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Gamma irradiation during gametogenesis in young adult zebrafish causes persistent genotoxicity and adverse reproductive effects

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ABSTRACT

The biological effects of gamma radiation may exert damage beyond that of the individual through its deleterious effects on reproductive function. Impaired reproductive performance can result in reduced population size over consecutive generations. In a continued effort to investigate reproductive and heritable effects of ionizing radiation, we recently demonstrated adverse effects and genomic instability in progeny of parents exposed to gamma radiation. In the present study, genotoxicity and effects on the reproduction following subchronic exposure during a gametogenesis cycle to ^{60}Co gamma radiation (27 days, 8.7 and 53 mGy/h, total doses 5.2 and 31 Gy) were investigated in the adult wild-type zebrafish (*Danio rerio*). A significant reduction in embryo production was observed one month after exposure in the 53 mGy/h exposure group compared to control and 8.7 mGy/h. One year later, embryo production was significantly lower in the 53 mGy/h group compared only to control, with observed sterility, accompanied by a regression of reproductive organs in 100% of the fish 1.5 years after exposure. Histopathological examinations revealed no significant changes in the testis in the 8.7 mGy/h group, while in 62.5% of females exposed to this dose rate the oogenesis was found to be only at the early previtellogenic stage. The DNA damage determined in whole blood, 1.5 years after irradiation, using a high throughput Comet assay, was significantly higher in the exposed groups (1.2 and 3-fold increase in 8.7 and 53 mGy/h females respectively; 3-fold and 2-fold increase in 8.7 and 53 mGy/h males respectively) compared to controls. A significantly higher number of micronuclei (4–5%) was found in erythrocytes of both the 8.7 and 53 mGy/h fish compared to controls. This study shows that gamma radiation at a dose rate of ≥ 8.7 mGy/h during gametogenesis causes adverse reproductive effects and persistent genotoxicity (DNA damage and increased micronuclei) in adult zebrafish.

1. Introduction

The aquatic environment is a primary recipient of ionizing radiation as the consequence of increasing amounts of gamma emitting radionuclides from various anthropogenic and non-anthropogenic activities (nuclear accidents, nuclear power plant waste discharge, cosmic radiation, naturally occurring primordial radionuclides). Gamma radiation is a potent agent for breaking bonds in the genetic material or causing cellular damage through the induction of oxidative stress, particularly in dividing cells having high active metabolism. As such, it has the potential to induce reprotoxicity and genetic defects (Adam-Guillermin et al., 2012; Hurem et al., 2017a) and impair reproductive function in aquatic fauna (Won et al., 2015). Germ cells are the precursors of the gametes (oocytes and sperm), and due to their characteristics of rapid cell division and high active metabolism are particularly vulnerable to ionizing radiation. Ionizing radiation-induced cell damage can result in a variety of deleterious effects during the lifetime of an organism, and as germ cell damage has been found to be transmissible and inherited by future generations, such damage can also result in more long-term population effects (Kong et al., 2016).

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To date, the effects of ionizing radiation on the reproductive performance in fish have only been studied following exposure to either acute (Michibata, 1976; Hyodo-Taguchi and Egami, 1976; Kuwahara et al., 2003) or very high chronic doses (Hyodo-Taguchi and Etoh, 1983). In addition, DNA damage was analyzed in adult fish with single high dose exposures, but not chronic exposure scenarios (Lemos et al., 2017).

Although doses in the environment tend to be lower than those used in laboratory experiments, previous studies have reported exposure of aquatic biota to high doses of ionizing radiation after nuclear accidents. In the contaminated Ural lakes (near Mayak PA) following the Kyshtym accident, in 1957 doses to fish were estimated to 30–40 mGy/day (Sazykina and Kryshev, 2003). Furthermore, fish and other aquatic organisms in the Chernobyl reactor cooling pond accumulated doses of up to 10 Gy during the first 60 days of the accident (Hinton et al., 2007).

Studies of genotoxic and reprotoxic effects in fish from ionizing radiation exposure that covers the entire gametogenesis cycle are still scarce. The zebrafish (*Danio rerio*) has proven to be a good vertebrate model to assess reproductive effects (Hoo et al., 2016; Laan et al., 2002) due to its developmental and physiological advantages such as a short reproduction cycle, high fecundity, transparent embryos and a high degree of similarity with other vertebrates. A pair of adult zebrafish can reproduce approximately two times per week over its breeding cycle, and yield 200–300 eggs at each spawning. In addition, the maximal reproductive capacity in zebrafish is known, and can be achieved by young sexually mature fish between three and six months of age (Skidmore, 1965). The United Nations Scientific Committee for the Effects of Atomic Radiation 1996 report stated that aquatic organism populations including fish would not be negatively affected by a chronic dose rate of 400 μGy/h (0.4 mGy h), although a reduction of spermatogonia at this dose rate can be found (UNSCEAR, 1996). However, the span of dose rates known to inflict damage to the reproductive organs is quite broad as a total dose of 10 Gy caused minimal effects on the maturation of oocytes in fish (UNSCEAR, 1996).

The present work assessed the effects of subchronic gamma radiation exposure (27 days, ^{60}Co, dose rates 8.7 and 53 mGy/h, total 5.2 and 31 Gy) in adult zebrafish during a gametogenesis cycle on the overall health, reproduction, and genotoxicity. In order to determine whether reproductive function is impaired in later life following radiation exposure, effects on reproduction were evaluated both one month and one year after irradiation. Histopathological examination of the gonads was performed in order to determine possible deleterious reproductive effects in irradiated adults, while the genotoxic effects in the form of DNA damage and the number of micronuclei (MN) in red blood cells were assessed in both male and female zebrafish one year after gamma irradiation.

2. Materials and methods

2.1. Fish husbandry

Adult zebrafish (ZF, aged 6 months) from the AB wild type strain (30 males and 30 females per exposure group) were obtained from the Zebrafish Facility at the Norwegian University of Life Sciences (NMBU). The exposure of ZF to external gamma radiation took place at the FIGARO Co-60 irradiation facility (source activity ~420 GBq) at NMBU and is schematically depicted in Fig. 1. Recirculating system water was prepared from particle and active charcoal filtered reverse osmosis kept sterile by UV irradiation water of pH 7.5 and temperature 28 ± 1 °C with regular weekly or daily water changes depending on the water quality described in Hurem et al. (2017b). The light regime of 10–14 light-dark cycle (250–320 lx) was used and fish were fed dry feed Gemma Micro 300 (Skretting, Stavanger, Norway) twice a day and live artemia (Scanbur, Copenhagen, Denmark) once a day, both during and after the experimental periods.

After exposure, fish were maintained according to standard operating procedures at the NMBU Zebrafish Facility until sampling for histopathology, genotoxic effects and measurement of weight and length.

2.2. Ethical statement

This research was performed in accordance with the Norwegian Animal Protection Act (implemented EU Directive 2010/63/EU). Approval number FOTS ID 5793 was issued on December 12, 2013 by IACUC of Norwegian School of Veterinary Science (since 2014 Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences, Oslo, Norway).

2.3. Biometric parameters

Weight and length were measured 1.5 years after exposure, in 22 male and 22 female anesthetized fish from both the control and 8.7 mGy/h groups. In the 53 mGy/h group, weight and length were measured in 10 males and 10 females and in 24 fish of undetermined sex. The condition factor of unexposed and gamma irradiated fish was calculated according to the formula ($K = [\text{mass (g)} \times 100]/[\text{length (cm)}]^3$) (Jones et al., 1999).

2.4. Reproduction assessment

Thirty adult irradiated male and female zebrafish of the AB wild type strain were used in the breeding trials. The mating experiments took place during six consecutive breeding weeks one month after gamma irradiation and during five consecutive breeding weeks one year after irradiation. One breeding trial was performed in each week for all groups simultaneously. For maintenance during the reproduction experiments, males and females from each exposure were divided into two groups, kept in 12 holding tanks of 2 L volume, with 12 fish per tank and used intermittently over even and odd numbered breeding weeks. In each breeding trial, six standard (conservative) 1 L breeding tanks with a meshed bottom for separation of eggs (Aquatic Habitats, Apopka, FL, USA) were used with one breeding pair per tank. The setup and male/female separation took place in the late afternoon and breeding pairs were formed using one male and female from the same exposure group. The morning after, barriers were removed and the breeding pairs were allowed to mate for 30 min. Egg collection and counting was performed immediately after breeding, followed by the separation of sexes and transfer of fish to holding tanks.

2.5. Fish anesthesia and euthanasia

For anesthesia of the fish, 0.2% Tricaine Methanesulfonate (MS-222) (Sigma-Aldrich, Oslo, Norway) in dH_2O adjusted to pH 7.0 with 1.0 M Tris (pH 9.5) combined with iced system water was used. Briefly, fish remained in this solution until no visible movement was observed. For euthanasia, an overdose of tricaine was used in iced system water, and the fish were observed until failing to react to external stimuli and/or following cessation of opercular (gill) movement.

2.6. Histopathological analysis

Whole fish were fixed individually in 4% paraformaldehyde for a minimum of 4 days and then processed according to standard histological procedures using Hematoxylin and Eosin (H&E) stain. Histopathological examination was performed blindly using a Zeiss Axioskop microscope equipped with a digital camera (Leica SFC 420). Eight males and eight females from the two exposed groups and controls were processed, examined and analyzed 1.5 years after gamma exposure.