Aquatic Toxicology 150 (2014) 17–26

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Gamma radiation induces growth retardation, impaired egg production, and oxidative stress in the marine copepod *Paracyclopina nana*

Eun-Ji Won, Jae-Seong Lee*

Department of Biological Sciences, College of Natural Sciences, Sungkyunkwan University, Suwon 440-746, South Korea

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 16 February 2014
Accepted 18 February 2014
Available online 26 February 2014

Keywords:
Gamma radiation
Marine copepod
Oxidative stress
Growth retardation
Antioxidant mechanism
DNA repair

ABSTRACT

Accidental nuclear radioisotope release into the ocean from nuclear power plants is of concern due to ecological and health risks. In this study, we used the marine copepod *Paracyclopina nana* to examine the effects of radioisotopes on marine organisms upon gamma radiation, and to measure the effects on growth and fecundity, which affect population and community structure. Upon gamma radiation, mortality (LD50 – 96 h = 172 Gy) in *P. nana* was significantly increased in a dose-dependent manner in ovigerous *P. nana* females. For developmental impairment of gamma-irradiated nauplii, we observed growth retardation; in over 30 Gy-irradiated groups, offspring did not grow to adults. Particularly, over 50 Gy-irradiated ovigerous *P. nana* females did not have normal bilateral egg sacs, and their offspring did not develop normally to adulthood. Additionally, at over 30 Gy, we found dose-dependent increases in oxidative levels with elevated antioxidant enzyme activities and DNA repair activities. These findings indicate that gamma radiation can induce oxidative stress and DNA damage with growth retardation and impaired reproduction.

1. Introduction

Increased energy consumption worldwide causes an increase in the need to build nuclear power plants as an alternative option to generate electricity and heat. However, the increasing frequency of nuclear radiation accidents threatens human and environmental health. For example, in 1986, the Chernobyl accident clearly demonstrated the risk of nuclear power. After the Chernobyl accident, Krivolutzkii and Pokarzhevskii (1992) reported a dramatic decrease in earthworm number and population composition in a zone three kilometers from the reactor. The frequency of germline mutations in human minisatellite loci was doubled in children who were born in polluted areas compared to the control group after the Chernobyl accident (Dubrova et al., 1996). After the recent nuclear disaster in Fukushima, Japan in 2011, the accidental release of radioactive materials into the marine environment threatened a wide area. In fact, high radionuclides were measured in particles in the Korean atmosphere (Kim et al., 2012). Other countries were impacted after being exposed to nuclear contamination by consuming marine organisms such as seafood (Fisher et al., 2013).

Radionuclides are sources of ionizing radiation (IR) (Sobolev et al., 1994). IR such as α-, β-, and γ-rays, which are generated by the decaying process of high-energy states of atomic nuclei, induce adverse effects on organisms including growth reduction, DNA damage, and morphological changes (Daly and Thompson, 1975; Kovalchuk et al., 1999; Zaka et al., 2004). In particular, gamma radiation induces severe damage in diverse organisms at multiple levels from molecules to individuals (e.g. DNA, chromosomal aberrations, cells, proteins, growth and reproduction) (Aquino, 2012; Sudprasert et al., 2006; Rhee et al., 2012). Of them, double strand breaks (DSBs) of DNA are the most common symptom, leading to severe residual effects on irradiated organisms (Karran, 2000; Rhee et al., 2012, 2013). In human blood cells, Sudprasert et al. (2006) reported that gamma radiation induces chromosomal aberrations such as dicentrics and deletion of chromatids. In fish larvae, Rhee et al. (2012) found increased expression of DNA repair-related genes in gamma-irradiated *Kryptolebias marmoratus*, suggesting that IR strongly affects DNA, induces DNA instability, and recruits DNA repair mechanisms to recover the impaired DNAs. IR also produces free radicals in cells through Compton effects (Cassidy et al., 2007), increasing the levels of reactive oxygen species (ROS), which are highly damaging to cells (Riley, 1994). Aquino (2012) reported that gamma radiation interacts with water in cells, leading to the direct formation of ROS. However, organisms have

* Corresponding author. Tel.: +82 31 290 7011.
E-mail address: jslee2@skku.edu (J.-S. Lee).

http://dx.doi.org/10.1016/j.aquatox.2014.02.010
0166-445X/

innate defense mechanisms to protect cells from ROS attack (Regoli et al., 1997) that use several enzymes such as glutathione *S*-transferase (GST; EC 2.5.1.18), glutathione reductase (GR), catalase (CAT; EC 1.11.1.6), and superoxide dismutase (SOD; EC 1.15.1.1), and non-enzymatic molecules like glutathione (GSH). In DNA repair mechanisms, several key proteins including DNA-dependent protein kinase (DNA-PK), Ku70, and Ku80 act as DNA ligases to recover unequal DSBs caused by gamma radiation (Rhee et al., 2012); these genes are good indicators of DNA damage induced by gamma radiation. Agarwal et al. (2012) and Rhee et al. (2012) suggest that ROS induction is useful, along with other physiological parameters, in predicting the effects of environmental stressors on aquatic organisms (Regoli et al., 1997; Won et al., 2012). While many studies have been conducted in fish, in vivo and/or in vitro studies of the effects of gamma radiation-induced oxidative stress in aquatic microorganisms are incomplete (Grygoryev et al., 2013; Kuwahara et al., 2003; Mothersill et al., 2013; Mitani and Egami, 1982; Rhee et al., 2012; Smith et al., 2013).

Of marine invertebrates, copepods are the most abundant taxa and are a promising model species in ecology and ecotoxicology (Raisuddin et al., 2007). In addition, copepods play prominent roles in the food web as they link producers to consumers and play a key role as important food sources for higher trophic levels in the estuarine marine environment and aquatic culture systems (Lee et al., 2006; Pinto et al., 2001; Sun and Fleeger, 1995). Among them, the marine planktonic copepod *Paracyclopina nana* (Copepoda, Cyclopoida) is widely distributed in estuaries and shows high tolerance to a wide range of salinity and temperature fluctuations (Lee et al., 2006). *P. nana* are ideal for ecotoxicological studies, as they are of small size (<1 mm), have high fecundity, are easy to maintain and have a short life cycle of less than two weeks, allowing for easy observation of their life table parameters across several generations (Hwang et al., 2012; Lee et al., 2006, 2012). Moreover, a massive RNA-Seq database consisting of 67,179 contigs with a total assembled length of 96,966,561 bp (N50 = 2310 bp) was available from our recent study (unpublished) in *P. nana*.

To extend our knowledge to the toxicity of gamma radiation-induced oxidative stress, we examined in vivo effects on *P. nana*. In this paper, we analyzed several endpoints such as mortality, egg production, and growth rate after gamma irradiation at different doses. We also investigated oxidative stress-induced antioxidant enzymes with modulated gene expression to demarcate biochemical and molecular changes after gamma radiation. This will provide a better understanding of the molecular mechanistic defenses after gamma radiation in the copepod *P. nana*.

2. Materials and methods

2.1. Culture and maintenance of P. nana

The copepod *P. nana* was maintained under controlled incubator conditions with a 12 h light/12 h dark cycle at a temperature of 25 °C. The salinity of the culture medium was 15 psu with a pH of about 7.8–8.2. *P. nana* was fed a diet of green microalgae, *Tetraselmis suecica*, once every day. The copepod species identity was verified by morphological characteristics and sequence analysis of mitochondrial DNA cytochrome oxidase 1 (*CO1*) as the barcoding gene for animals (Ki et al., 2009).

2.2. Harvesting ovigerous P. nana females and nauplii and gamma radiation

To obtain the same developmental stages of ovigerous *P. nana* females, we used a two-generation culture. First, ovigerous *P. nana* females were gathered using 200 μm sieves and released into fresh medium. After 12 h, newly hatched nauplii were harvested by removing adults (150 μm sieve). Then the nauplii were raised for about two weeks to obtain ovigerous *P. nana* females of the same age, which were used to evaluate mortality and new egg production. To analyze physiological indices including antioxidant enzyme activities, non-enzymatic compounds, and molecular endpoints, we used only adults.

For gamma irradiation of copepods, we used a ^{137}Cs (Cesium) gamma ray source (2 Gy/min; Department of Radiation Oncology, College of Medicine, Hanyang University, Seoul, South Korea).

2.3. Lethal dose, growth retardation, and egg production

To determine the lethal dose (LD) of gamma rays to *P. nana*, we initially tested 12 different doses of gamma radiation (0–400 Gy). The LD50 was calculated 96 h after gamma irradiation (Finney, 1971) and the damage to the eggs was measured by counting the number of dropped egg sacs.

To measure the growth rate of *P. nana*, we used nauplii. For each dose, we irradiated *P. nana* (30 individuals total) to 0, 20, 30, and 50 Gy, then transferred them into 6-well plates (10 individuals/well), feeding them with the microalga *T. suecica* throughout the experiment. Growth rates were observed under a stereomicroscope every 24 h for two weeks. The developmental stages of *P. nana* were divided into five different stages: N1–N2, N3–N5, N6, C1–C3, and adult.

To evaluate respawning ability, we irradiated ovigerous *P. nana* females at six different doses (0, 10, 20, 30, 50, and 100 Gy). After 1 h of gamma radiation, approximately 10 individuals (5 individuals × 2) of *P. nana* were isolated from each of the irradiated groups to observe respawning ability at each dose. Egg sac formation was observed every 24 h for five days, and the requiring day to have new eggs was counted after first hatching. New egg production was calculated by percentage of fertile individuals among irradiated experimental groups at each dose.

2.4. Measurement of physiological indices-ROS, GSH, and antioxidant enzyme activities

To analyze physiological indices, we irradiated *P. nana* (approximately 200 adult individuals) to 0, 10, 20, 30, 50, and 100 Gy. ROS were measured as described in Kim et al. (2011). Briefly, samples were homogenized in buffer containing 0.32 mM sucrose, 20 mM HEPES, 1 mM $MgCl_2$, and 0.5 mM PMSF (pH 7.4). The supernatants obtained after centrifugation (10,000 × *g* for 20 min at 4 °C) were allowed to react with H_2DCFDA. The wavelengths for measurements were 485 nm for excitation and 520 nm for emission (Thermo Scientific Co., Varioscan Flash). The total sulfhydryl (—SH) group contents representing glutathione (GSH) were determined by an enzymatic method using BIOXYTEC GSH-420™ kit (OxisResearch®, Portland, OR, USA). Samples were homogenized in trichloroacetic acid and the upper aqueous layer was collected by centrifugation at 3000 × *g* for 10 min at 4 °C, then measured at an absorbance of 420 nm using a spectrophotometer (Ultrospec 2100 pro, Amersham Bioscience). The standard curves were generated using GSH equivalents (0, 150, and 350 μM).

We measured superoxide dismutase (SOD) activity using a SOD determination kit (Sigma–Aldrich, St. Louis, MO, USA). The samples for analysis were collected according to the manufacturer's protocol. The SOD activity was calculated by measuring the decrease in color development at 440 nm compared to the control. Glutathione reductase (GR) was measured by the BIOXYTEC® GR-340™ kit (OxisResearch®, Portland, OR, USA). The samples were homogenized using diluent buffer containing BSA/EDTA and the supernatants obtained after centrifugation were used for analysis. GR activity was measured indirectly by measuring the consumption