Morphological changes of rat jejunum after whole body $\gamma$-irradiation and their impact in biodosimetry

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**Short title:** radiation-induced enteritis
Summary

Introduction: Gastrointestinal form is the second stage of the Acute Radiation Syndrome (ARS) with a threshold dose of 8 Gy. It represents an absolutely lethal clinical-pathological unit, enteritis necro-haemorrhagica (resp. duodenitis, jejunitis, ileitis) with unknown causal therapy.

Objectives: The purpose of our study has been to evaluate the morphological changes in a model of radiation-induced enteritis in rats and estimate the significance of changes in biodosimetry.

Materials and Methods: The laboratory rats were randomly divided into 21 groups, 10 animals per group. Samples of jejunum were taken 24, 48, 72, and 96 hours after the whole body γ-irradiation with the doses of 1, 5, 10, 15, and 20 Gy, and routinely stained with haematoxilin and eosin. Five morphometric markers - intercryptal distance, enterocytal height on the top and base of villus, length of basal lamina of 10 enterocytes and enterocytal width - in irradiated rat jejunum were examined by means of microscope. The results were compared with sham-irradiated control group.

Results: After lethal doses of irradiation, all morphometric parameters of jejunum significantly changed. With the exception of intercryptal distance, they might be considered as suitable biodosimetric markers under experimental condition.

Conclusion: Our results of morphometry of radiation-induced jejunitis are in concordancy with those in another studies. We firstly quantified morphological post-radiation changes in jejunum in the animal trial. We suggest that some of them might be used under experimental condition. Experimental study is a predecessor of any clinical assessment of a specific marker. Under clinical practice, the sensitive biodosimetric parameter would serve as one of the guidances of evaluation of the
absorbed dose in irradiated troops as well as rescue workers. This is in concordancy
with tasks and Standardization Agreement of the North Atlantic Treaty Organization.

Key words: Radiation-induced enteritis; Threshold dose; Biodosimetric marker.
**Introduction**

Large devastating effects of ionizing radiation on cell renewal systems, e.g. intestinal epithelium, have been known for more than 100 years (Regaud *et al.* 1912, Walsh 1897). Radiation-induced enteritis is an absolutely lethal clinical-pathological syndrome whose effective causal therapy remains unknown. Target radiosensitive cells are represented by hitherto immuno-histochemically undefined enterocytal stem cells localised on the bottom of the crypts of Lieberkühn. Ascending from here the cells lose the proliferative property and mature into differentiated enterocytes, which cover the top of the villus (Classen *et al.* 1998). Under physiological condition, the matured enterocytes are successively eliminated into the intestinal lumen and replaced by the new cells from the crypt. In 5-8 days after irradiation with the dose of 8 Gy and higher, the inflammatory cascade, denudement of epithelium, and other morphological changes of the intestinal surface develop – see Figures 1 and 2. The damaged intestinal epithelium loses its functions and septic shock becomes the most common cause of death (Brennan *et al.* 1998, Buell and Harding 1989, Fajardo 1982, MacNaughton 2000).

However, the quantification of radiation-induced enteropathy has not been described yet. The objective of our study was to evaluate via standard procedure of radiation-induced enteritis the morphological changes in rat jejunum after whole body γ-irradiation with the doses of 1, 5, 10, 15, and 20 Gy in 24, 48, 72, and 96 hours after exposure. Secondly, to estimate the significance of morphological changes in biodosimetry.

Authors chose the jejunal epithelium because that due to its anatomy and physiology enables more detectable cell kinetics and minute description of enterocytes than other parts of gastro-intestinal tract. The second reason for having chosen jejunum
was that it represents a very dynamical part of gastro-intestinal tract with an active enzymatic secretion. In sequence of this study, the assessment of changes of enzymatic secretion depending on ionizing radiation will be examined, which is impossible in other parts of gastro-intestinal tract.

**Material and methods**

A total of 210 adult male Whistar rats aged 12-16 weeks weighing 250-300 g were randomly allocated to 21 groups. Under anaesthesia with thiopental, the animals were irradiated with the whole body γ-rays of $^{60}$Co unit (Chisotron Chirana) at a dose rate of 1.0 Gy/min at a distance of 100 cm from the skin with the doses of 1, 5, 10, 15, and 20 Gy. The animals were killed by cervical dislocation under the ether anaesthesia 24, 48, 72, and 96 hours after irradiation. Sham-irradiated control group was sacrificed after 72 hours. All the procedures involving animals were approved by the Ethics Committee of Faculty of Military Health Sciences in Hradec Králové.

**Histological examination**

Samples of proximal jejunum (approximately 2 cm aborally from duodeno-jejunal flexure) were extirpated and routinely processed for microscopical examination. The samples were fixed with a 10 % neutral buffered formalin up to 24 hours and embedded into paraffin. The one µm thick tissue sections of jejunum were stained with haematoxylin and eosin. Samples of each animal were stained with Gram’s staining for excluding bacterial invasion, too.

**Measurement of morphometric parametres**
Stained samples were evaluated using a BX-51 microscope (Olympus Company, Prague, Czech Republic). Under the microscope, the intercryptal distance (magnified 200-fold), height of enterocyte on the top and at the base of villus, length of basal lamina of 10 enterocytes and width of enterocyte (magnified 800-fold) were measured. Ten measurements were performed per one animal, so we had 100 measurements per one group. All values (mean ± 2 x standard error of mean, S.E.M.) were calculated in micrometers (µm).

Data processing
The Mann-Whitney test was used for the statistical analysis, giving mean ± 2 x S.E.M., that express 95 % confidence interval.

Results
1. Intercryptal distance in irradiated jejunum
The results of intercryptal distance in rat jejunum are shown in Figure 3. In comparison with non-irradiated animals, the values of intercryptal distance in rats 72 hours after irradiation with the dose of 10 Gy and in all animals irradiated with the doses of 15 and 20 Gy were significantly higher with maximum 96 hours after irradiation with the dose of 20 Gy.

A significantly lower value was found in rats irradiated with the dose of 1 Gy after 72 hours.

2. Enterocytes’ height on the top of villus in jejunum
The results of enterocytes’ height on the top of villus in rat jejunum are shown in Figure 4. In comparison with non-irradiated animals, the values of enterocytes’ height
were significantly lower in rats examined 24-48 hours after irradiation with the dose of 5 Gy and in all time intervals in all animals irradiated with the dose of 10 Gy and higher with maximum decrease observed at the time interval of 96 hours after irradiation with the dose of 20 Gy. The dose-related diminishing of the enterocytes’ height was observed 48-96 hours after irradiation with the doses of 5-20 Gy.

3. Enterocytes’ height on the base of villus in jejunum
The results of enterocytes’ height on the base of villus in rat jejunum are shown in Figure 5. In comparison with non-irradiated animals, the values of enterocytes’ height were significantly lower in rats examined 24-48 hours after irradiation with the dose of 1 Gy, 24 hours after irradiation with the dose of 5 Gy, in all time intervals after irradiation with the dose of 10 Gy and higher, and 48-96 hours after irradiation with the dose of 15 Gy. Maximum decrease was observed at the time interval of 96 hours after irradiation with the dose 20 Gy. From the dose of 15 Gy and higher, the time-related decrease of enterocytes’ height was observed.

4. Length of basal lamina of 10 enterocytes in jejunum
The results of length of basal lamina of 10 enterocytes in rat jejunum are shown in Figure 6. In comparison with non-irradiated animals, the values of basal lamina were significantly higher in rats examined 48 hours after irradiation with the dose of 5 Gy, and in all time intervals in all rats irradiated with the dose of 10 Gy and higher. Maximum increase was measured at the time interval of 96 hours after irradiation with the dose of 20 Gy. The value measured at the time interval of 72 hours after irradiation with the dose of 1 Gy was significantly lower.
5. Enterocytes’ width in jejunum

The results of the enterocytes’ width in rat jejunum are shown in Figure 7. The changes of the enterocytes’ width in rat jejunum are very similar to those of the previous parameter. In comparison with non-irradiated animals, the values of the enterocytes’ width were significantly higher in rats examined 48 hours after irradiation with the dose of 5 Gy, 48-96 hours after irradiation with the doses of 10 and 15 Gy and at all time intervals after irradiation with the dose of 20 Gy. A maximum increase was measured at the time interval of 96 hours after irradiation with the dose of 20 Gy. The value measured at the time interval of 24 and 72 hours after irradiation with the dose of 1 Gy was significantly lower.

Discussion

Fajardo described a flattening of villus in developed radiation-induced intestine injury (Fajardo 1982). In our animal experiment, irradiation with sublethal doses did not influence the intercryptal distance in rat jejunum. After irradiation with lethal doses, the intercryptal distance increased in most groups proportionally with the dose. Besides the influence of reparating mechanisms, there is an enormous physiological variability in morphology of villus, therefore the intercryptal distance cannot be considered as a suitable biodosimetric parameter.

According to Fajardo, the main microscopic sign of radiation-induced intestine injury is flattening of enterocytes on the surface of villi (Fajardo 1982). In our laboratory trial, we recognised that irradiation with sublethal (subthreshold) doses does not significantly change the height of the enterocyte both on the top and on the base of the villus. The early decrease of height of the enterocyte on the top of the villus irradiated with the dose of 5 Gy is completely compensated in 3 days. On the
contrary, the early decreasing height of the enterocyte on the base of the villus irradiated with the dose of 5 Gy is completely compensated in 2 days. Even after irradiation with a supralethal dose of 10 Gy, the height of the enterocyte on the base of the villus is temporarily compensated in 2 days, then significantly decreased. After irradiation with supralethal doses, the height of the enterocyte on the top of the villus significantly decreased. The height of the enterocyte on the base of the villus, with the exception of temporarily compensation 48 hours after irradiation with the dose of 10 Gy, significantly decreased, too. We suppose that the basal enterocytes attain higher and more rapid property of compensation (repopulation) than the enterocytes on the top of villus.

The height of the enterocyte is dependent on the dose of irradiation and might be considered as a very sensitive and suitable biodosimetric marker after irradiation with the dose of 10 Gy and higher. Besides the number of crypts on the transverse section of jejunum which is supposed as a fundamental biodosimetric marker (Österreicher et al. 2005, Potten 2004), probably, the height of enterocyte might be another useful parameter under experimental condition.

The flattening of enterocytes in intestinal radiation-induced pathology is a compensatory mechanism enabling lower amount of cells to cover a larger surface of villus (Fajardo 1982). According to our results, irradiation with sublethal doses does not influence the length of basal lamina of 10 enterocytes. Forty-eight hours after irradiation with the dose of 5 Gy the length became significantly longer, but by the third day the changes were compensated by repopulation of cells. After irradiation with supralethal doses, the length of basal lamina of 10 enterocytes significantly and dose-dependently increased. Tendency towards proliferation in the crypts retards the
developing intestinal damage, while later and after the higher doses the reparative mechanism of repopulation is insufficient.

Similarly, the width of one enterocyte increased after irradiation with supralethal doses. In comparison with the width of one enterocyte, the length of basal lamina for 10 enterocytes partially equilibrates the intercellular and intracellular variability and is more accurate. Moreover, the length of basal lamina of 10 enterocytes in all measurements was shorter than 10 fold of one enterocyte’s width. We suppose that it might be a good marker of radiation-induced intestine injury under experimental condition.

Experimental study is a predecessor of any clinical assessment of a specific marker. In clinical practice and, especially, under military field condition, the suitable biodosimetric parameter would serve as one of the guidances of evaluation of the absorbed dose in irradiated troops as well as rescue workers. This is in concordancy with tasks and Standardization Agreement of the North Atlantic Treaty Organization (Stanag 2002). Based on our other and hitherto unpublished experiments, biodosimetric markers detectable in proliferating cells cannot be used in non-proliferating cells (e.g. native lymphocytes). For clinical practice, the buccal or anal epithelium seems to be more accessible proliferating cell populations of gastro-intestinal tract that might reveal the gravity of radiation-induced damage.

**Conclusion**

In our animal experiment, the large morphological changes in small intestine mucosa develop rapidly and progressively during the first 4 days after irradiation with supralethal doses. The threshold dose for appearance and development of radiation-induced enteropathy in rats falls between 5 and 10 Gy. Thus the threshold dose in
rats seems to be very similar to that in human, as well as the course of disease resembles the clinical manifestation in patients. The threshold dose for radiation-induced enteritis is lower than 10 Gy, which agrees with data listed in documents of the North Atlantic Treaty Organization (Stanag 2002).

In the laboratory trial, we firstly quantified morphological post-radiation changes in jejunum (Fajardo described these morphological changes, however, he did not quantify them). With the exception of intercryptal distance, they might be considered as suitable biodosimetric markers under experimental condition.
References:


STANAG 2374: NATO Standardization Agreement No. 2374 (Allied Medical Publication No. 6), NATO Standardization Agency, 2002, pp 120.

Figure 1. Sham-irradiated jejunum, 800-fold, HE

Figure 2. Jejunum 72 hours after irradiation with the dose of 20 Gy, 800-fold, HE
Figure 3. Intercryptal distance in jejunum in µm

Figure 4. Height of enterocyte on the top of villus in µm
Figure 5. Height of enterocyte on the base of villus in µm

Figure 6. Length of basal lamina of 10 enterocytes in µm
Figure 7. Width of one enterocyte in µm