DNA Damage in Melania Snail (*Semisulcospira libertine*) Irradiated with Gamma Radiation

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1. Introduction

Generally radiological protection has focused on human [1]. But International Commission on Radiological Protection (ICRP) [2] requires the effect data of ionizing radiation on nonhuman biota for the radiological protection of the environment [3]. The choice of a melania snail as a model for environmental biomonitoring of radiation genotoxicity took into account that invertebrates represent one of aquatic species [4].

The comet assay or single cell gel electrophoresis (SCGE) assay, first introduced by Östling and Johanson [5], was used to detect DNA single strand breaks and to investigate the application of this technique as a tool for aquatic biomonitoring. Comet assay offers considerable advantages over some other assays used in DNA damage detection, such as chromosomal aberrations, sister chromatid Exchange and the micronucleus test, since there is no need for cells to be in a dividing state. Other advantages are its rapidity, relatively low coast, and wide applicability to virtually any nucleated cell type [6].

In this study, we evaluated DNA damage in cells of *Semisulcospira libertina* after irradiation with ^{60}Co gamma radiation by using the comet assay.

2. Materials and Methods

2.1 Animals

Melania snails (*S. libertina*), 15-20 mm in length, were collected in July 2010 from an uncontaminated site in the Churyeong stream, Ssangchi-myon, Sunchang-gun. In the laboratory, the animals were maintained in plastic aquaria in a flow of dechlorinated, well-aerated water at 23±2 ℃.

2.2 Exposure

A group of three melania snails was transferred to plastic conical tube with a small amount of water and then irradiated with 5, 10, 20, 50, 100, 200 and 400 Gy. One negative non-exposed control group was maintained under the same conditions as the exposed group. Gamma radiation was provided by a ^{60}Co source at the Korea Atomic Energy Research Institute.

2.3 Harvesting of cells

Each animal was wiped and placed on a plastic petridish to which a phosphate-buffered saline (PBS) was added. To obtain a cell suspension, individual feet were excised and then chopped, using clean razor blade. The cell was placed directly into 1.5 ml Eppendorf tubes, all on ice. Thereafter the cells were collected by centrifugation for 5 min at 1,593 x g. Supernatant was discarded and cell pellets were resuspended in 100 μl cold PBS.

2.4 Comet assay

The comet assay was performed under alkaline conditions following Singh et al. [7] procedure, with modifications. A volume of 20 μl of cell suspension mixed in 200 μl of 0.5% low melting-point agarose dissolved in PBS were spread onto fully frosted microscope slides pre-coated with 1% normal melting-point agarose. The third layer of 0.5% LMA agarose was added and solidified. The cells were then lysed for 2 hr at 4 ℃ in lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO, pH 10.0). After lysis, slides were placed in an electrophoresis tank and the DNA was allowed to unwind for 20 min in the electrophoresis buffer (300 mM MaOH and 1 mM EDTA, pH > 13.0). Electrophoresis (25 V, 300 mA, 25 min) was performed in the same buffer. Slides were washed twice during 5 min in neutralization buffer (0.4 M Tris, pH 7.5) before debydration in absolute ethanol for 10 min. The slides were dried in darkness and stained with 100 μl ethidium bromide (20 μg/ml). The comets were analyzed using an image analysis system (Komet 4.0 from Kinetic Imaging Ltd, Liverpool, UK). Fifty cells per slide were scored out of a total of 100 cells per dose.

2.5 Statistical analysis

The statistical tests are performed using the Version 7.5 of OriginPro. The statistical significance tests ($p < 0.05$) between the exposure groups were

determined by one way analysis of variance (one-way ANOVA).

3. Results

DNA damage as measured by the comet assay has been linked to exposure to a wide spectrum of genotoxic and cytotoxic compounds, as well as to physical agents like sunlight and radioactivity [8]. The level of DNA damage in the melania snails was shown as the olive tail moment. The DNA tail moment is defined that both the length of tail and the intensity of the DNA in the tail [8]. The results of DNA damage induced by ionizing radiation in *S. libertine* were shown in Fig. 1.

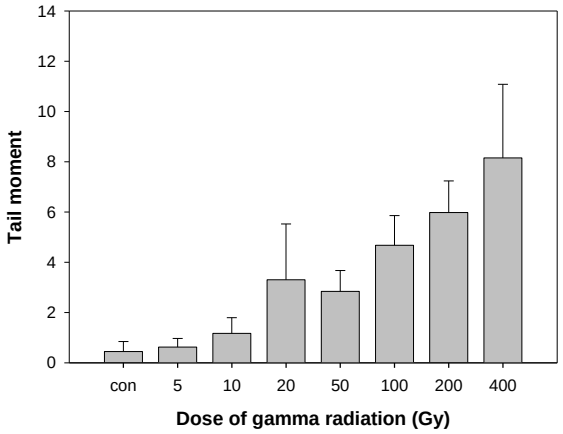

Fig. 1. Results of the comet assay expressed in terms of the median tail moment of DNA in the comet tail in the feet cell suspensions isolated from the melania snails after gamma irradiation (5 to 400 Gy). Olive Tail Moment = (Tail mean – head mean) X tail % DNA / 100. Data are expressed as mean ± S.D.

At low doses of radiation a number of cells remained unchanged, but at higher doses most of the cells showed moderate or strong DNA damage (Fig. 1). Analysis of comet assay showed differences in the tail length dependng on the radiation dose. Genotoxic effect of ionizing radiation was detected at all doses as a dose-related increase in DNA migration.

4. Conclusions

These results therefore suggest that the melania snails may be useful indicator organisms to assess the genotoxic risks of ionizing radiation to terrestrial environments and that the comet assay is a useful tool to use as biomarker of genotoxic effects on invertebrates in water. Also, this study may be useful for indicating the toxicity levels at which physical agents and variety of chemical compounds can damage the DNA of living cells.

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