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BLEOMYCIN INDUCED INHIBITION OF DNA SYNTHESIS IN ATAXIA-TELANGIECTASIA CELL LINES

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SUMMARY: Exposure of fibroblast and lymphoblastoid cell lines from normal subjects to bleomycin (30-300 μg/ml) results in a dose dependent biphasic inhibition of DNA synthesis when compared with untreated cells. Using alkaline sucrose density gradient sedimentation we have confirmed that bleomycin decreases both DNA replicon initiation and chain elongation in normal lymphoblastoid cells. Doses of up to 100 μg/ml which caused a marked decrease in the rate of DNA synthesis in normal lymphoblastoid cells had much less effect on the inhibition of DNA synthesis in lymphoblastoid cell lines from ataxia-telangiectasia patients. The inhibition of DNA replicon initiation and chain elongation in these cell lines occurred only at high doses of bleomycin (100 μg/ml).

INTRODUCTION

Ataxia-telangiectasia (A-T) is a human autosomal recessive disease that exhibits an increased predisposition to cancer (1). Cells from A-T patients have been shown to be radiosensitive by both colony survival (2) and cytogenetic (3,4,5) methods. It has been reported that some A-T strains are deficient in repair replication when γ-irradiated under hypoxic conditions (6), whereas under aerobic conditions A-T cells are able to excise some γ-ray induced products from exogenous substrates (7). Recently it was reported that cell free extracts from A-T fibroblasts had a lower capacity for enhancing the priming activity of γ-irradiated colicin E1 DNA for DNA polymerase I from Micrococcus luteus (8). We have confirmed these observations using cell free extracts from two A-T lymphoblastoid cell lines (9).

Following γ-irradiation of normal mammalian cells the rate of DNA synthesis decreases in a biphasic manner (10). It was shown that the inhibitory effects of ionizing radiation on DNA synthesis was caused by

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blocking DNA replicon initiation (10,11). We have reported that A-T lymphoblastoid cells do not show an inhibition of DNA synthesis following moderate doses of γ-rays (12,13).

Here we report the effect of the anti-tumour antibiotic bleomycin on DNA synthesis in normal and A-T lymphoblastoid cell lines. Bleomycin has a radiomimetic action on chromosomes (14), and induces both single and double DNA strand scissions in bacterial and mammalian cells (15,16). It has been shown to release bases from purified DNA and destroy the deoxyribose moiety (17). As with ionizing radiation (2,5) it has been shown that following bleomycin treatment A-T cells have high levels of chromosome aberrations and low survival when compared with normal cells (18).

MATERIALS AND METHODS

Cells and Culture Conditions: Lymphoblastoid cell lines from three ataxia-telangiectasia homozygote patients (GM1525/AT2BI, GM1526/AT8BI, GM717) and a normal individual (GM558) were obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, N.J., U.S.A. A normal lymphoblastoid cell line (LIZ) was obtained from Dr. C.M. Steele, MRC Clinical and Population Cytogenetics Unit, Edinburgh, U.K. Cells were grown in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland, U.K.) supplemented with 15% (W/V) heat inactivated foetal calf serum in a humidified atmosphere of 5% CO_2 in air at 37°C. Fibroblasts from a normal individual (BAK) and an A-T patient (AT5BI) were grown in DME medium supplemented with glutamine, 15% foetal calf serum, penicillin (100 Iu/ml) and streptomycin (100 µg/ml) in 9 cm. dishes under a humidified atmosphere of 5% CO_2 in air at 37°C.

Measurement of DNA synthesis: Lymphoblastoid and fibroblast cells (1.5-3.5 x 10^5 cells/ml) from normal individuals and three ataxia-telangiectasia patients in exponential growth were incubated for 24h. at 37°C in growth medium containing 0.01 µCi/ml [^{14}C]thymidine (56 mCi/mmol). The medium was removed and the cells incubated for 60 min at 37°C in medium containing various doses of bleomycin (0-300 µg/ml). Medium containing bleomycin was removed and the cells resuspended in non-radioactive medium and incubated for 30 min at 37°C. The medium was again removed and both bleomycin treated and mock-treated cells were incubated in medium containing 20 µCi/ml of [^{3}H] thymidine (25 Ci/mmol) for 10 min. at 37°C. The radioactive medium was removed, the cells washed in ice cold Dulbecco's A phosphate buffered saline and ice cold 20% (W/V) trichloroacetic acid was added. The acid-insoluble precipitate was washed four times in 5% (W/V) TCA and the precipitate dissolved in 0.1M NaOH. Both [^{14}C] and [^{3}H] radioactivity was measured in Insta-Gel (Packard Ltd.) and the ratio of [^{3}H/^{14}C] used as a measure of DNA synthesis.

DNA replicon initiation and chain elongation: Lymphoblastoid cell suspensions (3.5 x 10^5 cells/ml) in exponential growth were incubated for 24h at 37°C in the presence of 0.02 µCi/ml [^{14}C] thymidine (56 mCi/mmol). Radioactive medium was removed and the cells incubated for 90 min. at 37°C in medium containing bleomycin (100 µg/ml). The bleomycin was removed and the cells incubated for 10 min. at 37°C in medium containing 20 µCi/ml of [^{3}H] thymidine (25 Ci/mmol).

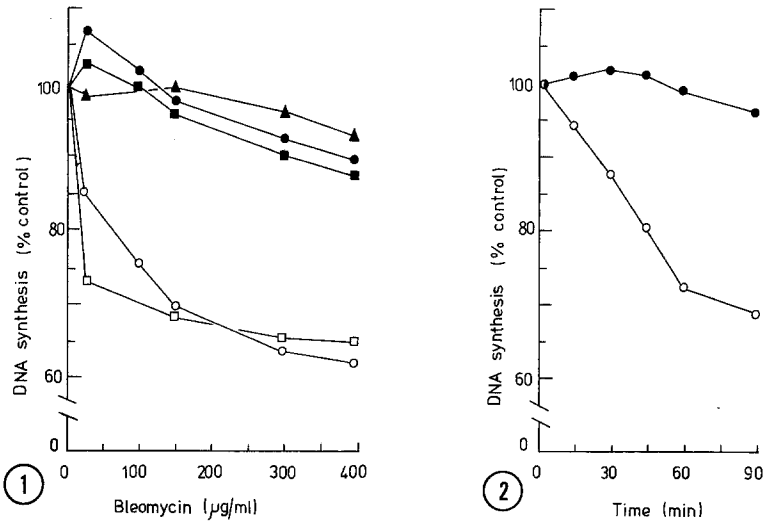

Fig. 1. Effect of increasing bleomycin dose on the rate of DNA synthesis in i) lymphoblastoid cell lines from a normal subject GM558 (○) and two A-T patients GM1525/AT2BI (●), GM1526/AT8BI (■), ii) fibroblasts from a normal subject BAK (□) and an A-T patient AT5BI (▲). The experimental procedure is as described in the Methods section. The rate of DNA synthesis ($^{3}H/^{14}C$ ratio) in each cell line is expressed as a percentage of untreated (100%) control cells. Each point represents the mean of triplicate samples.

Fig. 2. The rate of DNA synthesis in lymphoblastoid cell line from a normal subject GM558 (○) and an A-T patient GM1525/AT2BI (●) following exposure to bleomycin (30 μg/ml). Details as in Fig. 1 and Materials and Methods.

Radioactive medium was removed and the cells washed in ice cold Dulbecco's A phosphate buffered saline containing 242 μg/ml thymidine. Cells were placed in 1 ml of lysis solution (containing 0.5M NaOH, 0.02M EDTA and 0.1%NP40) and placed on top of a 5-20% alkaline sucrose gradient (0.1M NaOH, 0.9M NaCl, 0.01M EDTA) for 4h at 25°C. Gradients were centrifuged at 20°C in a SW 25.2 rotor (Beckman) at 14,000g_{av} for 15h. Fractions containing 1.5 ml were then collected, 100 μg of calf thymus DNA added to each fraction and the DNA precipitated with 0.5 ml of 20% (W/V) TCA containing 2% (W/V) $Na_4P_2O_7$. The acid-insoluble material was collected on GF/C filters (Whatman) and the filters washed in 5% (W/V) TCA containing 2% (W/V) $Na_4P_2O_7$ (5 x 5 ml). Both [^{14}C] and [^{3}H] radioactivity was measured by liquid scintillation counting in Insta-Gel (Packard Ltd).

RESULTS

Figure 1 illustrates the effect of increasing bleomycin dose on the rate of DNA synthesis in lymphoblastoid (GM558) and fibroblast (BAK) cell line from normal individuals and three cell lines (GM1525/AT2BI, GM1526/AT8BI, AT5BI) from A-T patients. An inhibition of [^{3}H] thymidine incorporation into the DNA of the normal cell line was observed with increasing bleomycin dose (30-300

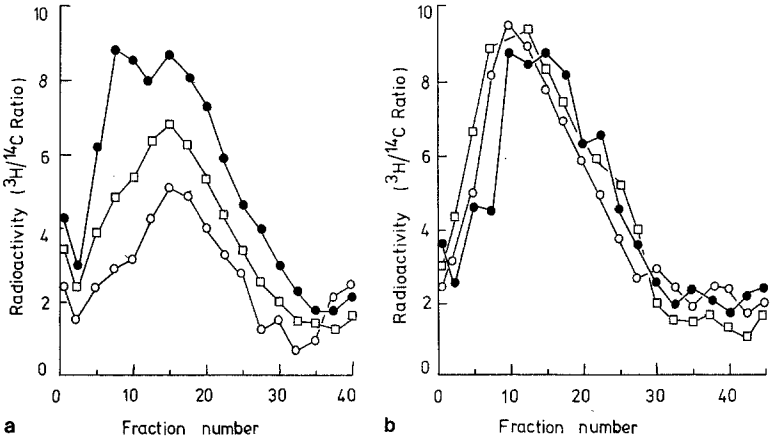

Fig. 3a. Alkaline sucrose density gradient profile of DNA from a normal subject (GM558) after treatment with bleomycin for 90 min. at 37°C. Untreated (●), 30 µg/ml (▣) and 100 µg/ml (○). Experimental procedure is as described in the Methods section. Results are expressed as the ratio of $^3H/^{14}C$ acid-insoluble radioactivity in each of the gradient fractions.

Fig. 3b. Alkaline sucrose density gradient profile of DNA from an A-T patient GM717 after treatment with bleomycin for 90 min. at 37°C. Untreated (●), 30 µg/ml (○) and 100 µg/ml (□). See Fig. 3a and Materials and Methods for details.

µg/ml). The inhibition of DNA synthesis in the normal cell lines is biphasic, showing an initial steep component at doses up to 100 µg/ml following by a shallow decline at higher doses. It has been reported that the initial steep decline is due to the inhibition of DNA replicon initiation and the shallow component due to blocked chain elongation (19). The dose response in both the A-T cell lines, GM1525/AT2BI and GM1526/AT8BI, showed no decrease in the rate of DNA synthesis at bleomycin doses up to 100 µg/ml, at the higher dose (300 µg/ml) DNA synthesis was slightly inhibited. Figure 2 shows that the rate of DNA synthesis in the normal cell line (GM558) continued to decrease for up to 90 min after bleomycin (30 µg/ml) treatment. In the A-T cell line GM1525/AT2BI a much slower decrease in DNA synthesis was observed.

Using velocity sedimentation in alkaline sucrose density gradients we investigated the effect of bleomycin on DNA replicon initiation and chain elongation in a normal (GM558) and an A-T (GM717) lymphoid cell line. Fig. 3a illustrates a typical gradient profile of the DNA from a normal individual.

The ratio of $^{3}H/^{14}C$ radioactivity in both low and high molecular weight fractions decreased with increasing bleomycin dose. Therefore, following bleomycin treatment both replicon initiation and chain elongation were inhibited. Fig. 3b illustrates the effect of bleomycin upon replicon initiation and chain elongation in an A-T lymphoid cell line (GM717). The gradient profile of DNA from the bleomycin treated A-T cell line was quite different from that obtained with the normal cell line. No reduction in the low or high molecular weight DNA fractions was obtained. However, a slight increase in replicon initiation was observed.

DISCUSSION

The results presented here show that in normal human cells both replicon initiation and chain elongation are inhibited by bleomycin in a dose dependent manner, whereas no inhibition of either replicon initiation or chain elongation occurred in the A-T cell lines following low doses of bleomycin. These results are in agreement with those recently obtained using normal and A-T fibroblasts (20). These results are similar to those previously obtained using ionizing radiation as the DNA damaging agent (12,13,21). The reason for this defect in DNA synthesis is unknown, but may be related to the likely defects in DNA repair. Peripheral lymphocytes from A-T patients, X-irradiated prior to culturing (G_0) show at mitosis an increased amount of both chromosome and chromatid type damage. The chromatid interchanges induced in A-T cells by irradiation at G_0 are present in greater numbers than in irradiated normal cells (22). DNA damage induced by ionizing radiation in A-T cells at G_0 can be potentiated to chromosome damage by caffeine added to cultures up to 24h following irradiation (23). There is, therefore, evidence from cytogenetic studies of a much greater level of unrepaired and misrepaired damage in the DNA of A-T cells.

It has been proposed that in normal cells DNA strand breaks induce conformational changes in replicon clusters that inhibits replicon synthesis until the damage is repaired (21). This suggests that the chromatin structure

of A-T cells does not change in response to bleomycin induced DNA damage. However, it has been shown that both normal and A-T cells contain the same number of DNA strand breaks following damage induced by ionizing radiation (24).

Another possibility is that A-T cells are deficient in the repair of bleomycin induced DNA damage, and that a factor or process may exist which either recognises or is synthesised in response to DNA damage. This factor may function by altering chromatin structure which in turn may be responsible for the inhibition of DNA replicon initiation.

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