

Chromosome sensitivity to bleomycin in G₂ lymphocytes from Down syndrome patients

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ABSTRACT

Several studies have demonstrated that lymphocytes from patients with Down syndrome (DS) exhibit an increased frequency of chromosome aberrations when they are exposed to ionizing radiation or to chemicals at the G₀ or G₁ phases of the cell cycle, but not at G₂, when compared to normal subjects. To determine the susceptibility of DS lymphocytes at G₂ phase, bleomycin, a radiomimetic agent, was used to induce DNA breaks in blood cultures from 24 Down syndrome patients.

All the patients with DS showed free trisomy 21 (47,XX + 21 or 47,XY + 21). Individuals that showed an average number of chromatid breaks per cell higher than 0.8 were considered sensitive to the drug. No control child showed susceptibility to bleomycin, and among the 24 patients with DS, only one was sensitive to the drug.

No significant difference was observed between the two groups, regarding chromatid break frequencies in treated G₂ lymphocytes. The distribution of bleomycin-induced breaks in each group of chromosomes was similar for DS and controls. No significant difference was found in the response to bleomycin between male and female subjects. Probably, the main factor involved in chromosome sensitivity of lymphocytes from patients with DS is the phase of the cell cycle in which the cell is treated.

INTRODUCTION

Lymphocytes from patients with Down syndrome (DS) are more sensitive to ionizing radiation and to chemicals than lymphocytes of normal individuals (Lambert *et al.*, 1976; Morimoto *et al.*, 1984). Frequencies of induced aberrations, especially dicentrics, in G₀ and G₁ phases of the cell cycle are substantially higher in patients with DS (Preston, 1981; Morimoto *et al.*, 1984). Few studies have been made on G₂ phase of the cell cycle (Dekaban *et al.*, 1966; Leonard and Merz, 1983; Shafik *et al.*, 1988), and no significant increase of breaks in DS lymphocytes in relation to lymphocytes of normal individuals has been found.

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Several suggestions have been made to explain the high frequency of induced aberrations in DS cells irradiated in G₀ or G₁. Countryman *et al.* (1977) observed a more rapid repair of chromosome breaks in DS lymphocytes X-irradiated in G₁ phase than in normal ones. The authors proposed a mechanism by which reduced rejoining times would increase aberration frequencies in competition between a (hypothetical) error-free repair system and the error-prone repair system that generates chromosomal aberrations.

Preston (1981) also observed a more rapid repair of DNA damage converted into chromosome aberrations in DS lymphocytes X-irradiated in G₀ than in normal cells, what would increase the probability of interaction (or misrepair) of two lesions present at the same time and close together in space.

Lambert *et al.* (1976) suggested that the higher frequency of dicentric chromosomes in DS lymphocytes as compared to control cells after X-irradiation is the result of a higher yield of X-ray-induced lesions, or of a higher proportion of chromosome breaks joined by ligase activity.

It is possible that an altered DNA repair mechanism is related to the higher frequency of leukemia in DS. Several types of leukemia are associated with primary (specific) chromosome changes (Sandberg, 1986), and for this reason an altered DNA repair mechanism in DS lymphocytes could be responsible for the occurrence of chromosome combinations that would favor cancer initiation.

Leukemia is the only cancer type that appears at a higher frequency in DS (about 8-19 times greater than expected), and the radiosensitivity of those individuals seems to be restricted to lymphocytes (Leonard and Merz, 1987).

Cytogenetic experiments with mutagens can provide an estimation of the individuals' DNA repair capacity. According to Hsu (1987), the higher the number of induced breaks not repaired, the more deficient the repair mechanisms.

Bleomycin (BLM) is a radiomimetic chemical that has been used to induce breaks in DNA of cultured lymphocytes, to verify the individual capacity of repair mechanisms. Cultured lymphocytes from patients with different types of cancer present high frequencies of chromatid breaks induced by BLM in G₂ phase of the cell cycle when compared to normal controls (Hsu *et al.*, 1989).

MATERIAL AND METHODS

Peripheral blood samples were drawn from 24 patients with Down syndrome (12 males and 12 females) and 24 healthy controls, matched by age and sex. Since the risk of leukemia development is higher among patients with DS below 10 years (Fong and Brodeur, 1987), all the individuals recruited for this study were between three months and 10 years and five months old.

Two simultaneous short-term cultures (72 h) for each individual were set up in RPMI 1640 media supplemented with 20% fetal bovine serum and phytohemagglutinin (PHA). Five hours prior to harvest, bleomycin (Blenoxane, Bristol Laboratories) was added to a final concentration of 0.03 U/ml in one of the cultures. Two hours prior to harvest, 0.05 ml of colchicine (0.008%) was added. Conventional air-dried preparations were stained with Giemsa without application of any banding procedure.

Fifty cells from each type of culture (without and with BLM) were scored for chromatid-type aberrations, following the protocol of Cherry and Hsu (1983). According to Hsu (1987), chromatid gaps increase the total frequency of lesions, but the overall picture (sensitivity to BLM) was not substantially altered. Therefore, chromatid gaps or attenuated regions were disregarded. The number of chromatid breaks of each sample was converted into the number of chromatid breaks per cell (ctb).

Hsu *et al.* (1985) suggested that a ctb value of 0.80 could be taken as an arbitrary cut-off line to separate the BLM-sensitive from the less sensitive (or resistant) classes. Individuals with a ctb value higher than 1.00 were considered as hypersensitive to BLM (Hsu *et al.*, 1989). This classification was adopted in the present study. Chromatid breaks per cell were scored in a blind test.

Statistical analysis

The Mann-Whitney nonparametric test (Zar, 1984) for two independent samples was used to verify a possible difference between males and females in DS patients and in control subjects, in the frequency of spontaneous and BLM-induced chromatid breaks.

The nonparametric two-way analysis (Zar, 1984) was used to determine the group and treatment effects. The homogeneity test (Gattás, 1978) was used to compare the distribution of BLM-induced chromatid break frequencies in chromosome groups between DS patients and normal controls and to verify if the proximal, median and distal chromosome regions break at similar frequencies in both experimental groups.

The adherence test (Gattás, 1978) was used to verify if the proximal, median and distal chromosome region breaks occurred at similar frequencies or if there was a preferential occurrence of breaks in one of them. The Lilliefors test (Campos, 1983) was used to determine if the chromatid break frequencies per cell had a normal distribution. Regression analyses were used to verify if the distribution of BLM-induced breaks is related to the relative length of each chromosomal group, using the chromosome length measurement established by the Paris Conference (1972).

RESULTS

Table I summarizes the age, sex and the chromatid breaks per cell (ctb) values for spontaneous and BLM-induced chromatid breakage from 24 patients with DS and 24 normal controls. All the patients with DS showed free trisomy 21.

Table I - Sex, age and mean frequency of spontaneous and bleomycin-induced (BLM) chromatid breaks per cell (ctb) for Down syndrome (DS) patients and normal controls. (F = Female; M = male; Y = years; Mo = months).

Patient with DS	Sex	Age	ctb		Control	Sex	Age	ctb	
			Without BLM	With BLM				Without BLM	With BLM
1	F	4Mo	0.00	0.36	1	F	1Mo	0.00	0.36
2	F	1Y 2Mo	0.04	0.44	2	F	1Y 2Mo	0.00	0.22
3	F	3Mo	0.00	0.28	3	F	1Mo	0.00	0.18
4	F	5Mo	0.02	0.70	4	F	1Y 4Mo	0.00	0.20
5	F	1Y 6Mo	0.00	0.68	5	F	1Y 11Mo	0.02	0.62
6	F	2Y 2Mo	0.00	0.68	6	F	3Y 8Mo	0.02	0.20
7	F	2Y 5Mo	0.00	0.64	7	F	4Y 1Mo	0.00	0.26
8	F	6Y 4Mo	0.00	0.20	8	F	7Y 11Mo	0.00	0.62
9	F	10Y 2Mo	0.06	0.42	9	F	10Y 3Mo	0.00	0.46
10	F	3Mo	0.02	0.48	10	F	1Y	0.00	0.34
11	F	10Y 1Mo	0.00	0.22	11	F	8Y 6Mo	0.04	0.22
12	F	3Y	0.04	0.30	12	F	3Y 8Mo	0.00	0.42
13	M	3YA	0.00	0.44	13	M	1Y 9Mo	0.00	0.12
14	M	8Y 7Mo	0.00	0.24	14	M	7Y 8Mo	0.00	0.58
15	M	2Y 7Mo	0.00	0.52	15	M	1Y 6Mo	0.02	0.28
16	M	8Y 9Mo	0.00	0.26	16	M	6Y 10Mo	0.04	0.52
17	M	10Y 5Mo	0.02	0.94	17	M	10Y 7Mo	0.00	0.36
18	M	6Y 7Mo	0.00	0.36	18	M	6Y 8Mo	0.02	0.28
19	M	5Y 8Mo	0.00	0.38	19	M	3Y 9Mo	0.00	0.54
20	M	10Y	0.00	0.28	20	M	10Y 2Mo	0.00	0.50
21	M	7Mo	0.00	0.22	21	M	5Mo	0.02	0.20
22	M	9Y	0.00	0.18	22	M	7Y 10Mo	0.00	0.18
23	M	9Y	0.00	0.28	23	M	8Y 9Mo	0.00	0.34
24	M	3Mo	0.00	0.14	24	M	7Mo	0.00	0.34

Spontaneous ctb values ranged from 0.00 to 0.06 in patients with DS and from 0.00 to 0.04 in the control group. No control child showed BLM-induced ctb values higher than 0.80, and only one patient with DS was sensitive to the drug.

Using the Mann-Whitney nonparametric statistic, no significant difference was found between males and females, for cultures without and with BLM in patients with DS and control subjects (DS patients, cultures without BLM: $U = 46.5$, $P > 0.10$; DS patients, cultures with BLM: $U = 46.5$, $P > 0.10$; control individuals, cultures without BLM: $U = 66.5$, $P > 0.10$; control individuals, cultures with BLM: $U = 69$, $P > 0.10$). The treatment effect was similar in patients and controls ($H = 0.150$, $P > 0.50$) and also, no group effect (in the whole group without and with BLM) was found ($H = 0.084$, $P > 0.50$). The number of BLM-induced breaks was significantly higher than the number of spontaneous breaks ($H = 74.969$, $P < 0.001$) in patients and controls.

No significant difference was found in the distribution of BLM-induced breaks in chromosome

groups between males and females for patients with DS ($\chi^2 = 5.37$, $P > 0.10$) or controls ($\chi^2 = 2.46$, $P > 0.10$) (Table II).

Induced and expected break frequencies in each chromosome group, estimated on the basis of chromosome length (Paris Conference, 1972), for patients with DS and controls, are shown in Table III. In both populations the distribution of induced breaks was related to the lengths of each chromosomal group. Regression analysis of the number of breaks and chromosome lengths for the two groups are shown in Figures 1 and 2. Among males, both populations had a positive regression coefficient: DS = 0.979 and control = 0.927 and the same was found among females: DS = 0.920 and control = 0.946.

There was no difference in the distribution of induced breaks at proximal, median and distal chromosome regions for male DS patients and controls ($\chi^2 = 1.63$, $P > 0.10$). However, for females, there was a significant difference between DS patients and controls ($\chi^2 = 32.71$, $P < 0.01$), with a higher number of breaks in proximal and distal regions for controls and in the

Table II - Observed and expected bleomycin-induced chromatid break frequencies for chromosomes 1, 2, 3, B, C, D, E, F, and G for male (M) and female (F) patients with Down syndrome (DS) and controls.

Group	Chromosome or chromosome group									Total
	1	2	3	B	C	D	E	F	G	
DS (M)										
Observed	20	24	9	25	74	19	5	2	1	179
Expected	24.03	21.09	9.32	29.92	67.68	15.69	7.85	2.45	0.98	
Control (M)										
Observed	29	19	10	36	64	13	11	3	1	186
Expected	24.97	21.91	9.68	31.08	70.32	16.31	8.15	2.55	1.02	
Total	49	43	19	61	138	32	16	5	2	365
DS (F)										
Observed	33	22	13	41	78	27	5	1	1	221
Expected	33.77	22.51	12.44	39.70	79.99	23.70	7.11	1.18	0.59	
Control (F)										
Observed	24	16	8	26	57	13	7	1	0	152
Expected	23.23	15.49	8.56	27.30	55.01	16.30	4.89	0.82	0.41	
Total	57	38	21	67	135	40	12	2	1	373

Table III - Observed and expected bleomycin-induced chromatid break frequencies for chromosome groups estimated on the basis of chromosome length of male (M) and female (F) patients with Down syndrome (DS) and controls.

Frequency	Chromosome or chromosome group									Total
	1	2	3	B	C	D	E	F	G	
DS (M)										
Observed	20	24	9	25	74	19	5	2	1	179
Expected	14.45	13.73	11.69	21.19	64.03	18.42	16.33	8.95	10.21	179
Relative length to the lot 2n*	16.88	16.04	13.66	24.76	74.82	21.52	19.08	10.46	11.93	209.15
DS (F)										
Observed	33	22	13	41	78	27	5	1	1	221
Expected	17.59	16.71	14.23	25.80	83.29	22.42	19.88	10.89	10.19	221
Relative length to the lot 2n*	16.88	16.04	13.66	24.76	79.94	21.52	19.08	10.46	9.78	212.12
Control (M)										
Observed	29	19	10	36	64	13	11	3	1	186
Expected	15.15	14.40	12.26	22.22	67.15	19.31	17.12	9.39	9.00	186
Relative length to the lot 2n*	16.88	16.04	13.66	24.76	74.82	21.52	19.08	10.46	10.03	207.25
Control (F)										
Observed	24	16	8	26	57	13	7	1	0	152
Expected	15.15	14.40	12.26	22.22	67.15	19.31	17.12	9.39	9.00	152
Relative length to the lot 2n*	16.88	16.04	13.66	24.76	79.94	21.52	19.08	10.46	7.88	210.22

*Based on Paris Conference (1972).

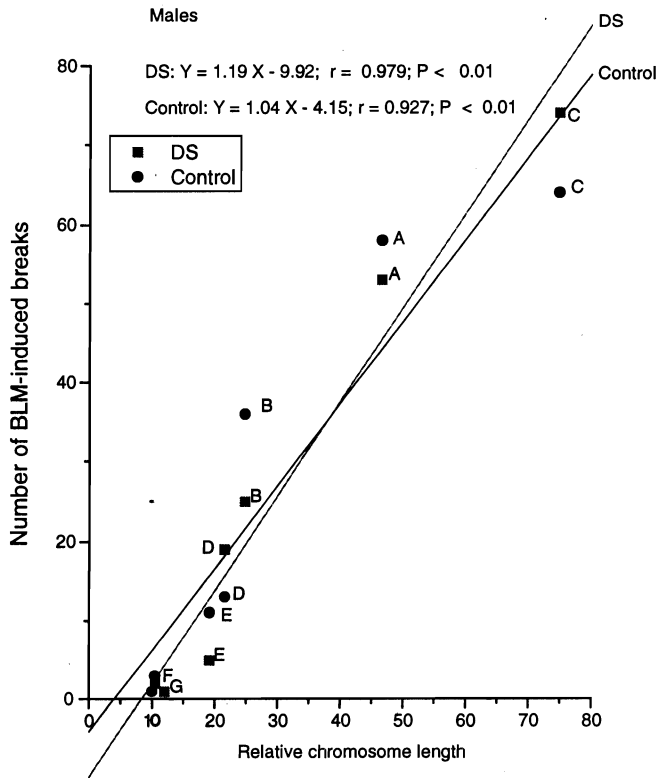


Figure 1 - Sample regression of bleomycin-induced breaks on relative lengths of chromosomal group in the male patients with Down syndrome (DS) and controls. A, B, C, D, E, F, G, Chromosome group.

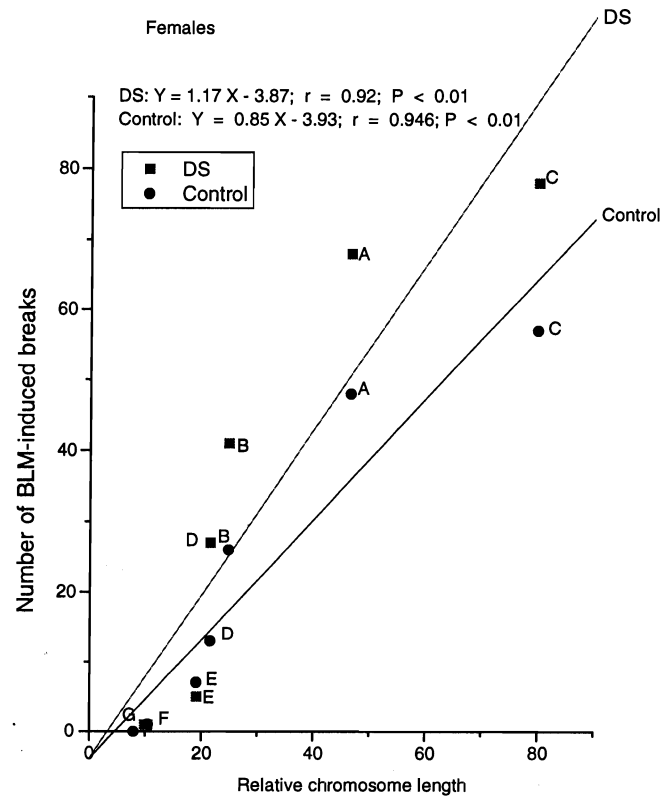


Figure 2 - Sample regression of bleomycin-induced breaks on relative lengths of chromosomal group in the female patients with Down syndrome (DS) and controls. A, B, C, D, E, F, G, Chromosome group.

median region for DS patients. In all cases, the median chromosome region presented the highest break frequencies (Table IV).

The Lilliefors test showed that the frequencies of BLM-induced chromatid breaks per cell are distributed in a normal curve for male DS patients and controls ($P > 0.10$).

Table IV - Observed (O) and expected (E) bleomycin-induced break frequencies in proximal (p), median (m) and distal (d) chromosome regions for male (M) and female (F) patients with Down syndrome (DS) and controls.

Group	Chromosome region			Total
	p O (E)	m O (E)	d O (E)	
DS (M)	24 (28.44)	89 (86.80)	66 (63.75)	179
Control (M)	34 (29.56)	88 (90.20)	64 (66.25)	186
Total	58	177	130	365
DS (F)	12 (25.48)	152 (126.79)	57 (68.73)	221
Control (F)	31 (17.52)	62 (87.21)	59 (47.27)	152
Total	43	214	116	373

DISCUSSION

In the present study, the frequencies of spontaneous chromosome breaks found in DS and in controls did not differ significantly and they were in agreement with the expected frequencies for the normal population (0.00 to 0.07), according to Hopwood *et al.* (1989). Other studies also relate absence of spontaneous fragility in DS (Preston, 1981; Vijayalaxmi and Evans, 1982).

No significant difference was found in the BLM-induced break frequencies between males and females for patients with DS and controls. These results are in agreement with those of Pelz *et al.* (1988), who found no sex difference between methotrexate sensitivity for DS vs. controls. However, Shubber *et al.* (1991) observed an increased frequency of sister chromatid exchanges (SCE) in male in relation to female DS children.

Among the 24 patients with DS, only one male presented sensitivity to BLM. No control presented ctb values higher than 0.80. No significant difference was found between the two groups.

Some individuals presented very low responses to BLM (DS 8, DS 22, DS 24, controls 3, 4, 6, 13, 21 and 22). In human population, resistant to this

drug have been found (Hsu *et al.*, 1989). Studies with individuals with a long smoking history but no symptoms of cancer indicate that this group belongs to this population, because they presented very low responses to BLM (Hsu *et al.*, 1989).

Hsu *et al.* (1989) analyzed 335 normal subjects and they found 22.69% to be BLM-sensitive. In the present study, if we consider only patients with DS, the percentage of BLM-sensitive individuals was 4.2%. Taking into account all the 48 individuals analyzed, this percentage is 2.1%. The difference between these results could be related to the small number of individuals in the present work or to genetic differences in the population studied.

Leonard and Merz (1987) suggested that trisomy of chromosome 21 does not necessarily confer increased chromosomal radiosensitivity, because several studies that associated chromosomal radiosensitivity with DS were realized with cultured lymphocytes irradiated in the G₀ or G₁ phases of the cell cycle and so, these data should not be generalized to other tissues or agents, nor even to lymphocytes in other phases of the cell cycle.

Studies of chromosomal sensitivity to BLM in lymphocytes of patients with DS incubated with the drug for 72 h (Vijayalaxmi and Evans, 1982) or treated in G₀ (Iijima *et al.*, 1984; Morimoto *et al.*, 1984) showed significant differences between DS and normal cells. Possibly, the disagreement between those studies and the present results is related to the phase of the cell cycle in which the cells were treated.

Our results are in agreement with those of Dekaban *et al.* (1966) and Leonard and Merz (1983), who did not observe chromosomal radiosensitivity in the G₂ phase of the cell cycle in trisomy 21 lymphocytes. Different proliferation kinetics may explain this. The DS lymphocytes start the cell cycle before normal ones after PHA stimulation, and the result is loss of the initial period of minor sensitivity observed in normal lymphocytes, and for this reason, the G₂ phase would not be affected (Leonard and Merz, 1983).

Pelz *et al.* (1988) and Shubber *et al.* (1991) found higher chromosomal sensitivity to several agents in lymphocytes of patients with DS and of their parents, when compared to lymphocytes of normal controls and their parents, which suggested that this sensitivity is genetically inherited. There is evidence that genetic factors are involved in chromosomal sensitivity to BLM (Hopwood *et al.*, 1989; Bartholomei-Santos and Lucca, 1995) and the type of inheritance may be autosomal dominant (Sales, 1991).

The distribution of BLM-induced breaks in each chromosome group was similar for DS patients

and controls, for both males and females. Similar results were found by Voreschovsky and Juraskova (1990) for BLM-induced breaks in lymphocytes of patients with tuberous sclerosis, and normal controls, and also by Yanagisawa (1978) for SCE in male DS cells and male controls with mental retardation.

The distribution of BLM-induced breaks in proximal, median and distal chromosomal regions was similar in male patients with DS and controls, but it was discordant in females. Yanagisawa (1978) found similar results in male patients with DS and controls, in relation to the distribution of SCE, however females were not studied.

The mean frequencies of BLM-induced breaks per cell presented a normal distribution in both the DS and control group. Cherry and Hsu (1983) also observed a normal distribution of BLM-induced breaks in lymphocytes of medullary thyroid carcinoma patients and their family members.

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RESUMO

Inúmeros trabalhos têm demonstrado que linfócitos de pacientes com síndrome de Down apresentam uma maior frequência de aberrações cromossômicas quando expostos a radiação ionizante ou agentes químicos nas fases G₀ ou G₁ do ciclo celular, mas não em G₂, quando comparados com controles normais.

Para determinar a sensibilidade de linfócitos de pacientes com síndrome de Down, na fase G₂, usou-se o radiomimético bleomicina em culturas de linfócitos de 24 pacientes.

Todos os pacientes mostraram trissomia livre do cromossomo 21 (47,XX + 21 ou 47,XY + 21). Indivíduos que apresentaram frequência média de quebras cromatídicas por célula superior a 0,8 foram considerados sensíveis à droga. Nenhum controle apresentou suscetibilidade à bleomicina e entre os 24 pacientes com síndrome de Down somente um foi sensível à droga.

Não se observou qualquer diferença significativa entre os dois grupos em relação às frequências de quebras cromatídicas em linfócitos em G₂, o que está de acordo com outros trabalhos. A distribuição das quebras induzidas pela bleomicina, em cada grupo cromossômico, foi igual para pacientes e controles. Nenhuma diferença significativa foi observada na resposta à bleomicina entre homens e mulheres,

nos dois grupos. Provavelmente, o principal fator envolvido na sensibilidade cromossômica de linfócitos de pacientes com síndrome de Down seja a fase do ciclo celular na qual a célula é tratada.

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