Proceedings of the Pakistan Academy of Sciences 51 (2): 139–144 (2014)

ISSN: 0377 - 2969 (print), 2306 - 1448 (online)

Pakistan Academy of Sciences

Research Article

Cellular Stress Response Induced by Low Dose Gamma Radiation

Inayat ur Rahman[1]*, Naeem Ullah Jan[1], Hamid Rasul Niazi1[2] and Haroon Rahim[3]

[1*]Gandhara College of Pharmacy, Gandhara University, Peshawar, KPK, Pakistan
[2] Wazir Mohammad Institute of Paramedical Technology, Gandhara University, Peshawar, KPK, Pakistan
[3]Department of Pharmacy, Sarhad University of Science and Technology, KPK, Pakistan

Abstract: Low dose radiation-induced damages to DNA are mainly repaired by poly (ADP-ribose) polymerase (PARP-1 and PARP-2) system present in the cell. Suppression of neoplastic transformation of human hybrid cells by low doses of low linear energy transfer (LET) radiation is abrogated by PARP enzyme inhibitors and this presents a new tool in radiotherapy for cancers. The aim of this study was to investigate DNA damages by low doses of gamma rays (2.4 mGy/h) in the presence of inhibitors, 3-aminobenzamide (3-AB) and amino naphthalimide (ANI), with control. Cultured human fibroblast (VH10 cells) were irradiated with Cs^{137} γ-source at a dose rate of 2.4 mGy /hour for total doses of 50 mGy or 100 mGy in the presence of 3-AB and ANI and cell damage was detected through Comet assay. Compared to control, the presence of ANI did not inhibit PARP function at 50 mGy of 2.4 mGy/hr but there was increased tail moment in the presence of 3-AB and this might be due to differences in their concentrations. However, at 100 mGy dose, the tail moment increased in the presence of both 3-AB and ANI with the later showing greater increase. These results show that PARP inhibitors sensitized the cells to low dose γ radiation by abrogating the activity of PARP enzyme. DNA repair process reduced and the tail moment increased.

Keywords: Gamma radiation, PARP, 2.4 mGy, 100 mGy

1. INTRODUCTION

Ionizing radiations are atomic particles or electromagnetic waves that cause ionization of atoms and molecules. Particulate radiation is alpha-particle, beta particle, and neutron while electromagnetic waves (photons) include ultraviolet radiation, x-rays and gamma rays. Sources of ionizing radiations are radioactive materials, particle accelerator, x- ray tubes and the environment [1]. The Ionizing radiation energy is transferred to atoms and molecules in the cellular structure and ionizes or excites them. Both ionization and excitation produces free radicals which break chemical bond of molecules and form new chemical bonds. Vital molecules of cells (DNA, RNA and proteins) are damaged as a result of such energy transfer mechanism. Production of reactive oxygen species (ROS) is the result of water radiolysis in cells that cause a major damage to these macro molecules. This oxidative stress is the cause of different biological phenomena and diseases [2, 3]. Low level of radiation effects are small that could be repaired by different ways of DNA repair mechanisms. DNA damages induced by low dose rate radiation are repaired or cell may go through apoptosis to eliminate the potential genetic lesions. If the lesion is not so large because of low dose, it may lead to cancer due to error prone DNA repair processes [4].

Poly (ADP-ribose) polymerase or PARP is a family of proteins of 17 members involved in different functions. There are four domains of PARP including, catalytic domain, DNA binding domain, cleavage domain and auto-modification domain. These domains are responsible for different functions of the PARP enzyme. PARP-1 and PARP-2 are most important in DNA repair process. DNA-ribose polymer is synthesized from nicotinamide adenine dinucleotide (NAD) on PARP-1 activation. DNA single strand break (SSB)

Received, July 2013; Accepted, May 2014
*Corresponding author: Inayat ur Rahman; Email: marwax07@yahoo.com

is detected by PARP-1 and binding to SSB recruits base excision repair proteins (BER), i.e., XRCC1, DNA pol beta, DNA ligase and kinase to break sites. PARP-1 is oligomerized by PARP-2 in the repair mechanism [5]. It has been noted that low doses of low linear energy transfer (LET) radiation could suppress neoplastic transformation of human hybrid cells in vitro. This suppression is abrogated by using 3- aminobenzamide (3-AB), an inhibitor of PARP enzyme [6]. PARP inhibitors, 3-AB and 4-Amino-1, 8-naphthalimide (ANI) competitively inhibit the catalytic domain of PARP from binding to NAD and prevent ribosylation. In this way, the DNA breaks will not be repaired by PARP enzyme and cells will die. In radiotherapy for tumor cells, the effect of ionizing radiation is potentiated by using PARP inhibitors [7, 8]. Inhibitors act by increasing the sensitivity of mammalian cells to low doses of ionizing radiation [9, 10]. The use of PARP inhibitors in cancers in which BRCA 1/2 are mutated is a novel approach in cancer therapy [8, 11]. In treatment of glioblastoma, the sensitivity of rapidly proliferating glioma cells to ionizing radiation is enhanced by using PARP inhibitors [12, 13]. Inhibition of PARP enzyme represents a new tool in radiotherapy for cancers and gives promising results [14, 15]. The aim of this study was to investigate the induction of DNA damages by low doses of low dose rate gamma radiation by inhibiting poly (ADP-ribose) polymerase (PARP) protein, a DNA repair enzyme.

2. MATERIAL AND METHODS

2.1 Cell Culture

The adherent human fibroblast VH10 cell line was cultured in Minimum Essential Medium Eagle (MEM) supplemented with antibiotics (1 % penicillin-streptomycin 10000 IU) and 10 % bovine calf serum. For experiments, cells were washed 2x with Hanks Balanced Salt Solution (HBSS) and trypsinized with 1 ml trypsin-EDTA for 5-7 min at 37 °C. Then 10ml MEM was added to neutralize trypsin and the cell suspension was put on shaker in order to prevent cell attachment. The number of cells was counted 3 times by cell counter and 0.2 x 10^6 cells were seeded into 25 cm^2 flasks containing 8 ml MEM. Cells were grown overnight in a humidified 95 % air and 5 % CO_2 atmosphere at 37 °C.

2.2 Irradiation Conditions

After overnight incubation, cell medium was changed with fresh 8 ml MEM containing 5 mM 3-AB or 10 µM ANI, respectively. One sample was used without inhibitor as control. Flasks were incubated in the specially designed low dose rate radiation incubator with the Cs^{137} γ-source underneath at a dose rate 2.4 mGy /hour for total doses of 50 mGy or 100 mGy. Irradiation was carried out as described earlier [16].

2.3 Comet Assay

After irradiation, cells were washed with HBSS and trypsinized. The cell suspension was kept on ice and cell number was counted for each sample. Then 200µl of cell suspension with 0.2 x 10^6 cells/ml was mixed with 2 % low melting agarose in same ratio (1:1), 80 µl of each sample were applied on the slides coated with 0.5 % normal melting agarose and covered with cover slips. In cold room, all slides were put in lysis buffer (2.5M NaCl + 100mM Na2EDTA + 10 mM Tris Base) of pH 10 for 1 hour. After one hour, slides were washed, shifted to electrophoresis tank filled with unwinding buffer (3M NaOH + 10 mM Na2EDTA) of pH 10 and voltage to 32.5V, left for 1 hour for unwinding process. After one hour, keeping the voltage constant, switched on the power of electrophoresis for 25 min. Then rinsed slides 3 x 5 min in neutralizing buffer of pH 7.5 (0.4M Tris Base). The slides were stained with 50µl DAPI stain and covered with cover slips. All slides were analyzed on next day.

2.4 Statistical Methods

The values are expressed as means ± SD. Statistical analyses were performed using SAS statistical software, version 8.0 (SAS Institute, Cary, NC, USA) for Windows®. A p-value <0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of Inhibitors on VH10 Cell at 50mGy of 2.4 mGy/h Gamma Rays

The present study was conducted to detect the effect of low dose of low dose rate gamma rays (2.4 mGy/h) in the presence of inhibitor, 3-AB

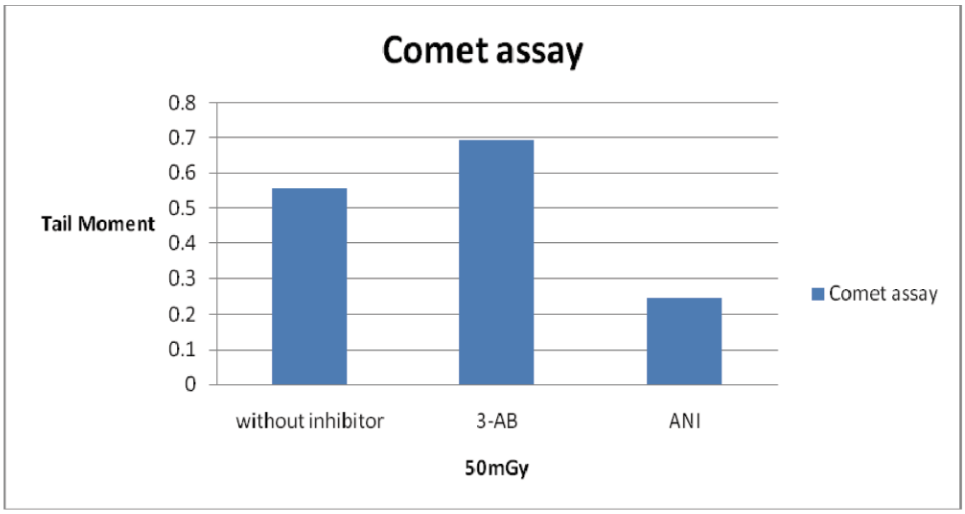

Fig. 1. Effect of 50 mGy dose of low dose rate (2.4 mGy/h) gamma radiation on cell DNA in the absence and presence of inhibitors, 3-aminobenzamide (3-AB) and ANI. Data are mean of 2 independent experiments.

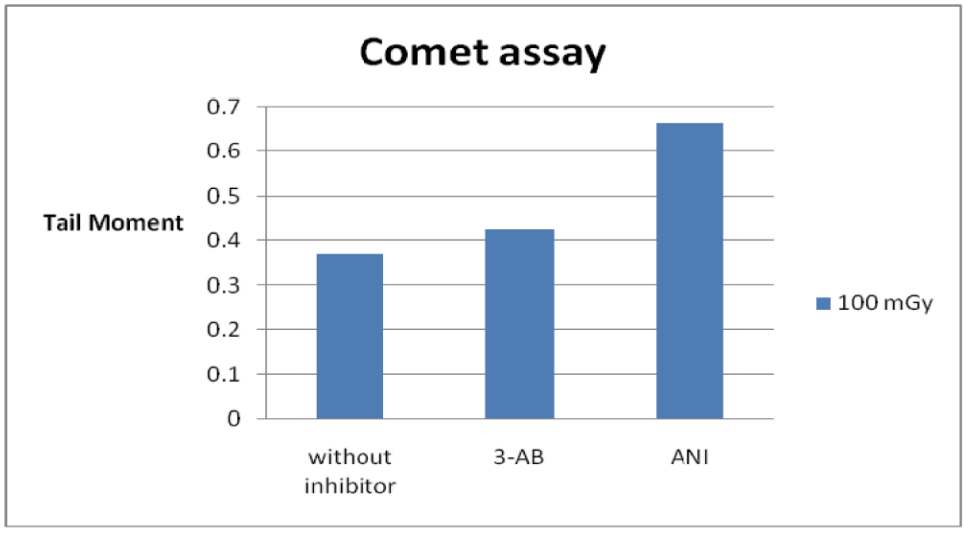

Fig. 2. Effect of 100 mGy dose of low dose rate (2.4 mGy/h) gamma radiation on cell DNA in the absence and presence of inhibitors, 3-aminobenzamide (3-AB) and ANI.Data are mean of 2 independent experiments.

and ANI with control. Comet assay was used to detect cell damage. In this technique, individual cell DNA damage and its migration was identified in the form of tail moment. There was increased tail moment in the presence of 3-AB as compared to control (Fig. 1). These results are supported by previous study that shows increased sensitization of cells to gamma radiation by treatment with 3-AB [17]. This method is more flexible and sensitive, requires less number of cells and has low cost. In genotoxicity, it is frequently used to identify mutagenic agents to human i.e. ionizing radiation [18]. Incubation with 3-AB (100 mM) on 50 mGy dose reduced DNA repair process (tail moment increased). In the presence of inhibitors the tail movement was more than without inhibitor. In contrast, ANI did not show inhibition of PARP function due to some unclear error (Fig. 1, 3). This is probably due to low concentration of ANI compared to 3-AB (8µl and 400µl respectively) used with the dose of 50 mGy. The effect of ANI is shown to be concentration dependent [18].

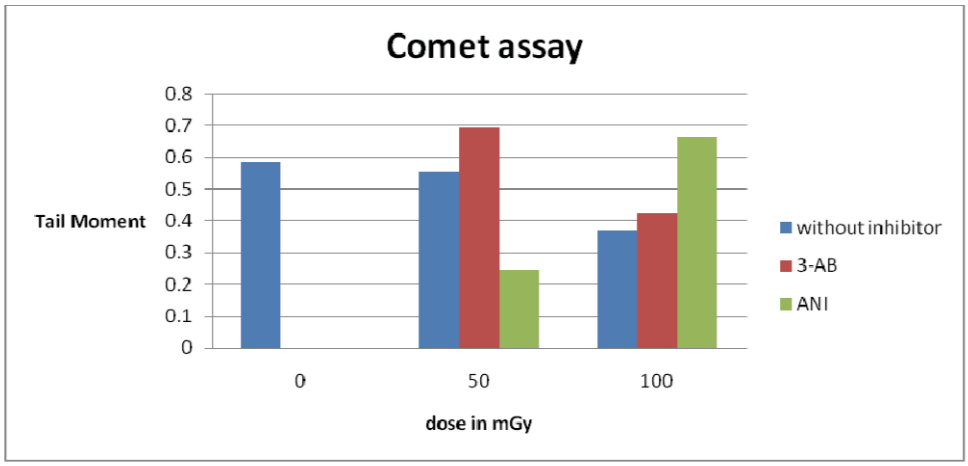

Fig. 3. Effect of low dose rate γ–radiation on cells in the presence and/or absence of PARP 3-AB and ANI. Data are mean of 2 independent experiments.

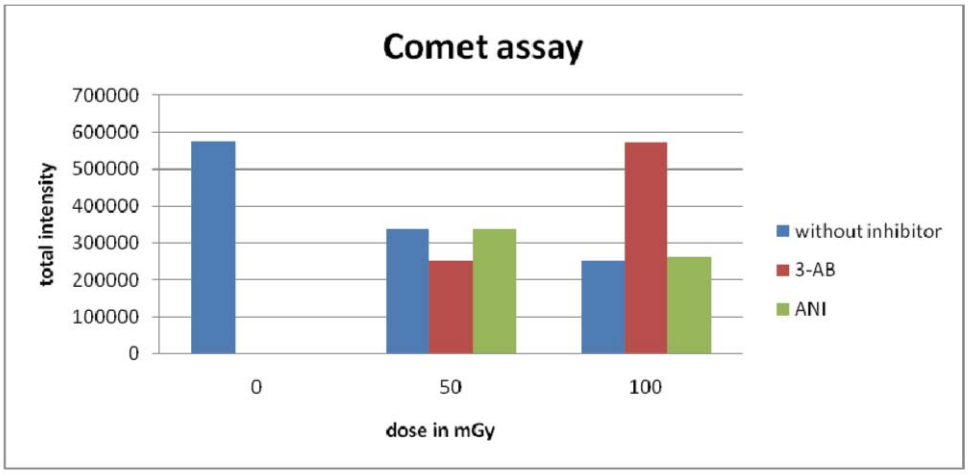

Fig. 4. Total intensity of DNA of γ-irradiated cells in the presence and/or absence of 3-AB and ANI. Data are mean of 2 independent experiments.

Radiosensitiztion mostly depends on the phase of cell cycle. Moreover, inhibiting DNA repair by ANI radiosnsitization is studied specifically in S phase. In this experiment, confluent VH10 cells were used that repaired DNA damage irradiated before S phase or on G2-M and G1 phases [7, 19].

3.2 Effect of inhibitors on VH10 cell at 100 mGy of 2.4 mGy/h Gamma Rays

Figure 2 represents the radiosensitizing effect of both PARP inhibitors during irradiation of 100 mGy dose. Compared to control, tail moment of cell DNA was more with inhibitors ANI and 3-AB. Further, the effect of ANI on PARP inhibition was much larger at 100mGy dose (Fig. 2, 3). It has been shown that 3-AB and ANI are radiosensitizing the culture cells which abrogate the activity of PARP enzyme, ANI is highly specific to inhibiting PARP activity in DNA repair and more potent than 3-AB [20, 21].

3.3 Effect on Total Intensity

Low dose and low dose rate of gamma radiation effect showed decreased total intensity of cellular DNA. It also decreased with inhibitors, but in the presence of 3-AB (0-100mGy), the total intensity was equal to control sample (without radiation and inhibitors). The irradiated cells were mostly examined for apoptosis, which might then be proposed that total intensity was affected (Fig.

4). This study of radiosensitization with PARP inhibitors on confluent fibroblasts is generally agreed with previous observations [7, 16, 17, 22, 23].

4. CONCLUSIONS

The study concludes that PARP inhibitors are concentration dependent and the dose and dose rate affects the DNA damage to large extent. Moreover, the killing of cells by low dose of low dose rate gamma radiation is improved in the presence of PARP inhibitors.

5. ACKNOWLEDGEMENTS

The authors are thankful to Gandhara College of Pharmacy, Gandhara University, Peshawar for providing the required facilities and supporting the research project.

6. REFERENCES

1. Hall, E.J. & A.J. Giaceia. *Radiobiology for the Radiobiologist,* 6th ed. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA, p. 1-15 (2005).
2. Goodhead, D.T. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. *International Journal of Radiation Biology* 65: 7-17 (1994).
3. Robert, A. Fosbinder & D. Orth. *Essentials of Radiologic Science*, Part-1. Lippincott Williams & Wilkins, Pennsylvania, Pennsylvania, USA, p. 1-20 (2011).
4. Little, J.B. Radiation Carcinogenesis. *Carcinogenesis* 21: 397-404 (2000).
5. Chalmers, A.J., M. Lakshman, N. Chan & R.G. Bristow. Poly (ADP-ribose) polymerase inhibition as a model of synthetic lethality in developing radiation oncology targets. *Seminars in Radiation Oncology* 20(4): 274-281 (2010).
6. Pant, M.C., X. Y. Liao, Q. Lu, S. Molloi, E. Elmore & J.L. Redpath. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. *Carcinogenesis* 24(12): 1961-1965 (2003).
7. Noel, G., C. Godon, M. Fernet, N. Giocanti, F. Meqnen-Channet & V. Favaudon. Radiosensitization by the poly(ADPribose) polymerase inhibitor, 4-amino-1,8-naphthalimide is specific of the S phase of the cell cycle and involves arrest of DNA synthesis. *Molecular Cancer Therapeutics*. 5: 564-574 (2006).
8. Davar, D., J.H. Beumer2, L. Hamieh & H. Tawbi. Role of PARP inhibitors in cancer biology and therapy. *Current Medicinal Chemistry* 19(23): 3907–3921 (2012).
9. Chalmers, A., P. Johnston, M. Woodcock, M. Joiner & B. Marples. PARP-1, PARP-2, and the cellular response to low doses of ionizing radiation. *International Journal of Radiation Oncology* 58: 410-419 (2004).
10. Chalmers, A.J. Poly (ADP-ribose) Polymerase-1 and Ionizing Radiation: sensor, signaller and therapeutic target. Clinical Oncology 16: 29-39 (2004).
11. Evers, B., R. Drost, E. Schut, M. de Bruin, E. van der Burg, P.W.B. Derksen. Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. *Clinical Cancer Research* 14: 3916-3925 (2008).
12. Dungey, F.A., D.A. Loser & A.J. Chalmers. Replication-dependent radiosensitization of human glioma cells by inhibition of poly (ADP-Ribose) polymerase: mechanisms and therapeutic potential. *International Journal of Radiation Oncology Biology Physics* 72(2): 1188-1197 (2008).
13. Zheng, Y.D., X.Q. Xu, F. Peng, J.Z. Yu & H. Wu. The poly (ADP-ribose) polymerase-1 inhibitor 3-aminobenzamide suppresses cell growth and migration, enhancing suppressive effects of cisplatin in osteosarcoma cells. *Oncology Reports 25:* 1399-405 (2011).
14. Albert, J.M., C. Cao, K.W. Kim, C.D. Willey, L. Geng. D. Xiao & H. Wang. Inhibition of poly (ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. *Clinical Cancer Research* 13(10): 3033-3042 (2007).
15. Zhang, J., K. Yanyan, T. Yongjie, W. Zhe & Z. Jie. Effects of poly (ADP-ribosyl) polymerase (PARP) inhibitor on cisplatin resistance & proliferation of the ovarian cancer C13 cells. *Indian Journal of Medical Research* 137: 527-532 (2013).
16. Gilbin, R., A. Frederic & G. L. Jacqueline. Effects of chronic external gamma irradiation on growth and reproductive success of Daphnia magna. *Journal of Environmental Radioactivity* 99: 134-145 (2008).
17. Thraves, P., K.L. Mossaman, T. Brennan & A. Dritschilo. Radiosensitization of human fibroblasts by 3-aminobenzamide: an inhibitor of poly (ADP-ribosylation). *Radiation Research* 104 (2 Pt 1): 119-127 (1985).
18. Tice, R. R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann & H. Kobayashi. Single cell gel/Comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis* 35: 206-221 (2000).
19. Staff, E., B. Karl, H. Siamak, C. Joanna & W. Andrzej. Gamma-H2AX foci in cells exposed to a

mixed beam of X-rays and alpha particles. *Genome Integrity* 3: 8 (2012).

20. Schlicker, A., P. Peschke, A. Bürkle, E.W. Hahn & J.H. Kim. 4-Amino-1,8-naphthalimide: a novel inhibitor of poly(ADP-ribose) polymerase and radiation sensitizer. *International Journal of Radiation Biology* 75: 91-100 (1999).
21. Pereira, S., V. Malard, R.J. Luc, H.D. Anne, A. Jean, F. Nicolas & A.G. Christelle. Low doses of gamma-irradiation induce an early bystander effect in Zebra fish cells which is sufficient to radioprotect cells. *PLOS ONE* 9(3): e92974 (2014), doi:10.1371/journal.pone.0092974
22. Hirai,T., H. Shirai, H. Fujimori, R. Okayasu, K. Sasai & M. Masutani. Radiosensitization effect of poly(ADP-ribose) polymerase inhibition in cells exposed to low and high liner energy transfer radiation. *Cancer Science* 103: 1045-1050 (2012).
23. Postiglione, I., A. Chiaviello & G. Palumbo. Twilight effects of low doses of ionizing radiation on cellular systems: a bird's eye view on current concepts and research. *Medical Oncology* 27: 495–509 (2010).