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Gamma radiation induces dose-dependent oxidative stress and transcriptional alterations in the freshwater crustacean *Daphnia magna*

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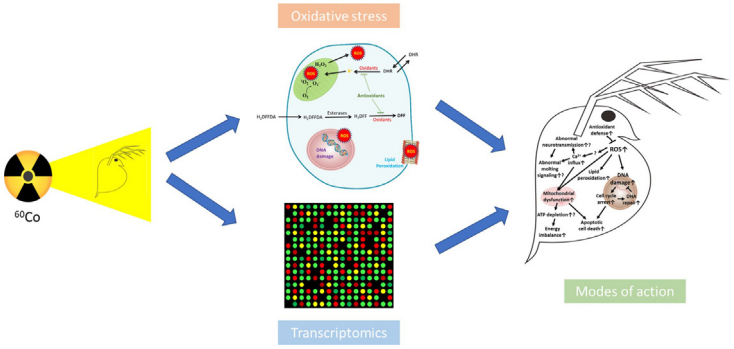
HIGHLIGHTS

- Oxidative stress as one of the main modes of action of gamma radiation
- Dose and time-dependent increase in ROS formation
- Cumulative dose dependent effects associated with LPO and DNA damage
- Multiple modes of action identified in response to gamma radiation

GRAPHICAL ABSTRACT



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ABSTRACT

Among aquatic organisms, invertebrate species such as the freshwater crustacean *Daphnia magna* are believed to be sensitive to gamma radiation, although information on responses at the individual, biochemical and molecular level is scarce. Following gamma radiation exposure, biological effects are attributed to the formation of free radicals, formation of reactive oxygen species (ROS) and subsequently oxidative damage to lipids, proteins and DNA in exposed organisms. Thus, in the present study, effects and modes of action (MoA) have been investigated in *D. magna* exposed to gamma radiation (dose rates: 0.41, 1.1, 4.3, 10.7, 42.9 and 106 mGy/h) after short-term exposure (24 and 48 h). Several individual, cellular and molecular endpoints were addressed, such as ROS formation, lipid peroxidation, DNA damage and global transcriptional changes. The results showed that oxidative stress is one of the main toxic effects in gamma radiation exposed *D. magna*, mediated by the dose-dependent increase in ROS formation and consequently oxidative damage to lipids and DNA over time. Global transcriptional analysis verified oxidative stress as one of the main MoA of gamma radiation at high dose rates, and identified a number of additional MoAs that may be of toxicological relevance. The present study confirmed that acute exposure to gamma radiation caused a range of cellular and molecular effects in *D. magna* exposed to intermediate dose rates, and highlights the need for assessing effects at longer and more environmentally relevant exposure durations in future studies.

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1. Introduction

The increased use of nuclear technologies in the past decades has increased the concern on the impacts of man-made radionuclides in the environment, especially after the nuclear accident in Chernobyl in 1986 and more recently at Fukushima. In addition, other anthropogenic activities as routine discharges from nuclear power plants, nuclear weapons testing, mining, and nuclear waste from research facilities enhance the discharge of radionuclides into the aquatic environment thereby causing significant exposure of aquatic organisms (UNSCEAR, 2008).

Most radionuclides are gamma emitting, and gamma radiation can result in direct damage to biomolecules, such as double-strand breaks in genomic DNA (Ward, 1995), genotoxic DNA alterations (Parisot et al., 2015), chromosomal aberrations and mutations (Dallas et al., 2012), or indirectly damage macromolecules through the production of free radicals and reactive oxygen species (ROS) (Reisz et al., 2014). As a consequence, effects on a genetic and cellular level can result in significant impacts at the individual and population level, such as increased mortality and morbidity, reproduction impairment, shortening of life span and growth inhibition (Dallas et al., 2012; Fuller et al., 2015; Won et al., 2014). Although gamma radiation is known to induce toxicity in several aquatic invertebrates, knowledge of low dose effects on this diverse group of organisms is still limited compared to more extensively studied organisms such as fish and mammals. An overview of the effects of ionizing radiation on aquatic invertebrates has already been carried out (Dallas et al., 2012; Fuller et al., 2015), highlighting the need for information regarding mechanisms of toxicity, early and sublethal effects in several groups of invertebrates, as for example the subphylum Crustacea. Crustaceans, such as the water flea *Daphnia magna*, have been identified as key models for the development of environmental radiation protection frameworks (ICRP, 2008).

Daphnia magna are small freshwater filter-feeding crustaceans that occupy a key position in the aquatic food web, not only as important phytoplankton grazers, but also as major food sources for fish and invertebrate predators (Shaw et al., 2008). Daphnids are one of the most used invertebrate species in freshwater ecotoxicology and ecology mainly due to their comparatively short generation time, ease of culturing under laboratory conditions, capacity to reproduce through parthenogenesis and sensitivity to various environmental stressors (Watanabe et al., 2008). Accordingly, daphnids have been routinely used as standard model organisms in regulatory toxicity testing and detailed test guidelines have been developed (OECD, 2004, 2008; US EPA, 1996). Knowledge of the ecology, phylogeny, toxicology, and physiology of daphnia species in combination with a fully sequenced genome (wfleabase.org) has enabled a high number of exposure studies with different stressors in this species. Recent development of genomic tools, such as genetic linkage maps, cDNA libraries, expressed sequence tags databases and microarrays, have further enhanced the understanding of environmental-induced modulation of gene functions that may give rise to effects of ecological relevance (Kim et al., 2015; Shaw et al., 2008; Watanabe et al., 2008).

Previous studies have shown that exposure to acute doses of gamma radiation can cause significant mortality (Fuma et al., 2003), cause reduction in mobility and growth in daphnids, as well as a decrease in carbon incorporation in connection to reduced activity, filtering and ingestion rates (Nascimento et al., 2015, 2016; Nascimento and Bradshaw, 2016). Chronic exposure to gamma radiation can negatively impact survival, growth (decrease in body mass and length), metabolic dynamics (reduced resistance to starvation, decrease in mean-life span, alterations in respiration rate and mitochondrial activity) and reproduction (reduction in fecundity, delay in brood release and reduction in brood size) in daphnids, effects that were aggravated in subsequent generations (Gilbin et al., 2008; Marshall, 1962, 1966; Parisot et al., 2015; Sarapultseva and Gorski, 2013; Sarapultseva et al., 2017). Radiation-induced genotoxicity after chronic exposure was also reported in *D. magna* in the form of significant DNA alterations and transmission to progeny across generations (Parisot et al., 2015).

One of the most well-known toxic mechanisms of gamma radiation is the generation of ROS (e.g. superoxide radicals, hydroxyl radicals and hydrogen peroxide), either through direct interaction with the water in cells (formation of free radicals, recombination of radicals) or indirectly by the generation of secondary ROS by subsequent chemical cascades. The production of these radicals in excess can overwhelm the antioxidant capacity of cells and lead to oxidative stress due to oxidization of cellular components, instigating cell damage and other deleterious effects (Reisz et al., 2014). Some of the most common examples of biochemical and physiological damages associated with oxidative stress are lipid peroxidation (LPO) (formation of malonaldehyde-like species and 4-hydroxyalkenals), protein oxidation (e.g. carbonylation and cysteine oxidation) and DNA damage (e.g. single and double-strand breaks, 8-hydroxydeoxyguanosine and other oxidized bases), that have been described as some of the mechanisms involved in the damage caused by gamma radiation (Dallas et al., 2012; Fuller et al., 2015; Reisz et al., 2014). Even though it is well documented that gamma radiation can cause oxidative stress responses in several aquatic organisms (Dallas et al., 2012; Fuller et al., 2015; Won et al., 2014), detailed knowledge about the mode of action (MoA) of gamma radiation and linkage to phenotypical effects in crustaceans are still limited. Thus, acute toxicity of gamma radiation-induced oxidative stress was examined in *D. magna* by focusing on ROS formation, lipid peroxidation and DNA damage. In addition, alterations in the global gene expression were investigated to identify potential MoAs of gamma radiation in *D. magna*.

2. Material and methods

2.1. Test organism

Daphnia magna used in this study have been maintained in the NIVA laboratory for >20 years (DHI strain NIVA, Oslo, Norway). *Daphnia magna* was cultured in EPA moderately hard media (MHRW, 96.0 mg/L $NaHCO_3$, 60.0 mg/L $CaSO_4.2H_2O$, 60.0 mg/L $MgSO_4$, 4.0 mg/L KCl, pH 7.2), which was renewed twice a week. Daphnids were fed daily with a suspension of the unicellular algae *Pseudokirchneriella subcapitata* and supplemented by an amount of dried baker's yeast (20 mg/mL). Cultures were kept in a climate room with light conditions set to 16:8 h light: dark photoperiod and temperature 20 ± 1 °C, according to the OECD 202 guidelines (OECD, 2004). Under these conditions, female daphnids reproduce by parthenogenesis every three days. All cultures and exposures were initiated using third to fifth brood neonates aged <24 h old.

2.2. Gamma radiation exposure

Gamma radiation exposures were conducted at the FIGARO ^{60}Co facility at the Norwegian University of Life Sciences (NMBU, Ås, Norway). *D. magna* neonates (<24 h old) were exposed for 24 and 48 h to external gamma radiation under controlled climate conditions in accordance with the OECD 202 guidelines (OECD, 2004), with slight modifications to accommodate the experimental conditions used in this study. Neonates were exposed in 24-well plates (FalconTM, Oslo, Norway) to 7 different gamma dose rates varying from 0.41 to 106 mGy/h (see Supplementary Table A1 for more information on dose rates and total doses), along with a control placed behind lead shielding in the same room (background radiation). Experiments were conducted at the same temperature as that used for maintenance of *D. magna* cultures and in the dark, and exposure conditions as temperature, pH and dissolved oxygen were monitored for each dose rate throughout exposure. Immobilization and molting frequency were recorded at 24 and 48 h. Due to relatively large sample size required for some of the parameters analysed, exposed daphnids were obtained across different experiments spaced in time, but subjected to the same experimental conditions. Three to six replicate plates were used for each endpoint, each