Received: 10 August 2018 / Accepted: 29 September 2018 / Published online: 28 February 2019

UDC: 616.341 + 615.849.12 + 611.018.825

RADIATION-INDUCED KI-67 PROLIFERATION IN THE SMALL INTESTINE OF RATS

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Abstract

Introduction. It is known that Hiroshima and Nagasaki were target of the first atomic bombs in the history of mankind. As a result, the population of Japanese cities was exposed to internal and external irradiation. Numerous authors maintain that primary neutron-activated radionuclides are chemical elements ^{56}Mn and ^{60}Co. Herewith, ^{56}Mn was determined the dominant role of neutrons induced by β-radiation during the first few hours after the atomic bombing. The systemic access in estimation of Ki-67 marker in radiosensitive small intestine defining the functional state and the interrelation of structural components serves as a combination of diagnostic criterion and prognosis.

The objective of study. To assess the prognostic significance of Ki-67 antigen for detection of cell proliferative activity in the small intestine of rats exposed to low dose of β− and γ–radiation.

Materials and methods. In experiment that held in February 2016, the male sex «Wistar» rats at 5 month old and in amount of 90, weighting approximately 270−350 g. Three groups were identified: 1) ^{56}Mn which obtained by neutron activation of 100 mg MnO_2 powder using the «Baikal−1» nuclear reactor with a neutrons fluence of 4×10^{14} n/cm²; 2) ^{60}Co γ−rays; 3) control group. Necropsy of the animals were on the 3rd, 14th and 60th days after irradiation, then the small intestine removed, after which it was fixed in 10% formalin. Paraffin sections were dewaxed and rehydrated using the method of D.Sarkisov and Yu.Perov (1996). To visualize the immunohistochemical reaction, the DAB+(DAKO) system was used. The number of Ki-67-positive cells, taking into account the colored nuclei of any intensity, expressing the results in percent. All the presented data and results were expressed as mean (M), median (Me) and interquartile interval (IQR). Statistical comparisons were made by Kruskel-Wallis test (SPSS 2,0). A $p<0,05$ was considered statistically significant.

Results. When comparing microscopic processes occurring in the rats small intestinal tissue after exposure to neutron-activated manganese dioxide and external irradiation, the most pronounced morphofunctional disorders which detected by the number of Ki-67-positive cells are noted in the later periods after ^{56}Mn effect. The studied parameters of the small intestine have statistically significant differences in the control and irradiated animal groups ($p<0,001$).

Conclusion. Morphofunctional disorders in the small intestine that result from internal and external irradiation are characterized by a change in the immunohistochemical indicator of Ki-67, indicative of cell proliferation in late terms. Comparing the level of Ki-67 antigen in the organ of various animal groups studied, a high level was observed in the late periods after exposure to neutron-activated manganese dioxide.

Key words: *^{56}Mn, small intestine, immunohistochemistry, Ki-67, rats.*

Резюме

РАДИАЦИОННО-ИНДУЦИРОВАННАЯ ПРОЛИФЕРАЦИЯ KI-67 В ТОНКОЙ КИШКЕ КРЫС

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Введение. В истории человечества мишенью первых атомных бомб, как известно, явились города Хиросима и Нагасаки. В результате население японских городов подверглось как внутреннему, так и внешнему излучения. Согласно мнению ряда авторов, основным нейтронно-активированным радионуклидом выступают химические элементы ^{56}Mn и ^{60}Co. Причем, ^{56}Mn определил доминирующую роль нейтронов, вызванных β-излучением в течение первых нескольких часов после атомной бомбардировки. Системный подход в оценке маркера Ki-67 в радиочувствительной тонкой кишке, определяющий функциональное состояние и взаимосвязанность структурных образований между собой служит по совокупности диагностическим критерием и прогнозом.

Цель исследования. Оценить прогностическую значимость антигена Ki-67 для выявления пролиферативной активности клеток в тонкой кишке крыс, подвергавшихся воздействию малых доз β– и γ–излучения.

Материалы и методы. В эксперименте, проводимом в феврале 2016 года, были использованы белые крысы-самцы линии «Wistar» в возрасте 5-ти месяцев в количестве 90, массой 270–350 гр. Были выделены 3 группы: 1) ^{56}Mn, полученный путём нейтронной активации 100 мг порошка MnO_2 на ядерном реакторе «Байкал–1» при флюенсе нейтронов 4×10^{14} н/см²; 2) ^{60}Co γ–излучение; 3) контрольная группа. Животных подвергали некропсии через 3, 14 и 60 дней после облучения, затем извлекали тонкую кишку, после чего фиксировали ее в 10% формалине. Парафиновые срезы депарафинировали и регидратировали по методике Д.С. Саркисова и Ю.Л. Перова (1996 г.) Визуализацию иммуногистохимической реакции проводили используя систему DAB+(DAKO). Количество Ki-67-позитивных клеток подсчитывали учитывая окрашенные ядра любой степени интенсивности, выражая полученные результаты в процентах. Представленные данные и результаты были описаны при помощи средней (M), медианы (Me) и межквартильного интервала (IQR). Статистические сопоставления проводились по критерию Краскела-Уоллиса (SPSS 2,0). $p<0,05$ считалось статистически значимым.

Результаты. При сравнении микроскопических процессов, возникающих в тканях тонкой кишки крыс после воздействия нейтронно-активированного диоксида марганца и внешнего облучения, наиболее выраженные морфофункциональные расстройства, выявляемые по количеству Ki-67-позитивных клеток отмечаются в поздние сроки после воздействия ^{56}Mn. Изучаемые параметры тонкой кишки имеют статистически значимые различия в группах контрольных и облученных животных ($p<0,001$).

Выводы. Морфофункциональные нарушения в тонкой кишке, возникающие в результате внутреннего и внешнего облучения, характеризуются изменением иммуногистохимического показателя Ki-67, свидетельствующего о клеточной пролиферации в поздние сроки. Высокий уровень антигена Ki-67 в тканях исследуемого органа в поздние сроки у экспериментальных животных в сравнительном аспекте отмечен после воздействия нейтронно-активированного диоксида марганца.

Ключевые слова: *^{56}Mn, тонкая кишка, иммуногистохимия, Ki-67, крысы.*

Түйіндеме

РАДИАЦИЯ ӘСЕРІНЕН ТУЫНДАҒАН ЕГЕУҚҰЙРЫҚТАР ЖІҢІШКЕ ІШЕГІНДЕГІ КІ-67 ПРОЛИФЕРАЦИЯСЫ

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Кіріспе. Адамзат тарихында Хиросима мен Нагасаки қалаларының алғашқы атом бомбаларының нысанасы болғаны баршамызға мәлім. Жапон қалаларындағы атом бомбалауын басынан өткірген тұрғындар ішкі мен сыртқы сәулелеу әсеріне ұшыраған болатын. Ғалымдардың пікірінше, негізгі нейтронды-белсендірілген радионуклидтердің бірі – ^{56}Mn пен ^{60}Co химиялық элементтері болып саналады. ^{56}Mn, негізінен атом бомбалауынан кейін алғашқы бірнеше сағат ішіндегі β-сәулелеумен тудырылған нейтрондардың доминантты рөлін құрағаны туралы дәлелдемелерді бірқатар ғалымдардың еңбектерінде байқауға болады. Радиосезімтал жіңішке ішектің құрылымдық пен функциялық жағдайлардың бір-бірімен өзара байланысын анықтайтын Кі-67 маркерін зерттеудің клиника жүзінде диагностикалық мәні мен болжамын іске асырудағы маңызы зор.

Зерттеу мақсаты. Шағын дозалы β– мен γ–сәулелеу әсеріне ұшыраған егеуқұйрықтардың жіңішке ішегінде жүзеге асатын жасушалардың пролиферациялық белсенділігін Кі-67 антигенінің деңгейін анықтау арқылы болжамдық маңыздылығын бағалау.

Материалдар мен әдістер. 2016 жылдың ақпан айында өткізілген тәжірибе жүзінде жасы 5 ай толған, 270-350 грамм салмағы бар 90 аталық жынысты ақ түсті «Wistar» тұқымдас егеуқұйрықтар қолданылған. Жануарлар үш топқа бөлінген: 1) ^{56}Mn, яғни 100 мг MnO_2 ұнтағын «Байкал–1» ядролық реакторы арқылы 4×10^{14} н/см² нейтрон флюенсінде нейтрондық белсендіру жүзінде алынған элемент; 2) ^{60}Co γ–сәулелер; 3) бақылау тобы. Жануарлардың некропсиясы сәулелеуден кейін 3-ші, 14-ші және 60-шы тәуліктерде орындалып, жіңішке ішегі алынғаннан соң 10% формалинде фиксацияланған. Парафиндік кесілімдер Д.С.Саркисов пен Ю.Л.Перовтың (1996 ж.) әдісі арқылы депарафинизацияланып, регидратацияланған. Иммунды гистохимиялық серпілістерді визуализациялау мақсатында DAB+(DAKO) жүйесі қолданылған. Пролиферациялық белсенділікті бағалауға арналған Кі-67-позитивті жасушалар саны анықталып, алынған нәтижелер пайыз мөлшері түрінде көрсетілген. Ұсынылған мәліметтер мен нәтижелер орта (M), медиана (Me) және квартиль аралық интервал (IQR) көмегімен аңғарылған. Статистика жүзіндегі салыстырмалы сипаттама Краскел-Уоллис нышаны арқылы жүргізілген (SPSS 2,0). $p<0,05$ статистика жүзінде мәнді деп бағаланған.

Нәтижелер. Нейтронды-белсендірілген марганец диоксиді мен сыртқы иондаушы сәулелеуден кейін егеуқұйрықтардың жіңішке ішек тініндегі микроскопиялық үдерістерді салыстырмалы түрде бағалау барысында, Кі-67-позитивті жасушалар саны бойынша анағұрлым айқын морфофункционалды бұзылымдардың ^{56}Mn әсерінен кейін жүзеге асатыны дәлелденген. Жіңішке ішектің зерттелген параметрлері бақылау мен сәулеленген жануарлар топтары арасында статистика жүзінде мәнді екені аңғарылған ($p<0,001$).

Қорытынды. Ішкі мен сыртқы иондаушы сәулелеуден кейінгі егеуқұйрықтардың жіңішке ішегіндегі морфофункционалды бұзылымдар кешеуілдеу мерзімі аясындағы қалыптасқан жасушалық пролиферацияны айқындайтын Кі-67 иммунды гистохимиялық көрсеткішінің өзгеруімен жүзеге асады. Тәжірибелік топтар арасындағы зерттеуге алынған ағза тінінде салыстырылған бұл көрсеткіштің нәтижелеріне сай, Кі-67 антигенінің сандық мөлшері нейтронды-белсендірілген марганец диоксиді әсеріне ұшыраған егеуқұйрықтарда нақты түрде жоғарылауымен сипатталады.

Негізгі сөздер: *^{56}Mn, жіңішке ішек, иммунды гистохимия, Кі-67, егеуқұйрықтар.*

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Introduction

It is well known that people who returned early to Hiroshima and Nagasaki after atomic bombing were reported to suffer from the symptoms of acute radiation effects [22]. Consequently, atomic bomb effects on the health of survivors have been correlated with delayed ^{56}Mn and ^{60}Co [25]. The accidental high-dose radiation exposure induces a series of injury levels in multiple organs of digestive system [26, 27]. Thereby, increasing attention has been given to the radiation effect on the gastrointestinal tract due to concerns about exposure to radiation after an accident [4, 9]. The small intestine is particularly sensitive to β– and γ– rays, rendering it vulnerable to the effects of collateral radiation from the radiotherapeutic treatment of intestinal cancer. Histologically, overexposure to ionizing radiation may result in the shortening of villi, disruption to the mucosal architecture, or even apoptosis and necrosis of the intestinal crypts [34].

The Ki-67 protein expression is associated with the proliferative activity of intrinsic cell populations in malignant tumors, allowing it to be used as a marker of tumor aggressiveness. The prognostic value of Ki-67 has been investigated in a number of studies with its potential as a reliable marker having been shown in cancer of the gastrointestinal tract [7]. Morphological changes of the intestinal mucosa after ionizing radiation have been well documented, but molecular events that regulate radiosensitivity of intestinal epithelial cells and radiation-induced intestinal injuries are not fully understood [30]. Little is known about the molecular mechanisms underlying intestinal damage and regeneration after exposure to ionizing radiation [32]. Presently, particular interest is a peculiarity of morphofunctional changes in the small intestine of persons exposed to ^{56}Mn and ^{60}Co, allowing in the future to work out the diagnostic criteria for assessing of radiation effect to the gastrointestinal tract [31].

The objective of study

To assess the prognostic significance of Ki-67 antigen for detection of cell proliferative activity in the small intestine of Wistar rats exposed to internal and external irradiation.

Materials and methods

A total of 90 white male Wistar rats, at 5 month old (a week before weaning) and weighed 270 to 350 g. The animals were reared in a specific pathogen free animal laboratory (Karaganda State Medical University, Kazakhstan) with controlled temperature (20 to 22 °C), humidity (40% to 60%), and photoperiod (8 h:8 h light–dark cycle). Healthy male rats were divided into 3 groups with 30 rats per group: 2 irradiated groups and one control group.

The first group of rats (n=30) were irradiated with low-dose (0,2 Gy) of neutron-activated manganese dioxide (^{56}Mn) by nuclear reactor located at «Baikal–1» (Kurchatov, Kazakhstan). Radioactive powder ^{56}Mn was obtained by neutron activation of 100 mg of MnO_2 (Rare Metallic Co., Ltd., Japan) powder 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 laboratory rats which were 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. General duration of rats exposition to radioactive powder was 4,0 hour [1].

The second group of rats (n=30) were irradiated by ^{60}Co γ−ray. The exposure time was 2,6 Gy/min using by czech radiotherapeutic device «Teragam K-2 unit», and the total cumulative dose of the electron beam irradiation was 2 Gy. During the exposure rats were placed in a specially engineered cage made of organic glass with individual compartments for each animal. After irradiation, the rats were all fed normally.

The third group consisted of control rats (n=30) which were placed on shelves in the same facility and shielded from the radiation. All applicable international, national, and institutional guidelines for the care and use of animals were followed by Ethical Committee of Semey State Medical University, Kazakhstan (Protocol №5 dated 16.04.2014).

Both irradiated and control rats were briefly anaesthetized by an intraperitoneal injection of 10% ketamine at 0,5 mg/kg. The animals were killed during necropsy after anesthesia on the 3rd, 14th and 60th day after irradiation and the small intestine was immediately surgically extracted for further immunohistochemical study. In order to analyze the radiation effect, we have chose the immunohistochemical method. After fixation, the fragments were paraffin embedded and sections with 4 μm thickness. Paraffin sections were deparaffinized and rehydrated in graded 10% formalin solutions.

Immunohistochemical staining of Ki-67, a widely-used proliferation marker, was performed on the primary lesion as described previously. Antigen retrieval was performed by heat treatment for 15 min. Antibodies against Ki-67 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were added and incubated overnight at 4°C. The Dako Real Envision Detection System and AEC peroxidase substrate (Dako, Glostrup, Denmark) were used to detect the primary antibody according to the manufacturer's instructions. To evaluate nonspecific binding, the primary antibody was substituted with PBS. Specially stained tissue sections and examined with Leica DM 1000 microscope (Germany) and

images were captured with a charge-coupled device camera. The Ki-67 labelling index (%) was calculated by dividing the total Ki-67–positive cells by the total numbers of cells multiplied by 40 and 100.

The data were presented as mean (M), median (Me) and interquartile interval (IQR) and analyzed by independent-samples the Kruskel-Wallis test between the experimental and control groups by significant difference using SPSS software (SPSS, V. 2,0). Differences were considered statistically significant at $p<0.05$ [19].

Results. In this study, we have performed experiment with Wistar rats exposed to internal (^{56}Mn) and external (^{60}Co) radiation. Tissue-proliferative response was evaluated by determining the number of Ki-67 cells. Although the radioactivity level received from ^{56}Mn was rather low, the observed biological effects were consistent in our experiment. That was previously reported the highest internal dose estimates in the small intestine of rats exposed to radioactive powder [26, 27]. To assess the health of rats after radiation, we have evaluated activity, posture, dehydration and pelage of the animals. Light microscopic study of the intestinal mucosa of control rats showed a normal architecture of villi, crypts and enterocytes. Intestinal tissues were collected on the 3rd, 14th and 60th day post-radiation, time associated with complete crypt ablation in small intestine after radiation exposure.

During the study of slide glasses of the rats small intestine on the 3rd day after ^{56}Mn effect we have observed presence the severe morphofunctional changes of glands, mild accumulations of proliferative cells. At rats exposed to ^{60}Co was found focal accumulations in the glandular lumen the cellular elements, preferably desquamated epithelial cells and reactive nature cell in compared with control rats. Scrutiny of animal intestines on the 3rd day after exposure to internal radiation reveals numerous enlarged hyperplastic crypts and small «cryptless» regions, features typical of actively regenerating intestinal epithelium. We quantified the crypt phenotype by counting regenerating crypts that we defined as containing adjacent Ki-67-positive cells contained within a crypt-like structure. Given that a little number of crypts apparently survive exposure to radiation and these remaining crypts would regenerate the intestinal epithelium.

Also, the animals' intestine was analyzed on the 14th day after exposure to external radiation we have observed complete regeneration. It was indistinguishable from unirradiated intestine. In contrast, animals exposed to internal radiation exhibit a dramatic deterioration of crypt-villus architecture on the 14th and 60th days after exposure. In addition to flattening of the epithelium and ectopic proliferation in «intercrypt» regions, the remaining crypts have an aberrant appearance, being grossly enlarged and multilayered. Normally, crypt expansion is followed by a fission event, whereby the crypt bifurcates to generate an supplemental crypt. In this case, probably, surviving crypts unable to share at the appropriate time and leads to an accumulation of cells in apparently hyperplastic, crypt-like structures.

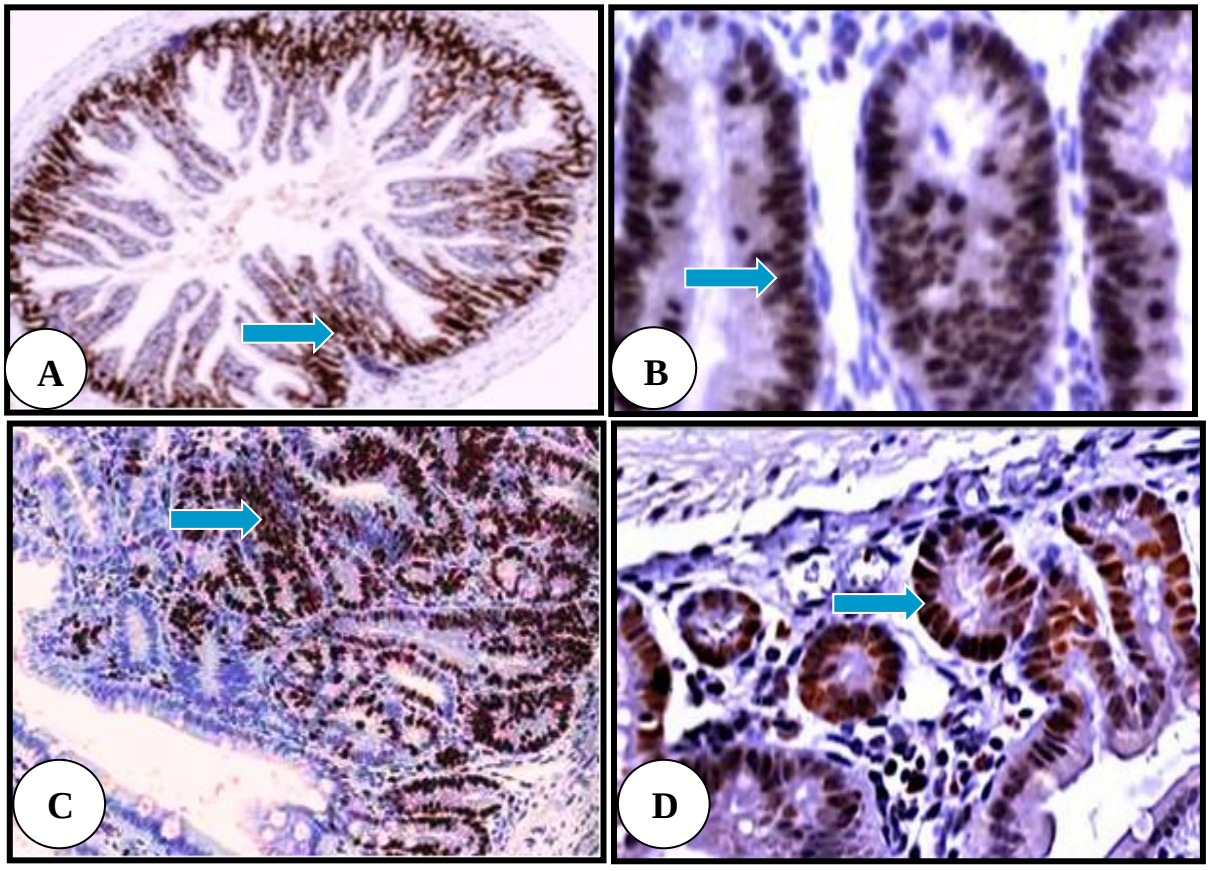

Fig. 1. Photomicrograph of proliferation marker Ki-67 in immunohistochemical staining of a section of the small intestine in ^{56}Mn– and ^{60}Co–exposed rats on the 60th day. Arrows show Ki-67 cells at the bottom of a crypt. Original magnification, ×10, ×40 and ×100

Microscopic picture of animals on the 60th day after ^{56}Mn exposure provided on figure 1–A, B. According to the morphological data we drew attention to the presence of more expressed proliferative activity of the cells and degenerative signs. Histological studies of ^{60}Co–exposed rats revealed radiation–induced prominent proliferation (Fig. 1–C, D).

The immunohistochemical indicators of the small intestine at different days after irradiation are presented in Table 1. As we can see it is evident that the numbers of proliferative cells in the villus of the intestine became statistically insignificant on the 14th day between the groups. The trend in the quantity of proliferative cells on the 14th day coincide with the indicators which specially for the 3rd day. Also, the greatest differences were noted after exposure to ^{56}Mn. Several Ki-67-positive cells remained in control rats where crypts had been located, but few Ki-67-positive cells remained in the intestinal mucosa. As can be seen, the number of proliferative cells in the mucosa is not statistically different when exposed to ionizing radiation. In compared with the control group the percentage of these cells in the intestinal villus were highest after ^{56}Mn irradiation. Moreover, this indicator more than in ^{60}Co-rats.

Table 1.

The number of Ki-67-positive cells (%) in the small intestine of rats.

^{56}Mn			^{60}Co			Control			Kruskal-Wallis test	p value
M	Me	IQR	M	Me	IQR	M	Me	IQR		
The 3rd day after exposure										
1,72	1,75	0,52	1,70	1,66	0,6	1,68	1,86	0,54	H=3,600	0,308
The 14th day after exposure										
2,02	1,94	0,21	1,72	1,66	0,34	1,98	2,03	0,21	H=24,200	<0,001
The 60th day after exposure										
4,24	4,09	1,22	3,88	4,08	0,86	2,08	1,98	0,26	H=39,026	<0,001

According to the table a number Ki-67-positive cells of in absolute values is lower than on the 3rd day, but the trend persists: the highest number after exposure to ^{56}Mn and ^{60}Co was 1,72% and 1,70%, respectively (p=0,308). The trend of the number of proliferative cells persists on the 14th day after irradiation. However, in comparison with the 3rd and 14th day, the amount increases after all irradiation methods and exceeds the control group values when exposed to ^{56}Mn by 2,02% and after ^{60}Co by 1,72% (p<0,001). Statistical analysis of immunohistochemical indicator based on the staining the quantity of Ki-67-positive cells gradually went up and reached a peak on the 60th day in comparison with acute and subacutes radiation effects. After exposure to γ-radiation its excess of the control group remains 2,08%, after β-radiation was 4,24% (p<0,001). As well as on the 3rd and 14th days the greatest deviations in comparison with the control group were observed after exposure to ^{56}Mn and ^{60}Co.

The line graph below illustrates that internal (^{56}Mn) radiation effect contributes moderate severe morphofunctional changes of the mucosa and submucosa on the 60th day. The sections are represented mainly by edematous stroma. In contrast to β– and γ–radiation in rats intestine after 2 month leads to the appearance narrowing and swollen glands, epithelial desquamation of some glands. Regarding experimental animals exposed to γ–radiation it should be noted the presence of marked morphofunctional changes of surface mucous layer (Figure 2).

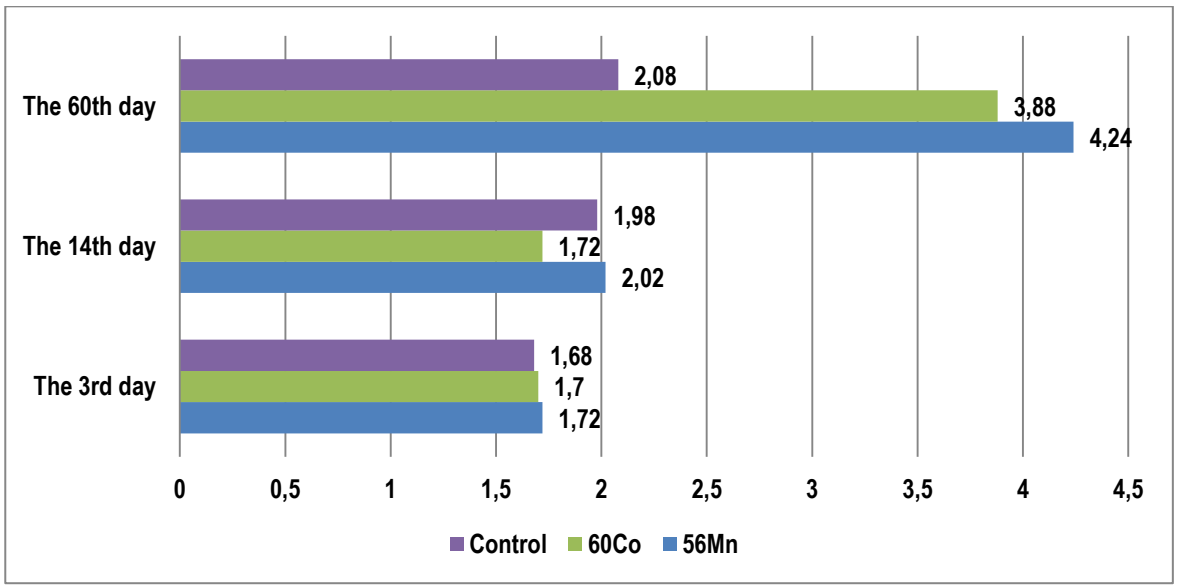

Fig. 2. The dynamics of immunohistochemical changes regarding the Ki-67–positive cells (%) in the small intestine of irradiated and control rats at different days.

The above data are consistent with our study results the small intestine in both ^{56}Mn– and ^{60}Co–exposed rats showed a little similar changes. Nevertheless, according to immunohistochemical examination the most pronounced changes were observed in the small intestine of rats from ^{56}Mn group, indicating that internal radiation has a significant biologic effect on examined organ.

The conducted research confirms the assumption that the controlled effect factor has a high degree of influence on all the resulting signs of immunohistochemistry. This suggests that a single exposure to small dose ^{56}Mn and ^{60}Co has a direct damaging effect to the small intestine of Wistar rats at a later date. A damaging mechanism acting of the small intestinal tissue can be the hyperactivation of lipoperoxidation under the influence of neutron-activated manganese dioxide capable of damaging the small intestine [32]. Another controlled exposure factor is external irradiation of rats which had the least pronounced effect on the parameters of the small intestine in comparison with internal irradiation.

The most prominent histologic picture characterized by presence the signs of morphofunctional changes on the 14th day, in particular, in rats exposed to ^{56}Mn compared to rats from ^{60}Co groups. The most pronounced neoplastic processes are observed in the late periods after irradiation of ^{56}Mn then studying histostructural processes occuring in the tissues of the studied animal organs after exposure to neutron-activated manganese dioxide and external irradiation. Morphofunctional disorders in the small intestinal tissues that result from irradiation are characterized by a change in the immunohistochemical indicator of Ki-67. The latter indicative of cell proliferation in late terms. Comparing the level of Ki-67 antigen in the small intestine of various animal groups studied. A high level was observed in the late periods after exposure to neutron-activated manganese dioxide.

Discussion

The Ki-67 expression is strongly associated with tumor cell proliferation and growth, and is widely used in routine pathological investigation as a proliferation marker. The nuclear protein Ki-67 is an established prognostic and predictive indicator for the assessment of biopsies from patients with intestinal cancer. Clinically, Ki-67 has been shown to correlate with metastasis and the clinical stage of tumors [10]. In addition, it has been shown that Ki-67 expression is significantly higher malignant tissues with poorly differentiated tumor cells, as compared with normal tissue. The Ki-67 labeling index is an independent prognostic factor for survival rate, which includes all stages and grade categories. There is a correlation between the ratio of Ki-67-positive malignant cells and patient survival. It has been shown that Ki-67 immunohistochemical staining is an effective method of assessing the prognosis in a number of tumor types [17]. Although Ki-67 is a key marker associated with proliferating cancer cells and a poor prognosis, its full potential in increasing proliferation has not been evaluated. In animal models with subcutaneous or orthotopic intestinal cancer, antisense oligonucleotides induced tumor growth inhibition [37, 38], potentially through the inhibition of Ki-67, indicating the involvement of Ki-67 in tumor cell proliferation [18].

In this study, we have shown the sequence of morphofunctional changes in the rat small intestine from early to late stage after a single influence of ^{56}Mn and ^{60}Co at small dose, which were the initiators of radiation–induced small intestinal injury. After the Ki-67–stained slide has been scanned at ×10 and ×40 magnification, a trained clinical laboratory scientist, who is blinded to the histological diagnoses and patient survival data, randomly selects at least twenty fields representative of the range of Ki-67 immunostaining in the previously encircled tumor for evaluation with an automated bright-field microscope at ×40. Proliferating cells of the small intestine were identified by immunohistochemical staining of Ki-67. The most Ki-67-positive cells were detected in the jejunal crypts. The number of Ki-67-positive cells are rosen in irradiated rats, corresponding to the increase in surviving crypts after internal irradiation. A greater number of Ki-67-positive cells was observed in the first and second groups compared with the control group. Findings of immunohistochemical studies have demonstrated that morphofunctional changes in the small intestine observed in irradiated rats little differed from the previously published results using different radiation models.

Morphological damages of radiation-induced enteropathy were known as architectural changes of intestinal mucosa such as villus shortening by cell death [26, 35]. The acute microscopic changes of intestine by irradiation were consisted of structural changes in the villus-crypt architecture and epithelial transformations [33, 36]. It is common known that small intestinal epithelium undergoes continual self-renewal; cells born in the proliferative crypt zone migrate upward, differentiate [11, 20]. Exceptions to this upward migration include the Paneth cells, which migrate down into the base of the crypt where they are maintained for up to 2 months and the resident stem cell population, which is critical for the maintenance of epithelial turnover [14].

The acute morphological changes of the small intestine by irradiation were consisted of structural changes in the villus-crypt architecture and epithelial transformations associated with radiation-induced degeneration [16, 23]. The most authors maintain that cell death resulting from Mn toxicity is combination with cessation of ATP synthesis due to mitochondrial damage [21, 24]. Dysfunction or death of intestinal epithelial cells caused by degeneration after radiation influence is considered as dangerous component in the pathogenesis of gastrointestinal syndrome [7]. The initiation and progression of radiation-induced intestine injury can be caused by disorder of metabolic processes and molecular mechanisms which form an compounded response [2, 3, 5].

The Ki-67 protein expression coincides with the transit of cells through mitosis and undergoes phosphorylation and dephosphorylation during mitosis in vivo, rendering it susceptible to protease degradation [8]. The characterization of the Ki-67 promoter region is essential for understanding gene transcription, and it is therefore important to investigate this in order to develop targeted interventions aimed at modulating gene expression [6, 10]. It was found that expression of p53 is correlated with that of Ki-67 in several types of cancer, including intestinal cancer. The p53 inhibits Ki-67 promoter activity via p53-dependent

pathways. It is hypothesized that there are at least two transcriptional regulatory mechanisms. One is that the p53-binding motifs affect the transcriptional repression of the Ki-67 promoter [15].

Multiple clinical laboratories have reported the successful use of the Ki-67 as a diagnostic tool. The Ki-67 expression, as evaluated by immunostaining has become the gold standard, with a cutoff level of positively-stained cells defined as high risk in terms of prognosis [12]. Nowadays, there are concerns with the scoring reliability of tissue microarrays due to tumor heterogeneity. Numerous pathologists have expressed the view that using a manual counting procedure will obtain a more reliable score that may lead to differences in interpretation between examiners with consequent variability in diagnoses [10]. A number of diagnostic applications for the Ki-67 protein have been described where latter was significantly more highly expressed in malignant than in normal tissues. The Ki-67 also tended to increase with decreasing tissue differentiation, and it was correlated with the presence of occult metastasis and the clinical stage of tumors [28]. Proliferative activity in tumors can be determined by mitotic counting, flow-cytometric determination of synthesis-phase fraction and immunohistochemistry using antibodies reactive against various proliferating cellular antigens [13, 17]. Its presence in a variety of tumors indicates that it may be possible to use the Ki-67 in routine grading of cancer. Notably, the expression of the Ki-67 reflects the tumor proliferation rate and correlates with initiation, progression, metastasis and prognosis of a number of tumor types [28].

It is known that Ki-67 is expressed in all cell-cycle phases outside of the resting phase G0. Numerous studies have similarly confirmed the utility of the Ki-67 proliferation index, because it shows a correlation with primary tumor size, lymphatic invasion, metastases, tumor proliferation activity. Intense immunohistochemical staining for Ki-67 is correlated with a poor prognosis in various malignancies [29].

Based on the data presented here, we hypothesize that proliferation marker to measure the growth fraction of cells in tumors the Ki-67 expression is strongly associated with cell proliferation and it is widely used in routine pathology [8]. The Ki-67 is well characterized at the molecular level and extensively used as a prognostic and predictive marker in cancer. The Ki-67 may be a promising molecular candidate for the diagnosis and treatment of a wide range of malignancies [15]. Some reported association of rosen Ki-67 expression with poor prognosis, others reported a good prognosis associated with Ki-67 expression. Although its prognostic value remains controversial [10].

Conclusion

According to immunohistochemical study, the most pronounced changes are detected in the small intestine of rats irradiated by ^{56}Mn, which indicates a significant biological effect of internal radiation to the studied organ. Crypt stem cells are highly sensitive to neutron-activated manganese dioxide, which causes a deficient supply of intestinal epithelial cells, villus denudation, crypt atrophy or disappearance and mucosal architecture disruption. The radiosensitivity of the small intestinal cells are directly proportional to its mitotic activity and inversely proportional to the degree of its differentiation. One of the types of cell death due to radiation can be mitotic death. The latter characterized by impossiblity for chromosomes to diverge into anaphase because of changes in DNA structure. In summary, our data obtained from in vivo experiments provide strong evidence that β–radiation causes formation of immunomorphological features. It can be typically for radiation-induced cell proliferation, namely the small intestinal pathology depending on radiation type.

Interest conflict

The authors report no conflict of interests.

Authors contributions:

Uzbekov D. – the practical implementation of rats necropsy and interpretation of data;

Shabdarbaeva D. – contributed to data analysis and manuscript preparation;

Chaizhunusova N. – administrative, technical and material support;

Sayakenov N. – histological analysis and interpretation of data;

Uzbekova S., Shalgumbayeva G.M.– statistical analysis;

Amantaeva G., Abeuova A. – collection of literature review;

Ruslanova B. – the practical implementation of histological staining, acquisition of data;

Aubakirova G. – the practical implementation of rats necropsy; – collection of literature review;

Hoshi M. – conceived and designed the experiments.

The study was conducted according to the scientific project: «Long-term effects of internal exposure at different levels of the body: a multicenter experimental study using a nuclear reactor».

Funding for the project was carried out by Semey State Medical University.

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