Radio-adaptive response of base excision repair genes and proteins in human peripheral blood mononuclear cells exposed to gamma radiation

Sneh M. Toprani and Birajalaxmi Das*

Low Level Radiation Research Section, Radiation Biology and Health Sciences Division, Bio-Sciences Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

*To whom correspondence should be addressed. Tel: +91-022-25595047/25590422; Email: biraj@barc.gov.in, birajalaxmi@yahoo.co.in

Received 28 September 2014; Revised 9 March 2015; Accepted 10 March 2015.

Abstract
Radio-adaptive response is a mechanism whereby a low-dose exposure (priming dose) induces resistance to a higher dose (challenging dose) thus significantly reducing its detrimental effects. Radiation-induced DNA damage gets repaired through various DNA repair pathways in human cells depending upon the type of lesion. The base excision repair (BER) pathway repairs radiation-induced base damage, abasic sites and single-strand breaks in cellular DNA. In the present study, an attempt has been made to investigate the involvement of BER genes and proteins in the radio-adaptive response in human resting peripheral blood mononuclear cells (PBMC). Venous blood samples were collected from 20 randomly selected healthy male individuals with written informed consent. PBMC were isolated and irradiated at a priming dose of 0.1 Gy followed 4 h later with a challenging dose of 2.0 Gy (primed cells). Quantitation of DNA damage was done using the alkaline comet assay immediately and expression profile of BER genes and proteins were studied 30 min after the challenging dose using real-time quantitative polymerase chain reaction and western blot, respectively. The overall result showed significant ($P \leq 0.05$) reduction of DNA damage in terms of percentage of DNA in tail (%T) with a priming dose of 0.1 Gy followed by a challenging dose of 2.0 Gy after 4 h. Twelve individuals showed significant ($P \leq 0.05$) reduction in %T whereas eight individuals showed marginal reduction in DNA damage that was not statistically significant. However, at the transcriptional level, BER genes such as $APRE1$, $FEN1$ and $LIGASE1$ showed significant ($P \leq 0.05$) up-regulation in both groups. Significant ($P \leq 0.05$) up-regulation was also observed at the protein level for $OGG1$, $APE1$, $MBD4$, $FEN1$ and $LIGASE1$ in primed cells. Up-regulation of some BER genes and proteins such as $APE1$, $FEN1$ and $LIGASE1$ in primed cells of resting PBMC is suggestive of active involvement of the BER pathway in radio-adaptive response.

Introduction
Humans are exposed to various types of genotoxic stress in their daily life including low doses of ionising radiation (IR). Low-dose radiation exposure to humans comes from natural as well as man-made sources. In recent years, efforts have been made to generate data from epidemiological and biological studies at low doses/low-dose rate radiation exposures, especially below 100 mSv. A number of epidemiological studies are available at such low-dose exposures on stochastic effects such as incidence of cancer and heritable effects (1–3). Epidemiological studies require a large population to be studied due to low statistical power. Due to higher values of spontaneous incidence of cancer, sometimes it is difficult to find out the effect of low-dose exposures in a population. Therefore, it is important to understand the biological mechanisms occurring in human cells exposed to low doses of IR.
The Linear No Threshold hypothesis is well debated as the data for low-dose exposures were extrapolated from high acute-dose exposures. Although there are plenty of data available from animal and human cells, the process of carcinogenesis at low-dose radiation exposures is still inconsistent and inconclusive. DNA repair processes are important in biological studies and efforts have been made to study dose response and adaptive response (AR) in human cells using different end points/parameters.

AR is a phenomenon where a small initial dose (priming) activates repair mechanism that reduces the response to a subsequent larger (challenging) dose (4–7). The molecular mechanism underlying radio-adaptive response is not yet clearly understood. It is assumed that AR may be either due to enhanced repair of DNA damage or due to protection against the adverse effects induced by high challenging doses (8). IR induces a variety of DNA lesions including oxidative base damage, abasic sites, single-strand breaks (SSB), double-strand breaks (DSB) and clustered damages. Depending on the amount of dose/dose rate and type of radiation; the severity varies. As compared to endogenous damages, induction of radiation-induced damage is comparatively low (9).

In general, low linear energy transfer radiation exposure of 1.0 Gy produces around 850 pyrimidine lesions, 450 purine lesions, 1000 SSB and 20–40 DSB per cell (9,10). The efficient DNA repair machinery in the human body constantly repairs these DNA lesions through biochemical and molecular pathways to maintain genome integrity. But if these IR-induced DNA damages are not repaired, they may lead to the accumulation of mutations or chromosomal aberrations in human cells. They may also lead to alteration of the expression profiles of several genes, miRNAs and proteins, activation of cell cycle check points and may even lead to cell death (apoptosis) (11–16). There is sufficient evidence that the effect of radiation is qualitatively and quantitatively different at low doses as compared to high-dose exposures (17). Considering all these factors, induction of repair processes and protection of radiation-induced damage by the AR in human cells are very complex. The dose, dose rate and time period between priming and challenging dose may be crucial for a cell to induce AR. Besides these factors, humans also display a high degree of inter-individual variation that may play an important role in radio-adaptive response as it is dependent on the radio-sensitivity of an individual.

ARs have been demonstrated in vitro and in vivo using various end points such as cellular damage, cell lethality, chromosomal aberrations, mutation induction, radio-sensitivity and DNA repair. It is not yet clear at what relevant dose and dose rate exposures the AR is functional in humans (18). A better understanding of the AR is needed before they can be confirmed as a risk estimate factor for radiation protection purposes. Olivieri et al. (19) have shown a reduced frequency of chromosome aberrations in peripheral blood lymphocytes cultured in the presence of [1H] thymidine followed by X-ray exposure. There are studies showing the involvement of adaptive DNA repair mechanisms such as nucleotide excision repair (NER) and non-homologous end joining (NHEJ) in mammalian cells but no DNA repair genes/enzymes have been directly implicated to this response (15,20–26).

DSB are known to be deleterious and are repaired by homologous recombination or NHEJ pathways. Similarly, SSB and base damages are repaired by base excision repair (BER) or NER mechanisms. There are several reports that suggest that SSB and base damages, if they remain unrepaired, may be associated with cancer and age-related diseases including Alzheimer’s disease and neurodegenerative disorders (27). Hence, BER also plays a crucial role in maintaining genome integrity. BER is initiated by DNA-specific glycosylases such as MBD4, MUTYH (monofunctional) and OGG1, NTH1 and NEIL1 (bifunctional) (28). Apurinic/apyrimidinic endonuclease 1 (APE1) processes AP (apurinic/apyrimidinic) sites (29,30). BER can be subdivided into short-patch and long-patch pathways. Short-patch BER involves genes/proteins such as XRCC1 (X-ray cross-complementing factor 1) and DNA LIGASE3, whereas in long-patch BER pathway, PCNA (proliferating cell nuclear antigen), FEN1 (Flap endonuclease 1) and DNA LIGASE1 are involved (31–34). Poly(ADP-ribose) polymerase (PARP) is also known to repair single-strand DNA nicks and play a crucial role in repairing damaged DNA (35). Our group has demonstrated active involvement of BER genes and proteins in repairing radiation-induced damage in G1/G0 stage of human peripheral blood mononuclear cells (PBMC) up to 4h (16).

AR has been demonstrated when both the conditioning and challenge doses are applied at late stages (S/G2 phases) of the cell cycle. However, there are disagreements as to whether or not the AR occurs if the conditioning dose is applied in the resting or early stages (G0/G1) of the cell cycle. This important point needs to be addressed because it has implications for the circumstances in which cells are chronically irradiated in vivo. Human PBMC are in the resting stage (G0/G1) of the cell cycle and are considered to be highly sensitive and thus an ideal choice to study ex vivo exposure (22,36–38). In the present study, an attempt has been made to find out the involvement, if any, of BER genes and proteins in AR, in resting human PBMC exposed to gamma radiation.

Materials and methods

Collection of blood samples

Venous blood samples (~12ml) were collected in ethylenediamine-tetraacetic acid (EDTA) containing vacutainers from 20 randomly selected healthy male donors aged 25–40 years. All the donors were non-smokers and without chronic illness. Written informed consent was obtained from all the donors for the study, which was approved by Medical Ethic Committee, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai.

Isolation of PBMC from human blood

PBMC were isolated using density gradient centrifugation using HiSep (HiMedia Laboratory Pvt. Ltd., Mumbai, India) at 400g for 30 min at room temperature. Theuffy layer containing PBMC were washed with ice cold 1x phosphate buffer saline (PBS) twice at 110g for 10 min and further divided into four aliquots. Each aliquot has two sub aliquots that were used as duplicates for each experiment. Each of the aliquots was used for DNA damage quantitation, gene and protein expression studies. Each sub-aliquot had a density of ~106 cells/ml.

Irradiation

Prior to irradiation, PBMC were re-suspended in RPMI 1640 medium (Hi Media Pvt. Ltd., Mumbai, India) and were exposed to gamma irradiation at room temperature using 60Co γ-teletherapy machine (Bhabhatron II, Panacea Medical Technologies, Bangalore, India) at a dose rate of 1.0 Gy/min. Four aliquots (in duplicates) were prepared and briefly the irradiation protocol is as follows:

The first aliquot of PBMC was unirradiated and considered as control which was processed simultaneously, along with the irradiated samples in all the experiments. The second aliquot of cells was exposed to a priming dose of 0.1 Gy and incubated for 4h. The third
 aliquot of cells was incubated for 4 h and after incubation, exposed to a challenging dose of 2.0 Gy, thus the cells are non-primed cells. The fourth aliquot of cells which was exposed to a combination of both priming and challenging dose, wherein initially the aliquot was exposed to priming dose of 0.1 Gy and then incubated for 4 h. After incubation, the same aliquot was irradiated with a challenging dose of 2.0 Gy, thus the cells are primed cells. For all the experiments, PBMC were incubated at 37°C, 5% CO₂ concentration and 90% humidity. DNA damage quantitation was done immediately after the challenging dose was given, whereas gene and protein expression was studied after 30 min of challenging dose (2.0 Gy).

**Cell cycle analysis using flow cytometry**

Cell cycle stages were analysed by propidium iodide (PI) staining using flow cytometry (PARTEC Cyflow space™ flow cytometer, Partec Private Ltd., Germany). The percentage of cells in different phases of cell cycle (G₁, S + G₂/M) and percentage of apoptotic cells were estimated. Cell cycle analysis was done for PBMC exposed to 0.1 Gy (priming dose), 2.0 Gy (non-primed cells) and 0.1 Gy of priming dose followed by a challenging dose of 2.0 Gy after 4 h (primed cells). An unirradiated sample was used as control. At the end of the incubation period, cells were washed with PBS and incubated with 1.0 ml of staining solution containing 50 μg/ml PI, 0.1% sodium-nitrate and 0.1% Triton X-100. A total of 20,000 cells were acquired in Partec Cyflow flow cytometry and analysed using Cylogic software (version 1.2.1, 2008, CyFlo Ltd., Finland). Too small objects including cell debris and the majority of non-adherent monocytes were excluded from analysis by a standard approach using the ‘lymphogate’ region in the forward and side scatter characteristics (FSC and SSC, respectively). Lymphogate was carefully set up to include smaller cells into the analysed population.

**DNA damage quantitation using alkaline comet assay**

DNA damage quantitation using the alkaline comet assay was performed on PBMC obtained from 20 individuals. Cells (~10⁶ cells/ml) were mixed with molten agarose (Sigma-Aldrich, USA) made in 1× Tris Borate Saline (TBS) and pH 10.0, 10% dimethyl sulfoxide and 1% Triton X-100. After lysis, the slides were immersed in freshly prepared Alkaline Solution, pH >13 containing sodium hydroxide (NaOH) pellets and 500 mM EDTA (pH 8.0) at 25 V, 300 mA for 20 min. The slides were then rinsed in neutralisation buffer (0.4 M Tris–HCl, pH 7.5) for 5 min, fixed in 70% ethanol for 5 min, stained with SYBR Green I (Sigma-Aldrich) and images were taken in dark at 20× magnification using a fluorescent microscope (Nikon Eclipse Ti-U inverted microscope, Japan). About 100 cells (50 from each duplicate slide) were randomly selected and quantified using TriTek Comet Score FreeWare™ version 1.5. Where percentage of DNA in tail was calculated as per the calculation:

\[
\text{Percentage of DNA in tail} \times 100 = \frac{\text{Total tail intensity}}{\text{Total comet intensity}}
\]

**Transcription profile of BER genes**

The transcription profile was studied for 12 BER genes, i.e. OGG1, NEIL1, NTH1, APE1, PARP1, XRCCL1, LIGASE3, MUTYH, MBD4, PCNA, FEN1 and LIGASE1 in PBMC of 20 individuals. Each aliquot of PBMC had a density of ~10⁶ cells/ml. After irradiation, PBMC were incubated in RPMI 1640 medium at 37°C (with 95% humidity and 5% CO₂ concentration) and gene expression pattern was analysed after 30 min of challenging dose administration.

**Total RNA isolation and cDNA preparation**

Total RNA was extracted using Hipura RNA isolation kit (Hi media Laboratory Pvt. Ltd., Mumbai, India) and quantified using Picodrop Microliter Spectrophotometer (Pico100, Pico drop Ltd., UK). RNA (500 ng) was reverse transcribed to cDNA using Transcriptor High Fidelity cDNA synthesis kit (Roche Diagnostics Pvt. Ltd., GmbH, Germany) and stored at −20°C.

**Real-time quantitative polymerase chain reaction of BER genes**

mRNA expression was quantitated for BER genes using real-time quantitative polymerase chain reaction (RT-qPCR) which was performed in 96-well microtiter plate using LC480 real-time PCR machine (Roche Diagnostics Pvt. Ltd., GmbH, Germany). PCR was performed in 12.5 μl mixture containing cDNA, 0.5U Fast start Taq DNA polymerase, 5.0 picomoles of both forward and reverse primer mixture containing dNTPs. Each PCR components were procured from Roche Diagnostics Pvt. Ltd., GmbH, Germany, whereas primer sets were procured from Sigma-Aldrich. The sequences of the primers used is given in Table 1. β-actin was used as a reference gene and the reactions were carried out in duplicates.

The PCR cycling conditions were as follows: a pre-incubation step at 95°C for 5 min followed by denaturation at 95°C for 10 s, annealing 58°C for 30 s and extension at 72°C for 30 s. Melting curve analysis was done in the following three steps: melting at 95°C for 5 min followed by an annealing step at 58°C for 1 min and an extension at 72°C followed by a final step at 40°C for 10 s. Melting curve analysis was done to ensure proper amplification of the product. Relative quantification was performed by using the LC480 software version 1.1. The results are expressed in normalised ratio as described by Pfaffle (41).

\[
\text{Normalised ratio} = \frac{\text{Concentration of target}_\text{Sample}}{\text{Concentration of reference}_\text{Sample}} : \frac{\text{Concentration of target}}{\text{Concentration of reference}}_\text{Calibrator}
\]

The relative expression was calculated with respect to control.

**Protein expression profile of BER using western blot**

Protein expression profile was studied for BER proteins such as OGG1, NTH1, NEIL1, APE1, PARP1, XRCC1, LIGASE3, MUTYH,
Table 1. Primer sequences of BER genes and β-actin used for RT-qPCR

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Gene name</th>
<th>Primer sequence</th>
<th>Base pair (bp)</th>
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<tr>
<td>1</td>
<td>β-Actin PR1</td>
<td>5′-CCAGAGCGGTACAGGGATAG-3′</td>
<td>20</td>
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<tr>
<td></td>
<td>β-Actin PR2</td>
<td>5′-CCAACCGCGAGAATGTA-3′</td>
<td>18</td>
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<tr>
<td>2</td>
<td>APEX1 PR1</td>
<td>5′-CAGGCTTGAATAGAAGAAG-3′</td>
<td>22</td>
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<td></td>
<td>APEX1 PR2</td>
<td>5′-TTTGGTCTTGGAAAGGCA-3′</td>
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<tr>
<td>3</td>
<td>PARP1 PR1</td>
<td>5′-CCAGTTCCCAGATGACT-3′</td>
<td>18</td>
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<tr>
<td></td>
<td>PARP1 PR2</td>
<td>5′-AGTCCCTTCTGTTGTCCGGATT-3′</td>
<td>21</td>
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<td>4</td>
<td>OGG1 PR1</td>
<td>5′-CTGATCTCCGTCGGAAGT-3′</td>
<td>18</td>
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<td>5′-CTGAGGATAAGTCTAGGA-3′</td>
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<tr>
<td></td>
<td>LIGASE1 PR2</td>
<td>5′-CCGTCCTGTGCTATTGG-3′</td>
<td>20</td>
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</table>

PR1: forward primer and PR2: reverse primer.

MBD4, PCNA, FEN1 and LIGASE1 in six individuals using western blotting. Each of the aliquots (2×10⁵ cells/ml) was irradiated and incubated at 37°C, 95% humidity and 5% CO₂ concentration along with the control and further processed 30 min post-irradiation after challenging dose administration. The PBMC were then lysed with Buffer A containing 10 mM HEPES (pH 7.4), 1.5 mM magnesium chloride (MgCl₂), 10 mM potassium chloride (KCl), 0.5 mM dithiothreitol (DTT) and 1x protease inhibitor mixture (Roche Diagnostics Pvt. Ltd., Gmbh, Germany). Nuclear pellet was obtained by centrifuging the cells at 2800×g for 10 min at 4°C and further dissolved in Buffer B containing 20 mM HEPES (pH 7.4), 25% Glycerol, 0.42 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA (pH 8.0), 1.0 mM DTT and 1x protease inhibitor mixture (Roche Diagnostics Pvt. Ltd., Gmbh, Germany). The cells were centrifuged at 8700×g for 10 min at 4°C to obtain nuclear extract from the supernatant. Bicinchoninic acid protein assay kit (Bangalore Genei India Pvt. Ltd., Bangalore, India) was used for estimation of the nuclear protein concentration and was calculated by plotting standard curve. Electrophoresis of 100 µg of nuclear protein per sample was carried out onto 12% sodium dodecyl sulphate–polyacrylamide gels. The protein was transferred to Poly-vinylidyne difluoride membrane (Sigma-Aldrich) by wet electro-blotting. Blocking of the membrane was carried out at 4°C for 2h using 5% blocking solution (bovine serum albumin) made in Tris-buffered saline with Tween-20 (TBST). The primary antibodies anti-OGG1, anti-NTH1, anti-NEIL1, anti-APEx1, anti-PARP1, anti-XRCC1, anti-LIGASE3, anti-MUTYH, anti-MBD4, anti-PCNA, anti-FEN1 and anti-LIGASE1 of dilution 1:10 000 each (Santa Cruz Biotechnology, Inc., USA) were prepared in 1% blocking solution and added overnight at 4°C. Further after washing, the membranes were incubated with secondary antibodies labelled with horseradish peroxidase (Santa Cruz Biotechnology, Inc.), diluted (1:10 000) in 1% blocking solution then subsequently washed and incubated with Super Signal West Dura Chemiluminescent substrate (Thermo Scientific, USA). β-actin was used as a loading control. The blots were scanned using Gel Capture (Version 5.3, DNR Bio imaging Systems Ltd., Israel).

Statistical analysis

The level of significance was taken at P ≤0.05. Coefficient of variation (CV) which represents the variability in relation to the mean was calculated by taking the ratio of standard deviation with the average mean (CV = standard deviation/average mean). For DNA damage analysis, gene expression and protein profile, paired ‘t’ test was performed to find out the significance between the aliquot receiving combination of priming dose of 0.1 Gy followed by a challenging dose of 2.0 Gy after 4h (primed cells) and aliquot receiving only a challenging dose (2.0 Gy) (non-primed cells). For protein expression profile, the bands were analysed using Image J 1.43m version software (Wayne Rushband, NIH, USA). Fold change at protein expression profile of irradiated samples were calculated as compared to unirradiated control. All the statistical analysis was performed using SPSS software version 16.0 (IBM Corporation, USA, 2008).

Results

In the present study, AR study was performed using DNA damage, transcription and protein expression profiles in PBMC of 20 individuals exposed to a priming dose of 0.1 Gy followed by a challenging dose of 2.0 Gy after 4h incubation. The DNA damage quantitation was measured immediately, whereas gene and protein expression profile were studied after 30 min of challenging dose administration. The CV was calculated for each end point among the individuals studied. In the present study, the CV among the individuals is observed to be a maximum of 34%.
Cell cycle analysis using flow cytometry

Cell cycle analysis was studied in human PBMC of five individuals. The percentage of cells in different phases of cell cycle (G\textsubscript{0}/G\textsubscript{1}, S + G\textsubscript{2}/M) and percent apoptotic cells (sub-G\textsubscript{1}) were estimated using flow cytometry as given in Figure 1A–D. Undivided cells (central peak) were in G\textsubscript{0}/G\textsubscript{1} phase of cell cycle (2n DNA content). The population showing more than 2n DNA represents cells in S + G\textsubscript{2}/M phase of cell cycle. Pre-G\textsubscript{1} peak represent percent of apoptotic cells. Majority of cells were in G\textsubscript{0}/G\textsubscript{1} (87–90%) phase while 2.4–3.2% cells were in sub-G\textsubscript{1} stage and minimal cells in S + G\textsubscript{2}/M (Figure 1E).

DNA damage quantitation using alkaline comet assay

DNA damage quantitation was carried out in all 20 individuals using the alkaline comet assay and the percentage of DNA in tail (%T) was correlated to radiation-induced DNA damage quantitation. Figure 2 presents %T at different doses. Our overall results have shown a
significant reduction \((P \leq 0.05)\) in %T ranging from 11.07% at 2.0 Gy (non-primed cells) to 9.42% in sample receiving a priming dose of 0.1 Gy followed by a challenging dose of after 4 h (primed cells).

However, the average %T varies from 7.87 to 11.55% in primed cells among the 20 individuals studied. Although there was overall reduction of DNA damage in the individuals studied, we observed inter-individual variation in AR and thus we stratified them into two groups.

In Group I \((N = 12)\), significant decrease \((P \leq 0.05)\) in %T ranged from 10.78% in non-primed cells to 8.39% in primed cells (Figure 3A). Among these 20 individuals, Group II \((N = 8)\) individuals, the average %T ranged from 11.49% in non-primed cells to 10.97% in primed cells although the decrease was not statistically significant (Figure 3B). There was a significant increase in %T in non-primed cells (2.0 Gy) both in Group I and Group II as compared to 0.1 Gy alone and unirradiated control.

Analysis of transcription profile of BER genes using RT-qPCR

The relative expression of 12 genes involved in the BER pathway, i.e. short-patch BER genes \((OGG1, NTH1, NEIL1, XRCC1 and LIGASE3)\), regulator BER genes \((APE1 and PARP1)\) and long-patch BER genes \((MUTYH, MBD4, PCNA, FEN1 and LIGASE1)\), was studied. As described earlier, among the 20 individuals, on the basis of average percentage of DNA damage, individuals were stratified into two groups: Group I \((N = 12)\) and Group II \((N = 8)\), which were further studied at mRNA level.

In Group I (Figure 4A and B), short-patch BER genes \((OGG1, XRCC1, LIGASE3)\), regulatory BER genes \((APE1)\) and long-patch BER genes \((FEN1 and LIGASE1)\) showed a significant \((P \leq 0.05)\) increase in mRNA expression in primed cells compared to cells exposed to 0.1 Gy and non-primed cells. However, no significant increase in mRNA expression was observed for \(NEIL1, NTH1, PARP1, MUTYH, MBD4\) and \(PCNA\) in primed and non-primed cells.

Group II (Figure 5A and B), the transcription profile of regulatory BER gene \((APE1)\) and long-patch BER genes \((FEN1 and LIGASE1)\) showed a significant \((P \leq 0.05)\) increase in mRNA expression in primed cells compared to cells exposed to 0.1 Gy and non-primed cells, but \(NEIL1, NTH1, XRCC1, LIGASE3, PARP1, MUTYH, MBD4\) and \(PCNA\) showed no significant increase in mRNA expression in Group II individuals. Among both the groups \(APE1, FEN1\) and \(LIGASE1\) showed significant \((P \leq 0.05)\) increase in mRNA expression.

Protein expression profile of BER using western blot

The expression profile of short-patch \((OGG1, NTH1, NEIL1, XRCC1 and LIGASE3)\), regulatory \((APE1 and PARP1)\) and long-patch BER proteins \((MUTYH, MBD4, PCNA, FEN1 and LIGASE1)\) was studied in irradiated PBMC of six individuals, three selected from each group (I and II). Protein expression profile was studied in nuclear extracts of primed, non-primed cells and cells exposed to 0.1 Gy alone along with unirradiated control (Figure 6A and B and Figure 7A and B).

In Group I, the protein expression profiles of \(OGG1\) (4.9-fold), \(XRCC1\) (2.5-fold), \(APE1\) (3.2-fold) and \(LIGASE3\) (2.7-fold) were significantly \((P \leq 0.05)\) increased in primed cells compared to control (unirradiated), 0.1 Gy and non-primed cells (Figure 6A and B). Long-patch BER proteins such as \(MBD4\) (2.0-fold), \(FEN1\) (1.72-fold) and \(LIGASE1\) (1.49-fold) showed a significant increase in protein expression in primed cells as compared to 0.1 Gy, 2.0 Gy (non-primed cells) and unirradiated control. However, no change in protein expression was observed for \(NTH1, NEIL1, PARP1, MUTYH\) and \(PCNA\) in this group.

In Group II, the protein expression profiles of \(OGG1\) (1.9-fold), \(APE1\) (4.3-fold), \(MBD4\) (1.7-fold), \(FEN1\) (1.9-fold) and \(LIGASE1\) (2.8-fold) showed significant increases \((P \leq 0.05)\) in protein expression in primed cells compared to 0.1 Gy, 2.0 Gy (non-primed cells) and unirradiated control (Figure 7A and B). However, no significant change in protein expression was observed for \(NTH1, NEIL1, XRCC1, LIGASE3, PARP1, MUTYH\) and \(PCNA\) in primed cells compared to 0.1 Gy, 2.0 Gy (non-primed cells) and unirradiated control. Interestingly, a significant reduction \((P \leq 0.05)\) in protein expression profile was observed for \(LIGASE3\) in primed cells compared to 0.1 Gy, 2.0 Gy (non-primed cells) and unirradiated control. The protein expression profile at 2.0 Gy was observed to be significantly increased for \(MBD4\) and \(FEN1\) compared to 0.1 Gy and control. In summary, the protein expression profiles of \(OGG1, APE1, MBD4, FEN1\) and \(LIGASE1\) showed significantly increased
expression in primed cells in both groups of individuals (Group I and Group II).

**Discussion**

IR is known to induce various types of DNA lesions that, if unrepaired or misrepaired, can lead to genomic instability, apoptosis or carcinogenesis (42, 43). The effect of high doses of IR in human cells is relatively well established (44) whereas at low dose and dose rate exposure of IR in biological systems is inconclusive. Understanding DNA repair processes at low-dose exposure in human cells is one of the mechanisms to know how the cells cope with the damage and leads to protection.

Radio-adaptive response is an important biological mechanism, which maintains the genome integrity by protecting the cells from adverse effects of radiation. It also prevents the initiation of cancer and thus is relevant to understanding environmental and occupational radiation exposure risks (45). However, this phenomenon is highly variable (46–48) depending upon the time, type of tissue or cells and the type of exposure. Sometimes, the damage occurred gets decreased after the challenging dose (AR), or it gives synergistic or additive effects (49).

AR studies have been conducted in several biological systems *in vitro* starting from mice to human (50–57). AR also varies on the basis of cell cycle at the time of irradiation. Induction of AR in the G1/G0 phase of the lymphocytes has inconclusive observations. In general, it is reported that the cell needs to pass S phase between the exposure of priming and challenging dose to result in AR (58). There are very few reports which have shown AR in G1/G0 irradiated lymphocytes (51). In the present study, the flow cytometric experiment has shown that most of the cell population is in G1/G0 phase of the cell cycle suggesting that primed and non-primed cells belong to same stage of the cell cycle. AR after a low-dose exposure is not instantaneous but requires about 4–6 h to develop full activity against a challenging dose exposure (14, 59–61). Thus, in the present study, we have analysed role of BER genes and proteins in resting PBMC after the challenging dose exposure at 4h.

The role of BER in AR is anticipated as there were observations showing that prevention of AR occurred when 3-aminobenzamide, an inhibitor of PARP, was added to cells (62–64). There are other studies that have shown the involvement of TNF-α, PARP1 and cyclin B1/CDK1 in radio-adaptive response (65–67). It has been observed that exposure of A549 cells (a human lung carcinoma cell line) to a low dose of radiation (0.25 Gy) for 4h before a clinically relevant dose (2.0 Gy) enhanced removal of thymine glycols after the higher dose. This provides evidence for an inducible repair response for radiation-induced damage to DNA bases (68). The present study is in agreement with the time period between the priming dose and challenging dose administration.

![Figure 3. Average percentage of DNA in tail (%T) at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy at 4h and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h in human PBMC using the alkaline comet assay. *Statistical significance (P ≤ 0.05) in primed cells compared to non-primed ones. Error bars represent mean ± SEM. (A) Average percentage of DNA in tail at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy at 4h and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h showing significant reduction in DNA in tail (%) in 12 individuals (Group I); (B) Average percentage of DNA in tail at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy at 4h and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h showing no significant reduction in DNA in tail (%) in 8 individuals (Group II).](image-url)
The complexity of AR in eukaryotic cells depends on the genotoxic agents used, time schedule, dose, cell type, experimental condition, adapting time given, cell cycle, physiological status of the donor and inter-individual variation which reflects contradiction in AR induction (24). One of the observations in radio-adaptive response is individual variation towards radio-sensitivity and variability of involvement of DNA repair process (69). Induction of DNA repair resulting in an AR is only visible through a narrow window of dose. The synthesis of repair proteins and the corresponding mRNAs is regulated in mammalian systems. There is a lack of information about activation of gene and protein expression in radio-adaptive response, although the role of NHEJ and the NER pathway has been reported (15,20,21,70).

In the present study, a radio-adaptive response experiment was conducted in human PBMC exposed to gamma radiation, where quantitation of DNA damage, the transcriptional and protein expression profiles of genes and proteins involved in BER pathway were studied. Our data revealed significantly reduced levels of DNA damage in primed cells, where a priming dose of 0.1 Gy was given followed by a challenging dose of 2.0 Gy after 4 h. There are similar studies on human PBMC exposed to a high level of natural background radiation areas in Ramsar, Iran (71). It is reported that one of the reasons for the reduced level of DNA damage is due to increased oxidative defence process induced by AR. Individual variation was observed at the level of DNA damage quantitation, in the present study, two groups of individuals were found. Group I (N = 12) showed significant reduction of DNA damage whereas Group II (N = 8) showed marginal reduction in DNA damage. The synthesis of repair proteins and the corresponding mRNAs is regulated in mammalian systems. There is a lack of information about activation of gene and protein expression in radio-adaptive response, although the role of NHEJ and the NER pathway has been reported (15,20,21,70).

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At the transcript level, Group I individuals showed significant up-regulation of short-patch BER genes such as OGG1, XRCC1 and LIGASE3 in primed cells whereas no change was observed in Group II individuals. This could be the major difference at gene expression level between Group I and Group II individuals. This is similar to the findings observed at the DNA damage level between the two groups. However, APE1 (a regulatory BER gene) and FEN1 and LIGASE1 (long-patch BER genes) showed similar expression between the two groups, which indicate activation of BER pathway for repairing radiation-induced damage.
Similarly at the protein level, regulatory protein (APE1) and long-patch BER proteins (MBD4, FEN1 and LIGASE1) showed significantly increased protein expression in primed cells whereas a significant induced expression of short-patch BER proteins (OGG1, XRCC1 and LIGASE3) was observed in Group I individuals. They showed a significant reduction in DNA damage (AR), thus indicating that involvement of short-patch BER proteins are required for the radio-adaptive response.

At the same time, Group II individuals showed increased expression of all long-patch repair proteins especially LIGASE1, which indicates that Group II individuals are repairing the damage through long-patch repair processes. Significant reduction in DNA damage (AR), thus indicating that involvement of short-patch BER proteins are required for the radio-adaptive response.

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An induced level of XRCC1 was observed at mRNA and protein levels (69) on exposure to IR indicating the involvement of XRCC1 in radio-adaptive response. PARP-1 is an abundant nuclear protein that binds rapidly and directly to both SSB and DSB. Cells lacking PARP do not affect the efficiency of BER process (73) but inhibition of PARP1 is known to affect AR. In the present study, we did not support the observation reported by others. In resting irradiated PBMC, PARP1 did not show any significant change either at transcript or at protein level. However, APE1 showed an increased expression both at mRNA and protein level, which is in agreement with other reports. For instance, transcriptional activation of APE1 was observed in rat liver hepatoma cells (H4) on exposure to gamma radiation and enhanced protein expression of APE1 was also observed in CHO and V79 cells (26,76). In the present study the gene and protein expression profiles of NTH1, MUTYH, PCNA and NEIL1 showed marginal increase or no significant change in expression in irradiated PBMC in primed cells. This is in agreement with the reports that increased expression has been reported at S phase compared to early G1 stage of these genes/proteins as they are associated with DNA replication processes (77).

BER is initiated by DNA-specific glycosylases which remove several structurally different damaged bases. Among them, 8-oxo-dG is the most abundant and potent pre-mutagenic lesion which is removed by hOGG1 (human 8-oxoguanine glycosylase protein 1) (78). Transcriptional and protein activation of OGG1 has been reported in HCT116 colorectal carcinoma cells (79) and in lung tissue of mice (80). Similarly, the degree of differential methylation varies with radiation dose and time. MBD4 (methyl-CpG binding domain protein 4) is involved in active DNA demethylation via BER. In the present study, increased expression of OGG1 and MBD4 both at transcript and protein levels indicated their active role in recognising and repairing specific base damages caused by gamma radiation in AR.

The transcription factors involved in BER gene regulation are E2F1, CREB-1, ATF-1, AP-1, p53 and NF-YA (69). An increased level of mRNA expression of p53 was reported at priming dose of 0.3 Gy and 0.6 Gy followed by 2.0 Gy of challenging dose at 4h (14). p53 is known to activate FEN1, which is the only factor responsible for the excision

Figure 5. Relative gene expression profiles of BER genes at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy (at 4h) and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h in human PBMC in Group II individuals (N = 8). Y-axis shows the relative gene expression of BER and X-axis shows the BER genes. *Statistical significance (P ≤ 0.05). Error bars represent mean ± SEM. (A) Relative gene expression of short-patch BER genes (OGG1, NTH1, NEIL1, XRCC1 and LIGASE3) and regulator BER genes (APE1) at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy (at 4h) and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h in human PBMC of eight individuals (Group II). (B) Relative gene expression of long-patch BER genes (MUTYH, MBD4, PCNA, FEN1 and LIGASE1) and regulator BER genes (PARP1) at 0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy (at 4h) and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4h in human PBMC of eight individuals (Group II).
step in the PCNA-dependent (long) BER. Up-regulation of FEN1 indicates the active role of the long-patch BER pathway that is capable of excising intact 5' flap structures in DNA by releasing 5'-dRP moiety (strand displacement DNA synthesis) (81). Significant up-regulation of LIGASE1 (long-patch BER) and LIGASE3 (short-patch BER) at mRNA and protein levels is associated with enhanced LIGASE activity, which is...
Figure 7. Expression profiles of BER proteins among Group II individuals. (A) A representative image of western blot of Group II individual showing protein expression profile of short-patch BER (OGG1, NTH1, NEIL1, XRCC1, LIGASE3), regulator BER (APE1, PARP1) and long-patch BER (MUTYH, MBD4, PCNA, FEN1 and LIGASE1) at various doses (Gy) [0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy (at 4 h) and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4 h] in human PBMC; (B) Histogram represents average protein expression (relative expression) among three individuals of Group II in post-irradiated human PBMC exposed to various doses [0.0 Gy (control), priming dose of 0.1 Gy, challenging dose of 2.0 Gy (at 4 h) and priming dose of 0.1 Gy followed by challenging dose of 2.0 Gy after 4 h]. *Statistical significance with $P \leq 0.05$. X-axis represents selected BER proteins and Y-axis represents relative expression of the protein.
in agreement with the study made in human primary fibroblasts exposed to UV-C light (82). Studies have shown that in human cells, LIGASE I is involved in alternative DSB repair (83) and in quiescent (G0/G1) cells. LIGASE III is involved in repair of diverse DNA lesions induced by IR, which even includes DSB (84). The activation of LIGASES in AR might give some new insight regarding the role of LIGASE in AR. In the present study, we have observed involvement of BER genes and proteins in radio-adaptive response. In spite of the individual variation, there are sufficient reports demonstrating the presence of the AR to suggest the involvement of low doses of radiation to subsequent high-dose exposures. Although several investigators have made an attempt to understand the mechanism involved in AR, the specific pathways and their possible relationship to carcinogenesis are yet to be fully investigated. Understanding cellular and molecular response mechanisms and their relationship to genotype may lead to improved ability to predict individual responses and to evaluate how individual risk associated with exposure to low-dose radiation varies (49).

Cells are under continuous environmental stress such as IR and internal metabolic stress such as reactive oxygen species. Mechanisms such as AR enhance cell survival and maintain genomic stability at low-dose exposures. The extent of AR in humans not only depends on dose or dose rate but even on the heterogeneity among the individuals exhibiting this response, which remains still unresolved. A deeper understanding of the radio-adaptive response and the interplay of all the repair pathways such as BER, NHEJ and NER is needed before estimating risk of low dose of IR for humans.

Acknowledgements

We are thankful to the volunteers who have participated in the study. We profusely thank Ms Prabhu J. A. and Mr Sangram Kamble for helping us in collecting the blood samples from BARC dispensary, Trombay, Mumbai. We thank Mr Sanjay Shinde and Mr Manjoor Ali for assisting in radiation work. We acknowledge the fellowship from CSIR.

Conflict of interest statement: None declared.

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