for the first time (to the best of our knowledge), an in-depth investigation of these complex changes on base of light-, electron-microscopic, and morphometric evaluations, and discuss their effects on respiratory function.

MATERIAL AND METHODS

The material presented here is specific, as it contains the results of animal experiments and of data acquired in a clinical setting.

Animal experiments

The investigation was performed on 16 female mice of BALB/c type (average weight 20.2 g and 8 weeks age, respectively). 8 animals were whole-body irradiated with 6.02 Gy by ^{60}Co cobalt source CHISOBALD B 75, Chirana, Prague, Czech Republic (giving bi-chromatic radiation with energies 1.17 MeV and 1.33 MeV). Excisions were taken 10 days after irradiation and evaluated as follows:

1. Transmission electron microscope (TEM) Zeiss-920, Germany at magnifications 5 000×–50 000×.
2. Scanning electron microscopes (SEM): BS-300 TESLA Brno, (Czech Republic) and
3. PHILIPS CM 20 (Holland) at magnifications 5 000×–50 000×.
4. Polarization microscope Reichart Polyvar Germany at magnification 2 000×.
5. Light microscope at magnification 200× (semi-thick sections stained by method after Richardson (Pearse 1985)).
6. Light microscope Reichart Polyvar, Germany at magnifications 200× (10 mm thick sections stained by trichrome method after Masson (Pearse 1985) and quantitatively evaluated by morphometry employing "Kslite" software from Kontron Electronik (Germany) without additional corrections.

Clinical investigation

4 patients, detailed in Table 1, with pulmonary metastases of osteosarcoma. The lungs were irradiated during the medical treatment in Bratislava, Slovakia. Surgery followed after radiotherapy. The metastases were surgically resected within a safety margin, wedge resection was performed in the lower lobe of the right lung (see Table 1). From the tissue inside the safety margin tiny excisions were evaluated using the same methodology like in animal experiments a).

RESULTS

At an initial step, all obtained excisions were investigated for alveolar macrophages and a significant proliferation of collagen ligaments in the barrier of the pulmonary gaseous exchange. These microscopic structures are responsible for an intact functioning of mucociliary clearance in its second phase, the alveolar-capillary phase (Brennan *et al.* 1998; Deetjen & Speckmann 1999; Junqueira *et al.* 1992; Weibel & Bachofen 1997; Yong & Heath 2000; Weibel 2013; Hogan *et al.* 2014; Westphalen *et al.* 2014). Alveolar macrophages sensitively react as the first line of defense of respiratory system and participate in a specific and non-specific immunity

Tab. 1. Patients information.

Patient			Irradiation		Surgery	Histology	Comments	
Nr.	Sex	Age [years]	Dose [mGy]	Duration [months]	Indication	Diagnosis	Interval radiation-excision [weeks]	Excised lung sector
1	m	16	108,2	23	metastasectomy	osteosarcoma (tibiae 1. sin.) cum mts. ad pulmo	6	
2	f	15	139,99	21	metastasectomy	osteosarcoma (femor 1. dx.) cum mts. ad pulmo	3	
3	m	12	138,18	28	metastasectomy	osteosarcoma (femor 1. sin.) cum mts. ad pulmo	2	
4	m	17	184,64	14	metastasectomy	osteosarcoma (femor 1. sin.) cum mts. ad pulmo	4	

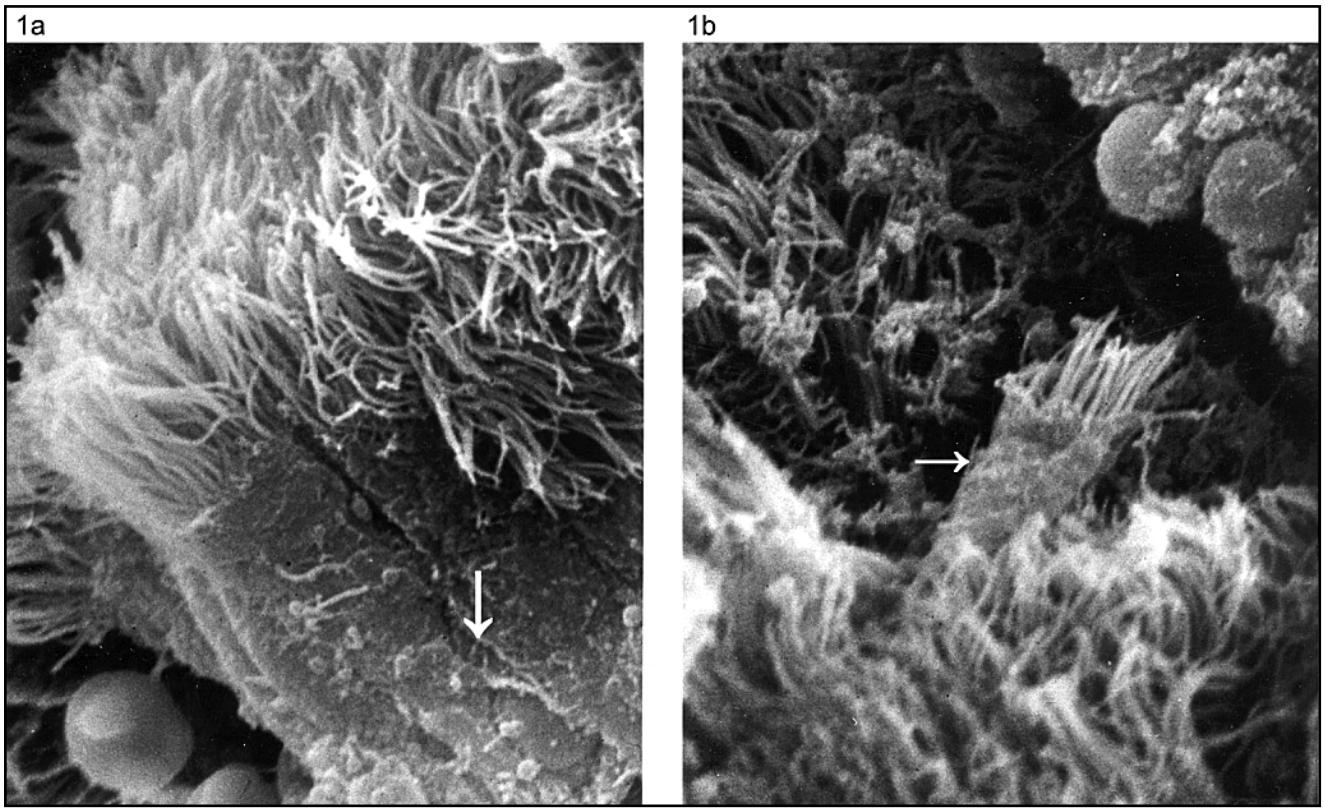


Fig. 1. Trachea in scanning electron microscope (SEM), mouse. Comparison of non-irradiated and irradiated tissue. Magnification 10 000×. **1a** – not irradiated tissue. Finding: Apical intact relief of cilia; Intercellular coupling can be simply identified (white ↓). **1b** – tissue after irradiation. Finding: Apical relief of cilia. Disengagement of cells due to damaged intercellular couplings; apoptosis of the cell (white →).

process by binding F_c fragment of immunoglobulin G on its F_c surface receptors, and by phagocyting inhaled particles, respectively. Alveolar macrophages use space ordering of collagen ligament in the barrier of gaseous exchange to cross this barrier. In the direction from the alveoli to the capillaries, this barrier is formed by an epithelial lining of alveoli, a basal membrane of epithelial alveolar lining, a thin layer of ligament with prevalence of collagen ligament, the basal membrane of the endothelial lining of capillaries, and endothelial lining of capillaries.

Comparing the two animal groups revealed significant cell apoptosis after irradiation (Figures 1a and 1b). Apoptosis counteracts mitosis and uncontrolled cell

proliferation, and cleans off unwanted cells. As such, it involves intracellular signaling and other critical processes that result in recognition and phagocytosis of unwanted cells (Henson *et al.* 1999; Intengan & Schiffrin 2001; Rock *et al.* 2009). The state of microscopic pulmonary structures (alveoli, the alveolar-capillary barrier, etc.) is shown in Figure 2. Figure 2a shows surface relief of lung alveoli of not irradiated tissue observed in SEM. Figure 2b shows structure of alveoli of experimental animal after irradiation. Figure 2c shows structure of alveoli of human child patient after irradiation. Light microscopy revealed that on sections stained for collagen ligament after Masson (Junqueira *et al.* 1992; Pearse 1985) this ligament fills lung alveoli. Further, there is remarkable dilatation of vessels with stagnating blood (Figure 3b). This fact gives evidence about reduction of respiratory area. This is missing in Figure 3a, in which the structure without irradiation is free of these changes. The set of Figures 4a, 4b shows the character of changes on semi-thick sections observed in light microscope. This type of sections is suitable for demonstrating cellularity and free space in the tissue. Figure 4a corresponds to the intact group and Figure 4b to the irradiated one. Our findings observed in light microscope have been quantified by means of morphometry (Hyde *et al.* 1992; Weibel 1990) in order to show the extent of changes in microscopic structure after irradiation. We have chosen morphometry for evaluating our results as it allows to determine the consequences of irradiation on the pulmonary structure. The evaluation of irradiated and non-irradiated groups was done under identical conditions (i.e., tissue probes, thickness of sections, staining, microscope, magnification, and evaluation method). Figures 3c and 4c quantify decrease of alveolar area and increase of the area of remaining tissue after irradiation based on samples from Figures 3a/b and 4a/b, respectively. In Figure 4c, the alveolar area decreased from 88.2% to 64.4% of the total area, which represents 27% reduction of alveolar area. Correspondingly, the area of the remaining tissue increased from 11.8% to 35.6% of the total area, i.e., about 3-times. Figure 3c shows similar trend. These data demonstrate a substantial decrease in the respirable pulmonary area due to radiation-induced proliferation of collagen ligament. Figures 5a and 5b show the membrane of gaseous exchange on an ultra structural level. Figure 5a corresponds to the intact group and Figure 5b is after irradiation. We found a significant difference in the thickness (i.e., 2.6 barrier thickness increase after irradiation, as per Figure 5c) and in the presence of microscopic structures occurring there. Figure 5b clearly shows the presence of proliferation of collagen ligament and capillaries in which blood is stagnating. This will severely interfere with O_2 and CO_2 transport as well as with nutrition of microscopic structures occurring here.

Fibroblast is responsible for the production of tropocollagen, which can be seen in Figure 6c and schematically in Figures 6a and 6b. Figures 7a–7d show how the extrafibrillar matrix net, important for inserting molecules of tropocollagen, is formed. This phenomenon of insertion can be seen in Figure 7e. The present results outline a picture of the consequences of radiation on juvenile human organism by the character of microscopic changes in the collagen ligament. Irradiation is an intervention into organism which is intended to ameliorate the patient's prognosis. Admittedly, each organism responds differently to stimuli, especially radiation, but in general a stimulus that is harmless for healthy organism may seriously affect a diseased organism. Tropocollagen, a product of secretion activity of fibroblasts, is a prolonged molecule, and the collagen fibers are result of its polymerization. Molecules in collagens I, II, and III aggregate into microfibrillary units which join and form fibers. The striation of collagen fibers (Figure 7e) reflects the way of overlapping molecular tropocollagen units in pulmonary barrier of gaseous exchange. Dark stripes are known (Junqueira *et al.* 1992; Yong & Heath 2000) to have more groups

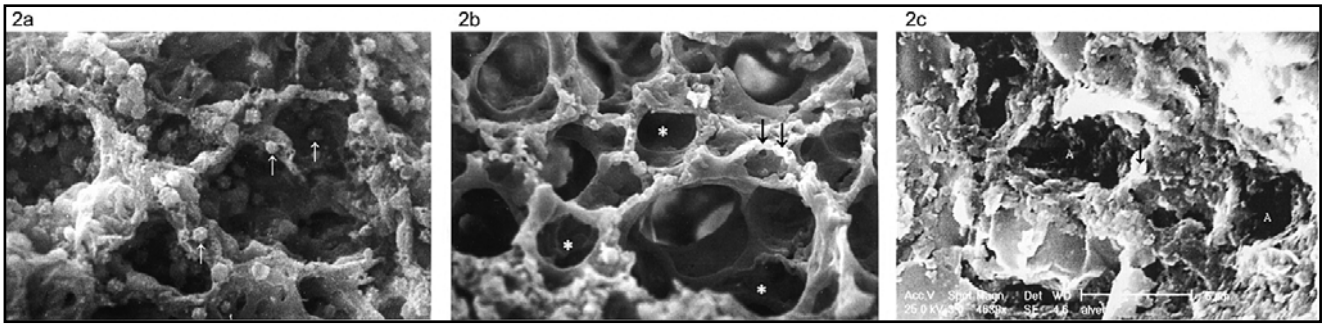


Fig. 2. Surface relief of lung alveoli in SEM. Mouse: non-irradiated and irradiated tissue (magnification 5 000×), Human: irradiated tissue (magnification 4 639×). **2a** – mouse tissue not irradiated. Finding: Intact structure; presence of alveolar macrophages (AM) (white ↑) responsible for sterility of the inner surface of alveoli. Presence of AM penetration through inter-alveolar pore. AM play important role in the second phase of mucociliary clearance. **2b** – mouse tissue after irradiation. Finding: In alveoli AM not present (white *) (due to irradiation; this is a crucial immune morphological finding); AM penetration through inter-alveolar pores not present; dilated capillaries filled in with stagnating blood (↓). **2c** – human tissue after irradiation – from patient (diagnosis: osteosarcoma). Finding: Singular presence of AM in alveoli (see white "**A**" labels near the center and near the right edge). AM penetration through inter-alveolar pores not present; dilated capillaries filled-in with stagnating blood (↓).

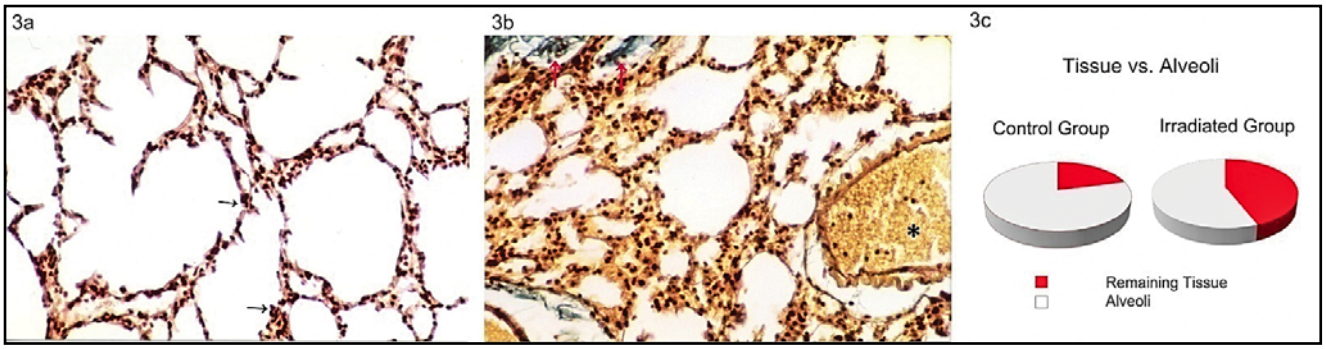


Fig. 3. Lungs in light microscope on semi-thick sections stained by method after Masson, mouse, Magnification 200×. **3a** – not irradiated tissue. Finding: Microscopic structure of intact collagen tissue (→) alveolar macrophages. **3b** – tissue after irradiation. Finding: – fibrosis in evolution (due to irradiation); – alveoli filled in with collagen ligament (↑ in upper left area) (due to irradiation); – stagnating blood (*) reduction of respiratory area (due to irradiation). **3c** – quantitative morphometric evaluation of Figs. 3a & 3b. Under the same conditions (concerning tissue, thickness of sections, staining, microscope, magnification, method of evaluation) we compared irradiated and not irradiated sections of lungs. Finding: In the control group the area of alveoli was 79% and the area of remaining tissue was 21%. In the irradiated group the area of alveoli was 56% and the area of remaining tissue was 44%. By other words – 2.095 times increase of the remaining tissue after irradiation compared to non-irradiated tissue.

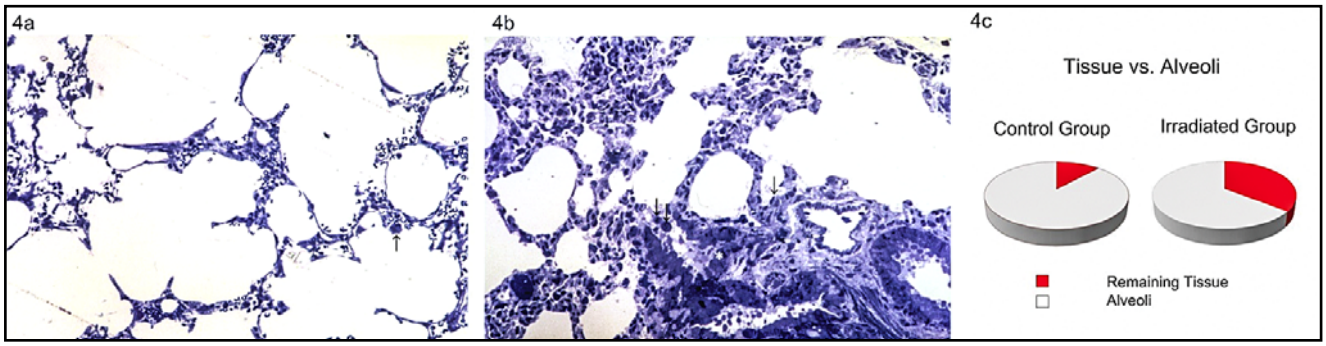


Fig. 4. Lungs on the semi-thick sections stained by method after Richardson *et al.* 1960, mouse, Magnification 200×. **4a** – not irradiated tissue. Finding: Intact microscopic structure, presence of AM on the inner part of alveolar wall. **4b** – structure after irradiation. Finding: Developing fibrosis; silhouette of collagen fibers is well depicted (white * in central bottom area). AM are present in the interstitium (↓). **4c** – quantitative morphometric evaluation of Figs. 4a & 4b. Under the same conditions (concerning tissue, thickness of sections, staining, microscope, magnification, method of evaluation) we compared irradiated and not irradiated sections of lungs. Finding: In the control group the area of alveoli was 88.2 % and the area of remaining tissue was 11.8%. In the irradiated group the area of alveoli was 64.4% and the area of remaining tissue was 35.6%. By other words – 3.017 times increase of the remaining tissue after irradiation compared to non irradiated tissue.

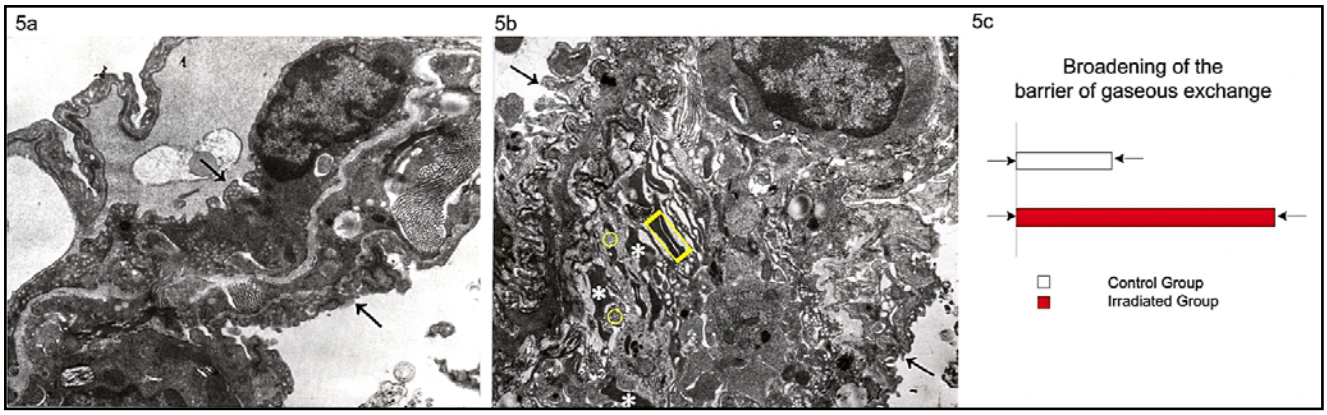


Fig. 5. Barrier of gaseous exchange in lungs on the level of transmission electron microscopy (TEM), mouse. Magnification 6 000×. **5a** – not irradiated tissue. Barrier of gaseous exchange (space marked between arrows → ←) Finding: Intact microscopic structure necessary for the exchange of O_2 and CO_2 gases. **5b** – tissue after irradiation. Broadening of the barrier of gaseous exchange (space marked between arrows → ←) Finding: presence of proliferation of collagen ligament (yellow ○) and capillary net (yellow box). Stagnation of blood in capillaries (white *). This will severely affect exchange of O_2 and CO_2 gases. **5c** – quantitative comparison of wall size. Under the same conditions we compared irradiated and not irradiated sections of lungs. Finding: 2.6 times increase of marked Barrier of gaseous exchange in irradiated tissue as compared with non irradiated tissue.

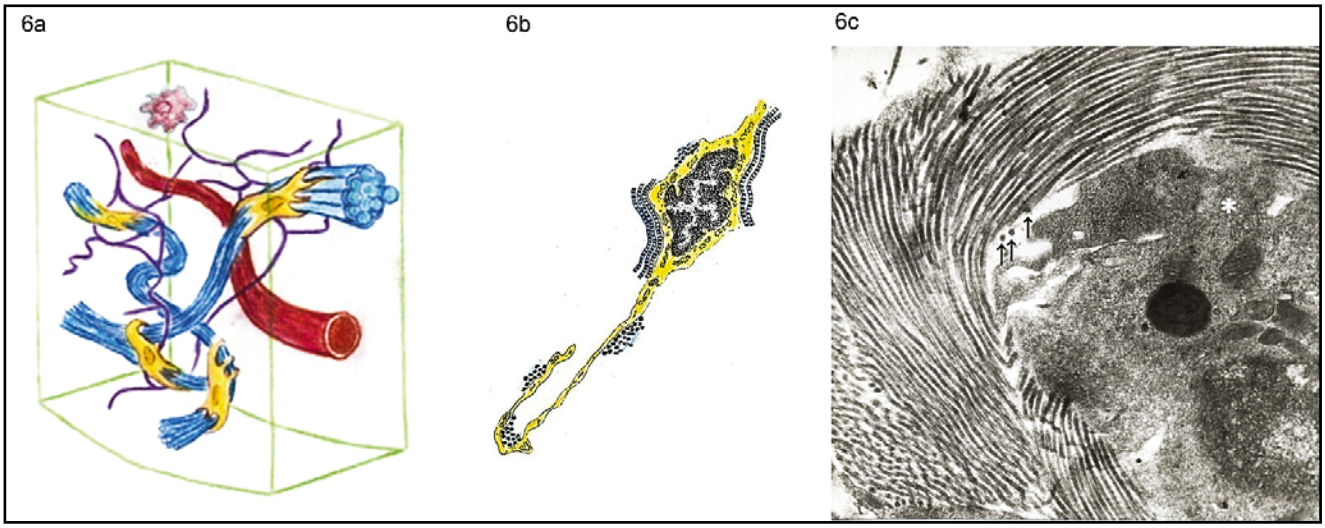
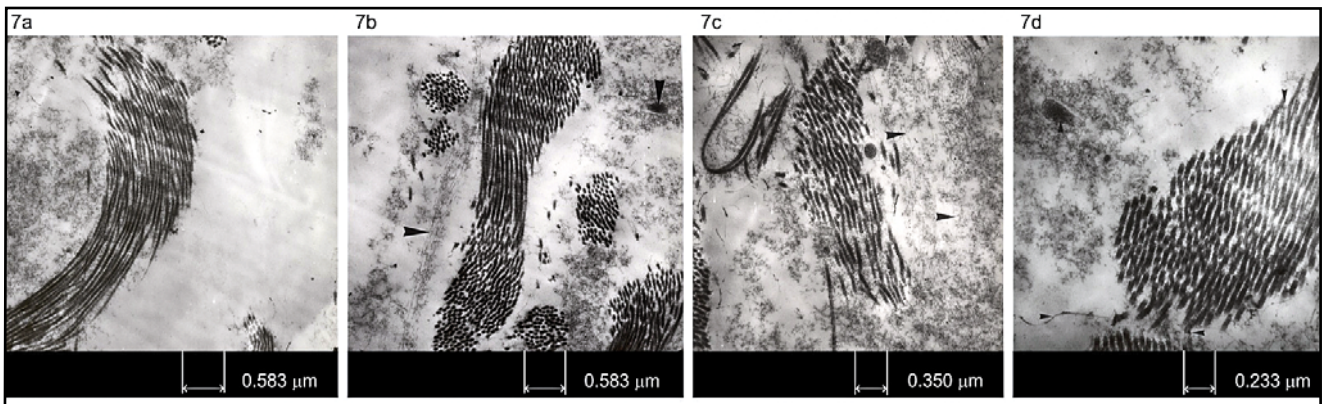


Fig. 6. Fibroblast in the barrier of gaseous exchange responsible for formation of collagen fibers. **6a** – schematic drawing of section. From upper right corner clockwise structures: blue – collagen bundle and fiber; pink – reticular fiber; red – capillary; yellow – fibroblasts & fybrocyte; magenta – macrophage. **6b** – schematic picture of fibroblast and its function, mouse. This schematic picture illustrates the silhouette of the fibroblasts and laying of the collagen ligaments shown in Fig. 6a. **6c** – tissue after irradiation, mouse – picture in TEM – Magnification 20 000×. Finding: This cell is irritated by radiation, which opens the way of rising fibrosis in lung alveoli; dilated cisterns of rough endoplasmatic reticulum (white *); Penetration of tropocollagen ( ); building of tropocollagen into the collagen fiber.



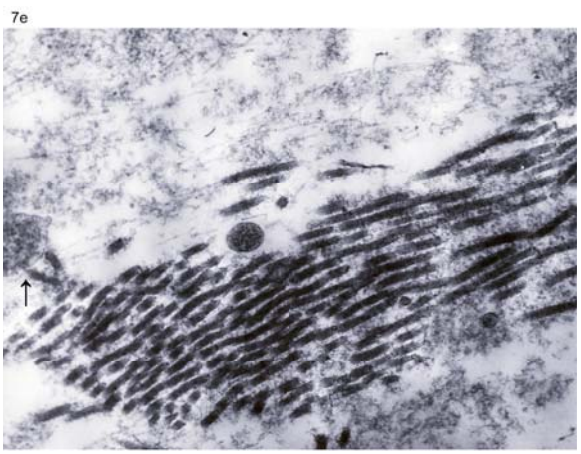


Fig. 7. Collagen ligament in the barrier of gaseous exchange of lungs – TEM pictures, mouse. Comparison of non-irradiated and irradiated tissue – same magnification 12 000× (7a,7b); irradiated tissue in a detail, sequences with increased magnification 12 000×, 20 000×, 30 000× in 7b, 7c, 7d respectively; irradiated tissue in a detail with magnification 50 000× (7e). **7a** – not exposed to irradiation. Magnification 12 000×. Finding: intact. **7b** – tissue after irradiation. Magnification 12 000×. Finding: Transport of tropocollagen is present (➧); Significant drawing of fibronectin coordinates (➤). Inset of tropocollagen into the coordinate matrix. **7c** – tissue after irradiation. Magnification 20 000×. Finding: Variety of size of tropocollagen droplets; Abundance of fibronectin coordinates (➤). **7d** – tissue after irradiation, Magnification 30 000×. Finding: Detail of fibronectin coordinate with transport of tropocollagen (➤); Cross-striped character of the collagen fibers (➧) and inset of tropocollagen droplet (➤). **7e** – tissue after irradiation, Magnification 50 000×. Finding: Unique picture (in detail) of tropocollagen transport to fibronectin structures (↑) in the process of creation of collagen fibers. Irritation due to irradiation extremely accelerates this process. The electron micrograph shows the dynamics of this process.

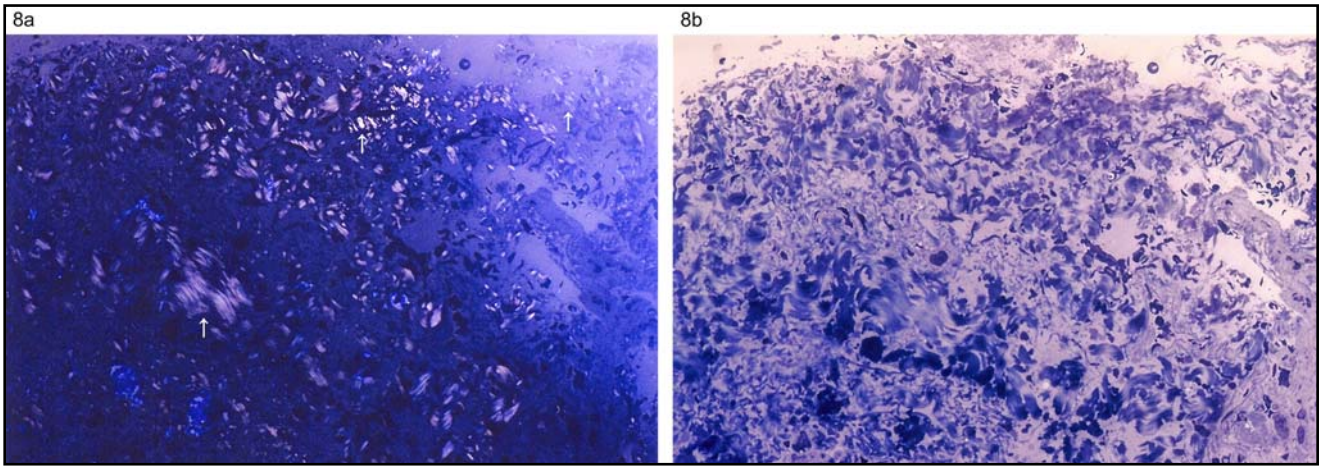


Fig. 8. Human lungs after irradiation in polarization and light microscope. Magnification 2 000×. Picture of lung alveoli of 15 year old patient with diagnosis of osteosarcoma with pulmonary metastasis. **8a** – irradiated tissue in polarization microscope. Finding: Structure of alveoli after irradiation of normal tissue of lungs of the so called safety zone. This zone is excited in the surgical action in order that whole metastasis is excised together with a part of healthy tissue. Marked presence of collagen fibers in the barrier of gaseous exchange of alveoli. Presence of collagen connective tissue (white ↑). **8b** – The same as in 8a, but in light microscope.

that can react with the lead solution during preparation of the samples for electron microscopy. Formation of collagen is a rather complicated process (MacFarlane *et al.* 2000; Weibel 1990; Weibel & Bachofen 1997; Yong & Heath 2000) that may fail at various levels and it is sensitive to environmental variations. Collagen ligament is part of microscopic structures occurring between *lamina basalis* of pneumocytes and *lamina basalis* of endothelial lining of capillaries. After irradiation, collagen ligament is dominant here owing to its proliferation. This hinders transport of O_2, CO_2 and motion of free cells of ligament, e.g. macrophages. This is reflected on the immunologic function of this part of lungs. We observed concurrently the same sections in polarization and standard light microscope (Figures 8a, 8b) and in this way we proved that the proliferating ligament is really a collagen ligament. This verified our conclusions from transmission electron microscope observations. The microscopic picture of fiber ordering resembles the ligaments of tendons. This restructuring of barrier of gaseous exchange increases the rigidity of lungs, so that a convenient environment for bacteria and viruses is formed. This is just one of the reasons responsible for post-radiative pulmonary complications. In summary, irradiation modifies the physiological picture of microscopic structure of collagen ligament, and we show here how it is projected onto the ultrastructural level.

DISCUSSION

Fibroblasts, fixed cells of collagen ligament, participate in forming the stroma of organs. These cells produce and secrete precursors of fibrous and amorphous basic matter of collagen ligament. Molecules of tropocollagen produced in the cell are secreted into intercellular space where they polymerize in the formed matrix as collagen fibrils. This process is influenced by frequent x-ray irradiation. We observed in this work that excisions from lungs studied in the light microscope and ultra thin sections investigated in the electron microscope both show an increased occurrence of collagen ligament that has different structure than elastic ligament and reticular ligament (Gartner & Hiatt 2001; Junqueira *et al.* 1992; Yong & Heath 2000; Lang *et al.* 1993; MacFarlane *et al.* 2000; Yong & Heath 2000). Hence we paid attention to collagen ligament. After irradiation, this type of ligament forms a barrier between basal membrane of pneumocytes and basal membrane of endothelial cells, which form lining of capillaries. Importance of this finding consists in the fact that in this case transport of gases is affected and surface is reduced for transport of nutrition to cells that belong under physiological conditions to air-blood barrier. Contrary to barrier of gaseous exchange of intact lungs, this barrier is filled with abundant collagen ligament after irradiation, what can be seen in Figures 8a, 8b and morphometrically quantified in Figure 3c and 4c. This ligament is compressing capillaries, which leads to stenosis of blood in them. Abundant ligament is blocking space for motion of free cells of ligament, e.g. for macrophages.

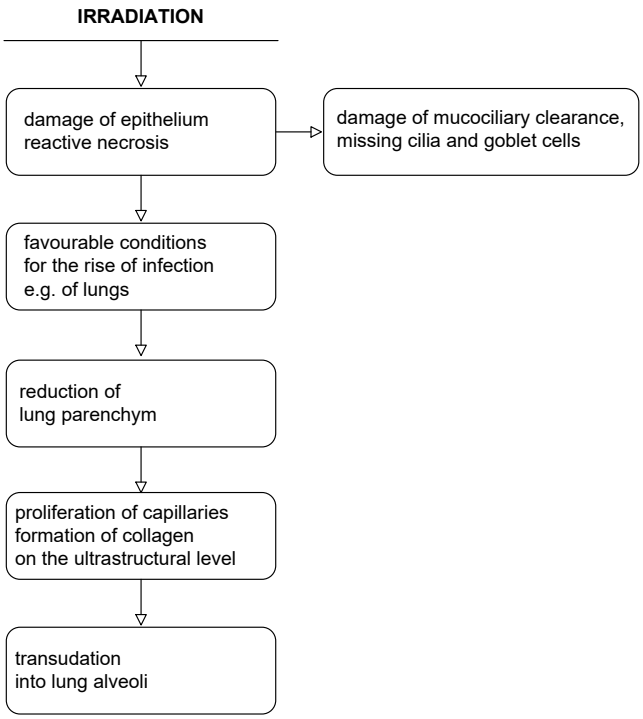
Owing to the continuity of the collagen fibrous network, the stroma is not only of mechanical importance, but it also assumes an additional role in clearing extravascular fluid that might interfere with pulmonary gas exchange. There is a continuous outward interstitial fluid flow in the lung that leaves the lung via lymph vessels. It is assumed that this interstitial fluid is formed by filtration from the microvasculature, predominantly from the expansive capillary bed in the alveolar septa. If this fluid was not drained continuously from the septa, it would lead to a thickening of the air-blood barrier (Weibel *et al.* 1997). This is also influenced by irradiation that irritates fibroblasts with following production of tropocollagen.

In the broadest sense, the pulmonary interstitium is the space bounded by the basal membranes of the air-space epithelium, the vascular endothelium, and the pleural mesothelium. Its structural constituents are those of connective tissue in general: a fiber system; a set of various interstitial cells, mainly fibroblasts and macrophages; some free extracellular fluid related to lymph; and a small amount of extracellular matrix that is increased after irradiation as can be seen in Figure 6. Besides abundance of collagen ligament, which is responsible for compression on capillaries with subsequent stenosis, also abundance of capillaries is observed. By the space distribution of capillaries, the microscopic picture resembles web like drawing of *varices cruri.*

The pulmonary interstitium appears to form a fluid continuum along the fiber system, from the alveolar walls to spaces enwrapping major airways and blood vessels and to the visceral pleura and septa emanating from it. This concept of fluid continuum is important in view of the fact that the major drainage pathway for interstitial fluid is via the lymphatics that originate in the connective tissue masses associated with larger vessels and airways and interlobular septa. Because lymphatics are missing in alveolar septa, one tends to conclude that interstitial fluid formed from alveolar capillaries needs to be drained to "juxta-alveolar" regions where it can be collected by lymphatic capillaries (MacFarlane *et al.* 2000; Weibel *et al.* 1997; Yong & Heath 2000). Due to irradiation, the collagen ligament network is abundant and the former process is blocked since abundant ligament changes the structure in the space of this network.

Tab. 2. Sequence of irradiation induced morphological changes and associated physiological conditions.

Functional Morphology of the sequence of changes found



CONCLUSIONS

Microstructural changes of lung tissue caused by human and animal exposure to radiation have been studied by electron microscopy, light polarization microscopy and morphometry, to get insights into the dynamics of morphological changes and associated post-radiative physiological conditions. Polarization microscopy results and quantitative morphometry data helped to validate conclusions of electron microscopic evaluation. Understanding of radiation damage dynamics may help in future studies on radiotherapy dosage and radioprotectives.

Mucociliary clearance has two phases assisted by four basic microscopic structures. The tracheo-bronchial phase involves an epithelial lining formed by the pseudostratified columnar epithelium with cilia and goblet cells, and an intact secretion cycle of mucine production in the goblet cells and seromucinous glands (Foltinová *et al.* 2002). Essential for the alveolar phase are alveolar macrophages and an intact barrier of gaseous exchange. Failure of one of these basic microscopic structures leads to disturbance of mucociliary clearance, manifesting itself as pathologic, relapsing changes, initializing a vicious circle which is often difficult to break.

We found that irradiation induces the appearance and proliferation of collagen ligament, thickening of the barrier of gaseous exchange, filling of alveoli with a parallel ordering of collagen fibers, reduction of respirable alveolar area, seriously affecting the exchange of O_2 and CO_2. Polarization microscopy proved the character of ligament responsible for these changes. They are collagen fibers responsible for the increased pulmonary rigidity. This finding agrees with the microscopic ultrastructure observed in electron microscope. Microscopic changes in ultrastructure of barrier of gaseous exchange originated by proliferation of ligament interfere also with blood circulation on places where vessels are proliferating due to damaged transport of O_2 and CO_2, and blood is stagnating due to compression of capillary walls by collagen ligament. Furthermore, post-radiatiative consequences are found to be a remarkable disappearance of alveolar macrophages from the inside of alveoli. This is important since in physiological conditions alveolar macrophages are responsible for maintaining sterility of the alveoli, which becomes problematic after irradiation. In this way we have shown why membrane of gaseous exchange is prone to post-radiative infections. Hence the disappearance of alveolar macrophages means a serious intervention into the immune system of mucociliary clearance and severe pneumonia can develop. The sequence of irradiation induced morpho-

logical changes and associated physiological conditions is shown in Table 2.

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