Recived: 5 May 2018 / Accepted: 10 June 2018 / Published online: 30 June 2018

UDC 616.341–614.876–616–092.4+576.344

MORPHOMETRIC INDICATORS OF THE SMALL INTESTINE OF IRRADIATED RATS

Darkhan E. Uzbekov [1], http://orcid.org/0000–0003–4399–460X
Dariya M. Shabdarbaeva [1], http://orcid.org/0000–0001–9463–1935
Nailya Zh. Chaizhunusova [2], http://orcid.org/0000–0002–6660–7118
Kairat A. Almissaev [1], http://orcid.org/0000–0001–6650–4971
Saltanat E. Uzbekova [3], http://orcid.org/0000–0001–9006–120X
Ruslan M. Saporov [1], http://orcid.org/0000–0003–3152–8759
Madina M. Apbasova [4], http://orcid.org/0000–0003–3215–1076
Masaharu Hoshi [5], http://orcid.org/0000–0001–6978–0883

[1] Department of Pathological anatomy and Forensic medicine,
[2] Department of Nutrition and Hygienic disciplines,
[3] Department of Anatomy and Histology,
[4] Department of Anesthesiology and Reanimatology,
Semey State Medical University, Semey, The Republic of Kazakhstan;
[5] Research Institute for Radiation Biology and Medicine, Hiroshima, Japan

Abstract

Introduction. It's known that persons exposed to β− and γ–rays, together with a different of damaging effects, particular importance is also attached to the digestive system. The dominant role of neutron−activated radionuclide Manganese−56 (^{56}Mn) was noted in the treatises of scientists who studied the atomic bomb effects of Japanese cities, deserving the interest today.

The research purpose. Investigate and compare the morphometric indicators in the small intestine of rats exposed to low dose by β– and γ–rays.

Materials and methods. In experiment, male sex «Wistar» rats 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» atomic 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. Tissues fragments embedded in paraffin, then sections are manufactured serial transverse 4 μm thickness, which were subsequently stained by hematoxylin and eosin (H&E). For stereoscopic changes, a microphotometric system with Avtandilov's ocular measuring grid was used. In each micropreparation, 20 fields with a total area of 181 points were counted. 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. On the basis of the histological research methods characterizing the structural state of the small intestine of irradiated rats, pronounced histomorphological changes are both reactive and destructive in later terms. Structural rearrangement of the tissue composition of the examined organ which persists several weeks after the termination of the influence of ^{56}Mn, indicates a toxic and sensitizing effect of internal irradiation as compared to ^{60}Co effects. The most pronounced dystrophic, inflammatory and necrotic changes are observed in the late periods after irradiation of ^{56}Mn when studying histostructural processes occurring in the tissues of the studied animal organs after exposure to neutron-activated manganese dioxide and external irradiation identified by the totality of morphometrical indicators. The studied parameters of the small intestine have statistically significant differences in the effect groups of the factors and the control group (p<0,001). It was found that the greatest difference in thickness of the mucosa compared to the control group after exposure to ^{56}Mn by 6,3 mcm, whereas after ^{60}Co effect at 6,25 mcm. The greatest decrease in the enterocytes number of the intestinal villi is observed in the I-group at 17,39%, and in the II-group at 3,99%. After exposure to ^{60}Co, the leukocytes number compared to the control group is 4,76% higher, and after ^{56}Mn at 3,77%. The greatest deviations in the lymphocytes number are observed after γ–, and then β–irradiation.

Conclusion. Thus, ^{56}Mn effect on the small intestine of rats showed a high level of risk β–ray exposure, which is confirmed by the morphometric indicators.

Keywords: *radioactive ^{56}Mn, gastrointestinal syndrome, intestinal cells, morphometry.*

Резюме

МОРФОМЕТРИЧЕСКИЕ ПОКАЗАТЕЛИ ТОНКОЙ КИШКИ ОБЛУЧЕННЫХ КРЫС

Дархан Е. Узбеков [1], http://orcid.org/0000–0003–4399–460X
Дария М. Шабдарбаева [1], http://orcid.org/0000–0001–9463–1935
Найля Ж. Чайжунусова [2], http://orcid.org/0000–0002–6660–7118
Кайрат А. Альмисаев [1], http://orcid.org/0000–0001–6650–4971
Салтанат Е. Узбекова [3], http://orcid.org/0000–0001–9006–120X
Руслан М. Сапоров [1], http://orcid.org/0000–0003–3152–8759
Мадина М. Апбасова [4], http://orcid.org/0000–0003–3215–1076
Масахару Хоши [5], http://orcid.org/0000–0001–6978–0883

[1] Кафедра патологической анатомии и судебной медицины, [2] Кафедра питания и гигиенических дисциплин, [3] Кафедра анатомии и гистологии, [4] Кафедра анестезиологии и реаниматологии, Государственный медицинский университет города Семей, г. Семей, Республика Казахстан;
[5] Научно–исследовательский институт радиационной биологии и медицины, г. Хиросима, Япония

Введение. Известно, что у лиц, подвергавшихся воздействию β– и γ–излучения наряду с различными повреждающими эффектами особое место отводится и пищеварительной системе. Доминирующая роль нейтронно–активированного радионуклида – Марганца–56 (^{56}Mn) отмечалась в трудах ученых, изучавших последствия атомной бомбардировки в японских городах и вызывает огромный интерес по сегодняшний день.

Цель исследования. Изучить и сравнить морфометрические показатели тонкой кишки крыс, подвергавшихся воздействию малых доз β– и γ–излучения.

Материалы и методы. В эксперименте использованы 90 крыс обоих полов линии «Вистар», массой 270–350 гр. Были 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–излучение; 3) контрольная группа. Животных подвергали некропсии через 3, 14 и 60 дней после облучения, затем извлекали тонкий кишечник, после чего фиксировали его в 10% формалине. Фрагменты тканей заливали в парафин, затем изготовливали поперечные серийные срезы толщиной 4 мкм, которые в дальнейшем окрашивали гематоксилином и эозином (H&E). Для стереоскопических изменений использовали микрофотометрическую систему с окулярной измерительной сеткой Автандилова. В каждом микропрепарате просчитывали 20 полей суммарной площадью 181 точка. 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 их сравнении 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. По совокупности гистологических методов исследования, характеризующих структурное состояние тканей тонкой кишки экспериментальных животных, выраженные гистоморфологические изменения в поздние сроки носят как реактивный, так и деструктивный характер. Структурная перестройка тканевого состава исследованного органа крыс, развивающаяся в ранние и поздние сроки после прекращения действия ^{56}Mn, указывает на токсическое и сенсибилизирующее действие внутреннего облучения по сравнению с эффектами ^{60}Co. При сравнении гистоструктурных процессов, возникающих в тканях тонкой кишки крыс после воздействия нейтронно-активированного диоксида марганца и внешнего облучения, наиболее выраженные дистрофические, воспалительные и некротические изменения отмечаются в поздние сроки после облучения ^{56}Mn, выявляемые по совокупности морфометрических показателей. Изучаемые параметры тонкой кишки имеют статистически значимые различия в группах воздействия факторов и контрольной группе ($p<0,001$). Установлено, что наибольшая разница по толщине слизистой оболочки по сравнению с контрольной группой после воздействия ^{56}Mn отмечается на 6,3 мкм, тогда как после экспозиции ^{60}Co на 6,25 мкм. Наибольшее уменьшение количества энтероцитов в ворсинках кишечника отмечается в I-ой группе на 17,39%, а во II-ой на 3,99%. После воздействия ^{60}Co количество лейкоцитов по сравнению с контрольной группой превышает на 4,76%, а после ^{56}Mn на 3,77%. Наибольшие отклонения по количеству лимфоцитов отмечается после γ–, а затем β–облучения.

Выводы. Таким образом, воздействие ^{56}Mn на тонкую кишку крыс выявил высокий уровень риска облучения, что подтверждено морфометрическими показателями.

Ключевые слова: *радиоактивный ^{56}Mn, желудочно–кишечный синдром, кишечные клетки, морфометрия.*

Түйіндеме

СӘУЛЕЛЕНГЕН ЕГЕУҚҰЙРЫҚТАР ЖІҢІШКЕ ІШЕГІНІҢ МОРФОМЕТРИЯЛЫҚ КӨРСЕТКІШТЕРІ

Дархан Е. Узбеков [1], http://orcid.org/0000–0003–4399–460X
Дария М. Шабдарбаева [1], http://orcid.org/0000–0001–9463–1935
Найля Ж. Чайжунусова [2], http://orcid.org/0000–0002–6660–7118
Кайрат А. Альмисаев [1], http://orcid.org/0000–0001–6650–4971
Салтанат Е. Узбекова [3], http://orcid.org/0000–0001–9006–120X
Руслан М. Сапоров [1], http://orcid.org/0000–0003–3152–8759
Мадина М. Апбасова [4], http://orcid.org/0000–0003–3215–1076
Масахару Хоши [5], http://orcid.org/0000–0001–6978–0883

[1] Патологиялық анатомия және сот медицина кафедрасы, [2] Тағамтану және гигиеналық пәндер кафедрасы, [3] Анатомия және гистология кафедрасы, [4] Анестезиология және реаниматология кафедрасы, Семей қаласының мемлекеттік медицина университеті, Семей қ., Қазақстан Республикасы
[5] Радиациялық биология және медицина ғылыми–зерттеу институты, Хиросима қ., Жапония

Кіріспе. β– мен γ–сәулелеу әсеріне душар болғандардың көптеген бүліндіргіш салдарымен қоса асқорыту жүйесіне да ерекше мән бөлінеді. Жапон қалаларындағы атомдық бомбалаудың салдарын зерттеген ғалымдардың еңбектеріндегі нейтронды–белсенді Марганец–56 (^{56}Mn) радионуклидінің басым рөлі заманауи жағдайда зор қызығушылық арттырады.

Зерттеу мақсаты. Шағын дозалы β– мен γ–сәулелеу әсеріне ұшыраған егеуқұйрықтардың жіңішке ішегіндегі морфометриялық көрсеткіштерді анықтау және салыстыру.

Материалдар мен әдістер. Тәжірибе жүзінде «Вистар» тұқымды 270–350 гр салмағы бар аталық және аналық жынысты 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–сәулелеу; 3) бақылау тобы. Жануарларға сәулелеуден кейін 3–ші, 14–ші және 60–шы тәуліктерде некропсия жүргізу барысында жіңішке ішегін алып, 10%–тік формалинде фиксацияладық. Тін фрагменттерін парафинге құйып, қалыңдығы 4 мкм көлденең сериялық кесінділер дайындап, әрі қарай гематоксилин мен эозинмен (H&E) боядық. Стереоскопиялық өзгерістер жүзінде Автандиловтың окулярлық өлшегіш тор сызығы бар микрофотометриялық жүйесі қолданылған. Әрбір микропрепаратта 181 нүктелі жиынтық көлемі жүзінде 20 алаңы есептелген. 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 критикалық деңгейі 0,05-ке тең деп саналған.

Нәтижелер. Эксперименттік жануарлар жіңішке ішегінің құрылымдық жағдайын анықтайтын гистологиялық зерттеу әдістері кешеуілдеу мерзімі аясында туындаған айқын гистоморфологиялық өзгерістердің реактивті және деструкциялық сипатын көрсеткен. ^{56}Mn әсеріне ұшыраған егеуқұйрықтардың зерттеуге алынған ағзасында қалыптасқан ерте және кешеуілдеу мерзімі аясындағы тіндік құрамының өзгеруі, негізінен ^{60}Co әсеріне қарағанда, ішкі иондаушы сәулелеудің анағұрлым улы және сенсибилизациялаушы ықпалын білдіреді. Нейтронды-белсендірілген марганец диоксиді мен сыртқы иондаушы сәулелеудің егеуқұйрықтардың ішкі ағзаларына әсерін салыстырмалы түрде бағалау барысында, морфометриялық әдісті қолдану арқылы сәйкес сандық көрсеткіштер көмегімен кешеуілдеу мерзімі аясында басқа тәжірибелік топтардағы жануарлар ағзаларында аңғарылған патологиялық өзгерістермен салыстырғанда, айқын дистрофиялық, қабынулық пен некроздық үдерістердің ^{56}Mn әсерінен кейін жүзеге асатыны дәлелденген. Жіңішке ішектің зерттелген параметрлері әсер ету факторлар топтары мен бақылау топтары арасында статистика жүзінде мәнді екені аңғарылған ($p<0,001$). Сілемейлі қабықшаның қалыңдығы бойынша анағұрлым айқын айырмашылық белгілері бақылау топтарына қарағанда, ^{56}Mn әсерінен кейін 6,3 мкм-ге, ал ^{60}Co экспозициясынан кейін 6,25 мкм-ге қалыңдағаны анықталған. Ішек бүрлеріндегі энтероциттер саны І-ші топта – 17,39%-ға, ал ІІ-ші топта – 3,99%-ға азайғаны құпталған. ^{60}Co әсерінен кейін лейкоциттер саны бақылау тобымен салыстырғанда 4,76%-ға, ал ^{56}Mn әсерін алғандарда 3,77%-ға жоғарылағаны дәлелденген. Лимфоциттер саны бойынша анағұрлым айқын ауытқулар γ–, содан соң β–сәулелеуден кейін байқалған.

Қорытынды. Сонымен, егеуқұйрықтардың жіңішке ішігіне ^{56}Mn әсері морфометриялық көрсеткіштермен расталатын β–сәулелену қаупінің жоғары деңгейін көрсетті.

Негізгі сөздер: *радиобелсенді ^{56}Mn, асқазан–ішек синдромы, ішек жасушалары, морфометрия.*

Библиографическая ссылка:

Узбеков Д.Е., Шабдарбаева Д.М., Чайжунусова Н. Ж., Альмисаев К.А., Узбекова С.Е., Сапоров Р.М., Апбасова М.М., Хоши М. Морфометрические показатели тонкой кишки облученных крыс / / Наука и Здравоохранение. 2018. 3 (Т.20). С. 5-19.

Uzbekov D.E., Shabdarbaeva D.M., Chaizhunusova N.Zh., Almissaev K.A., Uzbekova S.E., Saporov R.M., Apbasova M.M., Hoshi M. Morphometric indicators of the small intestine of irradiated rats. *Nauka i Zdravookhranenie* [Science & Healthcare]. 2018, (Vol.20) 3, pp. 5-19.

Узбеков Д.Е., Шабдарбаева Д.М., Чайжунусова Н.Ж., Альмисаев К.А., Узбекова С.Е., Сапоров Р.М., Апбасова М.М., Хоши М. Сәулеленген егеуқұйрықтар жіңішке ішегінің морфометриялық көрсеткіштері / / Ғылым және Денсаулық сақтау. 2018. 3 (Т.20). Б. 5-19.

Introduction

It's generally known that neutron–induced radioisotope such as ^{56}Mn was dominant element found after an atomic 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 ionizing radiation [23]. People who returned early to Hiroshima and Nagasaki after atomic bombing were reported to suffer from the symptoms of acute radiation effects [28]. Consequently, atomic bomb effects on health of survivors have been correlated with delayed β– and γ–rays [11]. The accidental high–dose radiation exposure induces a series of injury levels in multiple organs. Most of studies regarding the fast neutron effect have focused at intestinal changes [9, 31]. Thereby, increasing attention has been given to the radiation effect on the gastrointestinal (GI) tract due to concerns about exposure to radiation after an accident [24]. It's known that nuclear factor is pronounced in GI tract those that are exposed to the external environment, therefore one of outcomes of radiation effects is GI–syndrome [12], which characterized clinically by haemorrhage, endotoxemia, bacterial infection, anorexia, nausea, vomiting, diarrhoea, loss of electrolytes and fluid, dehydration, systemic infection, septic shock and even death. In spite of the significant advances that have occurred in research on underlying mechanisms over the last two decades, the overall morphogenesis of the gastrointestinal syndrome still remains unclear. According to morphologists, these symptoms are probably due to a rapid modification of the intestinal motility and to the structural alteration of the intestinal mucosa [16, 21, 37]. Presently, particular interest is a peculiarity of histomorphological 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 internal and external radiation effect on the digestive system [6].

The objective of study

To identify and compare the morphometric indicators in the rats small intestine after exposure by single small dose by ^{56}Mn and ^{60}Co.

Materials and methods

For this study, it was purchased and raised in a specific–pathogen–free facility six–month–old sexes «Wistar» rats from Animal Laboratory of Karaganda State Medical University in an amount of 90 with mean whole body weight 270–350 g. All animals were acclimatized for two weeks before initiation of experiments and kept under normal conditions and fed pellets concentrated diet. The rats were maintained at constant temperature (22±1°C) on 8 hour light–dark cycle. Then, animals were allocated into 3 groups. The first group of rats (n=30) were subjected to 0,2 Gy ^{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 $2,75×10^{8}$ Bq was sprayed pneumatically over animals 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 ^{56}Mn radioactive powder was 4,0 hour [2].

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 radiotherapy device «Teragam K–2 unit» (Czech Republic). After irradiation, rats were taken back to the animal facility and routinely cared. The experiment was followed our institution's guide for the care and use of laboratory animals. During the exposure, rats were placed in a specially engineered cage made of organic glass with individual compartments for each animal.

The third group consisted of control rats (n=30) which were placed on shelves in the same facility and shielded from the radiation. All animal procedures were approved by Ethical Committee of Semey Medical University, Kazakhstan (Protocol №5 dated 16.04.2014). The rats were housed in a moderate security barrier.

The rats were sacrificed on the 3rd, 14th and 60th day after irradiation and the small intestine was immediately surgically extracted for further histological and morphometric study. The small intestine sections were deparaffinized and dehydrated in graded 10% formalin solutions. Paraffin sections performed with 4 μm thickness. For routine pathology, sections were hydrated and stained by hematoxylin–eosin (H&E). The specimens were examined under a Leica DM 1000 microscope (Germany) and images were captured with a charge–coupled device camera. Qualitative histological assessment of intestinal injury was carried out to obtain an overall damage severity result.

For stereoscopic changes, a microphotometric system was used with Avtandilov's ocular measuring grid [1]. In the histological sections of the tissue of the small intestine, the thickness of the mucosa, the number and volume of enterocytes, the number of leukocytes and lymphocytes were analyzed. In each slide glasses, 20 fields with a total area of 181 points were counted. The studies were performed with ×10 and ×40 magnifications.

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 [18].

Results

In this study, we have performed experiment with ^{56}Mn-exposed Wistar rats. 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 internal dose estimates in the gastrointestinal organs of rats exposed to ^{56}Mn [30]. According to dosimetry study the highest doses were fixed in the small intestine [3, 29]. To assess the health of rats after radiation, we evaluated activity, posture, dehydration and pelage of the rats. Radiation induced a decrease in health score in all groups of irradiated animals.

Algorithm description of slide glasses of the small intestine included: presence of a layers in the intestinal wall; degree of vascular hyperemia; state of mucosa, submucosa, muscle membrane and serosa. Light microscopic examination 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. H&E sections of intestine revealed not damage in control groups. However, in segment radiated rats, partial loss of crypts were observed exclusively within the targeted segment, while the rest of the intestine was unaffected.

The study of slide glasses of the small intestine in rats on the 3rd day after ^{56}Mn effect shown presence the severe degenerative changes of glands, mild accumulations of leukocytes in the stroma and uneven hyperemia of the stromal capillaries (Fig. 1–B). In rats that exposed to ^{60}Co was found focal accumulations in the glandular lumen the cellular elements, preferably desquamated epithelial cells and reactive nature cells (Fig. 1–C) in compared with control rats (Fig. 1–A).

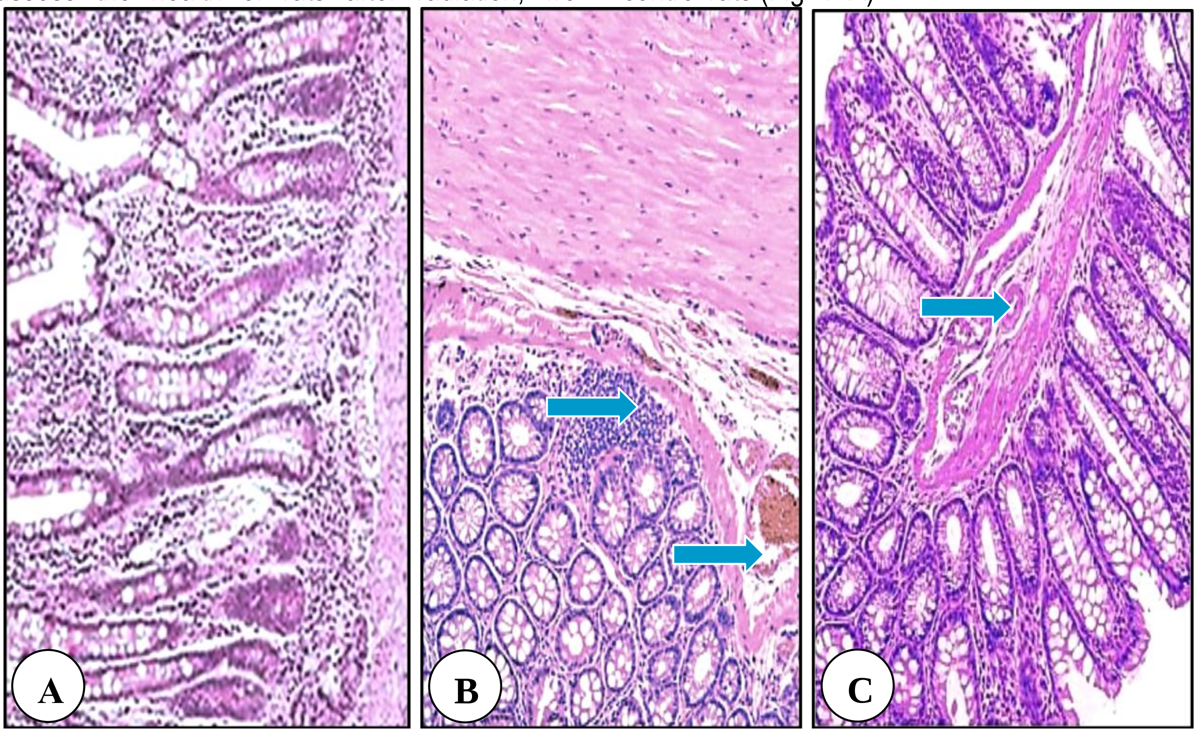

Fig. 1. Photomicrograph of rat small intestine on the 3rd day after exposure. Control (A), ^{56}Mn (B) and ^{60}Co (C); H&E staining, original magnification ×10.

The results of changes in the small intestine under the influence of the studied factors on the 3rd day are presented in Table 1. As can be seen, the thickness of the mucosa is not statistically different when exposed to ionizing radiation. The number of enterocytes in the villus of the intestine compared with the control group, it's the highest when ^{56}Mn irradiation and more than the parameters of the control group by 3,31%. When exposed to ^{60}Co, an increase of 1,48% is noted in comparison with the control group ($p<0,001$).

Table 1.

Morphometric indicators of the small intestine of experimental animals on the 3rd day.

Morphometric indicators	^{56}Mn			^{60}Co			Control			Kruskal-Wallis test	p value
	M	Me	IQR	M	Me	IQR	M	Me	IQR		
Thickness of the mucosa, mcm	36,16	35,92	2,96	35,56	35,32	3,02	35,02	35,36	2,52	H=2,802	0,422
Enterocytes number, %	76,42	76,54	1,98	74,58	74,54	1,72	73,10	73,22	0,88	H=31,106	<0,001
Enterocytes volume, mcm²	138,28	138,22	1,56	137,02	136,56	2,52	134,86	134,88	2,62	H=25,244	<0,001
Leukocytes number, %	20,46	20,28	1,46	19,96	20,06	1,72	11,28	11,26	2,04	H=47,258	<0,001
Lymphocytes number, %	24,06	24,22	1,4	24,14	24,28	1,58	25,02	24,88	1,16	H=27,688	<0,001

There were similar situation for leukocytes: irradiation by ^{56}Mn the number of leukocytes exceeds the control group by 9,14%; when exposed to ^{60}Co an increase of 8,67% that observed compared with the control group ($p<0,001$).

The number of enterocytes were the highest with irradiation of ^{56}Mn and ^{60}Co by 3,42% and 2,17%, respectively in comparison with the control group ($p<0,001$).

Changes of the number of lymphocytes depended on the studied factor. When exposure by ^{56}Mn and ^{60}Co the number of lymphocytes have decreased by 0,95% and 0,88%, respectively, relative to the control group ($p<0,001$).

Microscopic picture of animals on the 14th day after ^{56}Mn exposure provided on figure 2, where we drew attention to the presence of gaps semi–collapsing glands with degeneration, as well as optically empty vacuoles in the glands. Mild cluster of cellular elements, predominantly leukocytes. Considerable importance should be given to presence in the lumen of the individual glands accumulation of lymphocytes, modified leukocytes, mainly neutrophils. Histological studies of ^{60}Co–exposed rats revealed radiation–induced prominent degeneration. Moreover, we are registered the inflammatory response manifested by moderate of macrophages and lymphocytes infiltration in the stroma of the mucous and in lumen of some glands.

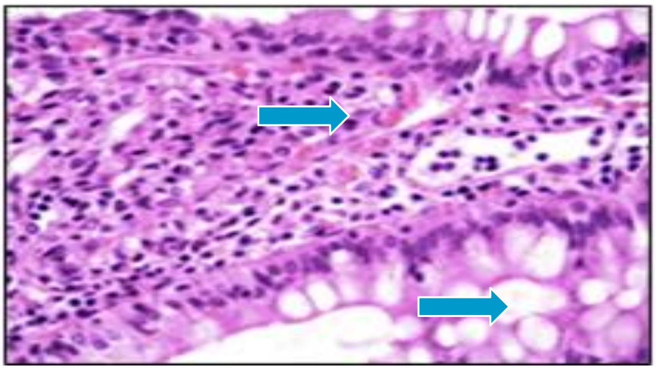

Fig. 2. Photomicrograph of the small intestine in ^{56}Mn–exposed rat on the 14th day. H&E staining, original magnification ×40.

The parameters of the small intestine on the 14th day after irradiation are presented in Table 2, from which it's evident that the numbers of enterocytes in the villus of the intestine became statistically insignificant on the 14th day between the groups. The thickness of the mucous membrane is different when exposed to various factors. It's the largest in comparison with the control group after irradiation by ^{56}Mn by 5,97 mcm, whereas after exposure to ^{60}Co more than the control group at 5,36 mcm. The trend in the number of enterocytes and on the 14th day coincide with the indicators of the 3rd day. Also, the greatest differences were noted after exposure to ^{56}Mn.

Table 2.

Morphometric indicators of the small intestine of experimental animals on the 14th day.

Morphometric indicators	^{56}Mn			^{60}Co			Control			Kruskal-Wallis test	p value
	M	Me	IQR	M	Me	IQR	M	Me	IQR		
Thickness of the mucosa, mcm	40,06	40,44	2,34	39,44	39,56	2,24	34,08	34,02	1,22	H=45,018	<0,001
Enterocytes number, %	73,08	73,82	3,98	73,58	73,58	1,96	73,30	73,42	2,24	H=0,4958	0,920
Enterocytes volume, mcm²	141,24	140,84	2,42	139,94	140,48	1,62	134,11	134,38	2,18	H=36,146	<0,001
Leukocytes number, %	20,84	20,48	1,54	20,02	20,28	1,36	13,10	13,34	2,52	H=42,862	<0,001
Lymphocytes number, %	27,48	28,46	5,42	27,54	28,44	5,73	25,31	24,36	3,74	H=20,322	<0,001

The same situation was specially for the leukocytes. Their number 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 7,74% and 6,93%, respectively ($p<0,001$). The trend of the number of lymphocytes persists and 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 2,17% and after ^{60}Co by 2,24% ($p<0.001$).

Figure 3 shows that β–radiation effect induces on the 60th day moderate severe degenerative changes of the mucosa and submucosa. The sections are represented mainly by edematous stroma. Epithelial glands exposed to collapse, in the lumen observed mild accumulations of cellular elements with a predominance the neutrophils. The glands are shrouded by optical empty vacuoles. Mostly celebrated the formation of necrotic foci. In contrast to the internal β– and external γ–radiation, inhalation of nonactivated manganese in rats intestine after 2 month contributes 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 degenerative and necrobiotic changes of surface mucous layer and uneven hyperemia of vessels. Here and there observed the foci of necrosis and reactive changes in the mucosa.

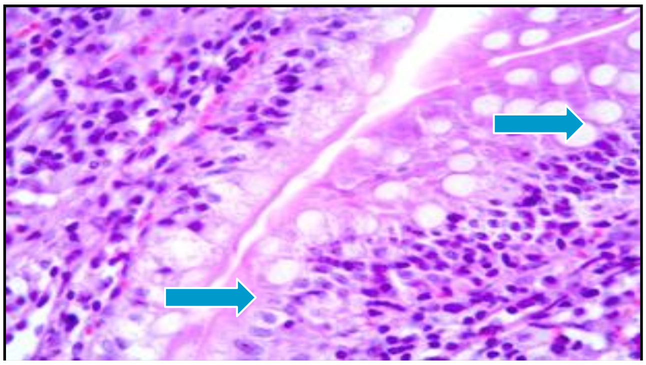

Fig. 3. Light microscopy of ^{56}Mn–induced rat small intestine on the 60th day after exposure; H&E staining, original magnification ×40.

The above data are consistent with our study results the small intestine in both ^{56}Mn– and ^{60}Co–exposed rats showed a similar changes. Nevertheless, according to results of histologic examination most pronounced changes were observed in the small intestine of rats from ^{56}Mn group, indicating that neutron radiation has a significant biologic effect on examined organ [32].

The parameters of the rat's small intestine after 60 days are presented in Table 3. As can be seen, all the studied parameters of the small intestine have statistically significant differences in the effect groups of the factors and the control group (p<0,001). The thickness of the mucosa continues to increase in comparison with 3 and 14 days. As before, the largest thickness difference with the control group after exposure to ^{56}Mn was 6,3 mcm, then after exposure to ^{60}Co at 6,25 mcm. The number of enterocytes in the intestinal villi continues to decrease in comparison with the control group. The greatest decrease is noted in the ^{56}Mn exposure group at 17,39% and after exposure to ^{60}Co by 3,99%.

Table 3.

Morphometric indicators of the small intestine of experimental animals on the 60th day.

Morphometric indicators	^{56}Mn			^{60}Co			Control			Kruskal-Wallis test	p value
	M	Me	IQR	M	Me	IQR	M	Me	IQR		
Thickness of the mucosa, mcm	40,56	40,22	1,38	40,52	40,22	1,64	34,26	34,48	1,6	H=36,348	<0,001
Enterocytes number, %	56,86	55,28	8,2	70,26	70,56	1,28	74,24	73,98	1,04	H=48,328	<0,001
Enterocytes volume, mcm²	138,34	138,64	4,86	138,02	137,78	3,34	133,78	133,34	1,4	H=29,392	<0,001
Leukocytes number, %	16,52	17,48	5,24	17,54	17,94	1,26	12,74	12,12	3,82	H=27,894	<0,001
Lymphocytes number, %	30,14	29,98	1,06	29,68	29,82	2,06	24,32	24,22	1,4	H=50,022	<0,001

The number of leukocytes gradually decreases and reaches the lowest values on the 60th day in comparison with acute and subacutes effects. After exposure to ^{60}Co, its excess of the control group remains 4,76%, after ^{56}Mn was 3,77%. The number of lymphocytes continues to increase by 60 days. 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 summary table of the ^{56}Mn effect on the parameters of the small intestine is presented in Table 4. As can be seen from this table, the thickness of the mucosa in the dynamics increases, the number of enterocytes in the villi of the intestine decreases, the enterocyte klemmi practically does not change, the number of leukocytes increases up to 14 days, then are decreasing. The number of lymphocytes in the dynamics is studied increases.

Table 4.

Morphometric indicators on different days in the ^{56}Mn–induced rat small intestine.

Morphometric indicators	3rd day			14th day			60th day			Kruskal-Wallis test	p value
	M	Me	IQR	M	Me	IQR	M	Me	IQR		
Thickness of the mucosa, mcm	36,16	35,94	2,96	40,06	40,44	2,34	40,56	40,22	1,38	H=28,462	<0,001
Enterocytes number, %	76,42	76,54	1,98	73,08	73,82	3,98	56,86	55,28	8,2	H=35,854	<0,001
Enterocytes volume, mcm²	138,28	138,22	1,56	141,24	140,82	2,4	138,34	138,64	4,86	H=16,732	<0,001
Leukocytes number, %	20,44	20,28	1,46	20,84	20,48	1,54	16,52	17,48	5,24	H=24,186	<0,001
Lymphocytes number, %	24,06	24,22	1,22	27,48	28,47	5,42	30,14	29,98	1,06	H=25,352	<0,001

The conducted research confirms the assumption that the controlled effect factor has a high degree of influence on all the resulting signs of morphometry. 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 gastrointestinal tract organs. 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 revealed indicators of the degree of influence of the controlled factor demonstrates that the effect of this factor doesn't affect the most significant indicators of morphometry but affect the most dynamic indicators of morphometry.

Discussion

In this study, we have shown the sequence of histomorphologic 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 intestinal injury (RIII). Results of morphologic studies have shown that structural changes in the small intestine observed in irradiated rats little differed from the previously published results using different radiation models.

In segment–radiated rats, the radiated intestinal segment was still identifiable by H&E staining at 14th day post–radiation and didn't exhibit complete normalization of architecture of the mucosa and bowel wall as compared with non–irradiated tissue adjacent to the radiated segment. Histological analysis showed that radiation could induce epithelial degeneration, that's characterized by the loss of intestinal structural integrity, on the 14th day post–radiation. A complete understanding of the mechanisms driving epithelial regeneration and repair, as well as the complications due to exposure of the small intestine to ^{56}Mn would benefit from the ability to study later phases of regeneration involving intestinal epithelial hyperplasia and hyper–proliferation and ideally, times associated with complete normalization of the intestinal epithelial architecture.

It's known that mechanisms of injury in normal tissues after irradiation include progenitor cell depletion, microvascular injury, inflammation and cell death [8]. The major pathological change caused by RIII is architectural disorganization including inflammatory cell infiltration, villitis, desquamation and necrosis [22]. Several evidences suggest that radiation-induced dysfunctions and either changes in subcellular, cellular, histological structure of the small intestine are mediated by concerted and interrelated changes of a plethora of various extracellular mediators and their intracellular messengers [37]. Data morphologic findings were consistent with radiation enteritis. Morphological damages of radiation–induced enteritis were known as architectural changes of intestinal mucosa such as villus shortening by cell death. The acute microscopic changes of intestine by irradiation were consisted of structural changes in the villus–crypt architecture and epithelial transformations [12, 19].

Whole–body radiation can cause severe damage to the digestive system causing inflammatory processes and immediate cell death. Radiation causes inflammation and dysregulation of immune homeostasis. These histomorphologic changes in examined organ of rats exposed to β– and γ–radiation make it possible to develop diagnostic criteria for assessing of radiation effect on the small intestine depending on cumulative dose [33].

Presently, great importance is attached to the role of Mn inducing cell death [26]. Experimentally confirmed that a certain percentage of Mn enters to organism through absorption in the gastrointestinal tract. If Mn not absorbed in the stomach, it's rapidly absorbed in the small intestine. Thus, in the literature we have examined the papers revealed regarding ability of Mn to cause histomorphologic changes in the small intestine of animals [20]. Evidence obtained using genetic modification technology has convincingly shown that intestinal stem cells are columnar cells at the crypt base intermingling with Paneth cells. The molecular determinants of intestinal radiosensitivity and gastrointestinal syndrome are not well understood. Some believe that damage to stem cells plays a critical role in this process [25]. Ionizing radiation leads to the exhaustion of the stem cells pool, increases the load on the differentiated cells [22].

Few studies have focused on a biopolymer whose manipulation significantly regulates gastrointestinal syndrome via securing stem cell zones and the integrity of intestinal epithelium [27].

Cell death after radiation should be noted that damaged cells are eliminated by the adjacent epithelial cells, endothelial, fibroblasts, macrophages [13, 17, 35]. The acute morphological changes of intestine by irradiation were consisted of structural changes in the villus–crypt architecture and epithelial transformations associated with radiation–induced degeneration [12, 36]. Most authors believe that cell death resulting from toxicity of Mn is combination with cessation of ATP synthesis due to mitochondrial damage [29]. Dysfunction or death of intestinal epithelial cells caused by degeneration after radiation influence is considered as dangerous component in the pathogenesis of gastrointestinal syndrome [14]. 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 [4–7, 10, 15, 34].

Summing up, the most pronounced typical pathological 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 identified by the totality of morphometrical indicators.

Conclusion

The most prominent histologic picture characterized by presence the signs of inflammation and degeneration on the 3rd and 14th day, in particular, in rats exposed to ^{56}Mn compared to rats from ^{60}Co groups. Our research results and their comparison with literature data leds to the conclusion that majority of experimental animals exposed to β– and γ–radiation more pronounced changes were observed on the 60th day after exposure consisting the appearance of chronic inflammation, signs of degeneration and necrotic foci. Consequently, like ^{60}Co, ^{56}Mn also promotes activation of inflammatory processes and stimulation of immune responses manifested by cellular infiltration.

Although whole–body radiation doses from ^{56}Mn was relatively low internal doses were noted in the small intestine, in addition to significant pathological changes that more severe and prolonged than ^{60}Co γ–irradiation effects. These data may indicate the potential for a high risk of internal exposure to ^{56}Mn which would have existed in airborne dust after atomic bomb explosions in Hiroshima and Nagasaki.

Thus, our data obtained from in vivo experiments provide strong evidence that β–radiation causes formation of morphologic features which typically for radiation enteritis that's a form of small intestinal injury depending on radiation type.

Interest conflict:

All authors declare that partial results of this experiment were described in the paper «Comparative characteristics of histomorphologic changes in the small intestine of rats exposed to gamma– and neutron radiation» from «European Journal of Natural History». 2017. №4. P. 38-42.

Authors contributions:

Uzbekov D. – the practical implementation of all phases of the experiment;

Shabdarbaeva D. – histological analysis and interpretation of morphometric data;

Chaizhunusova N. – administrative, technical and material support;

Almisaev K. – the practical implementation of histological staining;

Uzbekova S. – statistical analysis;

Saporov R. – the practical implementation of rats necropsy;

Apbasova M. – collection of literature review;

Hoshi M. – development of methodology.

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.

Литература:

1. *Автандилов Г.Г.* Основы количественной патологической анатомии / Г.Г. Автандилов. М.: Медицина, 2002. 240 с.

2. *Рахыпбеков Т.К., Хоши М., Степаненко В.Ф., Жумадилов К.Ш., Чайжунусова Н.Ж. и др.* Радиационно–биологический эксперимент на комплексе исследовательских реакторов «Байкал–1» // Человек. Энергия. Атом. 2015. № 2 (24). С. 43–45.

3. *Степаненко В.Ф., Рахыпбеков Т.К., Каприн А.Д., Иванов С.А., Отани К. и др.* Облучение экспериментальных животных активированной нейтронами радиоактивной пылью: разработка и реализация метода – первые результаты международного многоцентрового исследования // Радиация и риск. 2016. Т. 25, № 4. С. 112–125.

4. *Узбеков Д.Е., Ильдербаев О.З., Шабдарбаева Д.М., Саякенов Н.Б., Узбекова С.Е.* ^{60}Co әсеріне ұшыраған егеуқұйрықтардың әр түрлі жастағы ұрпағының жіңішке ішек лимфа түйіндеріндегі энергия алмасу үрдісінің салыстырмалы сипаттамасы // Наука и Здравоохранение. 2015. № 2. С. 72–81.

5. *Узбеков Д.Е., Ильдербаев О.З., Шабдарбаева Д.М., Саякенов Н.Б., Узбекова С.Е. и др.* Состояние обменных процессов в органах потомков крыс, подвергнутых воздействию γ–излучения // Наука и Здравоохранение. 2016. № 3. С. 79–82.

6. *Узбеков Д.Е., Кайрханова Ы.О., Hoshi M., Чайжунусова Н.Ж., Шабдарбаева Д.М. и др.* Влияние радиационного излучения на иммунную систему // Международный журнал прикладных наук и фундаментальных исследований. 2016, № 8 (4). С. 538–541.

7. *Узбеков Д.Е., Шабдарбаева Д.М., Саякенов Н.Б., Узбекова С.Е., Апбасова С.А.* Сәулелендірілген егеуқұйрықтардың І–ші ұрпағының иммундық қабілетті ағзаларындағы алмасу үрдістерінің жағдайы // Наука и Здравоохранение. 2014. № 6. С. 38–41.

8. *Andreyev H.J., Benton B.E., Lalji A., Norton C., Mohammed K. et al.* Algorithm–based management of patients with gastrointestinal symptoms in patients after pelvic radiation treatment (ORBIT): a randomised controlled trial // Lancet. 2013. Vol. 382. P. 2084–2092.

9. *Berbee M., Hauer–Jensen M.* Novel drugs to ameliorate gastrointestinal normal tissue radiation toxicity in clinical practice: what is emerging from the laboratory? // Current Opinion in Supportive and Palliative Care. 2012. Vol. 6, N 1. P. 54–59.

10. *Chen H., Min X.H., Wang Q.У., Leung F.W., Shi L. et al.* Pre–activation of mesenchymal stem cells with TNF–α, IL–1β and nitric oxide enhances its paracrine effects on radiation–induced intestinal injury // Sci. Rep. 2015. N 5. 8718 p.

11. *Cullings H.M., Fijita S., Funamoto S.* Dose estimation for atomic bomb survivor studies: its evolution and present status // Radiat. Res. 2006. Vol. 166, N 1. P. 219–254.

12. *Driak D., Osterreicher J., Vavrova J., Rehakova Z., Vilasova Z.* Morphological changes of rat jejunum after whole body gamma–irradiation and their impact in biodosimetry // Physiol. Res. 2008. Vol. 57. P. 475–479.

13. *Eriksson D., Stigbrand T.* Radiation–induced cell death mechanisms // Tumor Biology. 2010. Vol. 31. P. 363–372.

14. *Ghosh S.P., Kulkarni S., Perkins M.W., Hieber K., Pessu R.L. et al.* Amelioration of radiation–induced hematopoietic and gastrointestinal damage by Ex–RAD(R) in mice // J. Radiat. Res. 2012. Vol. 53, N 4. P. 526–536.

15. *Hauer–Jensen M., Denham J.W., Andreyev H.J.* Radiation enteropathy – pathogenesis, treatment and prevention // Nat. Rev. Gastroenterol. Hepatol. 2014. Vol. 11. P. 470–479.

16. *Hua G., Thin T.H., Feldman R., Haimovitz–Friedman A., Clevers H. et al.* Crypt base columnar stem cells in small intestines of mice are radioresistant // Gastroenterology. 2012. Vol. 143. P. 1266–1276.

17. *Janssen W.J., Henson P.M.* Cellular regulation of the inflammatory response // Toxicol. Pathol. 2012. Vol. 40. N 2. P. 166–173.

18. *Kruskal W.H., Wallis W.A.* Use of ranks in one-criterion variance analysis // Journal of the American Statistical Association. 1952. Vol. 47, N260. P. 583–621.

19. *Liu Z., Tian H., Jiang J., Уang У., Tan S. et al.* β–Arrestin–2 modulates radiation–induced intestinal crypt progenitor/stem cell injury // Cell Death and Differentiation. 2016. Vol. 23, N 9. P. 1529–1541.

20. *McMillan G.* Is electric arc welding linked to manganism or Parkinson's disease // Toxicology Review. 2005. Vol. 24, N 4. P. 237–257.

21. *Metcalfe C., Kljavin N.M., Ybarra R., de Sauvage F.J.* Stem cells are indispensable for radiation–induced intestinal regeneration // Cell Stem Cell. 2014. Vol. 14, N 2. P. 149–159.

22. *Onal C., Kayaselcuk F., Topkan E., Yavuz M., Bacanli D. et al.* Protective effects of melatonin and octreotide against radiation–induced intestinal injury // Dig. Dis. Sci. – 2011. – Vol. 56, N 2. – P. 359–367.

23. *Orlov M., Stepanenko V.F., Belukha I.G., Ohtaki M., Hoshi M.* Calculation of contact beta–particle exposure of biological tissue from the residual radionucides in Hiroshima // Health Physics. 2014. Vol. 107, N 1. 44 p.

24. *Qi Z., Chen У.G.* Regulation of intestinal stem cell fate specification // Science China Life Sciences. 2015. Vol. 58, N 6. P. 570–578.

25. *Qiu W., Carson–Walter E.B., Liu H., Epperly M., Greenberger J.S. et al.* PUMA regulates intestinal progenitor cell radiosensitivity and gastrointestinal syndrome // Cell Stem Cell. 2008. Vol. 2, N 6. P. 576–583.

26. *Roth J.A.* Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination // Biol. Res. 2006. Vol. 39, N 1. P. 45–57.

27. *Rentea R.M., Lam V., Biesterveld B., Fredrich K.M., Callison J. et al.* Radiation–induced changes in intestinal and tissue–nonspecific alkaline phosphatase: implications for recovery after radiation therapy // The American Journal of Surgery. 2016. Vol. 212, N 4. P. 602–608.

28. *Sasaki M.S., Endo S., Hoshi M., Nomura T.* Neutron relative biological effectiveness in Hiroshima and Nagasaki atomic bomb survivors: a critical review // J. Radiat. Res. 2016. Vol. 57, N 6. P. 583–595.

29. *Shichijo K., Fujimoto N., Uzbekov D., Kairkhanova У., Saimova A. et al.* Internal exposure to neutron-activated 56Mn dioxide powder in Wistar rats – Part 2: pathological effects // Radiation and Environmental Biophysics. 2017. Vol. 56, N 1. P. 55–61.

30. *Stepanenko V., Rakhypbekov T., Otani K., Endo S., Satoh K. et al.* Internal exposure to neutron-activated 56Mn dioxide dioxide powder in Wistar rats: part 1: dosimetry // Radiation and Environmental Biophysics. 2017. Vol. 56, N 1. P. 47–54.

31. *Uozaki H., Fukayama M., Nakagawa K., Ishikawa T., Misawa S. et al.* The pathology of multi–organ involvement: two autopsy cases from the Tokai–mura criticality accident // Br. J. Radiol. Suppl. 2005. Vol. 27. P. 13–16.

32. *Uzbekov D., Hoshi M., Shichijo K., Chaizhunusova N., Shabdarbaeva D., et al.* Comparative characteristics of histomorphologic changes in the small intestine of rats exposed to gamma– and neutron radiation. Materials of International scientific and practical conference «Diagnosis, therapy, prevention of socially significant diseases in humans» (4–10 March, 2017, Dubai, UAE) // European Journal of Natural History. 2017. – N 4. – P. 38-42

33. *Uzbekov D.E., Hoshi M., Shichijo K., Chaizhunusova N.Zh., Shabdarbaeva D.M. et al.* Radiation effects on morphofunctional state of the gastrointestinal tract (Literature review) // Вестник КазНМУ. 2017. N 2. P. 74–79.

34. *Uzbekov D.E., Ilderbayev O.Z., Shabdarbaeva D.M., Sayakenov N.B., Uzbekova S.E. et al.* Comparative characteristics of lipid peroxidation in small intestine at progeny irradiated rats // Вестник КазНМУ. 2016. N 3. P. 148–152.

35. *Uzbekov D.E., Shichijo K., Fujimoto N., Shabdarbaeva D.M., Sayakenov N.B. et al.* Radiation–induced apoptosis in the small intestine of rats // Science & Healthcare. 2017. N 3. P. 32–44.

36. *Wei L., Leibowitz B.J., Wang X., Epperly M., Greenberger J. et al.* Inhibition of CDK4/6 protects against radiation-induced intestinal injury in mice // Journal of Clinical Investigation. 2016. Vol. 126, N 11. P. 4076–4087.

37. *Zhu У., Zhou J., Tao G.* Molecular aspects of chronic radiation enteritis // Clinical and Investigative Medicine. 2011. Vol. 34, N 3. P. 119–124.

References:

1. Avtandilov G.G. Osnovy kolichestvennoi patologicheskoi anatomii [Fundamentals of quantitative pathological anatomy] / *G.G. Avtandilov. M.: Meditsina,* 2002. 240 s. [in Russian]

2. Rakhypbekov T.K., Hoshi M., Stepanenko V.F., Zhumadilov K.Sh., Chaizhunusova N.Zh. i dr. Radiatsionno–biologicheskii eksperiment na komplekse issledovatel'skikh reaktorov «Baikal–1» [Radiation–chemical experiment on complex of research reactors «Baikal–1»]. *Chelovek. Energiya. Atom* [Human. Energy. Atom]. 2015. N 2 (24). pp. 43–45. [in Russian]

3. Stepanenko V.F., Rakhypbekov T.K., Kaprin A.D., Ivanov S.A., Otani K. i dr. Obluchenie eksperimental'nykh zhivotnykh aktivirovannoi neitronami radioaktivnoi pyl'yu: razrabotka i realizatsiya metoda – pervye rezul'taty mezhdunarodnogo mnogotsentrovogo issledovaniya [Irradiation of laboratory animals by

neutron activated dust: development and application of the method – first results of international multicenter study]. *Radiatsiya i risk* [Radiation and risk]. 2016. Vol. 25, N 4. pp. 112–125. [in Russian]

4. Uzbekov D.E., Il'derbaev O.Z., Shabdarbaeva D.M., Sayakenov N.B., Uzbekova S.E. Sravnitel''naya kharakteristika energeticheskogo obmena v limfouzlakh tonkogo kishechnika potomkov krys razlichnogo vozrasta, podvergnutykh vozdeistviyu ^{60}Co [Comparative characteristics of energy metabolism in lymph nodes of small intestine of descendants of rats of different age exposed to ^{60}Co]. *Nauka i Zdravoohranenie* [Science & Healthcare]. 2015. N 2. pp. 72–81. [in Kazakh]

5. Uzbekov D.E., Il'derbaev O.Z., Shabdarbaeva D.M., Sayakenov N.B., Uzbekova S.E. i dr. Sostoyanie obmennykh protsessov v organakh potomkov krys, podvergnutykh vozdeistviyu γ–izlucheniya [State of metabolic processes in organs of rats progeny exposed to γ–radiation]. *Nauka i Zdravoohranenie* [Science & Healthcare]. 2016. N 3. pp. 79–82. [in Russian]

6. Uzbekov D.E., Kairkhanova У.O., Hoshi M., Chaizhunusova N.Zh., Shabdarbaeva D.M. i dr. Vliyanie radiatsionnogo izlucheniya na immunnuyu sistemu [Influence of radiation on the immune system]. *Mezhdunarodnyj zhurnal prikladnyh nauk i fundamental'nyh issledovanij* [International journal of applied and fundamental research]. 2016. N 8 (4). pp. 538–541. [in Russian]

7. Uzbekov D.E., Shabdarbaeva D.M., Sayakenov N.B., Uzbekova S.E., Apbasova S.A. Sostoyanie obmennykh protsessov v immunokompetentnykh organakh u 1–go pokoleniya potomkov obluchennykh krys [State of metabolic processes in immunocompetency organs at 1–st generation of descendants of rats exposed to radiation]. *Nauka i Zdravoohranenie* [Science & Healthcare]. 2014. N 6. pp. 38–41. [in Kazakh]

8. Andreyev H.J., Benton B.E., Lalji A., Norton C., Mohammed K. et al. Algorithm–based management of patients with gastrointestinal symptoms in patients after pelvic radiation treatment (ORBIT): a randomised controlled trial. *Lancet.* 2013. Vol. 382. pp. 2084–2092.

9. Berbee M., Hauer–Jensen M. Novel drugs to ameliorate gastrointestinal normal tissue radiation toxicity in clinical practice: what is emerging from the laboratory? *Current Opinion in Supportive and Palliative Care.* 2012. Vol. 6, N 1. pp. 54–59.

10. Chen H., Min X.H., Wang Q.У., Leung F.W., Shi L. et al. Pre–activation of mesenchymal stem cells with TNF–α, IL–1β and nitric oxide enhances its paracrine effects on radiation–induced intestinal injury. *Sci. Rep.* 2015. N 5. 8718 p.

11. Cullings H.M., Fijita S., Funamoto S. Dose estimation for atomic bomb survivor studies: its evolution and present status. *Radiat. Res.* 2006. Vol. 166, N 1. pp. 219–254.

12. Driak D., Osterreicher J., Vavrova J., Rehakova Z., Vilasova Z. Morphological changes of rat jejunum after whole body gamma–irradiation and their impact in biodosimetry. *Physiol. Res.* 2008. Vol. 57. pp. 475–479.

13. Eriksson D., Stigbrand T. Radiation–induced cell death mechanisms. *Tumor Biology.* 2010. Vol. 31. pp. 363–372.

14. Ghosh S.P., Kulkarni S., Perkins M.W. Hieber K., Pessu R.L. et al. Amelioration of radiation–induced hematopoietic and gastrointestinal damage by Ex–RAD(R) in mice. *J. Radiat. Res.* 2012. Vol. 53, N 4. pp. 526–536.

15. Hauer–Jensen M., Denham J.W., Andreyev H.J. Radiation enteropathy–pathogenesis, treatment and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 2014. Vol. 11. pp. 470–479.

16. Hua G., Thin T.H., Feldman R., Haimovitz–Friedman A., Clevers H. et al. Crypt base columnar stem cells in small intestines of mice are radioresistant. *Gastroenterology.* 2012. Vol. 143. pp. 1266–1276.

17. Janssen W.J., Henson P.M. Cellular regulation of the inflammatory response. *Toxicol. Pathol.* 2012. Vol. 40. N 2. pp. 166–173.

18. Kruskal W.H., Wallis W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association.* 1952. Vol. 47, N 260. pp. 583–621.

19. Liu Z., Tian H., Jiang J., Уang У., Tan S. et al. β–Arrestin–2 modulates radiation–induced intestinal crypt progenitor/stem cell injury. *Cell Death and Differentiation.* 2016. Vol. 23, N 9. pp. 1529–1541.

20. McMillan G. Is electric arc welding linked to manganism or Parkinson's disease. *Toxicology Review.* 2005. Vol. 24, N 4. pp. 237–257.

21. Metcalfe C., Kljavin N.M., Уbarra R., de Sauvage F.J. Stem cells are indispensable for

radiation–induced intestinal regeneration. *Cell Stem Cell.* 2014. Vol. 14, N 2. pp. 149–159.

22. Onal C., Kayaselcuk F., Topkan E., Уavuz M., Bacanli D. et al. Protective effects of melatonin and octreotide against radiation–induced intestinal injury. *Dig. Dis. Sci.* 2011. Vol. 56, N 2. pp. 359–367.

23. Orlov M., Stepanenko V.F., Belukha I.G., Ohtaki M., Hoshi M. Calculation of contact beta–particle exposure of biological tissue from the residual radionucides in Hiroshima. *Health Physics.* 2014. Vol. 107, N 1. 44 p.

24. Qi Z., Chen У.G. Regulation of intestinal stem cell fate specification. *Science China Life Sciences.* 2015. Vol. 58, N 6. pp. 570–578.

25. Qiu W., Carson–Walter E.B., Liu H., Epperly M., Greenberger J.S. et al. PUMA regulates intestinal progenitor cell radiosensitivity and gastrointestinal syndrome. *Cell Stem Cell.* 2008. Vol. 2, N 6. pp. 576–583.

26. Roth J.A. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biol. Res.* 2006. Vol. 39, N 1. pp. 45–57.

27. Rentea R.M., Lam V., Biesterveld B., Fredrich K.M., Callison J. et al. Radiation–induced changes in intestinal and tissue–nonspecific alkaline phosphatase: implications for recovery after radiation therapy. *The American Journal of Surgery.* 2016. Vol. 212, N 4. P. 602–608.

28. Sasaki M.S., Endo S., Hoshi M., Nomura T. Neutron relative biological effectiveness in Hiroshima and Nagasaki atomic bomb survivors: a critical review. *J. Radiat. Res.* 2016. Vol. 57, N 6. pp. 583–595.

29. Shichijo K., Fujimoto N., Uzbekov D., Kairkhanova У., Saimova A. et al. Internal exposure to neutron-activated 56Mn dioxide powder in Wistar rats – Part 2: pathological effects. *Radiation and Environmental Biophysics.* 2017. Vol. 56, N 1. pp. 55–61.

30. Stepanenko V., Rakhypbekov T., Otani K., Endo S., Satoh K. et al. Internal exposure to neutron-activated 56Mn dioxide dioxide powder in Wistar rats: part 1: dosimetry // *Radiation and Environmental Biophysics.* 2017. Vol. 56, N 1. pp. 47–54.

31. Uozaki H., Fukayama M., Nakagawa K., Ishikawa T., Misawa S. et al. The pathology of multi–organ involvement: two autopsy cases from the Tokai–mura criticality accident. *Br. J. Radiol. Suppl.* 2005. Vol. 27. pp. 13–16.

32. Uzbekov D., Hoshi M., Shichijo K., Chaizhunusova N., Shabdarbaeva D., et al. Comparative characteristics of histomorphologic changes in the small intestine of rats exposed to gamma– and neutron radiation. Materials of International scientific and practical conference «Diagnosis, therapy, prevention of socially significant diseases in humans» (4–10 March, 2017, Dubai, UAE). *European Journal of Natural History.* 2017. N 4. pp. 38-42

33. Uzbekov D.E., Hoshi M., Shichijo K., Chaizhunusova N.Zh., Shabdarbaeva D.M. et al. Radiation effects on morphofunctional state of the gastrointestinal tract (Literature review). *Vestnik KazNMU* [Bulletin of KazNMU]. 2017. N 2. pp. 74–79.

34. Uzbekov D.E., Ilderbayev O.Z., Shabdarbaeva D.M., Sayakenov N.B., Uzbekova S.E. et al. Comparative characteristics of lipid peroxidation in small intestine at progeny irradiated rats. *Вестник КазНМУ* [Vestnik KazNMU]. 2016. N 3. pp. 148–152.

35. Uzbekov D.E., Shichijo K., Fujimoto N., Shabdarbaeva D.M., Sayakenov N.B. et al. Radiation–induced apoptosis in the small intestine of rats // *Science & Healthcare.* 2017. N 3. pp. 32–44.

36. Wei L., Leibowitz B.J., Wang X., Epperly M., Greenberger J. et al. Inhibition of CDK4/6 protects against radiation-induced intestinal injury in mice. *Journal of Clinical Investigation.* 2016. Vol. 126, N 11. pp. 4076–4087.

37. Zhu У., Zhou J., Tao G. Molecular aspects of chronic radiation enteritis. *Clinical and Investigative Medicine.* 2011. Vol. 34, N 3. pp. 119–124.

Correspondence:

Uzbekov Darkhan – PhD, assistant of Department of Pathological anatomy and Forensic medicine of Semey Medical University, Semey, Kazakhstan.

Address: East Kazakhstan region, 071400, Semey city, Shakarim street, 13 A – 72.

Phone: 87222420532, +77055301026

E–mail: darkhan.uzbekov@mail.ru