

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

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For a broader understanding of long-term radiation pathology continues to represent the interest studying the patterns and degree of severity of intestinal structural changes and also its effects on occurrence of pathological processes in small intestine of human exposed to different type of radiation. The most prominent microscopic changes characterized by presence the signs of inflammation and degeneration on the 3rd and 14th day, in particular, in ⁵⁶Mn-exposed rats compared to rats from MnO₂ and ⁶⁰Co groups. The study results showed that in majority of experimental animals exposed to γ - and neutron radiation more pronounced changes were observed on the 60th day after exposure, consisting the appearance of chronic inflammation and foci of necrosis, whereas in MnO₂-inhaled rats dominated degenerative changes. The findings support a role of ionizing radiation in the formation of small intestinal damage depending on both dose and type of radiation.

Keywords: radioactive ⁵⁶Mn, γ -radiation, small intestine, morphologic changes, rats

It is known that among the neutron-induced radioisotopes ⁵⁶Mn is a dominant element found after an A-bomb explosion. Since ⁵⁶Mn and the other neutron-activated radioisotopes were present in dust after bombings, people have inhaled these radioactive materials and been internally exposed to radiation. People who returned early to Hiroshima and Nagasaki after A-bombing were reported to suffer from the symptoms of acute radiation effects [3]. 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 [10]. It is 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 [1], 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 GI-syndrome still remains unclear. Currently, 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 (cell loss and altered crypt integrity) [8]. The highly radiosensitive intestine is an important dose-limitative organ in both total body and abdominopelvic radiation [2]. Most of studies regarding the fast neutron effect have focused at intestinal changes [4].

Currently, particular interest is a comparative characteristic of microscopic changes in the small intestine of persons exposed to ⁵⁶Mn and ⁶⁰Co, allowing in the future to work out the diagnostic criteria for assessing of radiation effect factor on the GI tract, depending on the cumulative dose.

Objective of the study: identify and compare the microscopic changes in the rat small intestine after exposure by single dose of γ -radiation and neutron-activated ⁵⁶MnO₂ powder.

Materials and methods

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

The rats were allocated into four groups. The first group of animals ($n = 9$) were subjected to ⁵⁶Mn which was obtained by neutron activation of 100 mg of manganese dioxide (MnO₂) powder using nuclear reactor ("Baikal-1") with neutron flux 4×10^{14} n/cm². Activated powder with total activity of ⁵⁶Mn 2.75×10^8 Bq was sprayed pneumatically over rats placed in the special box. The moment of exposition beginning of experimental animals by ⁵⁶Mn powder is 6 minute after finishing of neutron activation. Duration of exposition of rats to radioactive powder was 3.5–4.0 hours (starting from the moment of spraying of ⁵⁶Mn powder till surgical extraction of the small intestine). The second group of rats ($n = 9$) were exposed to not irradiated MnO₂. The spray powder was carried out in a chemical box, which contained boxes of 9 rats. Each portion of MnO₂ was sprayed in box with lots of biologic objects. Then unirradiated powder and incubated biologic objects in a container for hour. The third group of rats ($n = 9$) were irradiated with a single dose of

2 Gy was performed at a dose rate of 2.6 Gy/min using ^{60}Co γ -ray by czech radiotherapeutic device "Teragam K-2 unit". During the exposure, animals were placed in a specially engineered cage made of organic glass with individual compartments for each rat. Irradiation doses were monitored using Accudose (Radcal) to deliver the exact dose to each animal. After irradiation, rats were taken back to the animal facility and routinely cared. The fourth group consisted of control rats ($n = 9$) which were placed on shelves in the same facility and shielded from radiation. All animal procedures were approved by Ethical Committee of Semey State Medical University, Kazakhstan (Protocol №5 dated 16.04.2014) in accordance with Directive of the European Parliament and the Council on the Office in animals protection.

All animals were anesthetized with an intraperitoneal injection of 100 mg/kg of ketamine hydrochloride. The rats were sacrificed on the 3rd, 14th, 60th day after irradiation and the small intestine was immediately surgically extracted for further histological study. A section from the small intestine was fixed in 10% formalin, embedded in paraffin wax, sectioned serially into 4 μm thick sections, 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 (Visitron Systems, Puchheim, Germany) at $\times 10$ and $\times 40$ magnifications. Qualitative histological assessment of intestinal injury was carried out to obtain an overall damage severity result.

Results of research and their discussion

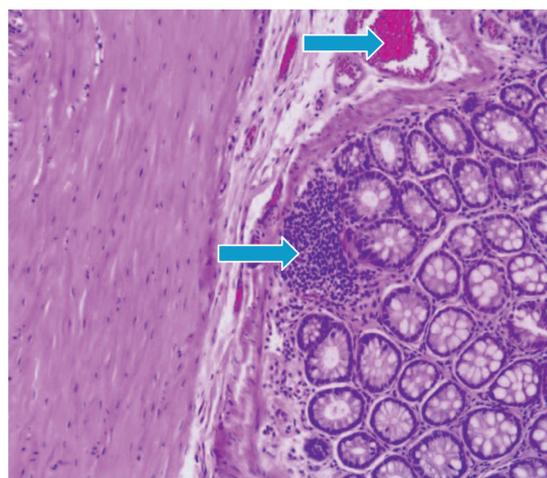
To assess the health of rats after radiation, we evaluated activity, posture, dehydration and pelage of the rats. Radiation induced a decrease in health score in all groups of irradiated animals.

We performed experiment with neutron–activated $^{56}\text{MnO}_2$ powder exposed rats. Although the level of radioactivity received from ^{56}Mn was rather low, the observed biological effects were consistent in experiment. It was previously reported the internal dose estimates in organs of ^{56}Mn –exposed rats. The highest doses were recorded in the small intestine [9].

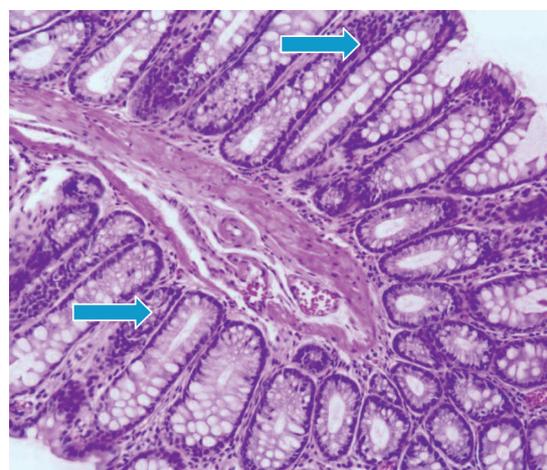
Algorithm description of slide glasses of the small intestine included: presence of layers of the intestinal wall; degree of vascular hyperemia; state of mucosa, submucosa, muscle membrane and serosa. Light microscopic examination of the intestinal mucosa of control rats showed a normal architecture of villi, crypts and enterocytes. Intestinal tissues were collected at day 3, 14 and 60 post–radiation, time associated with complete crypt ablation in small intestine after radiation exposure. H&E sections of intestine revealed no damage in control groups. However, in segment radiated rats, partial loss of crypts was 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 ef-

fect shown presence the severe degenerative changes of glands, mild accumulations of leukocytes in the stroma and uneven hyperemia of the stromal capillaries (Fig. 1–A), whereas in MnO_2 –inhaled rats observed only mild leukocytic infiltration. In rats exposed to ^{60}Co was found focal accumulations in the glandular lumen the cellular elements, preferably desquamated epithelial cells and cells of reactive nature (Fig. 1–B).



A



B

Fig. 1. Photomicrograph of the small intestine in ^{56}Mn –exposed rat (A) and ^{60}Co –exposed rat (B). H&E staining, original magnification $\times 10$

Microscopic picture of animals on the 14th day after ^{56}Mn exposure provided on fig. 2–A, 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. Figure 2–B shows that MnO₂ effect caused sign of degeneration severe swelling of the mucous membrane of the stroma and mild erythrocytes stasis. Histological studies of ⁶⁰Co–exposed rats revealed radiation–induced prominent degeneration. Moreover, we are registered the inflammatory response manifested by moderate of macrophages and lymphocytic infiltration in the stroma of the mucous and in lumen of some glands.

Fig. 3–A shows that neutron 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 ex-

posed 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 external γ –radiation and internal ⁵⁶Mn radiation, inhalation of not activated 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–B).

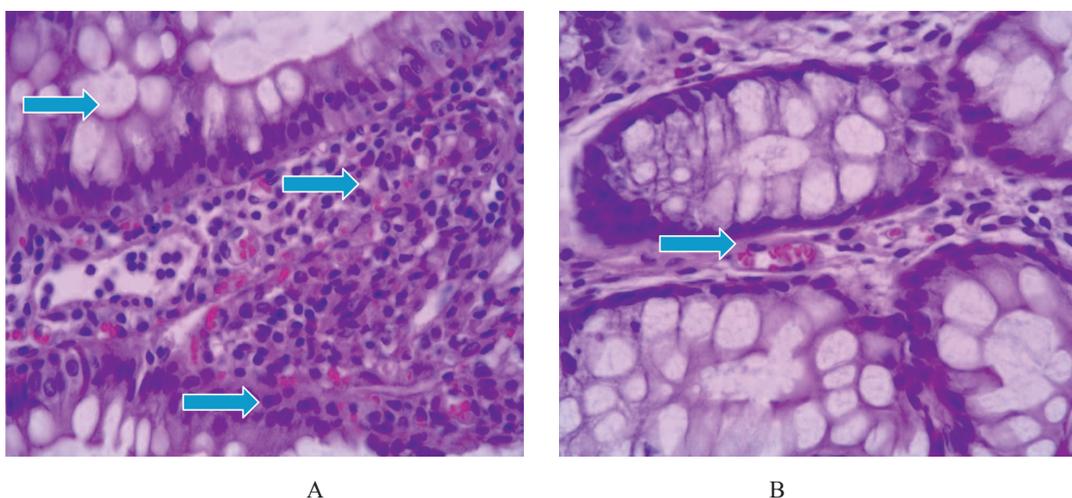


Fig. 2. Histologic sections of the small intestine in ⁵⁶Mn–exposed rat (A) and MnO₂–inhaled rat (B). H&E staining, original magnification $\times 10$ and $\times 40$

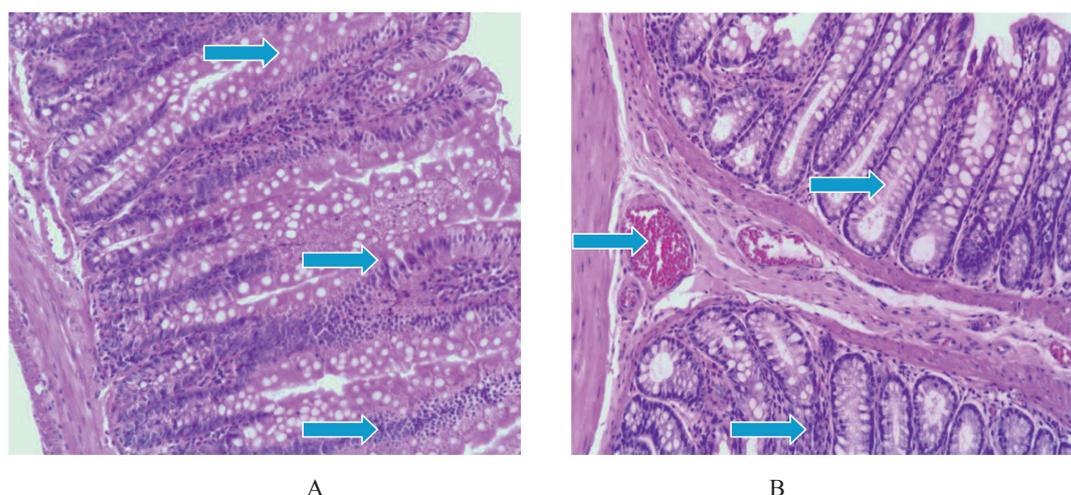


Fig. 3. Light microscopy of ⁵⁶Mn–induced (A) and γ –ray–induced rat small intestine (B) H&E staining, original magnification $\times 10$

Group	Pathological process	Day 3	Day 14	Day 60
⁵⁶ Mn	Inflammation	2 (+~++)	3 (++)	3 (++)
	Degeneration	3 (+~++)	3 (+~++)	2 (+~++)
	Necrosis			2 (+~++)
MnO2	Inflammation	3 (++)	2 (+~++)	2 (+)
	Degeneration		2 (+) ¹	2 (+)
	Necrosis			
⁶⁰ Co	Inflammation	2 (+~++)	2 (++)	3 (++)
	Degeneration	2 (+~++)	2 (+~++)	2 (+~++)
	Necrosis			2 (+~++)
Control	Inflammation			
	Degeneration			
	Necrosis			

Note. 1 – Incidence and pathological grades (in parenthesis).

The above data are consistent with our study results the small intestine in both ⁵⁶Mn– and ⁶⁰Co–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 ⁵⁶Mn group, indicating that neutron radiation has a significant biologic effect on examined organ (Table).

In this study, we have shown the sequence of morphologic changes in the rat small intestine from early to late stage after a single influence of ⁵⁶MnO2 and ⁶⁰Co at 2 Gy 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.

Currently, great importance is attached to the role of Mn, inducing cell death. Experimentally confirmed that a certain percentage of Mn enters to organism through absorption in the GI tract. If Mn not absorbed in the stomach, it is rapidly absorbed in the small intestine. Thus, in the literature we have examined papers revealed regarding ability of Mn to cause histomorphologic changes in the small intestine of animals [6].

In segment–radiated rats, the radiated intestinal segment was still identifiable by H&E staining at day 14 post–radiation and did not 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, which is characterized by the loss of intestinal structural integrity, at day 14 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 ⁵⁶Mn 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 is known that mechanisms of injury in normal tissues after irradiation include progenitor cell depletion, microvascular injury, inflammation and cell death [5]. The major pathological change caused by RIII is architectural disorganization, including inflammatory cell infiltration, villitis, desquamation and necrosis [7]. Several evidences suggest that radiation–induced dysfunctions and either changes in subcellular, cellular, and histological structure of the small intestine are mediated by concerted and interrelated changes of a plethora of various extracellular mediators and their intracellular messengers [8]. 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 associated with radiation–induced apoptosis [2].

Although whole–body radiation doses from ⁵⁶Mn were relatively low, higher internal doses were noted in the small intestine, in addition to significant pathological changes that were more severe and prolonged than the effects of ⁶⁰Co γ –irradiation. It should be noted that the most prominent microscopic changes were detected in ⁵⁶Mn–exposed rats after 2 weeks.

These data may indicate the potential for a high risk of internal exposure to ^{56}Mn , which would have existed in airborne dust after A-bomb explosions in Hiroshima and Nagasaki.

Summing up, whole-body radiation can cause severe damage to the GI tract, 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 γ - and neutron radiation make it possible to develop diagnostic criteria for assessing of radiation effect on the small intestine, depending on cumulative dose.

Conclusion

In 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 $^{56}\text{MnO}_2$ compared to rats from MnO_2 and ^{60}Co groups. Our research results and their comparison with literature data led to the conclusion that majority of experimental animals exposed to γ - and neutron radiation more pronounced changes were observed on the 60th day after exposure, consisting the appearance of chronic inflammation, signs of degeneration and foci of necrosis, whereas in MnO_2 -inhaled rats dominated degenerative changes. Consequently, like γ -rays, $^{56}\text{MnO}_2$ also promotes activation of inflammatory processes and stimulation of immune responses manifested by cellular infiltration.

Thus, our data obtained from in vivo experiments provide strong evidence that neutron radiation causes formation of morphologic features which typically for radiation enteritis, that is a form of small intestinal injury, depending on both dose and type of radiation.

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