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COMPARISON OF P53 PROTEIN IN THE PULMONARY TISSUE OF RATS EXPOSED TO INTERNAL AND EXTERNAL RADIATION

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Abstract

Introduction. It is known from literary review that in persons exposed to neutron-activated radionuclide − Manganese-56 (^{56}Mn) and external ionizing radiation (^{60}Co) along with dystrophic, inflammatory and necrotic phenomena in the respiratory system special attention is paid to the development of neoplastic processes.

The aim. To determine and compare the quantitative content of p53 protein in the pulmonary tissue of rats exposed to internal and external ionizing radiation.

Materials and methods. In experiment, male sex «Wistar» rats in amount of 90, weighting approximately 270−350 g. It was 3 groups identified: 1) ^{56}Mn which obtained by neutron activation of 100 mg MnO_2 powder using the «Baikal-1» atomic reactor with a neutrons fluence of 4×10^{14} n/cm²; 2) ^{60}Co γ−rays; 3) control group. Animals necropsy was made on the 3rd, 14th and 60th day after irradiation, then the lung removed, after that it was fixed in 10% formalin. Paraffin sections were dewaxed and rehydrated using a standard procedure. To visualize the immune histochemical reaction, DAB+(DAKO) system was used. For the purpose of calculating, respectively, the number of p53-positive cells, taking into account the colored nuclei of any intensity, expressing the results in percent. Statistical processing of the results was processed using licensed packages of application programs «SPSS 2,0». All quantitative variables are described using the mean (M), median (Me) and interquartile interval (IQR). In their comparison, depending on the factors studied, the Kruskel-Wallis criterion was used. The critical level of significance *p* in testing the statistical hypotheses in this study was taken to be 0,05.

Results. The number of p53-positive cells in the intra-alveolar septum of the pulmonary tissue increases in laboratory animals exposed to neutron-activated manganese dioxide from the 14th day, while in rats, this indicator increases significantly only on the 60th day after external irradiation. It should be noted that there was no statistical difference between the studied factors and the control group according to p53 protein level on the 14th day, whereas on the 60th day after exposure, the difference between experimental and control groups becomes significant ($p<0,001$). Apoptosis as a sign of DNA breaking chain correlates with cell injury observed late after irradiation. Immune histochemical analysis of lung tissue of rats exposed to internal and external radiation showed that the highest quantitative content of p53 protein was observed when exposed to ^{56}Mn.

Conclusion. Thus, ^{56}Mn effect to the rat lungs of revealed a high level of risk of exposure, which is confirmed by the presence of a high percentage of p53 indicating programmed cell death. The obtained data confirm the role of irradiation exposure in the formation of oncomorphological signs depending on the radiation type.

Keywords: *radioactive ^{56}Mn, pulmonary tissue, intra-alveolar septum, p53, apoptosis, rats.*

Резюме

СРАВНИТЕЛЬНАЯ ОЦЕНКА P53 БЕЛКА В ЛЕГОЧНОЙ ТКАНИ КРЫС, ПОДВЕРГАВШИХСЯ ВНУТРЕННЕМУ И ВНЕШНЕМУ ИЗЛУЧЕНИЮ

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Введение. Из литературных источников известно, что у лиц, подвергавшихся воздействию нейтронно-активированного радионуклида – Марганца-56 (^{56}Mn) и внешнего ионизирующего излучения (^{60}Co) наряду с дистрофическими, воспалительными и некротическими явлениями в дыхательной системе особое место отводится и развитию неопластических процессов.

Цель исследования. Определить и сравнить количественное содержание белка p53 в легочной ткани крыс, подвергавшихся воздействию внутреннего и внешнего ионизирующего излучения.

Материалы и методы. В эксперименте использованы крысы-самцы линии «Вистар» в количестве 90, массой 270–350 гр. Выделены 3 группы: 1) ^{56}Mn, полученный путём нейтронной активации 100 мг порошка MnO_2 на атомном реакторе «Байкал–1» при флюенсе нейтронов 4×10^{14} н/см²; 2) ^{60}Co γ–лучи; 3) контрольная группа. Лабораторных животных подвергали некропсии через 3, 14 и 60 дней после облучения, затем извлекали легкое, после чего фиксировали его в 10% формалине. Парафиновые срезы депарафинировали и регидратировали по стандартной методике. Визуализацию иммуногистохимической реакции проводили используя систему DAB+(DAKO). Количество p53-позитивных клеток подсчитывали учитывая окрашенные ядра любой степени интенсивности, выражая полученные результаты в процентах. Статистическую обработку результатов проводили с использованием лицензированных пакетов прикладных программ «SPSS 2,0». Все изучаемые количественные переменные показатели описаны при помощи средней (M), медианы (Me) и межквартильного интервала (IQR), при сравнении которых в зависимости от изучаемых факторов был использован критерий Краскела-Уоллиса. Критический уровень значимости *p* при проверке статистических гипотез в данном исследовании принимался равным 0,05.

Результаты. Количество p53-положительных клеток в межальвеолярной перегородке легочной ткани возрастает у лабораторных животных подвергавшихся воздействию нейтронно-активированного диоксида марганца начиная с 14-го дня, в то время как после внешнего облучения крыс данный показатель значительно повышается лишь на 60-й день. Следует отметить, что статистической разницы между изученными факторами и контрольной группой по уровню белка p53 на 14-й день не выявлено, тогда как на 60-й день после экспозиции разница между экспериментальной и контрольной группами становится значительной ($p<0,001$). Апоптоз как признак разрыва цепи ДНК, коррелирует с повреждением клеток, наблюдаемой в поздние сроки после облучения. Иммуногистохимический анализ легочной ткани крыс, подвергавшихся внутреннему и внешнему облучению показал, что наиболее высокое количественное содержание белка p53 отмечается при воздействии ^{56}Mn.

Выводы. Таким образом, воздействие ^{56}Mn на легкие крыс выявил высокий уровень риска облучения, что подтверждено наличием высокого процентного содержания p53, свидетельствующего о запрограммированной клеточной гибели. Полученные данные подтверждают роль радиационного воздействия в формировании онкоморфологических признаков, зависящих от типа излучения.

Ключевые слова: *радиоактивный ^{56}Mn, легочная ткань, межальвеолярные перегородки, p53, апоптоз, крысы.*

Түйіндеме

ІШКІ МЕН СЫРТҚЫ СӘУЛЕЛЕУ ӘСЕРІНЕ ҰШЫРАҒАН ЕГЕУҚҰЙРЫҚТАРДЫҢ ӨКПЕ ТІНІНДЕГІ P53 НӘРУЫЗЫН САЛЫСТЫРУ

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Кіріспе. Нейтронды-белсенді радионуклид – Марганец-56 (^{56}Mn) және сыртқы иондаушы сәулелеу (^{60}Co) әсеріне ұшырағандардың тыныс алу жүйесінде анықталған дистрофиялық, қабынулық пен некроздық құбылыстармен қатар неоплазиялық үдерістерге де ерекше мән бөлініп жүргені ғылыми әдебиеттерден мәлім.

Зерттеу мақсаты. Ішкі мен сыртқы иондаушы сәулелеу әсеріне ұшыраған егеуқұйрықтардың өкпе тініндегі p53 нәруызының сандық мөлшерін анықтап, өзара салыстыру.

Материалдар мен әдістер. Эксперимент жүзінде «Вистар» тұқымдас 270–350 гр салмағы бар аталық жыныстық 90 егеуқұйрық пайдаланылған. 3 топқа іріктеу жүргізілді: 1) ^{56}Mn, яғни 100 мг MnO_2 ұнтағын «Байкал–1» атом реакторы арқылы 4×10^{14} н/см² нейтрон флюенсінде нейтрондық белсендіру жүзінде алынған элемент; 2) ^{60}Co γ–сәулелер; 3) бақылау тобы. Жануарларға сәулелеуден кейін 3-ші, 14-ші және 60-шы тәуліктерде некропсия жүргізу барысында өкпесін алып, 10%-дық формалинде фиксацияланған. Парафиндік кесілімдер стандартты әдіс арқылы депарафинизацияланып, регидратацияланған. Иммунды гистохимиялық серпілістерді визуализациялау мақсатында DAB+(DAKO) жүйесі қолданылған. Жасушалардың бағдарламаланған өлімін анықтауға арналған p53-позитивті жасушалар саны анықталып, алынған нәтижелер пайыз мөлшері түрінде ұсынылған. Зерттеу нәтижелерінің статистикалық өңдеуі «SPSS 2,0» қолданбалы бағдарламаның лицензияланған пакеттері көмегімен жүзеге асырылған. Бүкіл зерттелген сандық көрсеткіштердің статистикалық өңдеуі кезінде олар орташа көрсеткіш (M) және медиана (Me), сондай-ақ квартиль аралық интервал (IQR) жүзінде сипатталған. Зерттеуге алынған факторлардың әсерін салыстырмалы түрде бағалау барысында Краскел-Уоллистің H-өлшемі қолданылған. Нөлдік статистикалық гипотеза нақтылығының *p* критикалық деңгейі 0,05-ке тең деп саналған.

Нәтижелер. Нейтронды-белсендірілген марганец диоксидіне ұшыраған зертханалық жануарлар өкпе тінінің альвеола аралық перделерінде анықталған p53-позитивті жасушалар санының 14-ші тәуліктен бастап жоғарылағаны, ал сыртқы сәулелеу әсерін алған егеуқұйрықтарда бұл көрсеткіштің 60-шы тәулікте ғана анағұрлым жоғарылағаны тіркелген. Зерттеуге алынған факторлар мен бақылау тобы арасында 14-ші тәулікте p53 нәруызы бойынша статистикалық айырмашылықтың анықталмағанын, ал енді 60-шы тәулікте экспозициядан кейін тәжірибелік пен бақылау топтары арасындағы айырмашылықтың анағұрлым болғанын айтып өткен жөн ($p<0,001$). Апоптоз үдерісі ДНҚ тізбегі бүлінуінің белгісі ретінде 60–шы тәулікте аңғарылған жасушалар зақымдануымен байланысты болған. Ішкі мен сыртқы сәулелеу әсеріне ұшыраған егеуқұйрықтар өкпе тінінің иммунды гистохимиялық талдауы, негізінен p53 нәруызының сандық көрсеткіші ^{56}Mn ықпалынан кейін анағұрлым жоғарылайтынын аңғарған.

Қорытынды. Сонымен, егеуқұйрықтардың өкпесіне ^{56}Mn әсері жасушалардың бағдарламаланған өлімін сипаттайтын p53 көрсеткішінің жоғары пайыздық мөлшерімен расталатын сәулелену қаупінің жоғары деңгейін көрсетті. Зерттеу нәтижелеріне сай иондаушы сәулелеу әсерінен туындайтын онкоморфологиялық өзгерістердің сипаты сәулеленудің түріне байланысты дамиды.

Негізгі сөздер: *радиобелсенді ^{56}Mn, өкпе тіні, альвеола аралық перделер, p53, апоптоз, егеуқұйрықтар.*

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Introduction

The factors for the evaluation of exposure to β– and γ–radiation at Hiroshima and Nagasaki are discussed in the external and internal doses from residual radiation exposure. Questions were asked about the conclusion that manganese–56 (^{56}Mn) is the most important radionuclide. Radiobiologists have concluded that the methodological guides on internal and external dose estimation developed for the public living near Semipalatinsk Nuclear Test Site can be applied with modifications to the conditions of residual radiation exposure to Japanese atomic bomb survivors. A view, based on an analysis using a multi-step pathologic process model, suggests that residual radiation doses in Hiroshima were approached to 2 Gy to match the modeled incidence [16]. The presence of numerous data on the results of morphofunctional study of the lung at the cellular and tissue levels in different radiation situations, according to the connection of increasing neoplastic processes in the respiratory system with the values of external and internal doses exposure during acute and long–term periods. At estimate the internal doses in rat organs exposed to neutron–activated ^{56}Mn using nuclear reactor (Experimental facility «Baikal–1», Kurchatov, Kazakhstan) with neutron flux 4×10^{14} n/cm² [4], the highest doses were recorded in the lung. Consequently, the cumulative absorbed dose of internal radiation exposure for with forced ventilation box with animals cumulative absorbed dose of internal radiation was 0,03 Gy for the lung, respectively [26, 27]. It is known that p53 is a nuclear phosphoprotein that acts as a transcription factor to control cell cycle checkpoints and induces apoptosis in response to ionizing radiation. It is known that wild-type p53 plays a role in the control of apoptotic pathways by downregulating Bcl-2 and upregulating Bax. Bcl-2 inhibits apoptotic cell death, whereas the expression of Bax and subsequent formation of Bax-Bcl-2 complex is thought to induce apoptotic cell death [19]. Therefore, currently, particular interest is a comparison of morphofunctional changes in the persons' lung exposed to ^{56}Mn and ^{60}Co, allowing to identify the informative criteria for assessing the effect of the internal and external radiation factor on the respiratory organs, depending on the acumulative dose [15, 30, 32].

The objective of study

Our goal has been to determine the content level of the p53 apoptosis regulatory protein in the pulmonary tissue of rats exposed to ^{56}Mn and ^{60}Co, followed by an evaluation of the diagnostic significance of morphofunctional changes.

Materials and methods

Six-month-old male Wistar rats (270–350 g) were purchased from Karaganda State Medical University (Kazakhstan). The rats were housed in groups of 2 to 3 per cage in an air-conditioned room at 22°C (lights on from 8 a.m. to 8 p.m.), and allowed free access to food and tap water at the Scientific Laboratory of Semey State Medical University. Food was removed one day before irradiation but water was available. Then, rats were allocated into 3 groups.

The first group of animals (n=30) were subjected to ^{56}Mn which was obtained by neutron activation of 100 mg of MnO_2 (Rare Metallic Co., Ltd., Japan) powder using the «Baikal–1» nuclear reactor with neutron flux 4×10^{14} n/cm². Activated powder with total activity of ^{56}Mn $2{,}75\times10^{8}$ 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. Duration of exposition of rats to radioactive powder was 3,5–4,0 hours (starting from the moment of spraying of ^{56}Mn powder till surgical extraction of the lung) [4].

The second group of rats (n=30) were irradiated with a total dose of 2 Gy was performed at a dose rate of 2.6 Gy/min using ^{60}Co γ−ray by czech radiotherapy device «Teragam K–2 unit». After irradiation, rats were taken back to the animal facility and routinely cared. All the experiments were followed our institution's guide for the care and use of laboratory animals. During the exposure, animals were placed in a plastic shell with lead shield (2 μm thickness) on the upper and lower sides.

The third group consisted of non-irradiated animals (n=30) which were placed on shelves in the same facility and shielded from the radiation. All animals were kept in a specific pathogen-free facility at the Scientific Laboratory in accordance with the rules and regulations of the 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 housed in a moderate security barrier. Laboratory animals in each group were sacrificed by deep anesthesia after exposure. They were sacrificed on the 3rd, 14th and 60th day after irradiation and the lung was immediately surgically extracted for further histological study (Table 1).

*Table 1.***The arrangement of experimental animals.**

№	Group	Dose (Gy)	The 3rd day after exposure	The 14th day after exposure	The 60th day after exposure	Animals number
1	^{56}Mn	0,15±0,02	10	10	10	30
2	^{60}Co	2	10	10	10	30
3	Control	0	10	10	10	30
Totally						90

The pulmonary tissue was resected and immersed in 10% neutral-buffered formalin, and embedding in paraffin blocks from which 4 μm sections were cut and stained. Identification of apoptosis was confirmed using a terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) technique (Apop Tag; Oncor, Gaithersburg, MD) which stains the oligofragmented DNA characteristically found in apoptotic nuclei. Intra-alveolar septum per group from complete pulmonary tissue that had been cut in the longitudinal plane were selected for analysis. The incidence of cell death in the lung was quantified by counting the number of dead cells in intra-alveolar septum stained sections at ×40 magnification by light microscopic analysis (Leica microscope DM 1000, Germany). For the purpose of calculating, respectively, the number of p53-positive cells, taking into account the colored nuclei of any intensity, expressing the results in percent. All fragments chosen were at least 20 cells in length, with cell position 1 located at the tissue.

All values were expressed as the mean (M), median (Me) and interquartile interval (IQR) of results obtained from animals per data point. Differences between groups were examined for statistical significance using the Kruskel-Wallis test (SPSS 2,0 program). A $p<0,05$ value was considered to be of statistical significance.

Results

In the present study, we have performed experiment with neutron–activated ^{56}Mn powder exposed Wistar rats. Although the level of radioactivity received from ^{56}Mn was rather low, the observed biological effects were consistent in experiment. It was previously reported the internal dose estimates in organs of ^{56}Mn–exposed rats. According to finding, p53 number in the lung was enhanced for an extended period after exposure to ^{56}Mn. For count of apoptotic cells in the pulmonary tissue was used longitudinal sections of the intra-alveolar septum.

On the 14th day after irradiation in rats from the first group, a large number of apoptotic cells was observed in the intra-alveolar septum, as determined by special staining. On the figure 1, there was a sharp increase the number of apoptotic cells in the intra-alveolar septum of β–ray–induced (A, B) and γ–ray–induced (C, D) rats on the 60th day after irradiation when compared with control rats. Light microscopy shows that apoptosis was observed in the intra-alveolar septum in the rats exposed to internal irradiation.

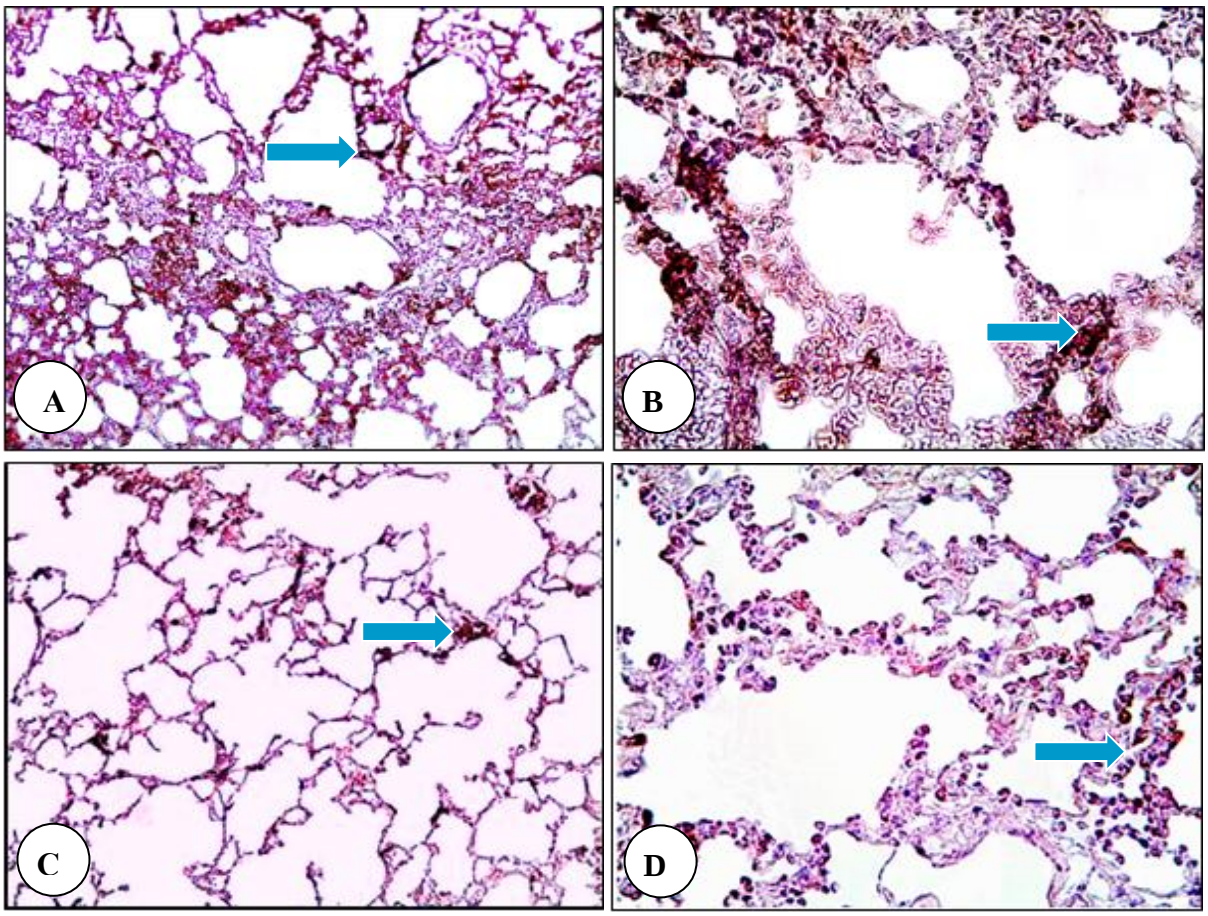

Fig. 1. Light microscopy of ^{56}Mn-induced (A, B) and ^{60}Co-induced rat lung (C, D). Original magnification ×10 and ×40

Apoptotic cells different small dimensions comparable with lymphocytes dimensions with high nuclear–cytoplasmic ratio, rounded contours and condensed chromatin and cytoplasm in experimental animals of the first group on the 60th day after irradiation. The distinctive morphological features of apoptosis were used to recognize apoptotic cells. Small clusters of dead cell fragments were assessed as originating from one cell and any doubtful cells were disregarded. Apoptosis was measured on the basis of nuclear image morphology and were able to correlate positive staining with measurable nuclear fragmentation.

Apoptotic cells look as the rounded or oval accumulations of intensively eosinophil cytoplasm with dense by the fragments of nuclear chromoplasm.

Table 2 shows the number of p53-positive cells in the intra-alveolar septum were increased in ^{56}Mn exposed rats from the 14th day after internal irradiation and in ^{60}Co exposed rats on the 60th day after external irradiation.

Table 2.

Number of p53-positive cells (%) in the intra-alveolar septum of laboratory rats.

^{56}Mn			^{60}Co			Control			Kruskel-Wallis test	p value
M	Me	IQR	M	Me	IQR	M	Me	IQR		
The 3rd day after exposure										
1,68	1,74	0,72	1,74	1,86	0,54	1,64	1,76	0,44	H=2,582	0,462
The 14th day after exposure										
2,28	2,32	0,82	2,02	2,08	0,22	1,78	1,94	0,54	H=5,862	0,116
The 60th day after exposure										
4,76	4,92	0,48	4,02	4,06	1,16	1,82	1,78	0,54	H=46,506	<0,001

Based on this table, it should be noted that there is no statistical difference between the studied factors and the control group for the p53 protein number on the 14th day, whereas on the 60th day after exposure the difference between experimental and control groups was significant ($p<0.001$).

Using the tagged consensus sequence of p53, we have showed that the increase in the DNA-binding activity of the p53 protein occurs independently of the level of this protein. Interestingly, in cells approaching aging, a significant number of chromosomes accumulate [25]. It is possible that critical shortening of telomeres in these cells leads to the accumulation of such chromosomes. Subsequent rupture of chromosomes in the next mitosis provokes formation of at least one rupture. These gaps are then perceived by the cell as a DNA damage signal, which induces to the p53 protein activation and then to stop in G1 [39].

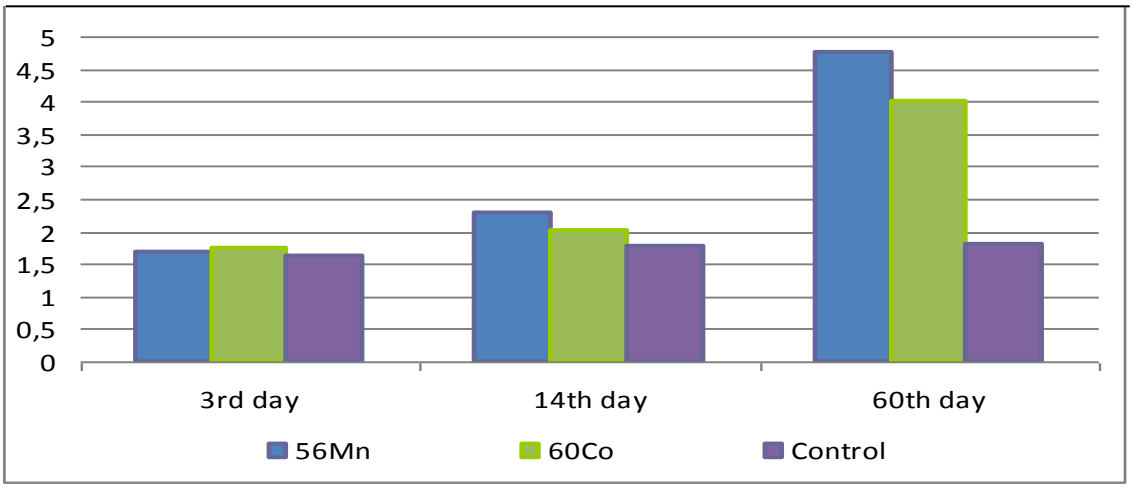

Fig. 2. Changes of p53 indication in the lung of experimental and control animals

The diagram shown in Fig. 2 shows that the studied immunohistochemical indicator increases after 2 months, because on the 3rd day there are low indices, on the 14th day the growth of this indicator is revealed, and in the later periods the quantitative content of the protein increases. The large increase of apoptotic cells on the 60th day mark in our first experiments revealed a higher turnover of intra-alveolar septal cells for the internal exposure model, as compared to the low level of apoptosis found in the external exposure model. As the half–life of ^{56}Mn is three hours, understanding the initial damage to pulmonary cells by internally deposited radioactive materials is crucial.

Immune histochemical method is used for a long time to verify the cancer of various localizations as well as in the diagnosis of predictor diseases. It is possible to use the obtained data to compose the nearest and remote predictions of course of the precancerous process [17]. In this respect, p53 biomarkers are of undoubted interest. The p53 protein encoded by a gene with the same name regulates apoptosis. Mutations in p53 result in cessation of apoptosis, which induces uncontrolled growth and development of pathological cells [39]. It can be assumed that in the cells of the intra-alveolar septum, there appears to be genetic instability, which on the one hand changes the cell cycle, and on the other hand the dysregulation of the apoptosis processes in the late periods after irradiation [40]. Thus, morpho-immunohistochemical study of pulmonary tissue of experimental animals revealed the predominance of apoptotic activity of cells in the first group. At the same time in some areas of affected tissues there were signs of necrosis with deposition of fibrin masses, leukocytes, which can be regarded as a result of secondary cell injury induced by internal radiation.

Discussion

Radiation-induced lung injury produces an eligible pre-metastatic microenvironment for cancer cells [1]. According to scientists' opinion one of the common neoplastic diseases ascribable to internal ionizing radiation in atomic bomb survivors and nuclear reactor workers are pulmonary cancers, which accounts for almost a quarter of radiotherapy-induced secondary malignant tumors [2, 3].

However, available data on histological alterations after radiotherapy human lung is limited, since patients are unlikely to give consent for diagnostic thoracotomy and autopsy. The existing histological data have mostly come from animal models. For this reason, animal models that reproduce radiation injuries in humans are mandatory. The rats and mice are the animal models of selection, because they are well characterized, easy to work with, and have genetically altered strains accessible for advanced research [35].

Organizing pneumonia is a form of lung toxicity that arises due to some interaction between radiotherapy and immune system. It is an important question why organizing pneumonia occurs after radiotherapy for breast cancer more frequently than after radiotherapy for other malignancies. The lungs are often exposed to radiation for the treatment for malignant tumor. Late damage to the lung, which usually manifests as fibrosis, is a radiation dose–dependent occurrence in patients undergoing radiotherapy for lung cancer. The incidence of organizing pneumonia after radiotherapy in patients with breast cancer significantly higher than another one [23]. In contrast, radiation pneumonitis occurs much more commonly after radiotherapy in patients with lung cancer [6]. Although the molecular mechanism for radiation pneumonitis is complex and obscure, involvement of cell adhesion molecules has been implicated [21]. It was experimentally confirmed that in the rats, morphologically, mild interstitial inflammatory cell infiltration was observed at 3rd day and intra-alveolar hyaline material was found at 2nd week after internal and external irradiation [22]. The alveolar inflammation score on the 14th day post-irradiation characterised by a small amounts of collagen which were detected in the intra-alveolar and interstitial areas [24, 31].

Ionizing radiation leads to the exhaustion of the stem cells pool, increases the load on the differentiated cells, resulting in enhanced processes of apoptosis. The immediate response to damaged DNA is the stimulation of DNA repair machinery and activation of cell cycle checkpoints, followed by down-stream cellular responses such as apoptosis [7]. It was observed that 2 Gy irradiation induced apoptosis and cell cycle arrest. Over the past decade, numerous studies have confirmed that multifunctional adaptor proteins have indispensable roles as scaffolds and adaptors in apoptosis–associated signal transduction [9]. In response to DNA damage, wild-type p53 accumulates in the nucleus and arrests cell cycle progression through the cyclin-dependent kinase inhibitor [8]. Using the markers for double-strand breaks, it was observed DNA damage accumulation during fractionated low-dose radiation with increasing cumulative doses [13]. The amount of radiation-induced varied significantly between bronchiolar and alveolar epithelial cells, suggesting that different cell populations in the pulmonary parenchyma had varying vulnerabilities to ionizing radiation [11]. The genetic background of DNA repair determined the extent of cumulative low-dose radiation injury. Moreover, increased DNA damage during external low-dose radiation affected replication, and apoptosis in the pulmonary parenchyma, which can influence to respiratory and metabolic functions of the lung [12].

The p53, a well-known tumor suppressor, becomes activated in response to a myriad of stresses, including DNA damage, ionizing radiation leading to diverse cellular responses, including cell cycle arrest, apoptosis [5]. It has been accepted that wild-type p53 increases the sensitivity to radiation, but for mutants, the results are controversial [10]. Apoptosis is the primary mechanism of radiation-induced cell death has emerged recently as an important mechanism of tumor cell death induced by radiation. Some investigations have demonstrated that the coregulation of both apoptosis can participate in mammalian cell death and apoptosis. Under some circumstances, apoptosis and radiation seem to be interconnected positively or negatively, and there might be a molecular switch between them. Undoubtedly, there are multiple connections between apoptotic process and lipid peroxidation that can jointly seal the fate of tumor cells [38]. In this study, we manage to elucidate the roles of p53 in the regulation of the radiosensitivity, if p53 would lead to different outcomes in the radiosensitivity or not, the results might contribute to the understanding of a potential regulatory mechanism of radiation-induced cell death and provide individual treatment aiming at p53 status and provide specific radiosensitizers for improving the efficacy of internal radiation [37]. The p53 has been shown to modulate generation of lipoperoxidation; therefore, it was measured reactive oxygen species levels in lung cancer cells. As a key tumor suppressor protein, p53 and its associated activities are tightly controlled by its interactions with other proteins, its subcellular localization and its post-translational modifications [14]. It is well known that p53 pathway function as central mediator of the cellular DNA damage response incurred by irradiation or chemotherapy drugs through regulation of DNA damage repair, cell cycle arrest, apoptosis and senescence. In recent years, miRNAs has

been demonstrated to target p53, leading to decreased sensitivity to ionizing radiation and chemotherapy drugs through rescuing the stress-induced cell cycle arrest and apoptosis [20].

The p53 transcription factor is frequently counterselected during tumor development due to its ability to trigger a multitude of tumor-suppressive effects in response to a wide variety of cellular stress signals, including DNA damage and oncogene activation [34]. The p53 mutations are present in lung adenocarcinomas and correlate with reduced survival. Most are missense mutations in the p53 DNA-binding region that can be classified as either contact or conformational mutations [25]. Moreover, p53 mutation inactivates the tumor suppressor gene, enabling the invasion, metastasis, proliferation, and cell survival of malignant cells [29]. Immune histochemical analysis for p53 showed clinical-morphological significance, further investigation is needed to verify its prognostic role in pulmonary neoplastic processes [28, 36].

It is generally known that cell death due to radiation occurs to apoptosis. It should be noted that apoptotic cells are eliminated by the adjacent epitheliocytes, endotheliocytes, fibroblasts, macrophages [6]. Apoptosis ensures the removal of dying cells by phagocytosis without inflammation [17]. Cell apoptosis is an important factor affecting progression of malignant tumors depends on the inhibition of cell death processes, and unlimited malignant hyperplasia of tumor cells. Therefore, interventions that may cause tumor cell apoptosis represent potential tumor treatment strategies. The most fully the apoptosis role was investigated at tumor growth. Intensification of apoptosis has implications for tumor regression [18]. If the cell is not able to produce apoptosis due the mutation it can start reproducing uncontrollably, resulting to tumors. The most authors believe that cell death resulting from Mn toxicity is not a classical apoptosis, and its combination with cessation of ATP synthesis due to mitochondrial damage [33].

Presently, association of apoptosis and many pathological conditions is no longer in doubt, therefore the detection of specific mechanisms of disturbance of apoptosis regulation with specific diseases will allow to determine the etiopathogenesis of these diseases [24]. And consequently it is possibility of correcting the disorder of regulation of programmed cell death [38]. The definition of apoptotic cell death has been used for a long time to verify the neoplastic processes of various localizations as well as in the diagnosis of predictor diseases. It is possible to use the obtained data to compose the nearest and remote predictions of the course of the precancerous process [22].

Conclusion

Immune histochemical determination of the p53 marker in the pulmonary tissue of irradiated rats showed a moderate level of diagnostic value regarding the possible development of neoplastic transformation. When comparing the quantitative indices of the protein content of the p53 regulator indicating the process of programmed death in the animal lung tissue, the highest values were noted in the late periods after ^{56}Mn exposure. Apoptosis is an indication of DNA strand breakage and most likely correlates to the continued cell injury observed beyond 60th day. In this study, apoptosis in sections increased steadily up to 14 days. The increased incidence of apoptosis from background levels was first observed at late period after β- and γ-irradiation. Internal radiation showed upregulation of p53 accumulation. In conclusion, ^{56}Mn has shown radiation-induced apoptosis in the rat lung increases in p53 accumulation, which is the region most sensitive to DNA damage. The determination of the p53 expression level is quite informative in predicting the course of the pathology after exposure to internal radiation. Collectively, our results suggest that low, yet damaging, doses of internal radiation increases the risk of ^{56}Mn toxicity to normal pulmonary tissue and the probability of developing predisposition to the neoplastic processes.

Interest conflict. All authors declare no conflict of interest.

Authors contributions:

Uzbekov D. – the practical implementation of all phases of the experiment;
Shichijo K. – acquisition of data;
Shabdarbaeva D. – immune histochemical analysis;
Sayakenov N. – interpretation of data;
Chaizhunusova N. – administrative, technical and material support;
Zhakipova A. – the practical implementation of histological staining;
Uzbekova S. – statistical analysis;
Saporov R. – the practical implementation of rats necropsy;
Ruslanova B. – preparation of paraffin blocks;
Apbasova M. – collection of literature review.

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