Biophysical Aspects of Auger Processes, AAPM Symposium Series No. 8 (1992) 080-090
Eds. RW Howell, VR Narra, KSR Sastry, DV Rao

DNA STRAND BREAKS IN IUdR CONTAINING CELLS AFTER IRRADIATION WITH LOW-ENERGY X RAYS

SYNNÖVE SUNDELL-BERGMAN and KARL J. JOHANSON

Department of Radioecology,
The Swedish University of Agricultural Sciences
P.O. Box 7031, S-750 07 Uppsala, Sweden

ABSTRACT

The induction and repair of DNA strand breaks were studied in control and IUdR labeled CHO cells after irradiation with low-energy X rays. More DNA strand breaks were found in IUdR labeled compared to control cells. If the data were least square fitted, the slope ratios (IUdR/control) were 1.23 immediately after irradiation and 1.22 after 60 min of repair. Using the linear quadratic model, the ratio between the two α-terms was 1.43 after 60 min repair. After 120 min of repair, the number of DNA breaks in control cells was close to zero for doses below 4 Gy while for IUdR labeled cells the number was significantly higher than zero.

INTRODUCTION

It is well known that incorporation of halogenated thymidine analogs into cellular DNA increases the radiosensitivity of mammalian cells as measured by survival (1-4), and may affect the ability of cells to repair radiation damage (2,5). Several reports have shown that the degree of radiosensitization increases as the percentage of thymidine replacement increases (6,7). Although the mechanism of sensitization has not been fully elucidated, it is probably not related to secondary effects such as inhibition of DNA polymerase or inhibition of enzymes involved in thymidine synthesis (8,9).

Recently Fairchild *et al.* (10) reported theoretical calculations showing that IUdR radiosensitization might be enhanced substantially by the use of photon energies just above 33.2 keV, the K absorption edge of iodine (10). According to Fairchild *et al.* (10) the enhancement would result from the effects of the Auger electron cascade which follows the creation of vacancy in the K shell of iodine via the photoelectric effect. These Auger electrons are very effective in causing subcellular damage in critical cell structures in a manner similar to ^{125}I decay in DNA (11,12).

Cells which have incorporated IUdR into their DNA show significant reductions of both the slope and the shoulder of their radiation survival curves (13,14). The α-coefficient in the linear-quadratic model, $y = \alpha D + \beta D^2$, which is supposed to account for the formation of double-strand breaks in one single radiation event (15), has been reported to be significantly higher for X irradiated IUdR labeled cells compared to control cells (13). Radiosensitization by IUdR has also been correlated with increases in chromosomal aberrations and micronuclei formation (16). Few studies have been reported on the induction and repair of DNA strand breaks in mammalian cells after IUdR incorporation, although the enhancement in sensitivity suggests the involvement of DNA damage and repair (17,18).

The effect on induction and repair of DNA strand breaks after irradiation with low-energy X rays has been examined using the DNA unwinding technique with double-labeling (19). Incorporation of IUdR into CHO cells followed by irradiation resulted in an increased number of DNA strand breaks in IUdR labeled cells. Furthermore the repair of the induced strand breaks in IUdR containing cells was less efficient than in the controls.

MATERIAL AND METHODS

Culturing Conditions

Exponentially growing CHO cells were cultured in plastic flasks (Nunc, Roskilde, Denmark) containing Ham's F10 (SVA, Uppsala, Sweden), supplemented with 10% newborn calf serum (Gibco, N.Y. USA), 1.0 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin and maintained in a 5% CO_2 atmosphere at 37°C.

Labeling of DNA

Iododeoxyuridine (IUdR) was added to cells to a final concentration of 10^{-6} M and the cells were incubated for three days in darkness. The IUdR solution was stored frozen in a weak alkaline stock solution of 1 mM. During the IUdR labeling, the cells were grown in medium prepared from thymidine depleted Ham's F10 medium. Cells were labeled with 7.4 kBq/ml of ^{3}H-thymidine (^{3}H-TdR) or 1.85 kBq/ml of ^{14}C-thymidine (^{14}C-TdR) (Amersham, U.K.) for 18 h before harvest. The cells were then washed 2-3 times in phosphate buffered saline (PBS) and, after the addition of new prewarmed medium, the incubation continued for an additional 3 h at 37°C.

Irradiation Conditions

The cells were irradiated at 0°C using a Siemens Stabilipan therapy X ray unit operating at 75 kV and 20 mA with a total filtration of 4 mm Al. The radiation dose and radiation quality were measured using an ionization chamber and thermoluminescence dosimeters. The half-value layer (HVL) for the 75 kV X rays was measured to be 4.3 mm Al corresponding to a mean photon energy of 38 keV (13). The dose rate was 0.16 Gy per min.

DNA Strand Break Analysis

The cells were detached from the flask with trypsin (0.25% in PBS). Prior to irradiation cells were mixed according to the following schedule: Cells which had incorporated IUdR and ^{3}H-TdR were mixed with only ^{14}C-TdR labeled cells. Furthermore ^{3}H-TdR labeled cells were mixed with IUdR and ^{14}C-TdR labeled cells (19,20). This implies that IUdR labeled and control cells were treated simultaneously during the DNA strand break analysis. The

number of DNA strand breaks was determined by the unwinding technique either directly after the irradiation or after a repair period of 60 or 120 min. Briefly, 100 µl of the cell suspension was added to 1 ml of a weak alkaline solution and left in the dark for 30 min. After neutralization the samples were sonicated and, after adding SDS, stored at -20°C until chromatography was performed. Single- and double-stranded DNA were separated on hydroxylapatite and the ^{3}H- and ^{14}C-activities were then determined in a liquid scintillation counter (Packard Instr. Co., USA) using Instagel (Packard Instr. Co., USA) as a scintillation solution. The number of DNA strand breaks, the sum of the single- and double-strand breaks, was calculated according to Rydberg (19).

RESULTS

In order to estimate the amount of cellular IUdR uptake, cells were incubated with ^{125}IUdR of known specific activity for 72 h. Following the incubation the DNA content and the incorporation of ^{125}IUdR per cell were determined. The replacement of thymidine with IUdR under these circumstances was estimated to be on average 10%.

Compared to control cells, some strand breaks were induced in IUdR labeled cells during the labeling period although care was taken to avoid light exposure to the cells. The experiments were repeated several times and the mean values based on 10 to 30 samples.

Figure 1 illustrates the induction of DNA strand breaks after irradiation with low-energy X rays. The number of DNA strand breaks increases in a dose-dependent manner up to 5 Gy where the curves approached saturation which probably is due to the limit of the method. The data were least-squares fitted and the slopes of the dose-response curves were found to be 0.32 ± 0.02 Gy^{-1} for IUdR labeled cells and 0.26 ± 0.02 Gy^{-1} for control cells. The relation between the slopes results in a ratio of 1.23 indicating a higher response for the IUdR labeled cells.

Figure 2 shows the DNA strand breaks that are remaining after 60 min of incubation at 37°C. The number of residual DNA strand breaks seems to be dose-dependent for both the control and the IUdR labeled cells. The slopes were found to be 0.037 ± 0.01 Gy^{-1} and 0.030 ± 0.007 Gy^{-1} for IUdR labeled and control cells, respectively, and the ratio between the two slopes was 1.23.

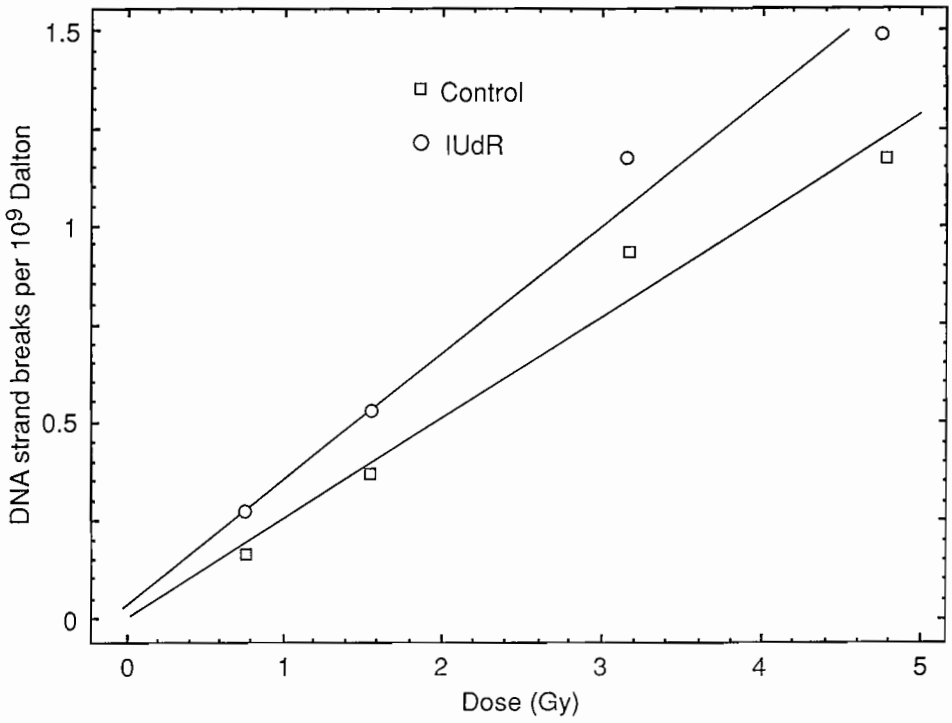


FIG. 1. The number of DNA strand breaks induced in control and IUdR labeled cells after irradiation with low-energy X rays. The dose rate was 0.16 Gy min^{-1}. The data were fitted by the least squares method.

The repair of DNA strand breaks was also fitted to the linear-quadratic relationship ($y = \alpha D + \beta D^2$) using an iterative least-square fit where D is the dose in Gy. The α-coefficients were $4 \times 10^{-3} \pm 0.4 \times 10^{-3}$ Gy^{-1} for IUdR and $2.8 \times 10^{-3} \pm 0.5 \times 10^{-3}$ Gy^{-1} for control cells which yields a ratio of 1.43. This indicated that the relative difference in the low dose range had increased during the 60 min repair phase.

The results obtained after 120 min of repair are presented in Fig. 3. In the low dose region (up to approximately 4 Gy), very few non-rejoined breaks were found in control and IUdR groups. For control cells the residual breaks in this region were not significantly different from zero, while for the IUdR labeled cells the number was significantly higher than zero. The difference

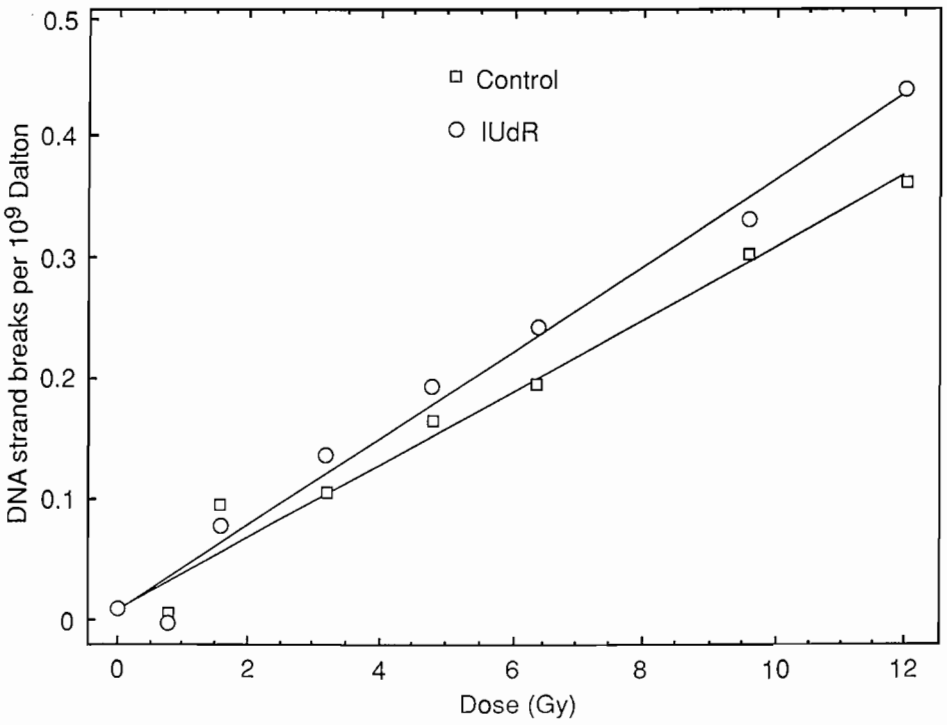


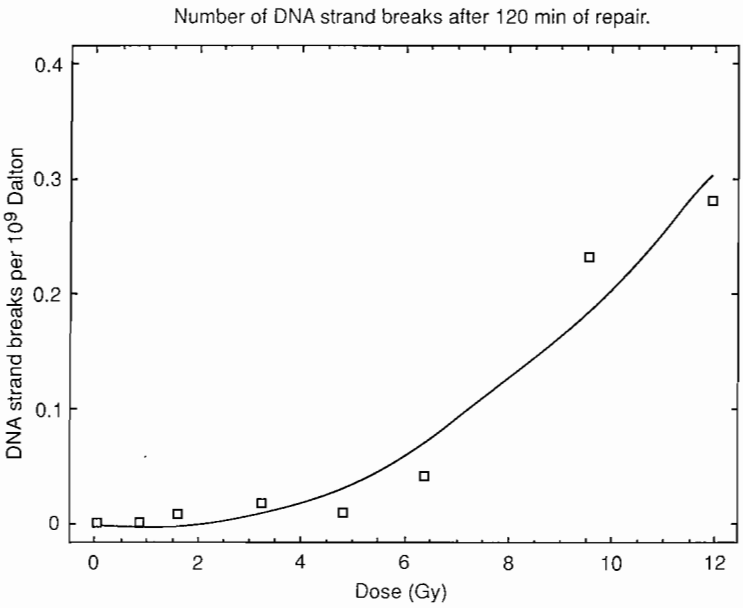
FIG. 2. Residual DNA strand breaks after 60 min of repair at 37°C in control and IUdR labeled cells as a function of radiation dose. The data were fitted by the least squares method.

between the two treatments after 120 min repair seems to be most pronounced in the low dose range.

DISCUSSION

The results show that replacement of thymidine by IUdR into cellular DNA sensitizes the cells to a subsequent exposure to low-energy X rays and this effect persisted after a prolonged incubation. The DNA strand breaks remaining after 60 min of incubation (repair phase) are assumed to be double-strand breaks or other severe DNA damage since the single strand breaks are repaired within 10 to 20 min of incubation (unpublished data). We therefore suggest that the observed increase in radiation damage per unit radiation dose

(A)



(B)

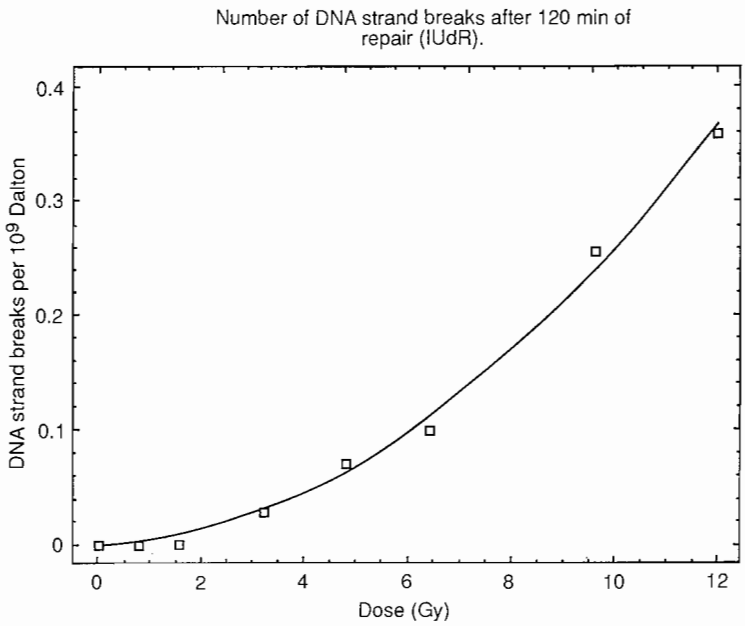


FIG. 3. Residual DNA strand breaks after 120 min of repair at 37°C in (A) control and (B) IUdR labeled cells as a function of radiation dose. The data were fitted to the linear quadratic model.

in IUdR labeled cells is an indication that more severe damage such as DNA double-strand breaks have been induced relative to the controls. This enhancement may be due to Auger processes, and previous survival studies support the possibility that a high-LET component might be involved in the radiation response of IUdR labeled cells (13). Our results are also consistent with findings by Shinohara *et al.* (21) who suggested that X irradiation of IUdR labeled HeLa cells induces critical DNA lesions for cell lethality.

Kinsella *et al.* (17,18) using the filter elution technique reported an enhancement factor of about 1.5 for double-strand break induction and repair in V79 cells after 23% replacement of thymidine bases. This ratio agrees fairly well with our results considering the differences in thymidine replacement. They also reported a higher enhancement factor for BrUdR than for IUdR which is not consistent with the photoactivation hypothesis proposed by Fairchild *et al.* (10).

There is growing evidence suggesting that double-strand breaks are the ultimate lesion involved in the formation of chromosome aberrations (22,23). In a previous study on micronuclei formation (16), a dose-modifying factor of 1.3 was obtained which is in agreement with the data from the DNA strand break studies. This similarity is particularly interesting because it suggests a cause-effect relationship between the two phenomena.

CONCLUSION

Irradiation with low-energy X rays was 1.2 times more efficient in inducing DNA strand breaks in IUdR labeled cells than in control cells. After repair, the corresponding value had increased and, according to the α/β model, more double-strand breaks seem to be induced in IUdR labeled cells. The results, however, give no ultimate answer to the possible involvement of Auger electrons in the radiation response after IUdR incorporation.

ACKNOWLEDGMENTS

We would like to thank Sven Richter for help with the dosimetry and irradiating of the cells, Ulla Johanson and Gertruda Ludwikow for performing the DNA strand break analysis. This work was supported by the Swedish Cancer Society.

REFERENCES

1. N. GELIHAS, M.A. RICH, and M.L. EIDINOFF, Radiosensitization of a mammalian cell line with 5-bromodeoxyuridine. *Radiat. Res.* **17**, 479-491 (1962).
2. J.T. LETT, G. PARKINS, and P. ALEXANDER, Mechanisms of sensitization to X rays of mammalian cells by 5-bromodeoxyuridine. *Nature* **203**, 593-596 (1964).
3. R.M. HUMPHREY and W.C. DEWEY, Radiosensitivity of normal and 5-bromodeoxyuridine treated mammalian cells during different phases of the cell cycle. *Exp. Cell Res.* **39**, 483-495 (1965).
4. W.C. DEWEY, L.E. STONE, H.H. MILLER, and R.E. GIBLAK, Radiosensitization with 5-bromodeoxyuridine of Chinese hamster cells x-irradiated during different phases of the cell cycle. *Radiat. Res.* **47**, 672-688 (1971).
5. W.U. SHIPLEY, M.M. ELKIND, and W.B. PRATHER, Potentiation of X ray killing by 5-bromodeoxyuridine in Chinese hamster cells: A reduction in capacity for sublethal damage accumulation. *Radiat. Res.* **47**, 437-449 (1971).
6. H.S. KAPLAN, K.C. SMITH, and P.A. TOMLIN, Effect of halogenated pyrimidines on radiosensitivity of *E. Coli*. *Radiat. Res.* **16**, 98-113 (1962).
7. T.L. PHILLIPS, W.J. BODELL, V. UHL, G.Y. ROSS, J. RASMUSSEN, and J.B. MITCHELL, Correlation of exposure time, concentrations and incorporation of IdUrd in V79 cells with radiation response. *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 1251-1255 (1989).
8. B. GOZ, The effects of incorporation of 5-halogenated deoxyuridines into the DNA of eukaryotic cells. *Pharmacol. Rev.* **29**, 249-272 (1985).
9. W. SZYBALSKI, X ray sensitization by halopyrimidines. *Cancer Chemother. Rep.* **58**, 539-557 (1974).
10. R.G. FAIRCHILD and V.P. BOND, Photon activation therapy. *Strahlentherapie* **160**, 758-763, (1984).
11. S. SUNDELL-BERGMAN, R. BERGMAN, and K.J. JOHANSON, Chromosome damage induced by decay of ^{3}H and ^{125}I incorporated into DNA of Chinese hamster cells. *Mutat. Res.* **149**, 257-263 (1985).
12. K.G. HOFER and W.L. HUGHES, Radiotoxicity of intranuclear tritium, iodine-125 and iodine-131. *Radiat. Res.* **47**, 94-109 (1971).
13. S. SUNDELL-BERGMAN, K.J. JOHANSON, R. RICHTER, and P. ÖSTBERGH, Enhancement of radiation sensitivity in iododeoxyuridine labeled cells exposed to low energy X rays. *Acta Oncol.* **29**, 623-626 (1990).
14. R. NATH, P. BONGIORNI, and S. ROCKWELL, Enhancement of IUdR radiosensitization by low-energy photons. *Int. J. Radiat. Oncol. Biol. Phys.* **13**, 1071-1079 (1987).
15. K.H. CHADWICK and H.P. LEENHOUTS, The molecular theory of radiation biology. *Monographs on Theoretical and Applied Genetics* 5. Berlin, Springer Verlag, 1981.
16. G. LUDWIKOW, C.G. STALNACKE, K.J. JOHANSON, S. SUNDELL-BERGMAN, and S. RICHTER, Microscopic and flow cytometric study of micronuclei in iododeoxyuridine labeled cells irradiated with soft X rays. *Acta Oncol.* **29**, 761-767, (1990).

17. T.J. KINSELLA, P.P. DOBSON, J.B. MITCHELL, and A.J. FORNACE Jr., Enhancement of x ray induced DNA damage by pretreatment with halogenated pyrimidine analogs. *Int. J. Radiat. Oncol. Biol. Phys.* **13**, 733-739 (1987).
18. A.J. FORNACE Jr., P.P. DOBSON, and T.J. KINSELLA, Enhancement of radiation damage in cellular DNA following unifilar substitution with iododeoxyuridine. *Int. J. Radiat. Oncol. Biol. Phys.* **18**, 873-878 (1990)
19. B. RYDBERG, Detection of induced DNA strand breaks with improved sensitivity in human cell. *Radiat. Res.* **81**, 492-494 (1980).
20. B. SANDSTROM and K.J. JOHANSON, A direct assay for detection of chemically induced changes in the rejoining kinetics of radiation induced DNA strand breaks. *J. Biochem. Biophys. Methods* **14**, 183-190 (1987).
21. K. SHINOHARA, H. NAKANO, and H. OHARA, Estimation of protectable fraction by cysteamine in 5-iododeoxyuridine labeled HeLa cells irradiated with X rays or Co-60-gamma-rays. *Abstr. Proc. of the 8th Intern. Congr. of Radiat. Res.* July, 1987.
22. G. OBE, A.T. NATARAJAN, and F. PALITTI, Role of doublestrand breaks in the formation of radiation-induced chromosomal aberrations. *Progress in Mutat. Res.* **4**, 1-9 (1982).
23. P.E. BRYANT, Enzymatic restriction of mammalian cell DNA using Pvu II and Bam H1: Evidence for the double-strand break origin of chromosomal aberrations. *Int. J. Radiat. Biol.* **46**, 75-65 (1984).

DISCUSSION

Martin, R. Have you compared OER for the two cell populations (re ± IUdR)?

Johanson, K. J. No. In order to study possible high-LET effects we have performed some studies using low dose rate.

Baverstock, K. F. What was the dose rate?

Johanson K. J. 0.16 Gy/min.

Halpern, A. It is customary to use the term "double labeling" for cases where the molecule contains two isotopes, not for the mixture of two singly-labeled molecules.

Johanson, K. J. When using liquid scintillation techniques for detection of radionuclides, the term double (or dual) labeling is often used when two radionuclides are mixed in the scintillation vials.

Goodhead, D. T. What do you suggest might be the reason for the curvature in the dose response for strand breaks after 120 min repair? Is this not surprising if you are measuring ssb?

Johanson, K. J. The unwinding technique measures both single strand breaks and double strand breaks. After 120 min of repair, there are mostly double strand breaks left unrepaired. I have no good suggestion which can explain the reason for the curvature. In the control cells it seems to be a

threshold and then a linear increase. In IUdR containing cells, the threshold seems to disappear but the linear part has a rather similar shape to the control. Thus, it seems that a more serious DNA damage is induced in IUdR containing cells.