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COMPARATIVE CHARACTERISTICS OF HISTOMORPHOLOGIC CHANGES IN THE LUNG OF RATS EXPOSED TO GAMMA- AND NEUTRON RADIATION

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It is known that persons exposed to ionizing radiation, together with a different of damaging effects, particular importance is also attached to the respiratory system. The dominant role of neutron-activated radionuclide Manganese-56 (^{56}Mn) was noted in the treatises of Japanese scientists who studied the A-bomb effects of Hiroshima and Nagasaki, deserving the interest today. In this regard, the research purpose was to study the microscopic changes in the lung of rats exposed to γ– and neutron radiation.

In experiment, both sexes «Wistar» rats in amount of 36, weighting approximately 220-330 g. Necropsy of the animals were on the 3rd, 14th and 60th days after irradiation, then the lung removed, after which it was fixed in 10% formalin. Tissues fragments embedded in paraffin, then sections are manufactured serial transverse 4 mm thickness, which were subsequently stained by hematoxylin and eosin (H&E). Our experimental studies have shown that the majority of animals exposed to ^{56}Mn and ^{60}Co starting from the 2nd week observed signs of fibrosis and chronic inflammation, foci of emphysematous dilated alveoli, whereas after inhalation by MnO_2 in rats prevailed thickening of interalveolar septa due to cellular elements. Foci of hemosiderosis, signs of vasculitis, lymphadenitis and hyalinosis were typically for neutron-irradiated rats.

Thus, ^{56}Mn effect on the rat lung revealed a high risk of neutron radiation, which confirmed the presence of inflammatory processes. The findings support a role of ionizing radiation in the formation of morphologic signs depending on both dose and type of radiation.

Key words: ionizing radiation, pulmonary tissue, morphologic changes, pneumonitis, fibrosis, rats

It is known that among the neutron-induced radioisotopes ^{56}Mn is a dominant element found after an A-bomb explosion. Since ^{56}Mn and the other neutron-activated radioisotopes were present in dust after bombings, people have inhaled these radioactive materials and been internally exposed to radiation. People who returned early to Hiroshima and Nagasaki after A-bombing were reported to suffer from the symptoms of acute radiation effects [16]. Currently, particular interest represents a comparative assessment of pulmonary morphologic changes in persons exposed to ^{60}Co and ^{56}Mn [10]. Most diseases attributable to radiation in A-bomb survivors and nuclear reactor workers are lung pathology [16]. Pulmonary damage was identified as an important sphere of interest in radiation research after a significant number of sacrifice died early from radiation induced lung injury (RILI) [3]. Scientists are continuing to develop the mitigators for radiation injuries to the lung caused by a single dose of ionizing radiation at doses pertinent to a radiologic attack or nuclear accident. Therefore, animal models that reproduce radiation injuries in humans are necessary [5]. Average or severe RILI significantly exerts on patients life quality, and may even lead to death. In spite of decennary of studying the morphogenesis for lethal RILI at the organ level remain insufficiently defined [3].

Objective of the study – to identify and compare the microscopic changes in the rat lung after exposure by single dose of γ-radiation and neutron–activated ^{56}Mn powder.

MATERIALS AND METHODS

For this study, it was purchased and raised in a specific-pathogen-free facility six-month-old both sexes «Wistar» rats in an amount of 36 with mean whole body weight 220-330 g. All experimental animals were acclimatized for 2 weeks before initiation of experiments and kept under normal conditions and fed pellets concentrated diet and vitamin mixtures. They were maintained at constant temperature (22±1°C) on 8 hour light -dark cycle.

The rats were allocated into 4 groups. The first group of animals (n=9) were subjected to ^{56}Mn which was obtained by neutron activation of 100 mg of manganese dioxide (MnO_2) powder using nuclear reactor («Baikal-1») with neutron

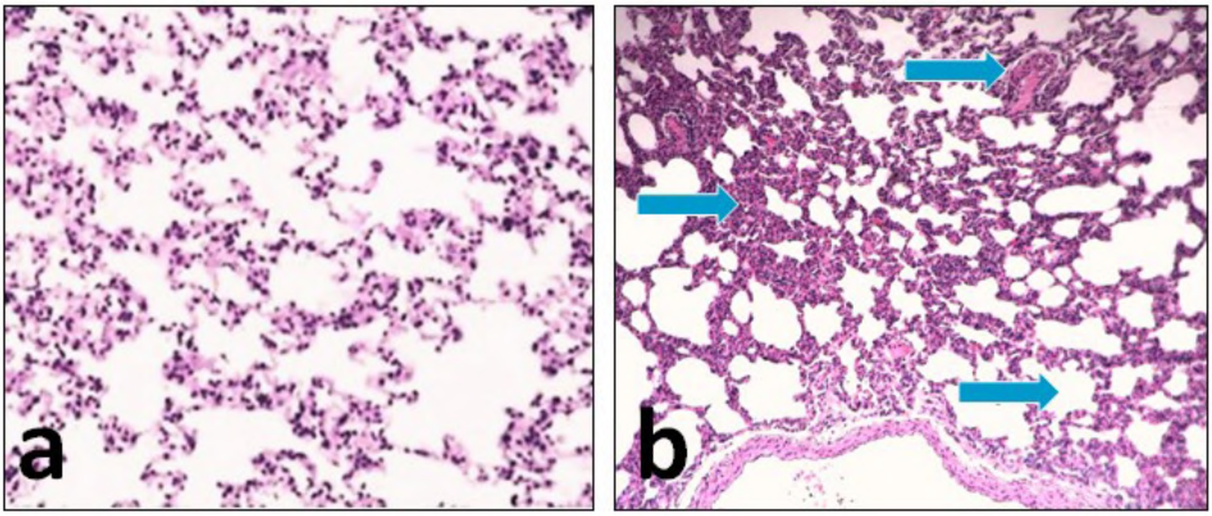

Figure 1 – Photomicrograph of uniform airiness in control rat lung (a).

flux $4×10^{14}$ n/cm². Activated powder with total activity of ^{56}Mn $2.75×10^{8}$ Bq was sprayed pneumatically over rats placed in the special box. The moment of exposition beginning of experimental animals by ^{56}Mn powder is 6 minute after finishing of neutron activation. The second group of rats (n=9) were exposed to not irradiated MnO_2. The spray powder was carried out in a chemical box, which contained boxes of 9 rats. Each portion of MnO_2 was sprayed in box with lots of biologic objects. Then unirradiated powder and incubated biologic objects in a container for hour. The third group of rats (n=9) were irradiated with a single dose of 2 Gy was performed at a dose rate of 2.6 Gy/min using ^{60}Co γ-ray by czech radiotherapeutic device «Teragam K-2 unit». During the exposure, animals were placed in a specially engineered cage made of organic glass with individual compartments for each rat. After irradiation, rats were taken back to the animal facility and routinely cared. The fourth group consisted of control rats (n=9) which were placed on shelves in the same facility and shielded from radiation. All animal procedures were approved by Ethical Committee of Semey State Medical University, Kazakhstan (Protocol №5 dated 16.04.2014) in accordance with Directive of the European Parliament and the Council on the Office in animals protection.

The rats were sacrificed on the 3rd, 14th, 60th day after irradiation and the lung was immediately surgically extracted for further histologic study. The lung sections were deparaffinized and dehydrated in graded 10% formalin solutions. Paraffin sections performed with 4 mm thickness. Stained by hematoxylin-eosin (H&E) slide glasses were examined under a Leica DM 1000 microscope (Germany) and images were captured with a charge–coupled device camera (Visitron Systems, Puchheim, Germany) at ×10 and ×40 magnifications. Qualitative histological assessment of lung injury was carried out to obtain an overall damage severity result.

RESULTS AND DISCUSSION

In the present study, we performed experiment with neutron-activated ^{56}Mn powder exposed rats. It was previously reported the internal dose estimates in organs of ^{56}Mn-exposed rats. The highest doses were recorded in the lung [13].

The study of lung components in control rats revealed an uniform airiness of pulmonary tissue, shown on figure 1 a. In the lung parenchyma of animals inhaled MnO_2, starting from the 2nd week reveals changes in the vascular walls with signs of productive vasculitis. Status of the pulmonary parenchyma is characterized by focal emphysema moderate severity. Intraalveolar septa are thickened due to lymphocytes, alveolar macrophages and less number of neutrophils. Changes in the structure of the interstitial tissue are manifested by presence of inflammatory foci (fig. 1 b).

Microscopic changes in the lung tissue of MnO_2-inhaled rat (b). H&E staining, original magnification ×10

Research of different pathologists established that radiation causes tissue damage via sensitization of autoreactive lymphocytes which react with lung tissue [18]. On the 3rd day after γ -radiation exposure, authors registers the inflammatory response manifested by moderate of cellular infiltration of interstitial lung tissue [7]. The above data are consistent with our study results the lung tissue in ^{60}Co-exposed rats showed a similar changes.

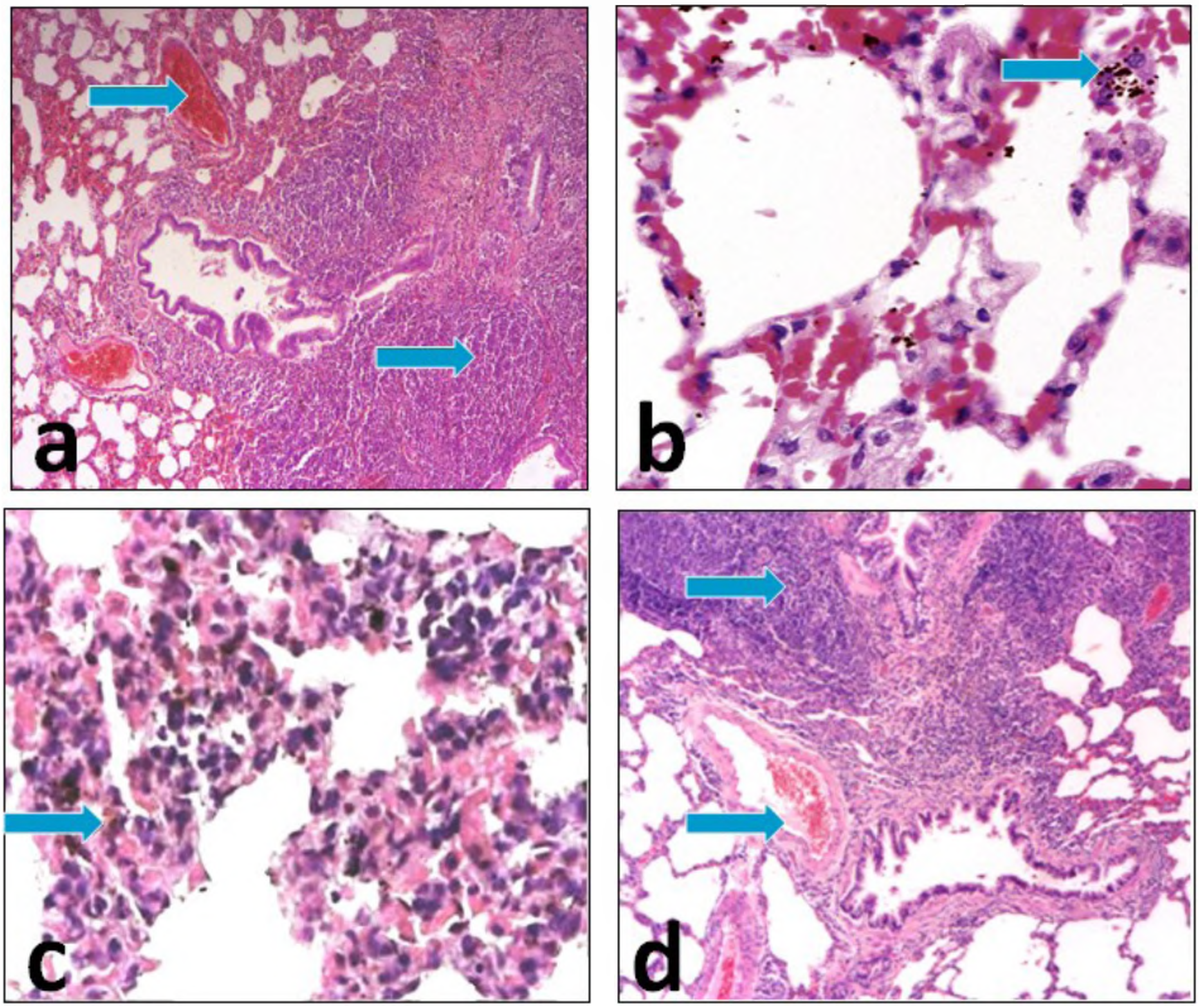

Figure 2 – Light microscopy of ^{56}Mn-induced (a, b, c) and γ-ray-induced rat lung (d). H&E staining, original magnification ×10 and ×40

According to histologic examination most pronounced changes were observed in the lung of ^{56}Mn-exposed rats, indicating that neutron radiation has a significant biologic effect on examined organ. In experimental animals exposed to ^{56}Mn and ^{60}Co were observed thickening of intraalveolar septa in virtue of leucocytes, erythrocytes, lymphocytes, histiocytes, alveolocytes, and rupture of the intraalveolar septa [10].

Figure 2 a shows an uneven blood filling with a predominance of capillary-venous plethora. It is necessary to pay attention to changes that occur in the bloodstream characterized by presence of erythrocytes in vascular lumen. Distruction of pulmonary parenchyma manifested by formation of fibrosis. It has been observed pronounced bronchitis with coverage of the lung parenchyma and lymph node, bronchial lumen contains stratum of desquamated ciliated epithelium, a small amount of mucus strands. It is also noted transition the inflammation to peribronchial tissue. By magnificating ×40, can be traced diapedetic microhemorrhage, several small focal intraalveolar hemorrhage of intensive red color with a small amount of leukocytes. Foci of acute alveolar emphysema varying degree of severity and prevalence with a thinning and defects of intraalveolar septa. In some fields of weak–moderate congestion hemosiderophages (fig. 2 b, c), observed on the 14th day in rats exposed ^{56}Mn. Comparative characteristics of changes in the lung after influence of γ- and neutron radiation showed uniformity of manifestation. Microscopic picture of animals on the 2nd week after ^{60}Co exposure provided on figure 2 d, where we are noted signs of thickening of the vascular walls, as confirmed by research which describes pulmonary vasculopathy after a single dose of γ-irradiation rats [8]. Considerable importance should be given

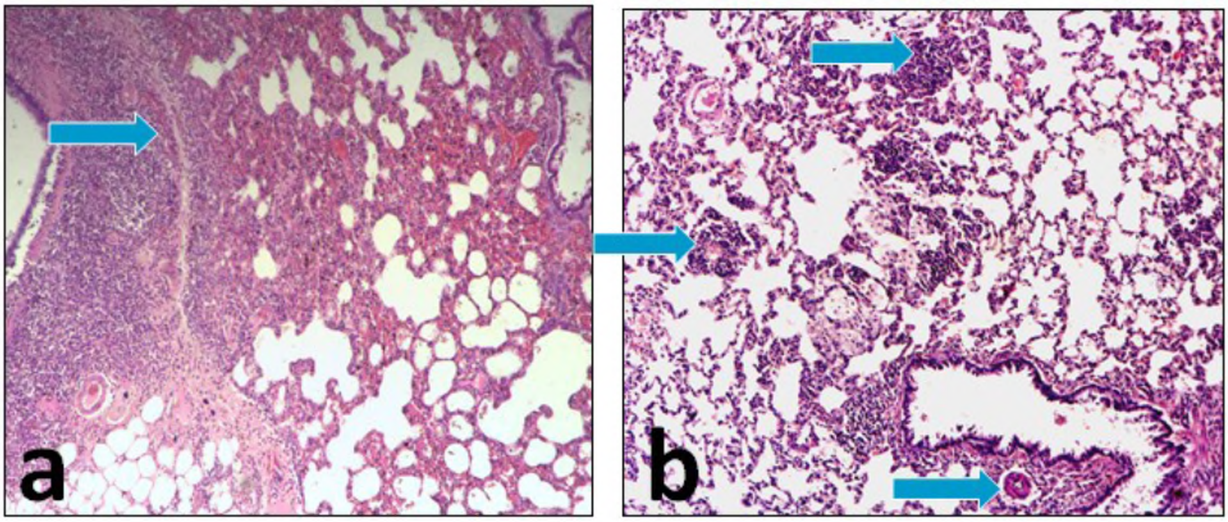

Figure 3 – Histologic sections of the pulmonary tissue in rats exposed to ^{56}Mn. H&E staining, original magnification ×10

to presence of the thickened walls of the interstitial tissue, alternating with emphysematous changed portions of the lung, presented keenly dilated alveoli. Mostly intra–alveolar septa are thickened. They determined cell infiltrates. Peribronchial infiltration is expressed. The sections are represented mainly by medium caliber bronchi which in a state of mild dystonia, a subtotal desquamation of ciliated epithelium and signs of mild mucus hypersecretion.

After radiation exposure in rat lungs on the 2nd month were found small focal mild hemorrhage, cellular response in the form of leukocyte infiltration and fibroblast proliferation (fig. 3 a).

Microscopic changes also characterized by presence of vasculitis, lymphadenitis, hyalinosis and peribronchial cell infiltration. According to our research all of above changes were typically for rats irradiated ^{56}Mn (fig. 3 b).

In this study, we have shown the sequence of morphologic changes in the rat lungs from early to late stage after a single influence of ^{56}Mn and ^{60}Co at 2 Gy dose, which were the initiators of radiation pneumonitis related to fibrogenesis. Results of morphologic studies have shown that structural changes in the lung observed in irradiated rats little differed from the previously published results using different radiation models. It should be noted that the most prominent microscopic changes were detected in rats exposed to ^{56}Mn after 2 weeks.

Currently, great importance is attached to the role of Mn, inducing apoptosis or necrosis. Thus, in the literature we have examined papers revealed regarding ability of Mn to cause histomorphologic changes in the lung tissue of animals [17]. In one of the papers, authors showed that the intoxication by Mn steams leads to distruction of the pulmonary parenchyma, the development of manganese pneumonia and lung edema [9], which corresponds to the data obtained in our experiment.

Identified us the signs of pneumonitis and fibrosis have been well described in models of irradiated rats [14]. According to the literature, the early phase after acute lung injury characterized by exudative inflammation complicated by interstitial pneumonia after few weeks post-radiation. The later phase is characterized by chronic inflammation and tissue restructuring [2]. There are studies indicating that accumulation and activation of monocytes, macrophages and lymphocytes are a key component of RILI [11]. Radiation damage contributes to the development of chronic inflammatory processes that may predispose irradiated individuals to the formation of lung fibrosis. Although the pathogenesis of radiation-induced lung fibrosis at the molecular and cellular levels is not completely understood, initial immune and inflammatory responses to repeated irritants lead to tissue damage and progressive fibrosis [14]. Histologic analysis of irradiated lungs showed the fibrosis development by progressive collagen deposition after several month, which characterized by formation of typical fibroblast foci and accompanied by a severe second outbreak of leukocyte infiltration. The later fibrogenesis phase characterized the fibrotic foci evolved and grow together into widespread fibrosis with the pulmonary architectonics remodelling [12]. During granulation growth and tissue repair, pulmonary interstitial fibrosis occurs in the late phase of RILI after fibroblast cell proliferation and collagen synthesis, inducing the development of respiratory failure, which is one of the major causes of death [15]. Our findings of pulmonary tissue changes in irradiated rats consistent with conclusion of some authors that marked thickening of alveolar walls and dilatation of intralobular septa, and also sealed foci of fibrosis, mainly in intralobular septa [6].

In contrast to the external γ-radiation, internal ^{56}Mn radiation contributes to the development of pathologic changes manifested by formation of foci of hemorrhage and emphysema [10]. The emphysema is characterized by dilatation of air space and destruction of the alveolar walls. Scientists have shown that the combination of lung fibrosis and emphysema have bad prognosis similar to idiopathic pulmonary fibrosis [4].

Our results showing about multifocal alveolar reactive changes, such as edema and moderate increase the number of cells in the alveolar walls, and also interstitial inflammation in the pulmonary tissue confirmed already known fact regarding γ-radiation effect to organism. These changes are most often found in the form of a thickening of the alveolar and vascular walls, sclerosis on the 3rd month after exposure [1].

Summing up, irradiation of normal lung tissue has two generally recognized adverse effects, including inflammatory and fibrotic processes [12]. These histomorphologic changes in the lung of rats exposed to γ- and neutron radiation make it possible to develop diagnostic criteria for assessing of radiation effect on the lung, depending on cumulative dose.

CONCLUSION

Our research results and their comparison with literature data led to the conclusion that majority of experimental animals exposed to γ- and neutron radiation more pronounced changes were observed from the 2nd week after exposure, consisting the appearance of fibrosis and chronic inflammation, foci of emphysematous dilated alveoli, whereas after inhalation by MnO_2 in rats dominated thickening of intra-alveolar septa on account of leukocytes, erythrocytes, lymphocytes, histiocytes and alveolocytes. Consequently, like γ-rays, ^{56}Mn also promotes activation of inflammatory processes and stimulation of immune responses manifested by cellular infiltration. It should be noted that the most prominent histologic picture characterized by presence of hemosiderosis foci, signs of vasculitis, lymphadenitis and hyalinosis were found, in particular, in rats exposed to ^{56}Mn.

Thus, experimental studies confirm the role of neutron radiation in the formation of morphologic features which typically for radiation pneumonitis, that is a form of acute or subacute lung injury, depends on both dose and type of radiation.

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СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА ГИСТОМОРФОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ В ЛЕГКИХ КРЫС, ПОДВЕРГАВШИХСЯ ГАММА- И НЕЙТРОННОМУ ОБЛУЧЕНИЮ

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Известно, что у лиц, подвергавшихся воздействию ионизирующего излучения, наряду с различными повреждающими эффектами особое место отводится дыхательной системе. Доминирующая роль нейтронно-активированного радионуклида (Марганца-56 (^{56}Mn) отмечалась в трудах японских ученых, изучавших последствия атомной бомбардировки в Хиросиме и Нагасаки, и представляет научный интерес по сей день. В связи с этим целью исследования явилось изучение микроскопических изменений в легких крыс, подвергавшихся воздействию γ- и нейтронного излучения.

В эксперименте использованы крысы обоих полов линии «Вистар» в количестве 36 особей, массой 220-330 гр. Животных подвергали некропсии через 3, 14 и 60 сут после облучения, затем извлекали легкие, после чего фиксировали в 10% формалине. Фрагменты тканей заливали в парафин, затем изготовливали поперечные серийные срезы толщиной 4 мкм, которые в дальнейшем окрашивали гематоксилином и эозином. Результаты экспериментального исследования показали, что у большинства животных, подвергнутых воздействию ^{56}Mn и ^{60}Co, начиная со 2 недели отмечались признаки фиброза и хронического воспаления, очаги эмфизематозно расширенных альвеол, тогда как после ингаляции MnO_2 в легких крыс наблюдалось утолщение межальвеолярных перегородок за счет клеточных элементов. Очаги гемосидероза, признаки васкулита, лимфаденита и гиалиноза преобладали у крыс, подвергавшихся воздействию нейтронного излучения.

Таким образом, изучение воздействия ^{56}Mn на легкие крыс выявило высокий уровень риска облучения, что подтверждено наличием воспалительных процессов. Полученные данные подтверждают роль ионизирующего излучения в формировании морфологических признаков, зависящих как от дозы, так и от типа излучения.

Ключевые слова: ионизирующее излучение, легочная ткань, морфологические изменения, пневмонит, фиброз, крысы

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ГАММА-МЕН НЕЙТРОНДЫ СӘУЛЕ ӘСЕРІНЕ ҰШЫРАҒАН ЕГЕУҚҰЙРЫҚТАР ӨКПЕСІНДЕГІ ГИСТОМОРФОЛОГИЯЛЫҚ ӨЗГЕРІСТЕРДІҢ САЛЫСТЫРМАЛЫ СИПАТТАМАСЫ

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Иондағыш сәуле әсеріне душар болғандардың көптеген бүліндіргіш салдарымен қоса тыныс-алу жүйесіне де ерекше мән бөлінеді. Хиросима мен Нагасакидағы атомдық бомбалаудың салдарын зерттеген жапон ғалымдарының еңбектеріндегі нейтронды-белсенді Марганец-56 (^{56}Mn) радионуклидінің басым рөлі заманауи жағдайда да қызығушылық арттырады. Осыған сүйене отырып, γ- мен нейтронды сәуле әсеріне ұшыраған егеуқұйрықтардың өкпесіндегі микроскопиялық өзгерістерді зерттеу мақсатымыз туындады.

Тәжірибе жүзінде «Вистар» тұқымды 220-330 гр салмағы бар аталық және аналық жынысты 36 егеуқұйрық пайдаланылған. Жануарларға сәулелеуден кейін 3-ші, 14-ші және 60-шы тәуліктерде некропсия жүргізу барысында өкпесін алып, 10%-тік формалинде фиксацияладық. Тін фрагменттерін парафинге құйып, қалыңдығы 4 мкм көлденең сериялық кесінділер дайындап, әрі қарай гематоксилин мен эозинмен (H&E) боядық. Тәжірибелік зерттеу нәтижелері ^{56}Mn мен ^{60}Co әсеріне ұшыраған жануарлардың көпшілігінде 2-ші аптадан кейін фиброз бен созылмалы қабыну белгілерін, альвеолалардың эмфизематозды кеңею ошақтары басым болғанын, ал MnO_2 ингаляциясынан кейін егеуқұйрықтар өкпесінде жасуша элементтерінің әсерінен альвеола аралық перделердің қалыңдауын көрсетті. Гемосидероз ошақтары, васкулит, лимфаденит пен гиалиноз белгілері нейтронды сәулелену әсеріне шалдыққан егеуқұйрықтарға тән болды.

Сонымен, егеуқұйрықтардың өкпесіне ^{56}Mn әсері қабыну үрдістерімен расталатын сәулелену қаупінің жоғары деңгейін көрсетті. Зерттеу нәтижелеріне сай иондағыш сәуле әсерінен туындайтын морфологиялық өзгерістердің сипаты сәулеленудің дозасы мен түріне байланысты дамиды.

Кілт сөздер: иондағыш сәуле, өкпе тіні, морфологиялық өзгерістер, пневмонит, фиброз, егеуқұйрықтар