Volume 8 Number 16 1980 Nucleic Acids Research

Effect of ionizing radiation on DNA synthesis in ataxia telangiectasia cells

J.Houldsworth and M.F.Lavin*

Department of Biochemistry, University of Queensland, Brisbane 4067, Australia

Received 7 May 1980

ABSTRACT

The effect of ionizing radiation on DNA synthesis in control and ataxia telangiectasia (AT) lymphoblastoid cell lines was determined. A dose dependent decrease in DNA synthesis was observed in control cells, and the rate and extent of this decrease in synthesis increased with time after irradiation. No decrease in DNA synthesis was obtained in AT cells, immediately following irradiation, at doses up to 400 rads. At longer times postirradiation, inhibition of synthesis increased but the extent of inhibition was less in AT cells than controls at all doses used. An immediate depression of DNA synthesis was evident in control cells after a radiation dose of 200 rads reaching a maximum at 90 min postirradiation. Little or no decrease in DNA synthesis was evident in AT cells up to 60 min after the same radiation dose, but a decrease occurred between 60 and 90 min after irradiation. The rate of recovery of DNA synthesis to normal levels was more rapid in AT cells than in controls.

INTRODUCTION

An increased sensitivity to ionizing radiation has been demonstrated both in fibroblasts and lymphoblastoid cells from patients with ataxia telangiectasia (1,2). This increased sensitivity can be correlated with a reduced level of DNA repair synthesis in a number of AT cells (3,4). It has been suggested that the basic biochemical deficit in AT cells is one involved in the repair of a limited number of strand scissions which arise as a result of a defective excision process (5). However, since AT is a multiform disease characterized by neurological, cutaneous and immunological abnormalities (6), it is possible that the defective process plays a broader role in development than in DNA repair *per se*.

Exposure of mammalian cells to ionizing radiation results in a depression

in DNA synthesis (7-9). A dose response curve is characterized by radioresistant and radiosensitive components of DNA synthesis (7-9). The radioresistant component appears to reflect the effect of irradiation on the rate of DNA chain growth (9), whereas the more sensitive component is due to an inhibition of initiation of DNA replication (10). An initial depression in the rate of DNA synthesis in cells exposed to moderate doses of ionizing radiation is observed, followed by a recovery of normal rates 3-4 hr postirradiation (7-9). We have determined the effect of ionizing radiation on DNA synthesis in control and AT homozygote cells as a means of investigating the nature of the defect in AT.

MATERIALS AND METHODS

Cell culture Epstein-Barr Virus (EBV) transformed lymphoblastoid cell lines were used in this study. All the cell lines were established at the Queensland Institute of Medical Research and we have adopted a nomenclature as described previously (2). The AT homozygote cells used in this study are designated AT1ABR, AT2ABR, AT3ABR, AT4ABR and AT5ABR. The control cells used are described as C1ABR - C6ABR. Cells were grown in suspension culture in medium (H18, Gibco) supplemented with 10% fetal calf serum, penicillin (10^5 units/l), and streptomycin (60 mg/l) in an atmosphere of 5% CO_2. Cultures were routinely checked for mycoplasma contamination (11).

Effect of γ-radiation on DNA synthesis

Cells were aliquoted into microwells in Tissue Culture Microtiter Trays (Disposable Products, Australia) at a concentration of 10^5 cells/100 µl/microwell, either 20 hr or 3 hr prior to irradiation. Cells were exposed to radiation (^{60}Co) in a Gamma Cell 220 (Atomic Energy of Canada Ltd.) at a dose rate of 5.20 - 4.69 Krads/min. The effective output was reduced to 10% by shielding the samples in a lead container. [^3H]thymidine (Radiochemical Centre, Amersham; 25 Ci/mmole, 1 µCi/microwell) was added in 100 µl of medium to give

a final volume of 200 µl/microwell. Incubation was carried out for 15 min at 37°C and terminated by placing the micro plates in a -70°C Revco. Samples were subsequently thawed at room temperature and acid-precipitable DNA was collected on GF/A glass fibre filters (Whatman) using a multicell harvesting device. Replicates were collected onto GF/A strips, which were then washed with 4 ml of water followed by 4 ml of ice-cold 5% trichloroacetic acid (TCA) per sample. The strips were washed in ethanol and dried. Individual samples were digested for 1 hr at room temperature with NCS Tissue Solubilizer (Amersham) prior to addition of the toluene scintillator. Samples were placed in the dark for 24 hr to avoid chemiluminescent interference, before counting in a Beckman LS-250 liquid scintillation counter. Incorporation of [^{3}H]thymidine into acid-precipitable DNA was expressed as dpm/10^6 cells. In the time course experiments the radiation dose employed was 200 rads, incubation and harvesting conditions were as described above.

Determination of single strand breaks in DNA

Cells were labelled with [^{3}H]thymidine (1 µCi/ml) for 24 hr, transferred to fresh unlabelled medium for 2 hr to deplete labelled precursor pools. Prior to irradiation cells were centrifuged, and resuspended in cold saline-EDTA solution (12) at a dilution of 5 x 10^5 cells/ml. Cells were irradiated in ice over a dose range, up to 5 Krads. Sedimentation was carried out on a isokinetic alkaline sucrose gradient (5 - 24% sucrose w/v in 1 M NaCl, 0.2 M NaOH and 1 mM EDTA). 100 µl of lysis layer (0.5 M NaOH, 10 mM EDTA) was applied to the top of each gradient and approximately 10^5 cells contained in 100 µl was added to this layer. After a 2 hr lysis period gradients were centrifuged in a Beckman L2-65B at 25,000 rpm for 4 hr using a SW41 rotor at 20°C. Fractions were collected by pumping onto strips of Whatman No. 1 chromatography paper. Strips were dipped in 5% TCA and washed in ethanol. After drying, strips were cut into individual fractions and counted in a Beckman LS-250 scintillation

counter using toluene scintillation fluid.

The number average molecular weight (Mn) of DNA was determined using an empirical relationship derived by Eigner and Doty (13). Numerical constants were determined for isokinetic sucrose gradients using bacteriophage lambda and T2 DNAs as markers. The molecular weight of sedimenting DNA was determined with the aid of a computer programme developed in this department.

RESULTS

The extent of DNA synthesis in control and AT lymphoblastoid cells after exposure to increasing doses of radiation appears in Fig. 1. In control cells

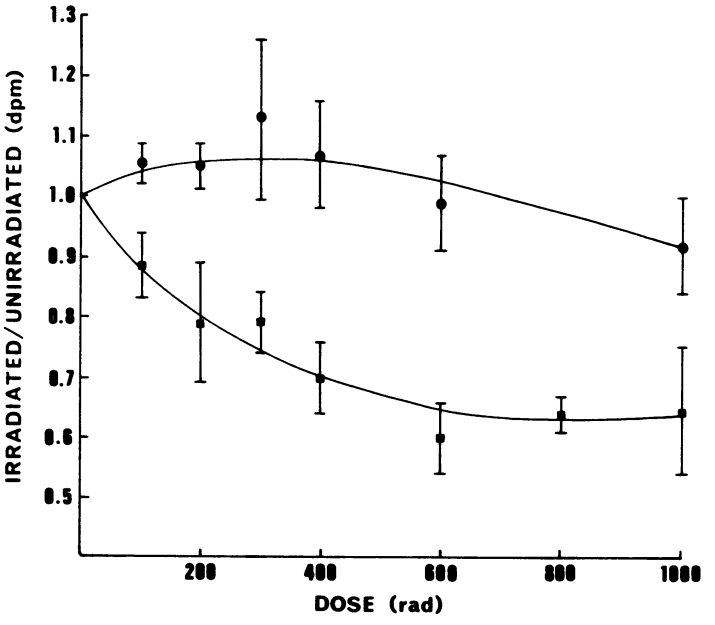

Figure 1. Effect of increasing radiation dose on DNA synthesis in lymphoblastoid cells labelled for 15 min with [^{3}H]thymidine immediately after irradiation, control cells (■), AT cells (●). Separate experiments were carried out using 4 AT cell lines (AT1ABR, AT2ABR, AT4ABR and AT5ABR) and 4 control lines (C1ABR, C3ABR, C5ABR and C6ABR). Ten replicates were used in each experiment and error bars represent S.E.M.

the level of DNA synthesis decreases in a biphasic fashion over the dose range 0 - 1000 rads, when DNA synthesis is determined immediately after irradiation. A rapid decrease in DNA synthesis is evident up to 400 rads which gives rise to a more resistant component at higher doses. These results are in keeping with a mode of inhibition of DNA synthesis by ionizing radiation in other mammalian cells (7-9). Surprisingly in AT cells no inhibition of DNA synthesis is evident up to 400 rads (Fig. 1). At higher doses a small decrease is evident. When DNA synthesis is determined at 30 min after irradiation a biphasic pattern of inhibition is again evident (Fig. 2). The initial rate of decrease of synthesis at lower doses is somewhat more rapid than that obtained when synthesis is measured immediately after irradiation. A decrease is also observed in AT cells but the extent of this decrease is less than that obtained with controls at all radiation doses employed (Fig. 2).

The effect on DNA synthesis is more dramatic in control cells at 90 min post-irradiation (Fig. 3). The level of DNA synthesis is reduced to approximately

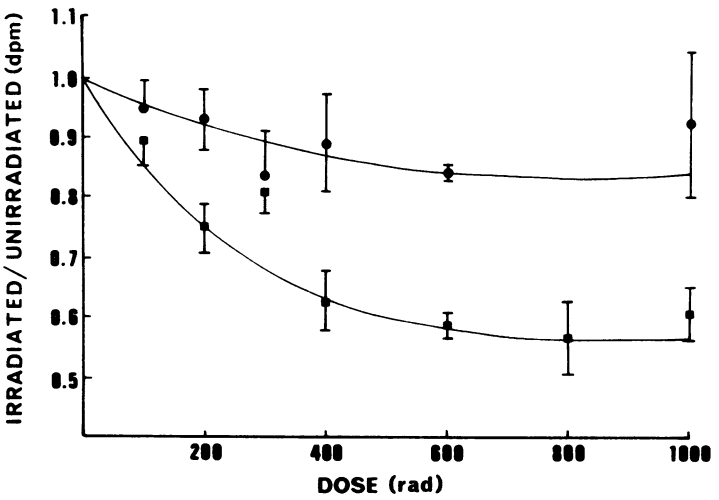

Figure 2. Effect of radiation dose on DNA synthesis in control cells (■) and AT cells (●) 30 min after irradiation. Incubation conditions are the same as those described in Methods and the legend to Figure 1. AT and control cells used are as described in legend to Figure 1. Error bars represent S.E.M.

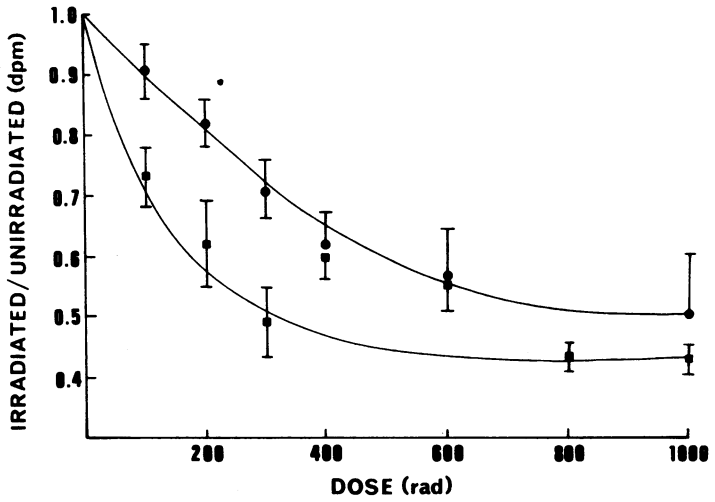

Figure 3. Effect of radiation dose on DNA synthesis in control (■) and AT (●) cells 90 min after irradiation. Conditions and cell lines are the same as described in legend to Figure 1. Error bars depict S.E.M.

50% of the unirradiated value at 400 rads levelling off at higher doses. In AT cells a rapid decrease in DNA synthesis also occurs at lower doses giving way to a more resistant component at doses above 400 rads (Fig. 3).

A dose of 200 rads was selected to investigate the extent of inhibition and the time course of recovery of DNA synthesis in control and AT homozygote cell lines. This dose was used since it lies within the radiosensitive component of the dose curve for control cells and would be expected to lead preferentially to an inhibition of initiation of DNA replication. The kinetics of the response of control and AT homozygote cell lines to radiation are presented in Fig. 4. In these experiments, immediately after irradiation, DNA synthesis was reduced to 84 ± 4% (normalized to 1.0 in Fig. 4) of the unirradiated value in control cells. DNA synthesis was virtually unchanged in AT cells at 99 ± 4% (also normalized) of that for unirradiated cells.

A rapid decrease in DNA synthesis levels with time is evident in control

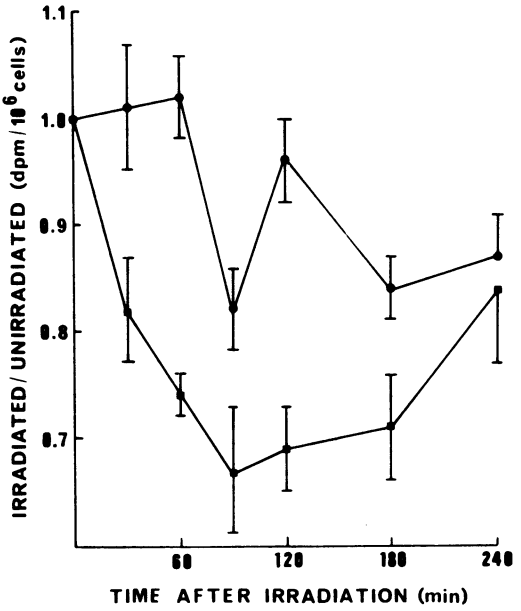

Figure 4. Inhibition and recovery of DNA synthesis in control (■) and AT (●) lymphoblastoid cells at various times after irradiation (200 rads). Six control cell lines were used with at least two separate experiments in each case. Four AT homozygote cells (AT1ABR, AT3ABR, AT4ABR and AT5ABR) were employed with at least three separate experiments for each cell line. In each experiment 10 replicates were used for each time point. Error bars represent S.E.M.

cells (Fig. 4). The level of DNA synthesis reached a minimum 90 min after irradiation followed by a gradual increase in synthesis up to 240 min post-irradiation. In contrast to these results no decrease in DNA synthesis is obtained in AT cells up to 60 min after irradiation (Fig. 4). A fall in the level of synthesis occurs between 60 and 90 min reaching a minimum at 90 min. The extent of inhibition in AT cells at 90 min is less that that in controls and the rate of recovery of synthesis is greater, reaching a value close to that of unirradiated cells at 120 min postirradiation. The significance of these results at the various times after irradiation is described in Table 1. In both cases at longer times after irradiation a gradual decrease is observed in DNA synthesis (results not shown).

Table 1. Inhibition and recovery of DNA synthesis in lymphoblastoid cells.

Postirradiation time (min)	Ratio[a)]		P value
	Controls	AT Homozygotes	
30	0.82	1.01	< 0.05
60	0.74	1.02	< 0.001
90	0.67	0.82	< 0.10
120	0.69	0.96	< 0.001
240	0.84	0.87	< 0.10

a) Refers to the ratio of incorporation of [^{3}H]thymidine in irradiated to that in unirradiated cells. Statistical significance was established in all cases using the Student's t Test based on a normal distribution of results.

It has been suggested that the introduction of single strand breaks into DNA by ionizing radiation alters the supercoiled structure and in this way inhibits replicon initiation (14). A possible, but perhaps unlikely, explanation for the difference in onset and degree of inhibition of DNA synthesis in control cells compared to AT cells is that less single strand breaks occur in AT cells after radiation. The number of single strand breaks appearing in DNA with increasing radiation dose was therefore determined in both cell types (results not shown). A similar number of strand breaks are observed in both cell types at all doses. At 200 rads, it is estimated that 0.76 single strand breaks per 10^9 daltons are obtained in control DNA, and the figure for AT DNA is 0.66 breaks per 10^9 daltons.

DISCUSSION

Lymphoblastoid cells from patients with AT show an increased sensitivity to ionizing radiation (2). In this study a dose dependent inhibition of DNA synthesis in control cells has been demonstrated at a number of times following irradiation. The response at all time points is biphasic, similar to

that reported for other cell types (7-9). In contrast AT cells continue to carry out DNA synthesis at the same level as that obtained in unirradiated cells at doses up to 400 rads and the decrease is minimal up to 1000 rads, when DNA synthesis is determined immediately after irradiation. At later times after irradiation some decrease in synthesis is observed but overall this decrease is less than that occurring in irradiated control cells.

The decrease in DNA synthesis and subsequent recovery in control cells with time after irradiation is similar to that in a number of other mammalian cells (7-9). However, DNA synthesis is not appreciably inhibited in AT cells up to 60 min after irradiation. Subsequently inhibition is observed, and a minimum in the level of synthesis is obtained at 90 min postirradiation. This minimum corresponds to that obtained with irradiated control cells. The radiation dose used in recovery experiments (200 rads) would be expected to inhibit initiation of DNA replication since it occurs in the radiosensitive component of the DNA synthesis inhibition curve (10). Furthermore preliminary results from this laboratory substantiate such an inhibition of initiation of DNA synthesis in control lymphoblastoid cells at 200 rads (Ford and Lavin, unpublished data).

Since the same number of single strand breaks are induced in the DNA of both cell types after exposure to 200 rads it seems unlikely that such breaks alone are responsible for inhibition of DNA replication. When target size is taken into consideration it has been estimated that one single strand break per 10^9 daltons may be sufficient to prevent initiation of DNA synthesis at all sites throughout such a region of DNA (14). The frequency of breaks obtained at the dose used in our experiments is approximately 0.7 per 10^9 daltons of DNA and would thus be expected to have a similar effect on DNA synthesis. Failure to detect inhibition of DNA synthesis in AT cells in the first 60 min after irradiation may be explained by a deficiency in excision of lesions resulting from irradiation or by activation of potential DNA re-

plication initiation sites which are not normally activated in unirradiated cells (15). The presence of an excision repair mechanism in normal cells would be expected to give rise to breaks in DNA which could cause inhibition of initiation of DNA replication. The response observed in AT cells at 60-90 min postirradiation may be indicative of either a slower rate of DNA repair or a later onset of the repair process at a reduced level. Previous results from this laboratory have demonstrated a reduced repair capacity in phytohaemagglutinin (PHA) stimulated lymphocytes from the AT patients employed in this study (4). It seems unlikely that, failure to observe inhibition of DNA synthesis in AT cells during the first hour postirradiation, can be accounted for by a less pronounced effect on the entry of G1 phase cells into S phase (16). The results of Saha and Tolmach (17), which show that low doses of X-rays do not appreciably affect thymidine nucleotide pools, would argue against fluctuations in pool size as an explanation for failure to obtain inhibition.

It is of interest to compare the results obtained here with the inhibition of DNA synthesis in Xeroderma Pigmentosum (XP) cells after exposure to ultraviolet (UV) radiation (18). Their results demonstrate a similar degree of inhibition in control and XP cells at 3 hr after UV irradiation and a failure of XP cells to recover normal levels of DNA synthesis. Park and Cleaver (19) have shown that excision repair plays a role in minimizing the inhibition of DNA chain growth, but resulting breaks inhibit replicon initiation. In XP cells DNA synthesis was inhibited to a greater extent than in control cells. In the results obtained in this study a delay in the inhibition of DNA synthesis and a more rapid recovery of normal rates is achieved in AT cells when compared to control cells. Unlike the results obtained with UV (18), inhibition of DNA synthesis cannot be correlated, in this study, with the extent of cell death. However failure to observe inhibition in the first 60 min, and the more rapid rate of recovery of synthesis could still be compatible with an

excision defect in AT cells.

An alternative explanation for the failure to observe inhibition of DNA synthesis in AT cells at short times after irradiation may relate to the susceptibility of chromatin structure in AT cells to radiation damage. If the structure of chromatin in AT cells was inherently less susceptible to ionizing radiation due to nucleosomal arrangements or packaging, then a less marked effect on DNA synthesis might be observed.

In summary we have shown that onset of inhibition of DNA synthesis after ionizing radiation in AT cells is delayed, extent of inhibition is less than that in controls, and recovery to near normal levels, though transient, is more rapid in AT cells.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Australian Institute of Nuclear Science and Engineering, and funds from the University of Queensland We wish to thank Mark Davidson for excellent technical assistance.

*To whom reprint requests should be directed.

REFERENCES

1 Taylor, A.M.R., Harnden, D.G., Arlett, C.F., Harcourt, S.A., Lehmann, A.R., Stevens, S. and Bridges, B.A. (1975) Nature 258, 427-429.
2 Chen, P.C., Lavin, M.F., Kidson, C. and Moss, D. (1978) Nature 274, 484-486.
3 Paterson, M.C., Smith, B.P., Lohmann, P.H.M., Anderson, A.K. and Fishman, L. (1976) Nature 260, 444-446.
4 Lavin, M.F. and Kidson, C. (1978) Clin. Immunol. Immunopathol. 10, 325-329.
5 Paterson, M.C. and Smith, P.J. (1979) Ann. Review Genetics 13, 291-318.
6 McFarlin, D.E., Strober, W. and Waldmann, T.A. (1972) Medicine 51, 281-314.
7 Olson, A.C. and Billen, D. (1978) Radiat. Res. 74, 199-204.
8 Walters, R.A. and Hildebrand, C.E. (1975) Biochem. Biophys. Res. Comm. 65, 265-271.

9 Watanabe, I. (1974) Radiat. Res. 58, 541-556.
10 Painter, R.B. and Young, B.R. (1976) Biochim. Biophys. Acta 418, 146-153.
11 Schneider, F.L., Stanbridge, E.J. and Epstein, D.J. (1974) Exp. Cell Res. 84, 311-318.
12 Setlow, R.B., Regan, J.D., German, J. and Carrier, W.L. (1969) Proc. Natl. Acad. Sci. U.S. 64, 1035-1041.
13 Eigner, J. and Doty, P. (1965) J. Mol. Biol. 12, 549-580.
14 Cleaver, J.E. (1978) Biochim. Biophys. Acta 516, 489-516.
15 Ockey, C.J. and Saffhill, R. (1976) Exptl. Cell Res. 103, 361-373.
16 Gerner, E.W. (1977) Radiat. Res. 71, 387-397.
17 Saha, B.K. and Tolmach, L.H. (1976) Radiat. Res. 66, 76-89.
18 Rude, J.M. and Friedberg, E.C. (1977) Mutation Res. 42, 433-442.
19 Park, S.D. and Cleaver, J.E. (1979) Nucleic Acid Res. 6, 1151-1159.