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Evaluation of γ-radiation-induced DNA damage in two species of bivalves and their relative sensitivity using comet assay

M.K. Praveen Kumar[a], S.K. Shyama[a,*], B.S. Sonaye[b], U Roshini Naik[a], S.B. Kadam[a], P.D. Bipin[a], A. D'costa[a], R.C. Chaubey[c]

[a] Department of Zoology, Goa University, Goa 403206, India
[b] Department of Radiation Oncology, Goa Medical College, Goa, India
[c] Radiation Biology & Health Science Division, Bhabha Atomic Research Centre, Mumbai, India

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ABSTRACT

Ionizing radiation is known to induce genetic damage in diverse groups of organisms. Under accidental situations, large quantities of radioactive elements get released into the environment and radiation emitted from these radionuclides may adversely affect both the man and the non-human biota. The present study is aimed (a) to know the genotoxic effect of gamma radiation on aquatic fauna employing two species of selected bivalves, (b) to evaluate the possible use of 'Comet assay' for detecting genetic damage in haemocytes of bivalves as a biomarker for environmental biomonitoring and also (c) to compare the relative sensitivity of two species of bivalves *viz. Paphia malabarica* and *Meretrix casta* to gamma radiation. The comet assays was optimized and validated using different concentrations (18, 32 and 56 mg/L) of ethyl methanesulfonate (EMS), a direct-acting reference genotoxic agent, to which the bivalves were exposed for various times (24, 48 and 72 h). Bivalves were irradiated (single acute exposure) with 5 different doses (*viz.* 2, 4, 6, 8 and 10 Gy) of gamma radiation and their genotoxic effects on the haemocytes were studied using the comet assay. Haemolymph was collected from the adductor muscle at 24, 48 and 72 h of both EMS-exposed and irradiated bivalves and comet assay was carried out using standard protocol. A significant increase in DNA damage was observed as indicated by an increase in % tail DNA damage at different concentrations of EMS and all the doses of gamma radiation as compared to controls in both bivalve species. This showed a dose-dependent increase of genetic damage induced in bivalves by EMS as well as gamma radiation. Further, the highest DNA damage was observed at 24 h. The damage gradually decreased with time, i.e. was smaller at 48 and 72 h than at 24 h post irradiation in both species of bivalves. This may indicate repair of the damaged DNA and/or loss of heavily damaged cells as the post irradiation time advanced. The present study reveals that gamma radiation induces single strand breaks in DNA as measured by alkaline comet assay in bivalves and comet assay serves as a sensitive and rapid method to detect genotoxicity of gamma radiation. This study further indicates that both *M. casta* and *P. malabarica* exhibit almost identical sensitivity to gamma radiation as measured by DNA damage.

Introduction

Applications of nuclear technologies have made a very significant contribution to modern civilization. Nuclear technology is useful to humans in several ways, including as a source of power, as a tool for medical diagnostics and also as an industrial tool. Exploding human population and the changing needs/lifestyle have resulted in a drastic increase in the production, consumption and disposal of chemical contaminants into our environment. The aquatic environment is often the ultimate recipient of a wide range of contaminants including chemical and radioactive wastes, a large proportion of which could be potentially genotoxic and carcinogenic (Moore et al., 2004; Jha et al., 2000a). Radioactive wastes emit radiation in different forms, e.g. α-, β-particles and gamma rays, which could pose a potential risk to human health and also to our environment (Dallas et al., 2012). Environment and man may be exposed to a moderate dose of 110 Gy of gamma radiation as a result of radiation accidents or nuclear/radiological terrorism alone or in conjunction with bioterrorism (Coleman et al., 2003). During nuclear accidents, radionuclides are released into the

Abbreviations: Gy, gray; h, hour; γ, gamma; EMS, ethyl methanesulfonate.

* Corresponding author. Tel.: +91 832 6519364.
E-mail addresses: here.praveen@gmail.com (M.K. Praveen Kumar), skshyama@gmail.com (S.K. Shyama).

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environment, either in modest amounts or on a larger scale, such as that caused by the Chernobyl NPP (Ukraine, formerly USSR) in 1986 or the earthquake-tsunami at Japan's Fukushima Daiichi NPP in 2011. The risk to non-human biota due to ionizing radiation exposure is of considerable current interest to both the International Commission on Radiological Protection (ICRP) and the International Atomic Energy Agency (IAEA) and they recommend the impact assessment of radiation on some natural organisms (IAEA, 1992; ICRP, 1991, 2007). Very little information is available on the potential detrimental effects of ionizing radiation on aquatic invertebrates (Dallas et al., 2012). They are very important human food source (Barnes and Rawlinson, 2009; Ren et al., 2010) and are also a source of food for various commercially important fish species (Pedersen et al., 2008). Further, they are frequently used as model organisms for toxicological tests (Depledge, 1998).

Haemocytes, the cells of the open vascular system of mussels, have been extensively used for genotoxicological studies, including for monitoring cytogenetic damage (Mersch et al., 1996; Pavlica et al., 2001). The usage of haemocytes provides a relatively non-invasive source of material for biomonitoring (Fossi et al., 1994; Mitchelmore and Chipman, 1998; Taddei et al., 2001). Further, these cell types are suitable for the comet assay and the MN assay because they can be rapidly and easily sampled without any need for cell dissociation (Belpaeme et al., 1998; Jha, 2008; Canty et al., 2009). These advantages have the benefit of shortening the time required for slide preparation and facilitating the sample process. The comet or single-cell gel electrophoresis assay is used as a very quick, sensitive method for measuring DNA damage in eukaryotic cells for the study of genetic damage associated with exposures to potentially genotoxic agents (Lovell and Omori, 2008). It is also used in regulatory and biomonitoring studies in a range of mammalian and non-mammalian both under in vitro and in vivo systems (Chaubey et al., 2001; Collins, 2004; Brendler-Schwaab et al., 2005; Malladi et al., 2007). It offers considerable advantages over several other cytogenetic methods used in DNA damage detection, such as sister chromatid exchange studies, micronucleus test and the chromosomal aberrations. Further, there is no need for cells to be in a dividing state. Other advantages include its rapidity, i.e. results can be obtained in a single working day and wide applicability to virtually any nucleated cell type (Jha, 2008; Canty et al., 2009).

In addition to the detection and quantification of contaminants in the environment in order to reveal their environmental impact, it is very essential to identify their toxic effects on living systems, which are the ultimate recipients of toxicant-induced mutation (Claxton et al., 1998). Mussels are globally used as bioindicators for pollution of coastal and estuarine environments by metals and radionuclides (Lonsdale et al., 2009). Hagger et al. (2005) have reported the β-radiation induced genotoxic, cytotoxic, developmental and survival effects of tritiated water in the early life stages of the marine mollusc, *Mytilus edulis*. Further, the genotoxic effects of tritium (^{3}H) in the adult life stage of *Mytilus edulis* have been evaluated by employing micronucleus (MN) test and comet assay (indicating DNA single strand breaks/alkali labile sites) in the haemocytes of exposed individuals (Jha et al., 2005, 2006). Tritiated water (HTO) and tritiated glycine (T-Gly) induced a significant number of micronuclei in the haemocytes of *Mytilus edulis* (Jaeschke et al., 2010). External and internal dose rates of ionizing radiation altered the DNA strand breakage and *RAD51* mRNA expression in marine mussel *Mytilus edulis* which was observed using gene expression study and comet assay (Alamri et al., 2012). However, studies on the genotoxic potential of radiation in the estuarine bivalves are lacking. Hence, the present study is aimed (a) to know of genotoxic effect of gamma radiation on estuarine bivalves (b) to evaluate the use of 'Comet assay' for detecting genetic damage in haemocytes of bivalves as a biomarker for biomonitoring and also (c) to compare the relative sensitivity of two species of bivalves *Paphia malabarica* and *Meretrix casta* to gamma radiation.

Materials and methods

Experimental bivalve specimens

Two species of estuarine bivalves, *P. malabarica* and *M. casta* which are abundant in Goa and used as a common sea food by locals were selected as the experimental animals. Healthy specimens were procured from an unpolluted site at Siolim, Goa and brought to the laboratory. Animals measuring an average length of 3.0 ± 0.4 cm were selected for the current study. They were maintained in aerated glass aquaria with sand, pebbles and estuarine water, procured from the same above cited unpolluted site. Water in the aquaria was replaced once in every day. These bivalves were acclimatized under the laboratory conditions for 2 weeks in semi static systems before exposure to reference agent as well as to different doses of gamma radiation.

Validation of comet assay

Prior to the evaluation of genotoxic effects of gamma radiation the comet assay was fully optimized and validated against a direct-acting reference genotoxin, ethyl methanesulfonate (EMS; Sigma, UK). The range of EMS concentrations used in the study was based on earlier studies (Jha et al., 2005). Both species of bivalves (10 animals per group) were exposed to different concentrations of EMS (18, 32 and 56 mg/L) dissolved in estuarine water.

Sample collection from bivalves

Four hundred (400) microliter of haemolymph was collected from the sinus region (located near the posterior adductor muscle) of each of the control and EMS-exposed bivalves using a hypodermic syringe under dim light at exposure times of 24, 48 and 72 h. Each sample was transferred to a microcentrifuge tube placed on ice in an ice box to prevent endogenous DNA damage occurring during sample preparation and also to inhibit DNA repair in the unfixed cells (Siu et al., 2004).

Cell viability assay

Prior to the comet assay, the cell count and the cell viability were checked to ensure that there were an optimum number of living cells to perform the assay. The cell count and viability assessment were conducted with a haemocytometer and trypan blue dye exclusion test. Haemolymph samples showing more than 90% viability and a cell count of 10^6 cells/mL were used for the comet assay.

Comet assay

The technique of lysis, unwinding and electrophoresis were standardized for haemocytes by adopting the protocol of Singh et al. (1988) and Tice (1995) with slight modifications, prior performing the comet assay. The comet assay was carried out in a dark room with dim red light. Haemolymph (15 μL) was mixed with 75 μL of 0.5% low melting point (LMP) agarose at 37 °C and rapidly spread on a frosted microscope slide (Fisherfinest premium) precoated with 1% normal melting point (NMP) agarose. A cover slip was applied on the smear and the slide was allowed to solidify for 5 min in a freezer at 0 °C. The cover slip was then gently removed and the slide was immersed in a fresh cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 10% DMSO 1% and Triton X-100) placed in a Petri dish for 2 h at 4 °C. Comet slides were later immersed in electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13) in a horizontal gel box for 30 min to allow the DNA to unwind. These slides were subjected to electrophoresis by applying 20 V and 275 mA for 20 min and later on the unwound DNA