Ecotoxicology and Environmental Safety 113 (2015) 18–22

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Gamma radiation induced cell cycle perturbations and DNA damage in *Catla Catla* as measured by flow cytometry

S. Anbumani *, Mary N. Mohankumar [1]

Radiological Safety Division, Indira Gandhi Centre for Atomic Research, Kalpakkam, Tamilnadu 603 102, India

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form
8 November 2014
Accepted 11 November 2014
Available online 5 December 2014

Keywords:
Cell cycle
DNA damage
Flow cytometry
Fish
Gamma radiation

ABSTRACT

Gamma radiation induced cell cycle perturbations and DNA damage in *Catla catla* were analyzed in erythrocytes at different time points using flow cytometry (FCM). Protracted exposure to radiation induced damage between days 12 and 45. Disturbances in cell cycle machinery, i.e., proportional increase and decrease in Gap0 or quiescent/Gap1 (G0/G1), Synthesis (S) and Gap2/Mitotic (G2/M) phases were observed at both acute and protracted treatments. Both acute and protracted exposures induced apoptosis with a notable significance between days 3 and 6 at protracted and on day 45 at acute doses. Fish exposed protractedly avail some DNA repair mechanisms than acutely exposed. This is the first study to analyze radiation induced DNA damage under laboratory conditions and suggests that flow cytometry can also be an alternate tool to screen genotoxicity induced by ionizing radiation in fish.

1. Introduction

Ionizing radiation induced DNA damage is a well known exposure biomarker. Cytogenetic techniques employed under field and laboratory conditions for detecting DNA strand breakages include chromosome aberrations, micronucleus test and comet assay etc. Flow cytometry (FCM), an instrumental tool has been widely used to quantify DNA damage by analyzing a high number of nuclei with reliable results in a short period of time. Ideally, all cells within an organism contain the same amount of DNA to maintain cellular homeostasis. Damage to DNA might result from the breakage, chromosome rearrangement and interference with the normal segregation during cell division. Further, double strand breaks are the important DNA lesions caused by ionizing radiation resulting in cells with an abnormally high or low DNA content. This DNA content variability can be detected as an increased coefficient of variation (CV) of cells in G_0/G_1 phase as measured by FCM.

Perturbed DNA content levels are reported in vertebrates inhabiting environments with radioactive and chemical contaminants (Bickham et al., 1988; Fertig and Miltenburger, 1989; Dallas and Evans, 1991; George et al., 1991; Lamb et al., 1991; Theodorakis et al., 1992; Custer et al., 1994). The CV has also been shown to increase in cells following radiation exposure under controlled laboratory conditions in mammalian systems (Otto and Oldiges, 1980; Van Dilla et al., 1980; Pinkel et al., 1983; Otto et al., 1984; McBee and Bickham, 1988; Lamb et al., 1991). Ionizing radiation induced DNA damage expressed as increased CV in fishes using FCM is not well studied under laboratory conditions.

Subtle changes under pollutant exposure results in an elevated CV with subsequent intercellular variation in DNA content that reflects the severity of DNA damage in exposed cell population. A few studies have addressed possible genotoxic effects of low level radiation exposure on non-humans using FCM by analyzing the CV of the G_0/G_1 peak (National Research Council (NRC), 1981; Whicker and Schultz, 1982; Hobbs and McClellan, 1986; Lamb et al., 1995; Dallas et al., 1998; Lingenfelser et al. 1997a,b).

Appearance of aneuploid cell populations as a result of mitotic machinery defects are also detectable by FCM analysis as additional peaks in the histogram, since these involve specific chromosomal deletions and rearrangements that are associated with several malignancies in chronic low dose exposures (Oshimura and Barrett, 1986). For instance, somatic cell aneuploidy has been found during the development of several types of cancers (Liang and Brinkley, 1995) considering as a useful prognostic marker in malignancies.

Cell cycle is a basic synchronizing event that takes place in three prominent phases to maintain the DNA integrity and any provoking disturbances leads to the malfunctioning of several

* Corresponding author. Present address: Ecotoxicology Division, CSIR – Indian Institute of Toxicology Research (IITR), Lucknow 226001, Uttarpradesh, India
E-mail addresses: aquatox1982@gmail.com (S. Anbumani), marynmk@gmail.com (M.N. Mohankumar).
[1] Present address: No: 40, D.J.Nagar, B.R.Puram, Peelamedu, Coimbatore – 641004, Tamilnadu, India

http://dx.doi.org/10.1016/j.ecoenv.2014.11.011
0147-6513/

metabolic processes. This reflects the survival pattern in their respective inhabiting ecosystem. Using FCM, changes in cell cycle perturbations can be analyzed by categorizing the percentage of cell populations residing in each phase of the cell cycle and apoptotic cascade phenomenon. Since ionizing radiation is known to affect erythro- andleuco-poiesis in vertebrates (Watson et al., 1962; Finstad et al., 1969), advantage of being nucleated erythrocytes in fish blood provide sufficient number of cells for FCM analysis thereby considering them as a suitable model system.

Gamma radiation induced DNA damage and cell cycle phase changes in fish exposed to short and long term exposures under laboratory conditions are scanty. Hence, in the present study gamma radiation delivered within a short duration (acute doses) and continuously over a relative long period (protracted doses) induced DNA damage and cell cycle phase perturbations were studied in the peripheral erythrocytes of *Catla catla* using FCM under controlled laboratory conditions.

2. Materials and methods

2.1. Experimental fish specimens

Freshwater fish *C. catla* (Hamilton, Family: Cyprinidae) was chosen due to its relative sensitivity to pollutants and high economic value in Asian market. Fingerlings weighing between 8 and 10 g and of length 8 ± 2.0 cm procured from Government approved commercial fish farm and transported to the laboratory in oxygenated bags and released into 50 L glass aquaria filled with dechlorinated tap water, acclimatized for 30 days under laboratory conditions with natural photoperiod and fed with oil cake. The fecal matter and other waste materials siphoned off daily to reduce ammonia content in water that was renewed once in two days with dechlorinated tap water. The water quality parameters analyzed and found within the normal range (pH – 7.1 ± 0.25, dissolved oxygen – 8.2 ± 0.36 mg/l, Temp. –25 ± 1 °C and hardness–220 ± 0.0 ppm of $CaCO_3$).

2.2. Exposure to radiation

A $LD_{50/30}$ value of 22.4 Gy deduced for this fish from previous study and a non-lethal dose of 5 Gy selected for the present investigation. The method of radiation exposure has been described in detail elsewhere (Anbumani and Mohankumar, 2012). Briefly, fish from the acclimatized set were placed in glass beakers of 1 L capacity containing dechlorinated tap water. The beakers were placed at a radial distance of 16 cm from a sealed Cs-137 gamma ray source (1 Ci, BRIT, Mumbai, India) and irradiated for 42 h at a dose rate of 0.002 Gy/min to a total dose of 5 Gy. The irradiation set-up could accommodate about 10–12 beakers in a circle to provide uniform irradiation. The size of the glass beakers was kept small to ensure that all fish were given approximately the same radiation dose. Another set of fish were irradiated at a dose rate of 3.2 Gy/min to a total dose of 5 Gy using gamma chamber with sealed Co-60 gamma ray source (GC-900, BRIT, Mumbai, India). After irradiation the fish were transferred to glass aquaria of 50 L capacity containing dechlorinated tap water. Fish exposed to a dose rate of 3.2 Gy/min is considered as acute whereas 0.002 Gy/min as protracted exposures.

2.3. Blood cell collection and staining

Approximately 0.1 ml of blood was taken from the caudal vein of anesthetized fish at different duration intervals (day 3, 6, 12, 18, 30, 45, 90 and 202) at the rate of 5 fish per duration into a syringe containing equal volume of citrate buffer (250 mM sucrose, 40 mM trisodium citrate-H_2O, dimethylsulfoxide, pH 7.6) (Vindelov et al., 1982) and processed for flow cytometry. Experimental related mortality not observed throughout the study. Samples prepared and nuclei were stained using Vybrant Dye Cycle Violet stain kit for cell cycle analysis and dead cell apoptosis kit containing Hoechst 33342 and Propidium iodide (PI) (Invitrogen) to study apoptosis according to the manufacturer's instructions.

2.4. Flow cytometry

Stained nuclei analyzed using a CyFlow space flow cytometer (Partec, Germany) was aligned with DNA-check fluorescent microsphere beads and checked periodically throughout the day. Vybrant Dye Cycle Violet stain bound to DNA complex has fluorescence excitation and emission maxima of 369/437 nm respectively. Hoechst 33342 stains the condensed chromatin of apoptotic cells more brightly than chromatin of normal cells with fluorescence excitation and emission maxima of ~350/461 nm and PI which is permeant only to dead cells has fluorescence excitation and emission maxima of ~535/617 nm respectively. The staining pattern resulting from the simultaneous use of these dyes makes it possible to distinguish normal, apoptotic and dead cell populations by flow cytometry. The flow rate was maintained at less than 200 nuclei throughout the sample run. The parameters include fluorescent intensity and time. A total of 30,000 nuclei counted for each sample in triplicates and data analysis done using the DNA analysis program software. This software calculated the coefficient of variation (CV) around the G_0/G_1 peak, measure of DNA damage and the percentage of cells in each phase.

2.5. Statistical analysis

INSTAT software is used and data tested for normality using the Shapiro–Wilk's test. Since data did not show normal distribution, one way ANOVA followed by Tukey–Kramer multiple comparison aposteriori was used. 1% and 5% levels of significance tested followed by Spearman Correlation and the level of significance set at 95% ($\alpha = 0.05$).

3. Results

3.1. Gamma radiation induced CV of G_0/G_1 peak

CV of G_0/G_1 peak in *C. catla* exposed to protracted and acute doses of gamma radiation are shown in Table 1. Both the exposures induced a significant increase in the CV of G_0/G_1 peak relative to sham irradiated fish specimens. Significant increase was noted between day 12 and day 90 for protracted dose with increment on days 12 and 30. However, in acute exposures this increase was noted between day 6 and 90 showing a peak on day 18 despite a trend for slight elevation on day 45 and 90 without any notable significance. Between the dose-rates, acutely exposed specimens showed a significant increase in the CV on day 18.

3.2. Gamma radiation induced cell cycle perturbations

Gamma radiation induced cell cycle phase alterations in fish (Table 2) at different post-exposure intervals. A significant decrease in the percentage of G_0/G_1 phase cells accompanied by a proportional increase in the G_2/M phase cell populations on day 6 after protracted exposure is noted. This is followed by an increase in the G_0/G_1 phase cells and a proportional decrease in G_2/M phase cells throughout the sampling period. Likewise, the percentage of S phase cells increased significantly between day 6 and 30 of post exposure period. Besides, acutely exposed fish