World Academy of Science, Engineering and Technology
International Journal of Health and Medical Engineering
Vol:17, No:09, 2023

Effect of Radioprotectors on DNA Repair Enzyme and Survival of Gamma-Irradiated Cell Division Cycle Mutants of Saccharomyces pombe

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Abstract : Introduction: The objective was to understand the effect of various radioprotectors on DNA damage repair enzyme and survival in gamma-irradiated wild and cdc mutants of S. pombe (fission yeast) cultured under permissive and restrictive conditions. DNA repair process, as influenced by radioprotectors, was measured by activity of DNA polymerase in the cells. The use of single cell gel electrophoresis assay (SCGE) or Comet Assay to follow gamma-irradiation induced DNA damage and effect of radioprotectors was employed. In addition, studying the effect of caffeine at different concentrations on S-phase of cell cycle was also delineated. Materials and Methods: S. pombe cells grown at permissive temperature (250C) and/or restrictive temperature (360C) were followed by gamma-radiation. Percentage survival and activity of DNA Polymerase (yPol II) were determined after post-irradiation incubation (5 h) with radioprotectors such as Caffeine, Curcumin, Disulphiram, and Ellagic acid (the dose depending on individual D 37 values). The gamma-irradiated yeast cells (with and without the radioprotectors) were spheroplasted by enzyme glusulase and subjected to electrophoresis. Radio-resistant cells were obtained by arresting cells in S-phase using transient treatment of hydroxyurea (HU) and studying the effect of caffeine at different concentrations on S-phase of cell cycle. Results: The mutants of S. pombe showed insignificant difference in survival when grown under permissive conditions. However, growth of these cells under restrictive temperature leads to arrest in specific phases of cell cycle in different cdc mutants (cdc10: G1 arrest, cdc22: early S arrest, cdc17: late S arrest, cdc25: G2 arrest). All the cdc mutants showed decrease in survival after gamma radiation when grown at permissive and restrictive temperatures. Inclusion of the radioprotectors at respective concentrations during post irradiation incubation showed increase in survival of cells. Activity of DNA polymerase enzyme (yPol II) was increased significantly in cdc mutant cells exposed to gamma-radiation. Following SCGE, a linear relationship was observed between doses of irradiation and the tail moments of comets. The radioprotection of the fission yeast by radioprotectors can be seen by the reduced tail moments of the yeast comets. Caffeine also exhibited its radio-protective ability in radio-resistant S-phase cells obtained after HU treatment. Conclusions: The radioprotectors offered notable radioprotection in cdc mutants when added during irradiation. The present study showed activation of DNA damage repair enzyme (yPol II) and an increase in survival after treatment of radioprotectors in gamma irradiated wild type and cdc mutants of S. pombe cells. Results presented here showed feasibility of applying SCGE in fission yeast to follow DNA damage and radioprotection at high doses, which are not feasible with other eukaryotes. Inclusion of caffeine at 1mM concentration to S phase cells offered protection and did not decrease the cell viability. It can be proved that at minimal concentration, caffeine offered marked radioprotection.

Keywords : radiation protection, cell cycle, fission yeast, comet assay, s-phase, DNA repair, radioprotectors, caffeine, curcumin, SCGE
Conference Title : ICMPRPR 2023 : International Conference on Medical Physics, Radiation Protection and Radiobiology
Conference Location : San Francisco, United States
Conference Dates : September 25-26, 2023

Open Science Index, Health and Medical Engineering Vol:17, No:09, 2023 publications.waset.org/abstracts/164879.pdf