Medical-biological aspects of radiation effects in Daphnia magna

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Abstract. We have shown that γ-irradiation at doses of 100 and 1000 mGy significantly compromised fecundity and reproductive success of the directly exposed D. magna. These effects were also observed among the non-exposed first-generation progeny of irradiated parents, thus implying the manifestation of transgenerational effects in Daphnia. We have also shown that compromised viability of irradiated D. magna can be attributed cytotoxic effects of irradiation. It would therefore appear that the compromised viability may be attributed to the cytotoxic effects resulted from epigenetic changes affecting some metabolic pathways involved in detoxification of free-radicals. Additionally we have analyzed more distant progeny of irradiated at doses of 10, 100 and 1000 mGy Daphnia. Our data demonstrated that multicellular crustacean D. magna represent a very useful experimental model for analyse of long-term effects of ionising radiation at the organismal level.

1. Introduction

Over recent years nuclear medicine has become one of the most important and fast-growing component of modern clinical practice. The widespread use of radiopharmaceutical (RFP) is an integral part of nuclear-medical technologies. The basic requirements for application include minimization radiation exposure for both patient and medical staff and safety for patient both RFP and products of its biotransformation in the body. The key characteristics of RFP such as activity per body weight, time and the mode of administration of them, as well as doses of exposure to tumor have been established in experiments with laboratory mice and rats, the species currently regarded in bio pharmacochemistry as gold standard. However, the development of simple and express tests is clearly warranted, as they can provide new insights into the mechanisms underlying the efficiency and toxicity of RFP and help establishing the optimal therapy regimens and improving the outcome of treatment. In our opinion, some alternative experimental models used in radiobiology may also be employed in bio pharmacology.

Over recent years, the work of our research group has been focused on the analysis of low-doses long-term effects of ionising radiation. Daphnia represent a very useful experimental model, which allows the very efficient and quick analysis of many aspects of long-term effects of ionising radiation. The life span of these animals is relatively short and seldom exceeds 10-11 weeks; they are also characterised by quite short durations of embryonic and postembryonic development (per 3-4 days
each) and gestation (5-8 days). Most importantly, Daphnia magna ‘enjoy’ parthenogenetic reproduction, which quickly allows establishing genetically identical strains of these animals. D. magna has been used in numerous studies aimed to analyse the effects of ionising radiation on mortality and fecundity [1]. The aim of our study was to analyse the long-term effects of low-LET irradiation on the survival, life span, fecundity and metabolic activity of directly exposed at low doses test-organism crustacean D. magna and their non-exposed generations’ offspring.

2. Long-term effects on survival and fecundity in Daphnia
The strain of Daphnia magna Straus used in our experiments was originally collected in the pond of the Moscow Zoo and maintained for several years at the laboratory in continuous parthenogenetic reproduction following the OECD guideline 211 [2] with modifications. D. magna were reared at density of 20 animals per 500 mL in aerated dechlorinated filtered tap-water (pH 7.5 – 8.2, O₂ ~9.0 mg/l; hardness: 128 mg L⁻¹, Ca:Mg 4:1, Fe 0.3 mg/l, Mn 0.1 mg/l) renewed a week. Daphnia were fed with green algae suspension (Chlorella vulgaris) at daily ration of 90 – 100 μg C Daphnia⁻¹. D. magna were incubated at 20 °C (±0.5 °C) on a 12 h / 12 h light/dark cycle photoperiod at light intensity 700-1200 lux (Climate Control model R2, Russia). Neonates were removed every weekday.

In our previous studies we evaluated the effects of high-dose acute exposure to gamma-rays on the survival of irradiated Daphnia [3]. According to our data, the survival rate of Daphnia exposed to 20 Gy is significantly compromised. Exposure to a higher dose of 100 Gy substantially compromises their survival, whereas the doses of 250 and 600 Gy can be regarded as lethal. According to our estimates the value semi-lethal dose for Daphnia is 63 Gy.

In our recent studies we exposed one-day-old Daphnia at doses of 10, 100, 1000 and 10000 mGy (28-1000 mGy min⁻¹) [4, 5]. Control and irradiated Daphnia were maintained individually with 50 mL of water in glass vials. Experimental units were checked in a day during 21 days for survival and neonatal release. On days of release, neonates were removed and counted as well as all dead daphnids.

To analyse the effects of parental exposure to the successive generation (F₁), one-day-old neonates from the third broods of generation F₀ were randomly taken from at least three females of irradiated or control groups and transferred to glass vials with 50 mL of water (one Daphnia per vial). Using the same protocol, a group of second-generation offspring (F₂) was also established. Following generations F₁ and F₂ were maintained as the original samples but without exposure to γ-irradiation. The survival and fecundity of the parthenogenetic progeny from these generations were measured on a daily basis over 21 days.

2.1 Long-term effect generations on survival in Daphnia
Using the Kaplan Meier approach, we estimated the mean life span for controls and irradiated Daphnia [4]. According to our results, the survival of Daphnia exposed to 10 mGy of gamma-rays does not significantly differ from that in controls. Thus, the mean life span of this group is approximately 50 days, which does not differ from the mean value in controls of 54 days. However, it is significantly compromised in the two groups exposed to 100 and 1000 mGy. The mean life span of these exposed groups was 43 and 36 days, respectively. Quite surprisingly, the effects of exposure to the two different doses of 100 and 1000 mGy are practically similar. It should also be noted that the results of our study were obtained within the dose-range far below the semi-lethal doses for Crustacean.

2.2 Long-term effect generations on fecundity in Daphnia
According to our data, acute parental exposure to γ-rays equally compromised the total fertility of irradiated F₀ Daphnia and their F₁ progeny (Figure 1A; [5]). The fertility of F₀ and F₁ Daphnia declined with the dose of parental exposure and significantly decreased at dose of 100 mGy and at higher doses. In contrast, the F₂ total fertility was compromised only among progeny of parents that received the highest dose of 10,000 mGy.
We also analysed the two main components of the total fertility – the brood size and number of broods. The effects of parental irradiation on the brood size were only observed among the F₀ Daphnia exposed to 1000 and 10,000 mGy (Figure 1B). On the other hand, the effects of parental γ-irradiation on the brood size were very close to those for the integrated measure of fertility estimated as the number of progeny per Daphnia (Figure 1C).

As we analysed parthenogenic strain of D. magna, even early F₀ irradiation resulted in exposure of primordial diploid eggs. In this respect, the design of our study is close to those aimed to analyse the effects of parental irradiation on the development of F₁ embryos in mammals [6]. According to their results, the developing mammalian embryos show extremely high sensitivity to irradiation, the lethal effects of which substantially exceed those following exposure during adulthood. Our data, summarised on Figure 1D also show that the effects of parental irradiation on the F₀ fertility, which are partially attributed to the early embryonic mortality, considerably surpass those on the life span of exposed adult Daphnia and their F₁ offspring.

As far as the results of our study on the transgenerational effects of parental irradiation on viability are concerned, it should be noted that we did not observe a significant shortening of the life span among the F₂ progeny. In this respect it is worth mentioning the results of transgenerational study by Luning and co-authors, showing that paternal irradiation can significantly compromise the viability of F₂ embryos [7].

3. Transgenerational alterations in biochemical activity in Daphnia

3.1. Long-term effect generations in free radical reactions in Daphnia
A violation of free radical reactions has been found by the method of free radical copolymerization in the two groups of *D. magna* exposed to 100 and 1000 mGy of γ-rays and their non-exposed first generation progeny [8]. The method is based on a quantitative radiometric registration of the polymerization process that develops in the cells of a multicellular organism in tissue in proportion to the existing number of free radicals. The fact of the increased level of free radicals may be indicative of the hereditary transgenerational effect and the cause of lower survival of *Daphnia* in both generations. However, the effect of a reduced survival rate does not persist in the second generation, which can be explained by the effect of radiation on the embryos of the first generation after acute irradiation of parents.

### 3.2. Long-term effect generations on mitochondrial activity in Daphnia

The modified *in vitro* MTT-assay [9] was used in our recent study. Four-days-old daphnids from at least first broods were used in the MTT assay. 200 µl of MTT solution (Sigma, St. Louis, USA) (0.5 mg/ml) was added to an Eppendorf tube containing 50 *Daphnia*. After 10-min incubation, *Daphnia* were homogenized. The optical density was measured at 492 and 630 nm on tablet immunoassay analyzer StatFux 2100 (Awareness Technology, USA, VIS-model) and corrected for background noise. The 630 nm OD background were subtracted from the 492 nm OD total signals. The average OD of the blank control wells (homogenized *Daphnia* in DMSO without MTT) was subtracted.

Metabolic test is one of the important diagnostic parameter for understanding the mechanisms underlying the effects of irradiation on mortality. It provides an opportunity to evaluate the oxidative stress that occurs in the body as a result of exposure. Metabolic test have been widely used for the analysis of cytotoxic *in vitro* effects of anticancer chemotherapeutic agents, as well as other drugs. They allow to evaluate the effect of test compounds on cellular metabolism, including the generation of reactive oxygen species (ROS), cells and DNA structure, determination of viability and cell proliferative activity [10; 11].

The methyl thiazol-diphenyl-tetrazolium bromide (MTT) cell viability assay is widely used in determining drug sensitivity profiles and in primary screening of potential efficacy of chemotherapeutic drugs in cell lines [9; 12; 13]. The MTT-assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in optical density (OD) using a plate reader at 540 and 720 nm.

In the cited articles was show that the *in vitro* MTT assay has been extensively applied to predict drug sensitivity *in vivo*. However, to date this test has seldom been used for the analysis of *in vivo* effects. Earlier MTT-assay was first applied for the investigation *in vivo* of the mechanisms of non-targeted effects of radiation and development of stress in multicellular crustaceans *D. magna*. In our recent study we analyzed the radiation-induced transgenerational alterations of mitochondrial activity at acute exposed in *D. magna* and their non-exposed offspring [14; 15].

According to our data, the cytotoxic effect is significantly elevated in the three groups exposed to 100, 1000 and 10,000 mGy of gamma-rays. Figure 2 shows that the optical density, reflecting changes in mitochondrial activity and expressed as a percentage of the total control, was lower in exposed *Daphnia* from F₀ generation than in control samples with the exception of irradiated at a dose of 10 mGy. The same result was observed in the F₁ generation of daphnids.
Figure 2. The direct and transgenerational effects of parental irradiation on mitochondrial activity in two generations of D. magna.

Thus, the MTT test showed that irradiation at doses of 100, 1000 and 10 000 mGy causes the cytotoxic effect in the directly exposed Daphnia and their non-exposed offspring of the first generation. The toxic effect is relatively weak after exposed Daphnia at a dose of 10 mGy. Apparently, some of the cells are killed, but the viability of Daphnia was not affected.

4. Conclusions
In this study we have analysed the long-term effects of low-LET irradiation at doses from 10 mGy to 10 Gy on functional and metabolic activity of directly exposed D. magna and their non-exposed progeny. We observed biological effect at doses at least 100 mGy and dose independence in a wide range of doses. The viability of non-exposed first-generation offspring of irradiated parents is also significantly compromised, thus implying the existence of transgenerational effects of parental exposed in Daphnia. Our data are consistent with the results of some previous studies on the transgenerational effects of paternal exposure in mammals.

Our results also imply that the increased level of free radicals can explain compromised viability of irradiated Daphnia and their non-exposed progeny. Given that the total activity of dehydrogenases steadily decreases following acute exposure to at least of 100 mGy of γ-rays, the toxic effects of such an exposure should manifest within this dose range. Our data also demonstrate that Daphnia represent a very useful and sensitive experimental model, which has a number of potential advantages for express analysis of the long-term effects of exposure ionizing radiation.

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