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Chemosensitivity of Hybridoma Cell Lines to Actinomycin D and Bleomycin Sulfate Compared to Non-hybridoma Cell Lines

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Abstract

Cytogenetic effects of two clastogenic antitumor agents, actinomycin D and bleomycin sulfate were assessed in G2 phase of cell cycle in two heterohybridoma cells, and two non-hybrid cell lines. Since heterohybridoma cells carry chromosome constitutions from different species, their responses to various environmental stimuli may vary and depend on their chromosomal origins. Two hybridoma cell lines, HF2x653 and F3B6, and two nonhybridoma cell lines, WIL2 and NS-1, as their parents were treated with bleomycin sulfate and actinomycin D in G2 phase of the cell cycle. Metaphase spreads were prepared and various types of chromatid aberrations were scored. The frequencies of chromatid aberrations of hybrid lines in both treatments were between the frequencies of WIL2 and NS-1. HF2 showed same chromosome aberration frequency with WIL2 and F3B6 with NS-1. The results indicated that chemosensitivity of hybridoma cell lines investigated in this study was nearly similar to their parental cell lines.

Keywords: Actinomycin D, Bleomycin Sulfate, Hybrid cell, Chromosome aberration

1. Introduction

Exposure to genotoxic compounds can induce mutations. Bleomycin-sulfate (BLM-S) and actinomycin D (Act-D) are two popular examples of mutagens that also have anticancer activity. BLM-S, an antitumor and antibiotic drug obtained from Streptomyces verticillus, is a radiomimetic agent and, in the presence of iron [Fe (II)] and O2, catalyzes single and double stranded cleavage of DNA [1]. BLM-S does not interfere directly with the DNA replication but acts as an S-independent fashion [2] and depending on the phase of the cell cycle, it produces chromatid-type (in G2 phase) or chromosome-type (in G0 and G1 phase) aberrations.

The actinomycins are a class of polypeptide antibiotics produced by the genus Streptomyces. The most significant member of actinomycins is actinomycin D which is primarily used as an investigative tool in cell biology to inhibit transcription. Act-D does this by making a complex with DNA at the transcription initiation site and thus prevents elongation by RNA polymerase [3]. This occurs largely through its guanine binding capacity [4] which has been found to induce chromosome breakage by Ostertag and Kersten in 1965 [5]

As mentioned previously, BLM-S and Act-D cause damage to the genetic material, but since mitotically dividing cells as cancer cells have a high metabolism rate, they show more DNA damage than non-dividing cells in response to these clastogenic agents which lead to the application of cancer therapy for BLM-S and Act-M.

The aim of the present study was to evaluate the induction of chromatid aberrations (CA) in two types of mouse- human hybridoma cells (i.e. HF2x653 and F3B6) by BLM-S and Act-D and to compare the frequency of various CAs with those induced in their parents, a human B-lymphocyte (WIL2.NS.6TG) and a myeloma mouse cell (NS-1) lines. Hybridoma cells are obtained from the fusion of two somatic cells with a similar or different origin which mostly have dissimilar chromosomal content in comparison to their parents. Therefore hybridoma cells have basically different nucleic acid content and consequently disparate cellular properties. This difference might be expressed in the amount of chromosome aberrations which are examined in this study. Various studies on the cytogenetic effects of BLM-S and Act-D in different cell systems [6-15] have already been conducted before but to the best

of our knowledge this is the first cytogenetic study of BLM-S and Act-D on the hybridoma cells

2. Methods

Mutagens

Bleomycin sulfate (Nippon Kayaku Co) is commercially available as sterile lyophilized powder which has been diluted with normal saline and used at concentrations of 20 and 40 µg/ml in this study. Actinomycin D (Merck, Lyovac Cosmogenen) which is composed of 0.5 mg dactinomycin and 20mg mannitol was also diluted by normal saline and employed at concentrations of 0.1 and 1 µg/ml.

Cells

Four cell lines were used, two of which were heterohybridoma cell lines including HF2x653 and F3B6 produced from the fusion of mouse myeloma cells and human normal lymphocytes while the other two cell lines were non-hybrid cells. A human B-lymphocyte line, WIL2.NS.6TG, as human parent, and a myeloma mouse cell line, NS-1, as mouse parent were two non-hybrid cell lines. All the cells were provided by National Cell Bank of Iran, Pasture Institute, (Tehran, Iran).

Cell cultures and treatments

After defreezing, the cells were grown in RPMI 1640 medium (Sigma) supplemented with 10% inactivated fetal calf serum (Gibco BRL), 2mM of L-glutamine, 100 U/ml of penicillin (Sigma) and 100 µg/ml streptomycin (Sigma), and cultured at 37oC in a humidified atmosphere with 5% CO2. During the exponential growth period (about 106 cells/ml), the cultures were treated with the mentioned concentrations of Act-D and BLM-S for 3 hours separately. Colchicine was added at 4 µg/ml concentration during the last 1.5 h in all the cultures to arrest the cells in metaphase. Metaphase preparation was done according to the standard protocol (0.075 M KCl hypotonic shock, methanol:acetic acid 3:1 (Merck) fixation and air dried method for slide preparation). Finally, the chromosomes were stained in 10% Giemsa stain (Merck).

Cytogenetic analysis

Chromatid aberrations were analyzed in the first cycle metaphase after treatments. Only well spread metaphases were examined per treatment to determine and score the frequencies of chromatid aberrations. One hundred well spread metaphases were scored for each sample. The chromatid aberrations recorded were chromatid gaps, chromatid breaks (including acentric fragments, minutes and isochromatid breaks) and chromatid exchanges (including multiradials, di- or multicentrics and rings). We have followed according to the Hsu (1987) [16] criteria and disregard gaps or attenuated regions. Thus the sum of chromatid breaks and exchanges as ″total chromatid aberration″ was used for statistical analysis.

Statistical analysis

Statistical comparisons were performed with the Kruskal-Wallis nonparametric and ANOVA with Duncan post hoc tests. The P value significance was set at 0.05. The data were analyzed by SPSS version 13.0 (SPSS Inc., Chicago, IL, USA)..

3. Results and Discussion

Table 1 and Figure 1 show the frequency of various chromatid aberrations in the control groups and groups treated with BLM-S and Act-D at different concentrations.

Since the studied cells in this research have different numbers of chromosomes, the analysis was done on the basis of aberration per chromosome which has been summarized in Table 2 for all the treated cell lines.

The results showed that in all cell treatments, both agents in both concentrations induce a significant increase of total chromatid aberrations compared to the control group ($P<0.05$). Additionally, treatments with higher doses (i.e. 40 µg/ml and 1 µg/ml for BLM-S and Act-D, respectively) in both agents differ significantly with the lower ones (i.e. 20 µg/ml and 0.1 µg/ml for BLM-S and Act-D, respectively) ($P<0.05$).

Results of pairwise Duncan post hoc test between the studied cells for both Act-D (Upper diagonal) and BLM-S (lower diagonal) treatments are also presented in Table 3 in a matrix format. Except for 1 µg/ml concentration of Act-D in which hybrid cells have not shown any significant difference in CA frequency with non-hybrid lines ($P>0.05$) (Table 3, right, upper diagonal), in the rest of the applied concentrations, the responses of hybridoma cell lines differ significantly in comparison to non-hybrid lines. In the control group, HF2x653 hybrid cells do not show any significant difference with non-hybrid cell lines (NS1 and WIL2) while F3B6 cells differ significantly with WIL2 but not with NS-1 (Table 3, left). The same pattern is observed for 20 µg/ml dose of BLM-S (Table 3, middle, lower diagonal) but for higher concentrations of BLM-S (40 µg/ml), HF2x653 and F3B6 hybrid cell lines are located with WIL2 and NS-1 non-hybrid cells in the same category respectively (Table 3, right, lower diagonal). In the case of Act-D, HF2x653 cell line has no difference with non-hybrid cells in both concentrations. On the other hand, F3B6 shows a significant difference with only WIL2 cell line in both applied doses (Table 3, middle and right upper diagonal).

Table 1. Frequency of various types of chromatid aberrations and percentage of damaged cells in the control group and cells treated with BLM-S and Act-D.

Drug	Cell	Concentration (µg/ml)	No. of Counted Cells	Chromatid Aberration ± S. E				
				Gap	Break	Exchange	Total Aberration	% of damaged cells
BLM-S	WIL2	Control	100	6±2.82	7±1.4	0	7±1.4	6
		20	100	7±1.4	22±5.64	0	22±5.64	22
		40	100	17±1.4	106±9.88	11±1.4	117±11.28	64
	HF2x653	Control	100	3±1.4	22±2.82	3±1.4	25±4.22	18
		20	100	18±2.82	62±8.48	3±1.4	65±9.88	42
		40	100	16±2.82	121±18.38	7±1.4	128±19.78	62
	NS-1	Control	100	9±1.4	51±9.8	3±1.4	54±11.2	30
		20	100	32±5.64	111±5.54	7±1.4	118±6.94	58
		40	100	41±4.24	224±31.10	8±2.82	232±33.92	76
	F3B6	Control	100	14±2.82	65±7.06	5±1.4	70±8.46	36
		20	100	45±4.24	132±16.96	4±2.82	136±19.78	64
		40	100	62±5.64	211±18.38	9±1.4	220±19.78	76
Act-D	WIL2	Control	100	6±2.82	7±1.4	0	7±1.4	6
		0.1	100	3±1.4	78±11.3	23±4.24	101±15.54	38
		1	100	19±1.4	210±11.3	19±4.24	229±15.54	70
	HF2x653	Control	100	3±1.4	22±2.82	3±1.4	25±4.22	18
		0.1	100	5±1.4	100±11.3	3±1.4	103±12.71	58
		1	100	29±5.64	220±15.54	5±1.4	225±16.94	80
	NS-1	Control	100	9±1.4	51±9.8	3±1.4	54±11.2	30
		0.1	100	21±1.4	180±9.72	6±2.82	186±15.54	70
		1	100	56±5.64	344±14.14	3±1.4	347±15.54	100
	F3B6	Control	100	14±2.82	65±7.06	5±1.4	70±8.46	36
		0.1	100	8±2.82	161±15.54	5±1.4	166±16.94	58
		1	100	41±2.24	311±15.54	9±1.4	320±16.94	80

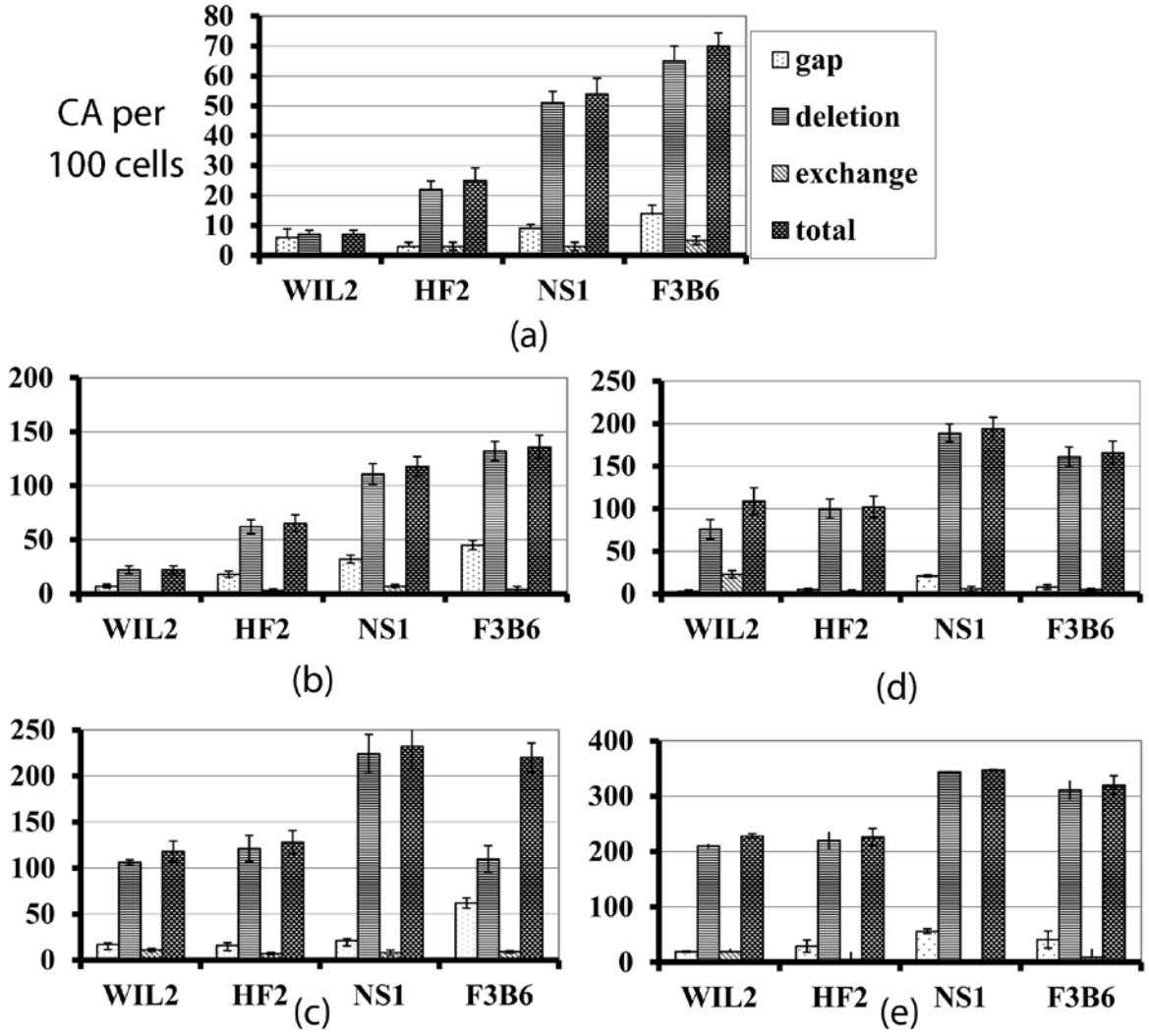

Figure 1. Column chart based on frequencies of various types of chromatid aberrations in different cell lines for the control group (a), BLM-S treatment at 20 µg/ml concentration (b), BLM-S treatment at 40 µg/ml concentration (c), Act-D treatment at 0.1 µg/ml concentration (d) and Act-D treatment at 1 µg/ml concentration (e).

Hybridoma cells are produced from the fusion of two or more somatic cells which may originate from the same or different species. The whole number of hybrid cell's chromosomes is not usually equal to the total number of parental chromosomes. After the fusion and in the early stages of cell growth all all parental chromosomes are present but in a few successive divisions some of them will be lost. When hybridoma cells are interspecies, chromosome loss occurs more frequently in the chromosomes of one of the parent cells that would cause the hybrid cell to be more similar to the other parent line [17]. Thus, nuclear environment of the hybridoma cells changes with chromosome loss which would probably alter the hybridoma cell specificities in comparison to the parental cells. The way of response to various types of chemotherapy drugs could be among these alterations. The present study was primarily performed to compare the clastogenic effects of BLM-S and Act-D on hybrid and non-hybrid cell lines.

BLM-S shows remarkable therapeutic efficacy for squamous cell carcinoma, malignant lymphoma and testicular carcinoma which with a radiomimetic mechanism exerts single and double strand breaks on DNA [2, 18-20]. Various cell lines show differential sensitivity to BLM-S. However, it is not known if this difference is truly intrinsic or is caused by differential permeability of the cell membranes. BLM-S is a large hydrophobic compound and most likely unable to diffuse across cell membranes [20]. Although there is no consensus mechanism for BLM-S cell uptake, it has been proposed that BLM-S transferring across plasma membranes of

Chinese hamster fibroblast and human head and neck carcinoma cell lines is mediating by binding to a receptor protein which leads it to the receptor mediated endocytosis pathway [21]. BLM-S resistance has been observed in those cells which had reduced BLM-S-membrane binding sites [22]. Moreover, lymphocytes with different BLM-S uptake rate show different responses to BLM-S treatment. Additionally, chromosome repair mechanisms have a major effect on the net chromatid aberration amount after drug treatment. Different and sometimes contradictory observations about the repairing ability of various cells have been reported. DNA unwinding method in different cell types have revealed that most of the DNA breakage by bleomycin is repaired within 5 min of discontinuation of the treatment when slight repair of DNA nicks occurs if the bleomycin remains in the medium [23, 24]. Pulsed field electrophoresis also showed a 50% rejoining of double strand breaks in Chinese hamster ovary wild type cells during 30 min after 1-h pulse bleomycin treatment [25] but G2 premature chromosome condensation technique did not measure the significant repair until 1-h after BLM-S removal [26].

Table 2. Total chromatid aberrations frequency per chromosome in the control and treated samples.

Drug	Cell line	No. of counted Chr.	Total chromatid aberration per chromosome		
			Control	1st treatment*	2nd treatment†
BLM-S	WIL2	4300	0.001	0.005	0.027
	HF2	4500	0.005	0.014	0.028
	NS-1	5200	0.01	0.022	0.044
	F3B6	5000	0.014	0.027	0.044
Act-D	WIL2	4300	0.001	0.025	0.05
	HF2	4500	0.005	0.022	0.05
	NS-1	5200	0.01	0.037	0.066
	F3B6	5000	0.014	0.033	0.064

*: 1st treatment doses for Act-D & BLM-S were 0.1 and 20 µgr/ml respectively
†: 2nd treatment doses for Act-D & BLM-S were 1 and 40 µgr/ml respectively

Table 3. Results of pairwise Duncan post hoc test between various studied cells based on aberration per chromosome in the control (left), 1st treatment (middle) and 2nd treatment (right) groups for Act-D (Upper diagonal) and BLM-S (lower diagonal).

	Control				1st treatment*				2nd treatment†			
	WIL2	HF2	NS-1	F3B6	WIL2	HF2	NS-1	F3B6	WIL2	HF2	NS-1	F3B6
WIL2	-	ns*	P<0.05	P<0.05	-	ns	P<0.05	P<0.05	-	ns	P<0.05	ns
HF2	ns	-	ns	P<0.05	ns	-	ns	ns	ns	-	ns	ns
NS-1	P<0.05	ns	-	ns	P<0.05	ns	-	ns	P<0.05	P<0.05	-	ns
F3B6	P<0.05	P<0.05	ns	-	P<0.05	P<0.05	ns	-	P<0.05	P<0.05	ns	-

*: 1st treatment doses for Act-D & BLM-S were 0.1 and 20 µgr/ml respectively
†: 2nd treatment doses for Act-D & BLM-S were 1 and 40 µgr/ml respectively
ns= non significant

Although 20 µg/ml treatment of BLM-S induced significant CA per chromosome in the treated cells compared to the control group, pairwise Duncan post hoc test matrix (Table 3) indicated that all the cell types were similar to the control group for 20 µg/ml dose of BLM-S treatment. On the other hand, for higher doses of BLM-S (40 µg/ml), hybrid cells had a completely different pattern of pairwise Duncan post hoc test. In other words, at 40 µg/ml of BLM-S treatment, HF2x653 and F3B6 hybrid lines responded exactly as their corresponding parents which were WIL2 and NS-1 respectively. Since repair ability or plasma membrane permeability of a hybrid cell as the other specificities of these cells are similar with their parent lines, this pattern of response is not unexpected. As mentioned previously, both concentrations of BLM-S treatment resulted in significant differences in the all studied cells, but it seems that higher doses of BLM-S are necessary to observe consistent reaction of hybrid lines as their corresponding parents. However this consistency somehow could be observable both in the control groups and lower dose treatments of BLM-S.

Act-D, marketed under the trade name Dactinomycin is one of the older chemotherapy drugs that has been successfully used in many cancer therapies such as gestational trophoblastic neoplasia, Wilms' tumor, rhabdomyosarcoma and Ewing's sarcoma [27-30]. Differential sensitivity of the cell lines also has been observed for actinomycin [31]. This differential sensitivity was also observed in our study relatively in the same way that had occurred for bleomycin treatment. In other words, cells responses are almost explainable by dividing them into two separate categories including HF2x653 and its corresponding parent line WIL2 versus F3B6, and its corresponding parent line NS-1. NS-1 is a myeloma cell line and has various types of genomic mutations and chromosomal damages including multiple translocations which could directly or indirectly involve those genes participating in cellular repair or drug resistance. Malfunction of these genes will ultimately increase the affected cell's sensitivity to clastogens. As can be seen in Table 3, in all the treatments and even in the control cell lines, NS-1 cells always show a significant difference in aberration per chromosome frequency compared to the normal lymphocyte line, WIL2. This is more evident in higher concentrations of Act-D treatment (Table 3, right, upper diagonal) in which the only two differing cells are NS-1 and WIL2. Conversely, heterohybridoma lines responses usually are similar to one of their parents. In fact, careful assessments of metaphasic spreads of the studied cells confirmed that HF2x653 and F3B6 hybridoma cells have similar genomic backgrounds as WIL2 and NS-1 respectively whether in the chromosome content or type.

4. Conclusion

Briefly, the studied cell lines in this study have shown a variety of different responses to the applied treatments. WIL2 and NS-1 non-hybrid cells are situated in two opposite extremes while the hybridoma cells were seen in moderate positions of the mentioned spectrum but with a significant inclination to their corresponding parent lines.

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