Combined Action of Radiation and Mercury on DNA Damage and Repair in Coelomocytes of Earthworms

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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. 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. Earthworms have been identified by the ICRP as one of the reference animals and plants to be used in environmental radiation protection. 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*.

MATERIALS & 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. 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

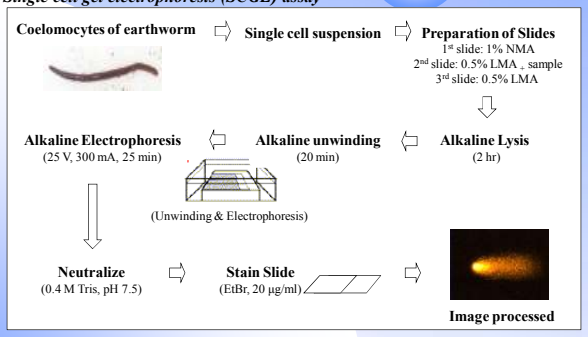

Fig. 1. Schematic diagram for the SCGE assay.

RESULTS

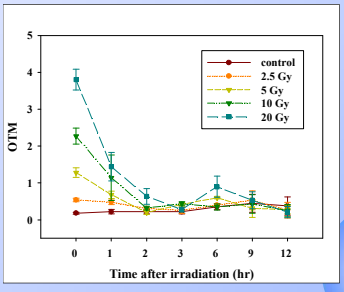

Fig. 2. DNA damage and repair kinetics in *E. fetida* irradiated with γ-rays. Figure shows average Olive tail moment (OTM). OTM = (tail mean - head mean) × tail%DNA / 100. Data are expressed as mean ± S.D.

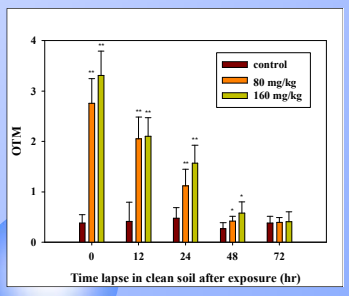

Fig. 3. DNA damage in coelomocytes of *E. fetida* after exposure to mercury chloride (0, 80 and 160 ㎎/㎏). Significant differences from the controls are indicated (*P < 0.005; **P < 0.001).

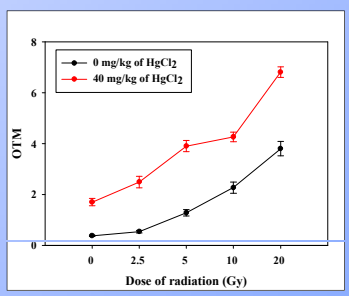

Fig. 4. DNA damage in coelomocytes of *E. fetida* irradiated with γ-rays (0, 2.5, 5, 10 and 20 Gy) after the treatments of $HgCl_2$ (0 and 40 mg/kg) for 48 hrs. Figure shows average Olive tail moment (OTM). OTM = (tail mean - head mean) × tail%DNA / 100. Data are expressed as mean ± S.D.

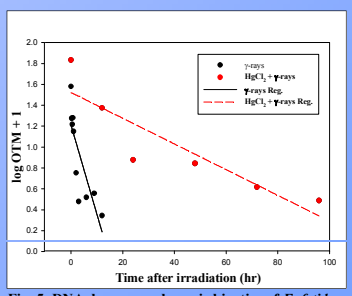

Fig. 5. DNA damage and repair kinetics of *E. fetida* irradiated with γ-rays (20 Gy), with or without presence of $HgCl_2$ (40 mg/kg).

- ❖ 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.
- ❖ 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.
- ❖ The repair time in the animals exposed to $HgCl_2$ and radiation in combination was nearly five times longer than that in the animals treated with radiation alone.

CONCLUSIONS

❖As confirmed by our studies, mercury inhibits the repair of radiation-induced DNA damage, and synergistically exerts their genetoxic effect with radiation on DNA molecules of the cells. Synergism due to the combined action of deleterious factors, even in the low intensity or dose, should be taken into the risk assessment.

ACKNOWLEDGMENT

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