# Effect of whole body proton or gamma irradiation on genetic damage and hematological variables

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## 1. Introduction

For the purpose of cancer therapy or spaceflight with mission or simple trip, a considerable concern about the absorbed amount of radiation and its deleterious effect on physiological system, if any, has been increased. Many efforts have been dedicated to estimate the risk, however, there is very little known about the spectrum of radiations during the flight through arctic zone as well as the effects of low-dose radiation. Here, we aimed to evaluate the effect of proton or gamma-irradiation at a recommended dose limit of occupational (20mGy per year) and the standardized radio-therapeutic fraction dose (2Gy) on gastro-intestinal damages, peripheral hematology, and the frequency of micronuclei formation.

## 2. Materials and Methods

### *2.1 Animals*

Six-week old, female C57BL/6 mice (n = 25) were purchased from Daehan biolink Co. (Daejeon, Korea) and acclimatized for 1 wk under the free access to food (NIH-7 open formula) and water. The Institutional Animal Care and Use Committee approved all procedures.

### *2.2 External beam irradiation*

Total-body proton irradiation was performed using 30 MeV protons from the accelerator housed at the Korea Institute of Radiological and Medical Sciences. The mice were exposed to the entrance plateau region of the proton beam, at the beginning of the Bragg Peak. Dose calibration was performed at depths corresponding to the center of the mice. Two groups of animals received total dose of 20 mGy or 2 Gy. Other additional groups of mice were irradiated with 20 mGy or 2 Gy γ-rays (single fraction) using $^{60}$Co theraton-780 (Atomic Energy of Canada, Ltd., Canada). A control group was not irradiated. Before irradiation, the mice were not anesthetized and placed into rectangular polystyrene boxes with air hole (25x25x5 cm).

### *2.3 Analysis of cells in peripheral blood*

The Blood was collected by retro-orbital bleed into K2EDTA tubes 24 h after irradiation and analyzed for hematological parameters using a Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan).

### *2.4 Micronucleus assay*

The animals were killed by cervical dislocation at 24 h after irradiation. The bone marrow cells were isolated from both femurs of each mouse and treated cytochalasin B (3μg/ml) to inhibit cytokinesis. The cells were further incubated for 3 h and then harvested onto glass slides using cytocentrifuge, air-dried, and fixed with methanol: glacial acetic acid (3:1 v/v). The slides were stained with a 4% Giemsa solution, and analyzed under a light microscope at a magnification of X 1,000. The micronuclei were scored when their diameter was <1/3 of the diameter of the main nucleus. The number of micronuclei in 200 binucleated cells per mouse was determined.

### *2.5 Terminal deoxynucleotide transferase labeling (TUNEL)*

Apoptosis identification was performed using the Apoptag method (Oncor, Gaithersberg, MD, USA) optimized for specific detection of apoptosis in intestinal epithelium [1]. After rehydration through an ethanol series, sections were permeabilized with proteinase K (20 mg/ml in PBS) for 20 min at room temperature and then washed in PBS. Two drops of equilibration buffer were applied to each section for 10 min at room temperature. The terminal deoxynucleotide transferase mixture (Apoptag) was prepared as directed, but then diluted 1: 2 with distilled water to reduce non-specific background staining. Slides were incubated with the enzyme mixture for 1 h at 37℃ before the reaction was terminated by washing sections for 10 min in the 'stop wash' solution provided. Digoxigenin-labelled dNTPs were treated with antidigoxigenin–peroxidase for 1 h at room temperature. Sections were washed in PBS, developed with DAB, and counter-stained with hematoxylin. Starting at the base of the crypt column, the cells were numbered up each side and the cell positions containing apoptotic fragments or immunopositive cells were recorded. For each experimental group, 40 crypts per animal were scored (200 crypts in total) and a distribution of apoptotic cells for each position was obtained.

## 3. Results

*3.1 Effect of radiation on peripheral blood cells*

During the process of the experiment, the body weight of animals has not changed before and after irradiation. The low dose of radiation at 20mGy did not cause any damage in the number of peripheral blood cells whatever the type of radiation. However, the therapeutic dose of radiation significantly decreased the number of white blood cells, especially in lymphocytes and neutrophils, and the degree of damages was slightly increased with $^{60}$Co-radiation exposure. The number of RBCs or platelets of irradiated mice exhibited no differences compared to un-irradiated control values.

*3.2 Apoptosis in intestinal crypts*

Because TUNEL staining of intestinal crypts conferred generally more convincing results for apoptosis than morphological scoring of H&E staining, it has been frequently used to detect apoptosis in gastrointestinal tissue. The apoptosis of intestinal crypts was markedly increased by the therapeutic dose of radiation, and the magnitude of apoptosis in gamma irradiated mice was severer than that of proton. However, the statistical significance was not detected in low-dose of radiation even though the incidence of increasing apoptosis was clearly shown.

*3.3 Micronuclei in the bone marrow cells*

The micronucleus assay is one of the most widely used in vivo screening tests for genotoxicity [2]. An increase of the frequency of micronuclei in the cytoplasm in interphase cells is an indication of induced chromosome damage [3]. The low dose gamma-ray or proton-induced frequency of micronuclei was represented the mean values from the pooled data of several experiment, showing no significant difference. In well accordance with above results, the therapeutic dose of radiation increased the frequency of micronuclei in bone marrow cells.

## 4. Conclusions

The aims of this study were to investigate the damages by different sources of radiation (proton vs. gamma radiation) and the doses of which one can be hardly detected the deleterious damage and the other is widely used as a standardized radiotherapy. As expected, exposure of low dose radiation has not shown any distinctive changes on gastrointestinal damages and hematological variables whatever the source of radiation is proton or gamma ray. However, it is worth to note that those results did not mean that this low dose of radiation might be safe and no harmful for living organisms.

The therapeutic dose of radiation showed significant changes on the parameters evaluated in the present study. The gamma ray caused severer damages than proton even the same total dose of radiation, suggesting that the physiological effects were dependent on the characteristic of the source of radiation.

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