DNA Repair Inhibition by Mercuric Chloride in Earthworms after Exposure to Radiation

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1. Introduction

All organisms are being exposed to harmful factors present in the environment. Ionizing radiation can damage DNA through a series of molecular events depending on the radiation energy. The biological effects due to the combined action of ionizing radiation with the other factor are hard to estimate and predict in advance [1]. Recently International Commission on Radiological Protection (ICRP) requires the effect data of ionizing radiation on non-human biota for the radiological protection of the environment [2]. Earthworms have been identified by the ICRP as one of the reference animals and plants to be used in environmental radiation protection [3]. Particularly, the earthworm *Eisenia fetida* can be used as a bio-indicator of pollution in soil. This study was performed to investigate the acute genotoxic effects of radiation and the synergistic effects between radiation and mercury in earthworm, *E. fetida*.

2. Materials and Methods

2.1 Test Animal

The species of *E. fetida* belongs to the taxa of phylum *annelida* and class *clitellata*, and is hermaphrodite and fertilizes its eggs inside a cocoon secreted by the clitella [4]. These worms live in the upper layer of the soils containing rotting vegetation, compost, and manure. *E. fetida* is native to Europe and found on every continent, except for Antarctica. Adult *E. fetida* with sexually matured and well-developed clitellum (average weight, 350 mg) was used for this experiment. Earthworms were maintained in dark in a 6:3:1 mixture of clean soil (gardening soil, Sanglim Co., Ltd., Korea), rice bran and cattle manure at 23 ± 2℃. The moisture content was adjusted to 65 ± 5% of the final weight with dechlorinated water.

2.2 Exposure

Experiments were done to identify the levels of DNA damage and the repair kinetics in the coelomocytes of *E. fetida* irradiated with ionizing radiation alone or with gamma rays after $HgCl_2$ treatment by means of the single cell gel electrophoresis assay. Mercuric chloride was mixed to artificial soil for final treatment concentrations of 40 mg of $HgCl_2$ per soil weight (kg^{-1}). The worms were exposed to these soils for a period of 48 hrs in the climate-controlled room. After $HgCl_2$ exposure test, the worms were transferred to a plastic Petri dish with moist filter paper and then acutely irradiated with 2.5, 5, 10, 20 Gy gamma radiation, respectively. External gamma radiation was provided by a ^{60}Co source (7.4 PBq, Korea Atomic Energy Research Institute, Korea).

2.3 Single cell gel electrophoresis (SCGE) assay

E. fetida coelomocytes were obtained by simple non-invasive technique described by Eyambe *et al.* (1991) [5] with slight modification. The SCGE assay was performed under alkaline conditions following Singh *et al.* [6] 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 NaOH 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 dehydration in absolute ethanol for 10 min. The slides were dried in darkness and stained with 100 μl ethidium bromide (20 g/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.

3. Results and Conclusions

The Olive tail moments (OTMs) were measured during 0 ~ 96 hours after irradiation. The results showed that the increase in DNA damage was depending on the dose of radiation. The more the oxidative stress was induced by radiation, the longer the repair time was required (Fig. 1).

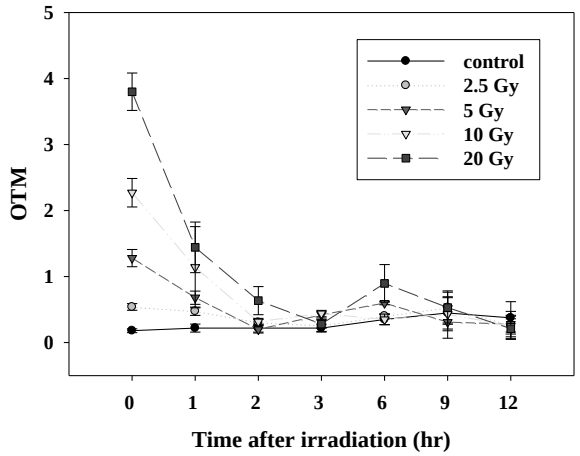

Fig. 1. DNA damage and repair kinetics in *E. fetida* irradiated with γ-rays. Figure shows average Olive tail moment (OTM). OTM = (tail mean - head mean) × tail%DNA / 100. Data are expressed as mean ± S.D.

When combination of $HgCl_2$ and ionizing radiation was applied, the OTMs were much higher than those treated with radiation alone, which indicated genotoxic effect, was increased after combined treatment of radiation and mercury (Fig. 2).

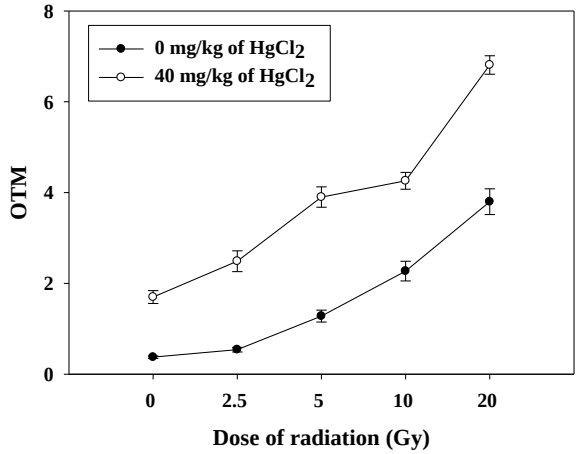

Fig. 2. DNA damage in coelomocytes of *E. fetida* irradiated with γ-rays (0, 2.5, 5, 10 and 20 Gy) after the treatments of $HgCl_2$ (0 and 40 mg/kg) for 48 hrs. Figure shows average Olive tail moment (OTM). OTM = (tail mean - head mean) × tail%DNA / 100. Data are expressed as mean ± S.D.

Earthworms were treated with 20 Gy gamma rays alone or with ionizing radiation combined with 40 mg/kg $HgCl_2$. The repair time in the animals treated with the combination of $HgCl_2$ and ionizing radiation was nearly five times longer than that in the animals treated with ionizing radiation alone. The results suggest that the mercury could even have deleterious effects on the DNA repair system. Therefore, influence of mercury on the DNA repair mechanisms is confirmed by this study.

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