
The response of ataxia telangiectasia cells to bleomycin

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ABSTRACT

The autosomal recessive disorder, ataxia telangiectasia (AT) is characterised by cellular sensitivity to ionizing radiation. The molecular basis of this radiosensitivity is the subject of controversy. We report here that cultured fibroblasts from AT patients are also sensitive to the lethal effects of bleomycin. As with ionizing radiation, no defect has been observed in the overall rejoining of single or double-strand breaks produced by bleomycin. Since, however, only apyrimidinic (and to a lesser extent apurinic) sites and strand breaks are known to be produced by bleomycin, we tentatively suggest that AT cells are unable to rejoin a very small fraction of the total strand breaks. We attribute our inability to detect such unrejoined strand breaks to the relative insensitivity of the sucrose gradient procedures normally used to detect strand breaks.

INTRODUCTION

Ataxia telangiectasia is an autosomal recessive disorder characterised by cerebellar ataxia, oculocutaneous telangiectasia, immune deficiency in most cases, and an increased predisposition to cancer especially of the lymphoproliferative system¹⁻⁴. There is an abnormally high frequency of chromosome aberrations in both lymphocytes and fibroblasts^{3,4}. Affected individuals are sensitive to ionizing radiation⁴, and this is reflected by hypersensitivity to X- and γ -radiation at the cellular and chromosomal levels^{5,6}. At the molecular level all AT cells tested can rejoin single^{5,7,8} and double-strand breaks⁹ produced in their DNA by ionizing radiations, at apparently normal rates. Paterson and coworkers have shown that cells from one group of AT patients have a reduced level of repair replication after anoxic γ -irradiation. In those cells tested, this was associated with a reduced rate of excision of an uncharacterised type of base damage produced by γ -rays⁷. This damage is manifested as sites in the DNA sensitive to a damage specific endonuclease present in extracts of Micrococcus luteus. In cells from another class of AT patients, which are

equally sensitive to the lethal effects of γ -rays, no such defect has been detected¹⁰, and we have speculated that the defect in excision of base damage may not be the cause of radiosensitivity even in the excision-defective class¹¹. To date the only molecular defect identified in an excision-proficient AT cell strain is a reduced level of an enzymatic activity present in human cell extracts which activates γ -irradiated DNA to make it a more efficient primer in a DNA polymerase reaction¹². The role of this activity in irradiated cells has not yet been identified.

In order to obtain more information on the defect in AT cells we have studied their response to the radiomimetic drug bleomycin (BM). Although many of the effects of BM are similar to those of ionizing radiation, the chemical lesions produced in DNA by BM are much less diverse than those produced by ionizing radiation. Bleomycin is an antibiotic which causes release of thymine (and to a lesser extent other) bases from DNA. In some cases this depyrimidination is accompanied by strand scission¹³. Both single- and double-strand breaks are produced in free DNA, the latter arising as single events rather than from two adjacent single-strand breaks on opposite strands of the DNA¹⁴. Both single and double strand breaks have been detected in the DNA of cells exposed to BM and both are rejoined in mammalian cells (see 13).

MATERIALS AND METHODS

Bleomycin was obtained from Lundbeck (Luton, England) and stored in aliquots in frozen aqueous solution at 5 mg/ml. Thawed aliquots were not refrozen.

1. Cell Strains

The normal cell strains, 2BI, 1BR and 4BR and the AT strains AT3BI and AT5BI⁵ were cultured in Eagle's MEM supplemented with 15% foetal calf serum. AT3BI was shown by Paterson *et al.* to have a reduced level of γ -ray induced repair replication, whereas AT5BI did not show this defect^{7,10}.

2. Cell Survival

The thin feeder layer technique of Cox and Masson¹⁵ was used as described by Arlett *et al.*¹⁶ 2.4×10^5 cells were incubated in a 25 cm² Falcon flask overnight, 1 ml of appropriate concentrations of BM was added and the cells incubated for 1 hr. They were then washed with buffered saline, trypsinized and appropriate dilutions plated onto the feeder layer.

3. Measurement of Strand Breaks

Cells were seeded at 10^5 per 5 cm dish on Day 1 and labelled with 1-5 $\mu\text{Ci/ml}$ ^3H -Thd (1-5 Ci/mole) or 0.05 $\mu\text{Ci/ml}$ ^{14}C -Thd (0.05 Ci/mole) on Day 2. On Day 3 the medium was removed and the cells treated with BM for 1 hr at 37°C in fresh medium. The BM was removed, the cells washed and incubated for different periods of time at 37° in fresh medium. They were then washed, scraped off the dishes into 0.3 ml EDTA in buffered saline and 0.1 ml layered on top of either alkaline or neutral sucrose gradients. For alkaline gradients the lysis layer contained 0.2 ml 2% sodium dodecyl sulphate/0.02 M EDTA on top of 4.7 ml 5-20% sucrose/0.1M-NaOH/0.1M-NaCl, and for neutral gradients 0.2 ml 2% lithium dodecyl sulphate on top of 5-20% sucrose, 1M-LiCl, 0.01M-sodium citrate, 0.02M EDTA pH9⁹. Centrifugation, fraction collecting and counting have been described previously¹⁷.

RESULTS

1. Cell Survival Experiments

In order to measure the cell-killing effects of BM, cells were exposed to the drug for 1 hr in medium and then plated at appropriate concentrations in the absence of drug. We found under these conditions that there was considerable variability in the amount of cell killing between experiments. We attribute this variability, in part at least, to small variations in pH which have been shown to have marked effects on the action of BM¹⁸. We have therefore only directly compared results between cell lines in the same experiment. Figure 1 shows survival curves from an experiment in which 1BR, AT3BI and AT5BI were treated with different doses of BM. As has been found by others using rodent cells^{18,19}, the dose-response was reproducibly non-linear in such experiments. The AT cells were more sensitive than the normal strains. A number of experiments similar to that shown in Figure 1 demonstrated that (1) both AT strains were more sensitive than the three normal strains 1BR, 2BI and 4BR; (2) At high doses AT3BI was significantly more sensitive than AT5BI; (3) The three normal strains showed comparable sensitivity.

2. Measurement of Single-strand Breaks

BM is known to produce both apyrimidinic sites and strand breaks in cellular DNA¹³. Both of these lesions are manifested as strand breaks under alkaline conditions and they can be measured by sedimentation of the DNA in alkaline sucrose gradients. Since AT3BI was the strain most sensitive to the

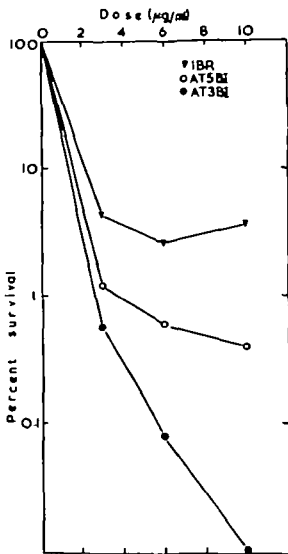


Fig. 1. Survival curves of normal and AT strains after treatment with BM (representative experiment)

lethal effects of BM we have compared the ability of AT3BI and normal cells to rejoin alkali-labile sites and strand breaks produced by BM. Figure 2 shows that they are repaired very rapidly after treatment with 100 µg/ml BM as shown by the increase in size of the DNA, and no difference could be detected between the rate of rejoining in the normal and the AT cell strain.

3. Double-strand breaks

BM has also been reported to produce double-strand breaks in cellular DNA²⁰. This is confirmed by the experiment of Figure 3 in which the DNA

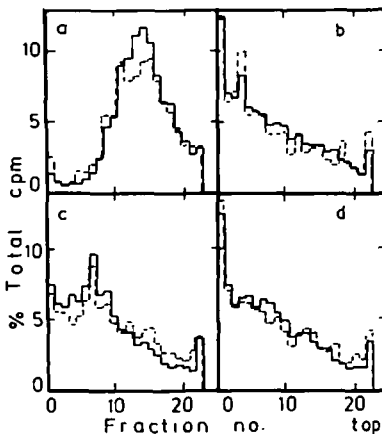


Fig. 2. Rejoining of single-strand breaks in 1BR (—) or AT3BI (---) after treatment for 1 h with 100 µg/ml bleomycin and subsequent incubation for 0'(a), 8'(b), 14'(c), 20'(d). The alkaline sucrose gradient profiles of the DNA are shown, with centrifugation at 35,000 rpm for 1 h.

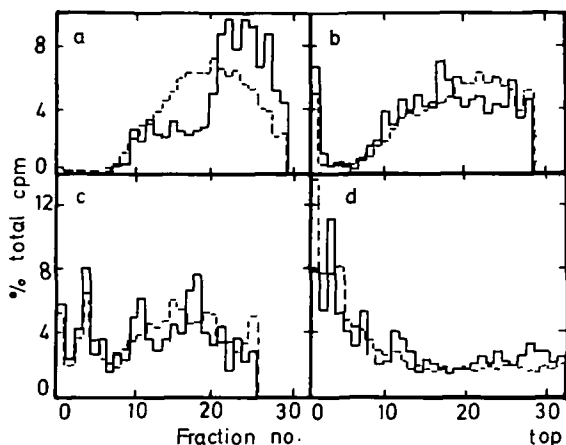


Fig. 3. Rejoining of double strand breaks in 1BR (—) or AT3BI (---) after treatment for 1 h with 500 $\mu\text{g}/\text{ml}$ bleomycin and incubation for 0 (a), 0.5 (b), 1 h (c), 2 h (d). Centrifugation at 7,000 rpm for 16 h on neutral sucrose gradients.

from BM treated cells was sedimented under "neutral" (non-denaturing) conditions. Under these conditions untreated DNA sedimented to the bottom of the tube. 500 $\mu\text{g}/\text{ml}$ BM reduced the weight-average molecular weight of the DNA to about 80×10^6 (Figure 3a) in both normal and AT3BI cells, showing that double-strand breaks were produced. On subsequent incubation the molecular weight of the DNA increased, and after 2 h the DNA was again found at the bottom of the tube (Figure 3d), showing that the double-strand breaks were rejoined. Although the kinetics of the rejoining varied between experiments, only small differences were seen between the profiles of the normal and AT3BI cells. Such minor differences showed no consistent pattern between experiments and are not considered significant.

DISCUSSION

Previous studies of Arlett and colleagues showed that AT cell strains were hypersensitive to the lethal action of ionizing radiation^{4,5}, but had a normal response to UV, methyl methanesulphonate, ethyl methanesulphonate and mitomycin C⁴. Har and Sargent on the other hand reported that some AT cell strains were sensitive to methyl methanesulphonate and some to mitomycin C²¹, and Scudiero found hypersensitivity to N-methyl-N'-nitro-N-nitrosoguanidine in five AT strains²². In the present work we have shown that two AT cell strains were sensitive to BM; similar results have been obtained independently by A. M. R. Taylor (personal communication). Thus AT cells are defective in repair of damage produced by either ionizing

radiation or BM. Both these agents induce base loss in DNA, but several workers have found normal or near normal levels of AP endonuclease activity in AT cell extracts^{12,23-25}. Both BM and ionizing radiation also produce strand breaks in cellular DNA. We have shown in the present work that, like rodent cells^{13,20}, human fibroblasts are able to rejoin both single- and double-strand breaks produced by the action of BM. No defect in rejoining of these breaks was found in AT cell strains, a situation very similar to that obtained with ionizing radiation⁷⁻¹⁰. Ionizing radiation causes a heterogeneous mixture of lesions in DNA, whereas base loss and strand breakage are the only types of damage known to be produced in DNA by BM¹³. (Reports however, suggesting that BM can act through a free radical mechanism (e.g. see 26) would lead one to expect that other types of damage may also be produced). A defect in the excision of γ -ray-induced base damage (endonuclease-sensitive sites) has been reported for some (but not all) AT strains studied^{7,10}, but we have argued that this is unlikely to be the primary cause of radiosensitivity in AT cells¹¹, and such lesions are not known to be produced by BM.

How can we reconcile the failure to find a DNA repair defect in some AT cells with the hypersensitivity to γ -rays and BM? One of us¹¹ and Taylor⁶ have recently proposed that there is a very small proportion of the total strand breaks (produced either by ionizing radiation or BM) which AT cells cannot rejoin. These strand breaks are envisaged as having some kind of unusual structure. It can be calculated¹¹ that the minimum number of unrejoined strand breaks necessary to account for the increased frequency of chromosome aberrations⁶ and lethality⁵ in AT cells would be too small to be detected biochemically even by the most sensitive procedures currently available²⁷. This hypothesis is consistent (1) with the observation that the oxygen enhancement ratio for ionizing radiation-induced cell killing of both normal and AT cell strains corresponds with that for production of strand breaks but not of base damage²⁸ and (2) with evidence suggesting that unrejoined strand breaks are the cause of high LET ionizing radiation-induced cell death in mammalian cells²⁹. Although the hypothesis is somewhat unsatisfactory in being unsupported by direct evidence and not easily accommodating the defect in excision of base damage, it does offer a reasonable explanation for most of the apparent inconsistencies in the available data on AT. Its verification must await a direct biochemical demonstration of the elusive defect in AT cells.

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