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Enhancement of bleomycin-induced chromosome damage in a human leukemic T-cell line

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ABSTRACT

A sensitivity to bleomycin was examined in a human leukemic T-cell line (CCRF-HSB2) established from the peripheral blood of a patient with acute lymphoblastic leukemia. At all concentrations of bleomycin tested, HSB2 showed reduced survival and increased chromosome breakage. A normal B-cell line (KS86) and a radiation-insensitive CCRF-HSB2-M cell line were found to be resistant against the effects of bleomycin. The increased sensitivity of the human leukemic T-cell line (HSB2) to bleomycin, as well as to radiation (Shiraishi *et al.* 1976, 1978; Taguchi and Shiraishi 1983), indicates that HSB2 resembles to ataxia telangiectasia cells in their susceptibility to chromosomal aberration-inducing treatments.

1. INTRODUCTION

Human leukemic T-cell line CCRF-HSB2 was established from the peripheral blood of a patient with acute lymphoblastic leukemia by Adams *et al.* in 1970. We have reported that this cell line is highly sensitive to radiation with regard to cell proliferation and chromosome damage, when compared with normal lymphocytes, a normal B-cell line and CCRF-HSB2-M which is a radiation-resistant mutant clone derived from HSB2 (Shiraishi *et al.* 1976, 1978; Taguchi and Shiraishi 1983).

So far, only a few cell lines of human origin which are characterized with high radiation sensitivity while having normal ploidy have been described. One well-known example is from a patient with ataxia telangiectasia (AT), a rare autosomal recessive disease (Harnden 1974). Cell lines from AT patients are sensitive to X-ray and bleomycin with respect to cell survival and chromosome damage (Cohen *et al.* 1981; Ishida and Buchwald 1982; Kohn *et al.* 1982; Morris *et al.* 1983; Shaham *et al.* 1983). Bleomycin has been shown to inhibit DNA synthesis (Suzuki *et al.* 1968) and cause single and double strand breaks of DNA both *in vivo* and *in vitro* (Nagai *et al.* 1969; Haidle 1971; Shirakawa *et al.* 1971; Haidle *et at.* 1972; Muller *et al.* 1972; Fujiwara and Kondo 1973; Miyaki *et al.* 1973; Umezawa *et al.* 1973; Muller and Zahn 1976). Phenomenologically, this drug thus appears to mimic the effects of X-irradiation that has been shown to produce DNA strand breaks and chromosome aberrations (Iqbal *et al.* 1976; Vig and Lewis 1978). However, the precise action mechan-

isms of bleomycin have not yet been elucidated.

Considering the high sensitivity of HSB2 to radiation (Shiraishi *et al.* 1976, 1978; Taguchi and Shiraishi 1983), it is of interest to examine the sensitivity of this cell line to bleomycin. The present paper demonstrates hyper-sensitivity of HSB2 to bleomycin in terms of a reduction in cell viability and an increase in chromosomal aberrations.

2. MATERIALS AND METHODS

Cell lines and cell culture: Two human leukemic T-cell lines (radiation-sensitive CCRF-HSB2 and radiation-resistant revertant clone CCRF-HSB2-M originated from CCRF-HSB2) and a normal B-cell line (KS86) which was derived from a normal healthy volunteer were used in the present study. The karyotypes were 46, XY, t(1; 7) (p32; q32), 47, XY, t(1; 7) (q32; p32), +iso6 (6; 6)(p11; q13), t(12; 16)(q24; q11), −16p, +22 (Taguchi and Shiraishi 1983) and 46, XX, respectively. Cells were maintained in RPMI 1640 (Nissui) medium supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere, and subcultured every second day. The generation time (cell cycle time) of these three cell lines was approximately 24 hr.

Cell growth inhibition experiments: For cell growth inhibition studies, cells (3×10^5/ml) were continuously incubated in the presence of bleomycin (Nippon Kayaku Co. Ltd., Tokyo, Japan) during the periods indicated. Bleomycin, dissolved in distilled water, was added to 10 ml culture in 100 μl volumes to yield final concentrations of 0, 0.03, 0.3, and 3.0 μg/ml. Viable cell counting was performed at 24 hr intervals up to 120 hr by a dye-exclusion method with 1% trypan blue using a hemocytometer.

Chromosome damage by bleomycin: Cells were treated with bleomycin at the three concentrations (3, 6 and 50 μg/ml) for 5 hr. Bleomycin treatment was followed by reincubating the cells in bleomycin-free medium in some experiments designed for assessing their repair ability. For chromosome analyses, cells were treated with 0.05 μg/ml Colcemid (Gibco) for the final 1 hr of incubation and processed according to standard techniques using 0.075 M KCl as a hypotonic solution and absolute methanol-glacial acetic acid 3:1 as a fixative. Flame dried slides were then prepared and stained with Giemsa (Merk). The changes in frequency of chromosomal aberration types in each cell line were compared.

Breaks per cell were scored and expressed according to the method of Cohen *et al.* (1981). Structural rearrangements, including dicentrics, multiradial configurations (tri- and quadri-radials, etc.), rings and obvious translocations, were considered as 2-break events, while chromatid and chromosome (isochromatid) breaks were scored as single-break events. The frequency of gaps was extruded from total aberration yield.

3. RESULTS

Effect of bleomycin on cell growth

Severe growth inhibition was observed in HSB2 with increasing concentration (0.03, 0.3 and 3.0 μg/ml) of bleomycin (Fig. 1). The normal B-cell line KS86 and the radiation-resistant cell line HSB2-M were less sensitive. HSB2-M were more resistant than KS86 at any concentrations tested.

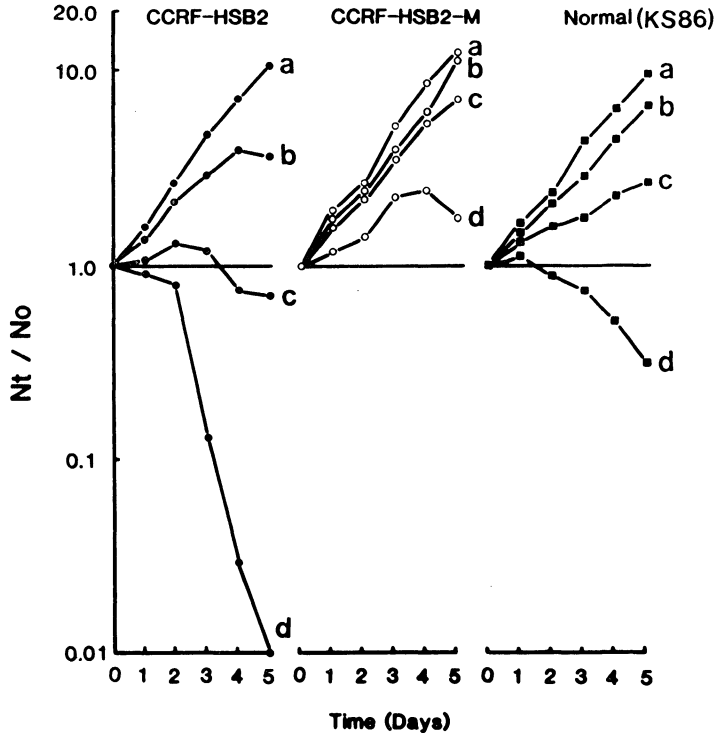

Fig. 1. Effect of three different concentrations of bleomycin on cell viability in three cell lines. a, control; b, 0.03 μg/ml; c, 0.3 μg/ml; d, 3.0 μg/ml.
No, viable cell number at 0 hr; Nt, viable cell number at given time. Nt/N_0 refers to viable cell number at given times divided by those at time zero.

Chromosomal sensitivity to bleomycin

Table 1 summarizes the chromosomal aberrations caused by bleomycin treatments. Incidence of chromosomal aberrations in the absence of bleomycin did not differ so much between the three cell lines, though there were a few breaks in HSB2 and HSB2-M (exp. 1).

In exps. 2 to 4 designed for assessment of the chromosome damage caused at G2 phase of the cell cycle, chromosomal aberrations induced by bleomycin treatments (3.0, 6.0 and 50.0 μg/ml) for 5 hr were compared between the three

Table 1. *Chromosomal aberrations produced by bleomycin treatment*

No. of Exp.	Conc. of[a] bleomycin (μg/ml)	Re-[b] incubation time (hr)	Cell line	Cells[c] with aberrations (%)	Number of aberrations/100 cells						Breaks per cell
					chromatid breaks	chromosome breaks	chromatid exchanges	dicentrics	translocations	rings	
1	0	0	HSB2	3	2	1	0	0	0	0	0.03
			HSB2-M	2	1	1	0	0	0	0	0.02
			KS86	0	0	0	0	0	0	0	0
2	3	0	HSB2	36	28	5	0	2	2	1	0.43
			HSB2-M	5	7	2	0	0	0	0	0.09
			KS86	18	10	2	0	0	0	0	0.12
3	6	0	HSB2	48	44	3	0	0	0	3	0.53
			HSB2-M	20	20	1	0	0	0	0	0.21
			KS86	19	19	0	0	0	0	0	0.19
4	50	0	HSB2	73	72	4	1	0	0	0	0.78
			HSB2-M	34	30	1	6	0	0	0	0.43
			KS86	34	34	0	5	0	0	0	0.44
5	50	24	HSB2	90	81	58	1	8	1	0	1.59
			HSB2-M	58	38	17	13	7	0	0	0.95
			KS86	41	35	8	11	5	1	0	0.77
6	50	48	HSB2	96	48	68	2	19	4	5	1.76
			HSB2-M	37	16	30	13	12	0	4	1.04
			KS86	32	8	44	10	19	2	0	1.14
7	50	72	HSB2	*	*	*	*	*	*	*	*
			HSB2-M	22	12	13	0	4	0	1	0.35
			KS86	19	7	8	0	8	1	0	0.33

* Chromosome analyses were not done because of severe cell death.

[a] Cells were treated with bleomycin for 5 hr.

[b] After the drug treatment, cells were washed twice with RPMI 1640 medium and then reincubated in a bleomycin-free medium for the periods indicated.

[c] A total of over 100 hundred metaphases per treatment was examined.

cell lines. In all cell lines an increase in the number of chromosomal aberrations was seen with increasing concentration of bleomycin and those chromosomal aberrations were predominantly composed of chromatid breaks. The frequency of chromatid breaks in HSB2 was over twice that in normal and HSB2-M cells.

In exps. 5 to 7, cells were treated with bleomycin at 50 μg/ml for 5 hr followed by reincubation (24, 48 and 72 hr) in a bleomycin-free medium. Such reincubation procedure was adopted to allow the cells to repair DNA breaks caused by the drug. Unexpectedly, the cells thus reincubated (exps. 5 and 6) showed more extensive damage than those with no reincubation in exp. 4 suggesting that bleomycin caused substantial breakage to the cells. The chromosome damage was composed of chromatid breaks, chromosome breaks, dicentrics, chromatid exchanges, translocations and rings. In all of the reincubation experiments carried out, the frequencies of both chromatid and chromosome breaks in HSB2 were over twice those in KS86 and HSB2-M. The

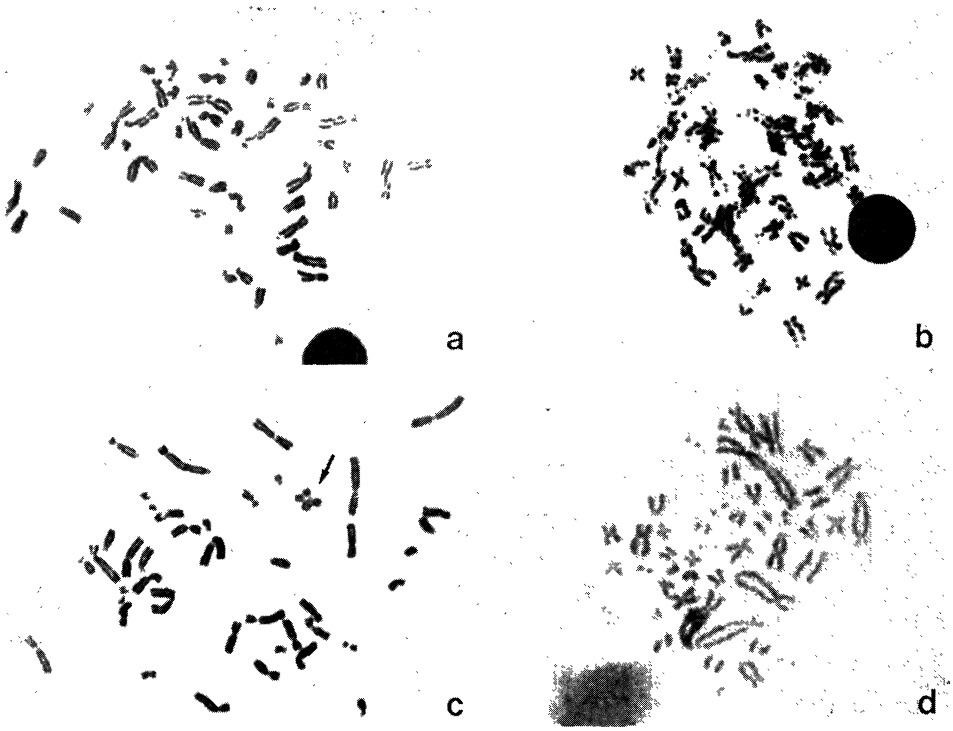

Fig. 2. a and b: Metaphase plates from a CCRF-HSB2 culture treated with bleomycin (50 μg/ml) for the final 5 hr (exp. 4). Note several chromatid breaks in a and multiple aberrations in b. c and d: Metaphase plates from CCRF-HSB2 exposed to bleomycin (50 μg/ml) for 5 hr, followed by reincubation for the subsequent 24 (c, exp. 5) or 48 hr (d, exp. 6). Note the chromosome-type aberrations in c and d. An arrow in c indicates quadriradial configuration.

frequencies of chromosome-type aberrations (chromosome breaks and dicentrics) in exps. 5 and 6 were markedly higher than those in exp. 4 in all cell lines tested. Typical metaphases with chromosomal aberrations in HSB2 are shown in Fig. 2. A remarkable reduction in frequency of chromosomal aberrations in KS86 and HSB2-M was observed in exp. 7 as compared with exp. 6. In exp. 7, chromosome analyses in HSB2 were unsuccessful because of severe cell death. The frequency of chromatid exchanges in KS86 and HSB2-M was over 5 to 10 times higher that of HSB2 in exps. 4, 5 and 6.

4. DISCUSSION

The results presented here demonstrate that a radiation-sensitive human leukemic T-cell line (HSB2) is also highly sensitive to bleomycin. The much higher frequency of chromosomal aberrations seen in HSB2 suggests a slower or poorer repair of DNA strand breaks in this T-cell line. This reinforces our previous suggestions that the high sensitivity of HSB2 to radiation is due to the defectiveness of repair mechanisms necessary to overcome the lesions produced by X-ray (Shiraishi *et al.* 1976, 1978; Taguchi and Shiraishi 1983).

There have been only a few human cell lines with normal ploidy which exhibit high sensitivity to bleomycin and X-ray, and to our knowledge, ataxia telangiectasia (AT) B-lymphoblastoid cell lines are the only example which has been extensively studied (Taylor *et al.* 1979; Cohen *et al.* 1981; Kohn *et al.* 1982; Morris *et al.* 1985). AT, an autosomal recessive genetic disease, has been known as a chromosome breakage syndrome on the basis of the fact that AT cells frequently show increased chromosome breakage in primary culture even without any addition of known clastogens (Harnden 1984; Kohn *et al.* 1982). The similarity of HSB2 and AT cells in sensitivities to bleomycin and X-ray suggests that the two types of cells should share similar or common defects in their DNA repair machinery. However, HSB2 cells did not show such a higher level of spontaneous chromosome breakage in control cultures as was observed with AT cells; the level in HSB2 was as low as that in the normal B-cell line (KS86). Thus, HSB2 is different from AT cells with respect, at least, to the level of spontaneous chromosome breakage in spite of their similar hyper-sensitivily to X-ray and bleomycin. HSB2 is, therefore, expected to provide another unique experimental system of human cells suitable for studying the mechanisms underlying the high sensitivity to X-ray and bleomycin.

A hypothesis has been proposed concerning how chromosome-damaging agents, especially ionizing radiation, induce different types of chromosomal aberrations in mammalian cells (Bender *et al.* 1974); each of the various types of chromosomal aberrations seen at metaphase can be explained as a result of breaks in DNA double helices and recombinations between them in relation to the stage of the cell cycle in which the treatment is performed. Double-

strand breaks caused during the G1 phase induce chromosome-type of aberrations. Single-strand breaks caused during G1 phase, in contrast, can not contribute the production of chromosomal aberrations because they are usually repaired before the cell cycle reaches mitosis excepting the single-strand breaks caused at G1-S border which can induce chromatid-type aberrations due to insufficient time for repair. Double-strand breaks during early S phase induce isochromatid (chromosome) breaks. As for breaks caused at late S and G2, not only double-strand breaks but also single-strand breaks, after being converted to double-strand breaks by a single-strand nuclease, induce chromatid breaks. Thus, double- and single-strand breaks result in distinct types of chromosomal aberrations depending on the cell cycle at which the breaks are caused. According to this hypothesis, chromatid breaks observed in exps. 2 to 4 reflect double strand breaks caused at late S and G2 phases because, judging from the doubling time of these three cell lines (about 24 hr), only cells at late S and G2 phases can reach mitoses during 5 hr treatment with bleomycin. The frequency of chromatid breaks remains similarly high even after 24 hr reincubation to allow the cells to repair DNA breaks (exp. 4 vs, exp. 5). The chromatid breaks in exp. 4, probably attributable to the double-strand breaks at late S or G2 phase as described above, might have converted to chromosome-type aberrations or have disappeared as a result of cell death during 24 hr reincubation, if remains unrepaired. Actually, the frequency of chromosome-type aberrations was increased after such reincubation period (compare exp. 4 and exp. 5). Then, if so this transformation of aberration from chromatid- to chromosome-type should be paralleled by a corresponding decrease in chromatid-type aberrations. However, the frequency of chromatid breaks after 24 hr reincubation (exp. 5) was not decreased as compared with that with no reincubation (exp. 4), This high frequency of chromatid breaks retained in exp. 5 can be explained by the combination of the delay of cell cycle and the failure of repairing single-strand breaks caused at G1 phase, the latter idea being in disagreement with the hypothesis by Bender *et al.* (1974) that most of the single-strand breaks at G1 phase can be repaired. This suggests that bleomycin can induce DNA breaks in cells at not only late S and G2 but also the other phases (probably G1 or early S) which becomes detectable as chromatid-type aberrations only after an appropriate period of reincubation, for example, for 24 hr as employed in exp. 5.

An obvious tendency was not recoignized that the two cell lines, KS86 and HS2-M, show more rapid recovery from bleomycin damage than HSB2 as far as judged from the percentage of the cells with aberrations (exps. 4 to 6). This might raise the possibility that there is no substantial difference in DNA repair abilities between them, but that their sensitivities to bleomycin differs so much that more DNA strand breaks can be caused in HSB2 than in the other two cell lines resulting in the higher incidence of chromosomal aberrations in

HSB2. Estimation of the number of causal breaks rather than their resulting chromosomal aberrations may be also of importance for further characterization of this phenomenon.

In the normal and the revertant cell lines, KS86 and HSB2-M, a drastic decrease of chromosomal aberration-frequency in exp. 7 as compared with those in exps. 5 and 6 may be due to the repair of the damage and/or the selective survival of undamaged cells in the bleomycin-treated culture during the long reincubation period (72 hr). In exps. 5 and 6, the increase in chromosome-type aberrations with prolonged reincubation time was paralleled by the decrease in chromatid breaks. This may again indicate that some of the chromatid-type aberrations, if remain unrepaired, can give rise to the additional chromosome-type aberrations during the cell cycles subsequent to bleomycin treatment, which in turn results in a corresponding reduction of chromatid-type aberrations.

Paika and Krishan (1973) reported a bleomycin-resistant characteristic of another human leukemic T-cell line (CCRF-CEM) which shows apparent polyploidy. Such distinct responses to bleomycin between HSB2 and CEM may be attributed to a difference in cellular DNA content. Taguchi and Shiraishi (1983) also suggested that a karyotypic alteration may play an important role in the change of radiation sensitivity.

Interestingly, the frequencies of chromatid exchanges in KS86 and HSB2-M were over 10 times higher than that of HSB2 (exps. 5 and 6). This suggests that a high frequency of chromatid exchanges can be an index of superior repair-ability of such normal and revertant cells, though chromatid exchanges themselves represent erroneous reunion of break points.

Shiraishi *et al.* (1978) reported an interesting observation using HSB2 that there is non-randomness in the distribution of X-ray-induced chromosome and chromatid breaks and their reunion between and within chromosomes or chromosome groups. Site-specific sensitivity of chromosomes to chemical agents including bleomycin in HSB2 remains to be studied.

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