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Effects of sodium butyrate on X-Ray and bleomycin-induced chromosome aberrations in human peripheral blood lymphocytes

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Summary

Peripheral blood lymphocytes from normal human volunteers or from Down syndrome patients were pre-treated with sodium butyrate (a compound which is known to induce structural modifications in the chromatin through hyperacetylation of nucleosomal core histones) and exposed to X-irradiation or treated with bleomycin in vitro in the G_0 and/or G_1 stage(s) of the cell cycle. The frequencies of chromosomal aberrations in the first mitosis after treatment were scored.

The results show an enhancement in the yield of aberrations in the butyrate pre-treated groups. However, the absolute frequencies of chromosomal aberrations as well as the relative increases with butyrate pre-treatment varied between blood samples from different donors suggesting the existence of inter-individual variations. There is a parallelism between the effects of X-irradiation or of combined treatments in G_0 and G_1 stages and between effects observed in the X-ray and bleomycin series. The increase in the yields of chromosomal aberrations in butyrate-treated and X-irradiated lymphocytes (relative to those which received X-irradiation alone) is interpreted as a consequence of the inhibition of repair of DNA damage by butyrate.

1. Introduction

Treatment of mammalian cells with sodium butyrate (hereafter to be referred to as butyrate) has been known to induce structural modifications in chromatin through hyperacetylation of nucleosomal core histones (Riggs *et al.* 1977; D'Anna *et al.* 1980; Bode *et al.* 1983; Pani *et al.* 1984). Smerdon *et al.* (1982) and Ramanathan & Smerdon (1989) showed that butyrate-treated human fibroblasts exhibited a marked increase in DNA-repair synthesis at early times after UV-irradiation and that this increase was primarily associated with nucleosome core regions in hyper-acetylated chromatin.

In our earlier study (Sankaranarayanan *et al.* 1985), we found that butyrate pre-treatment of unstimulated (G_0) human peripheral blood lymphocytes prior to X-irradiation (0·25–2 Gy) led to an increase in the frequencies of chromosomal aberrations as compared to cells that received only X-irradiation; however, the numbers of X-ray-induced DNA-strand breaks and the extent of repair (as measured by the nucleoid sedimentation technique) were the same in butyrate-treated and untreated cells at the dose level of 10 Gy used in these experiments. The enhancement in the frequencies of radiation-induced chromosomal aberrations was therefore tentatively interpreted as a consequence of butyrate-induced conformational change in the chromatin of G_0 lymphocytes; however, the question of whether at biologically meaningful doses, such as those used in cytogenetic experiments, alteration in DNA repair capacity might also play a role, could not be answered.

In this paper, we present the results of three sets of experiments. The first of these was designed to inquire whether X-irradiation of butyrate pre-treated lymphocytes at the G_1 stage of the cell cycle would elicit a response similar to that observed with G_0 lymphocytes. The rationale for this derives from the findings that (i) chromatin modifications normally occur when cells pass from the G_0 to G_1 stage of the cell cycle; (ii) G_1 lymphocytes have generally been found to be more sensitive than G_0 cells to the induction of chromosomal aberrations (e.g. Wolff, 1972; Beek & Obe, 1977) and (iii) actual measurements of histone acetylation in different stages of the cell cycle in CHO cells (D'Anna *et al.* 1977; Gurley *et al.* 1978) have shown that, normally, there is a substantial amount of histone acetylation in G_1.

The second set of experiments was aimed at

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examining whether the response of butyrate pre-treated G_0 lymphocytes to the induction of chromosomal aberrations by bleomycin, a radiomimetic compound, would be similar to that after X-irradiation. The third set of experiments was conducted to explore whether one can discriminate the role of butyrate-mediated chromatin modification from that of possible enhancement of DNA (mis)repair in butyrate-treated cells, through the use of G_0 lymphocytes from Down syndrome patients. As is well-known (e.g. Sasaki & Tonomura, 1969; Evans, 1972), G_0 lymphocytes from Down syndrome individuals can be more sensitive (by a factor of about 1·5–2) to the induction of dicentric and ring aberrations by X-rays than are lymphocytes from normal individuals, and this has been interpreted as due to more rapid (mis)-repair of DNA damage leading to chromosomal aberrations (Preston, 1981).

2. Material and Methods

Blood from healthy donors obtained from the Blood Bank of the Academic Hospital, Leiden or from volunteers in our laboratory, was used to set up whole blood cultures. For experiments using blood from Down syndrome patients, samples were obtained through the courtesy of Drs Timar Laszlo and A. Czeizel of the National Institute of Hygiene, Budapest, Hungary.

(*a*) *Comparison of chromosomal radiosensitivities of G_0 and G_1 lymphocytes*

Before embarking on experiments on the effects of butyrate, it was considered useful first to establish whether G_1 cells are more sensitive than G_0 cells to the X-ray induction of chromosomal aberrations. Therefore, in the first experiments, whole blood cultures were established each in 5 ml of PHA-containing Ham's F10 medium supplemented with 15% foetal calf serum. In the G_0 series, they were X-irradiated immediately and allowed to progress through the cell cycle; in the G_1 series, at about 14–15 h after PHA stimulation (this timing was based on the study of Beek & Obe, 1977), the cultures were irradiated and allowed to continue their progression through the cell cycle. Appropriate concurrent controls were run. Colcemid was added for the last 2 h in culture and all fixations were done at 50 h after PHA stimulation. Fixation, slide preparations and staining (Giemsa) were according to standard methods. The slides were scored for dicentrics, rings, and acentric fragments as well as for chromatid-type aberrations (chromatid-breaks and exchanges). Chromatid and isochromatid gaps were also scored, but were not included in the calculations since these showed no systematic differences between the different treatment groups.

(*b*) *The effects of butyrate pre-treatment on the frequencies of X-ray-induced chromosomal aberrations in G_0 and G_1 lymphocytes*

In the G_0 experiments, cultures were first set up in medium without PHA, treated with butyrate (5 mM final conc.) for 24 h after which they were irradiated, washed and resuspended in fresh medium containing PHA. 5-bromodeoxyuridine (BrdU) was added to one culture of each treatment group to check on cell cycle progression. The cells were fixed at 48 or 50 h after PHA stimulation, preceded by a 2 h colcemid treatment. In the G_1 experiments, cultures were set up in PHA-containing medium; 1 h later, butyrate was added (5 mM final conc.). After about 14–15 h, the cultures were X-irradiated, washed and resuspended in fresh PHA-containing medium. The remaining steps in the experimental protocol were the same as with G_0 cultures.

(*c*) *The effects of butyrate pre-treatment on the frequencies of bleomycin-induced chromosomal aberrations in G_0 lymphocytes*

Cultures were set up in medium without PHA, and butyrate treatment (final conc.: 3 and 5 mM) was for 24 h. An aqueous solution of bleomycin (Mack, Illertissen) was added to the cultures during the last two hours of this 24 h period, at final concentrations of 60 μg/ml, 120 μg/ml and 160 μg/ml. Following this, the cells were washed and resuspended in medium containing PHA and allowed to progress through the cell cycle. Fixations were done at 44–50 h after culture initiation, depending on the experiment and donor.

(*d*) *Comparison of the effects of butyrate pre-treatment on the frequencies of X-ray and bleomycin-induced chromosomal aberrations in G_0 lymphocytes from the same donors*

The experimental protocols were the same as those described above.

(*e*) *The effects of butyrate pre-treatment on X-ray-induced chromosomal aberrations in G_0 lymphocytes from Down syndrome patients*

Blood from two Down syndrome patients (DS1, a male born on 16-11-87 and DS2, a female born on 5-4-88) and two fixation times (46 and 48 h) were used, the protocols being the same as those described above.

All X-irradiations were carried out at room temperature with an ENRAF machine operated at 150 kV, 6 mA, 1 mm Al filtration at a dose rate of about 120 rad/min. Doses and dose-rates were monitored using a PTW dosimentor system.

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Table 1. *Frequencies of chromosomal aberrations induced in human lymphocytes after X-irradiation in G_0 or G_1 stage of the cell cycle. All fixations were done at 50 h after PHA stimulation*

Expt no.	Donor	Group	Dose (Gy)	No. cells scored	Dicentrics (D) and Rings (R)					Acentrics				Chromatid aberrations per 100 cells	
					D	R	D+R/100 cells	RI[a]	P[b]	n	%	RI	P	Breaks	Exchanges
1	A	Control	—	200	0	0	—			5	2·5			2·5	0
		G_0	1	100	16	0	16·0			14	14·0			5·0	0
		G_1	1	101	30	4	33·7	2·1	≪ 0·01	19	18·8	1·3	N.S.	6·0	0
		G_0	2	102	44[c]	2	45·1			21	20·6			3·0	0
		G_1	2	100	62[d]	5	67·0	1·5	≪ 0·01	32	32·0	1·6	0·05	9·0	0
	B	Control	—	100	1	0	1·0			1	1·0			3·0	0
		G_0	1	140	23	0	16·4			15	10·7			6·0	0
		G_1	1	100	34	2	36·0	2·2	≪ 0·01	14	14·0	1·3	N.S.	11·0	0
		G_0	2	200	77	8	42·5			47	23·5			8·0	0
		G_1	2	200	114[d]	15	64·5	1·5	≪ 0·01	72	36·0	1·5	≪ 0·01	11·0	0

[a] Relative increase in aberration frequency. [b] Determined by the χ^2 test (one-sided); N.S. = non-significant. [c] Includes 2 tricentrics each counted as 2 dicentrics.
[d] Includes 1 tricentric counted as 2 dicentrics.

3. Results and Discussion

(*a*) *Comparison of the frequencies of chromosomal aberrations induced in lymphocytes after X-irradiation in G_0 and G_1 stages of the cell cycle*

Data from these experiments are summarized in Table 1. It should be recalled that the radiosensitivity of G_1 lymphocytes was assessed by X-irradiating the cultures at 14–15 h after PHA stimulation, at which time-period, Beek and Obe (1977) found the sensitivity differences between the G_0 and G_1 stages to be maximal. Inspection of Table 1 will reveal that G_1 lymphocytes are in fact more sensitive to the induction of dicentrics and rings (by a factor of between 1·5 and 2·2) and this is true of the lymphocytes of both the donors. These results are in good agreement with those of Wolff (1972) and of Beek & Obe (1977); for the induction of acentric fragments, G_1 cells appear to have a slightly more (1·3x; 1 Gy, both donors) or about an 1·5-fold higher sensitivity (2 Gy, both donors). The frequencies of chromatid breaks, in general, are higher in G_1 than in G_0 cells.

(*b*) *Comparison of the effects of butyrate pre-treatment on the frequencies of chromosomal aberrations induced by X-irradiation of G_0 and G_1 lymphocytes*

The results are summarized in Tables 2 and 3. Considering first Table 2, it can be seen that with lymphocytes from donor A, two dose levels (1 and 2 Gy) were employed whereas with donor B, only one dose of 1·5 Gy was used. As will be clear, in all the experiments (i) with no butyrate treatment, the frequencies of dicentrics and rings are higher (by factors in the range of 1·2 to 1·6) in G_1 than in G_0 cells and (ii) in both cell stages, butyrate pre-irradiation treatment leads to an enhancement of these frequencies and this enhancement is approximately of the same magnitude. The situation with respect to acentrics is similar (relative increase in butyrate pre-treated groups in the range of 1·3 to 2).

The variation in the frequencies of dicentrics and rings with radiation treatment alone, and in the magnitude of increase with butyrate pre-irradiation treatment noted with blood samples from the same donors (compare Tables 1 and 2) prompted us to examine whether there are radiosensitivity differences between blood samples from different donors and whether the relative increase (with butyrate pre-irradiation treatment) seen with G_0 cells provides an indication of the enhancement expected with G_1 cells from the same donor studied simultaneously. The results are given in Table 3 and provide support for the existence of inter-individual variations with respect to (i) the sensitivity of G_0 and G_1 cells to X-ray-induced chromosomal aberrations and for (ii) a parallelism in response between G_0 and G_1 cells from

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Table 2. *Effects of sodium butyrate pre-treatment on the frequencies of chromosomal aberrations induced in human lymphocytes at the G_0 or G_1 stage of the cell cycle. Butyrate-treated cells were fixed at 52 h and those without butyrate treatment, at 50 h*

Expt no.	Donor	Group	No. cells scored	D	R	D+R/ 100 cells	RI	*P*	Acentrics		RI	*P*	Chromatid aberrations per 100 cells	
									n	%			Breaks	Exchanges
2	A	G_0 buty. control	100	0	0	—			3	3·0			5·0	0
		G_1 buty. control	100	0	0	—			0	—			7·0	0
		G_0: 1 Gy	100	16	3	19·0	1·4	N.S.	8	8·0	2·0	0·04	16·0	0
		G_0: buty. + 1 Gy	300	75	3	26·0			47	15·7			6·7	0·7
		G_1: 1 Gy	100	25[a]	1	26·0	1·5	0·02	14	14·0	1·4	N.S.	11·0	0
		G_1: buty. + 1 Gy	200	70	8	39·0			19	19·0			14·5	0
		G_0: 2 Gy	100	46	4	50·0	1·5	≪ 0·01	27	27·0	1·3	N.S.	13·0	0
		G_0: buty. + 2 Gy	300	203[b]	15	72·7			79	36·3			12·6	0·7
		G_1: 2 Gy	101	75	8	82·2	1·2	≪ 0·01	16	15·8	1·7	0·02	10·9	0
		G_1: buty. + 2 Gy	263	229[c]	22	95·4			71	27·0			8·7	0
	B	G_0 buty. control	100	0	0	—			1	1·0			5·0	0
		G_1 buty. control	103	0	0	—			1	1·0			1·0	0
		G_0: 1·5 Gy	200	71[a]	4	37·5	1·6	≪ 0·01	27	13·5	1·3	N.S.	12·5	0
		G_0: buty. + 1·5 Gy	200	112[a]	9	60·5			35	17·5			9·5	0
		G_1: 1·5 Gy	200	88[a]	2	46·0	1·7	≪ 0·01	38	19·0	1·8	≪ 0·01	14·0	0
		G_1: buty. + 1·5 Gy	300	224[d]	15	79·7			100	33·3			8·7	0

[a] Includes 1 tricentric counted as 2 dicentrics.
[b] Includes 2 tricentrics each counted as 2 dicentrics.
[c] Includes 2 tricentrics each counted as 2 dicentrics and 1 tetracentric counted as 3 dicentrics.
[d] Includes 3 tricentrics each counted as 2 dicentrics.

Table 3. *Variability of the effects of sodium butyrate pre-treatment on X-ray-induced chromosomal aberrations in G_0 or G_1 lymphocytes from different donors*

Expt no.	Donor	Group and fixation time	No. cells scored	D	R	D+R/ 100 cells	RI	*P*	Acentrics *n*	Acentrics %	RI	*P*	% 2nd division cells
3	C	G_1 control (48 h)	200	0	0	—			1	0·5			5
		G_1 buty. control (50 h)	200	1	0	0·5			3	0·7			7
		G_1, 1·5 Gy (48 h)	400	117	14	32·8	1·2	0·01	43	10·8	1·2	N.S.	2
		G_1, buty.+1·5 Gy (50 h)	400	152[a]	11	40·8			50	12·5			3
	D	G_1 control (48 h)	100	1	0	1·0			1	1·0			7
		G_1 buty. control (50 h)	100	1	0	1·0			1	1·0			8
		G_1, 1·5 Gy (48 h)	200	75[b]	2	38·5	1·2	0·03	19	9·5	1·5	N.S.	6
		G_1, buty.+1·5 Gy (50 h)	200	86[b]	10	48·0			29	14·5			8
4	E	G_0/G_1 control (48 h)	400	0	0	—			0	—			5
		G_0, buty. control (48 h)	200	0	0	—			0	—			5
		G_1, buty. control (48 h)	200	1	0	0·5			1	0·5			7
		G_0, 1·5 Gy (48 h)	400	147	18	41·3	1·2	⩽ 0·01	67	16·8	1·3	0·04	5
		G_0, buty.+1·5 Gy (48 h)	400	189	15	51·0			88	22·0			3
		G_1, 1·5 Gy (48 h)	400	194[c]	18	50·3	1·2	N.S.	62	15·5	1·6	N.S.	7
		G_1, buty.+1·5 Gy (48 h)	71	41	2	60·6			7	9·9			0
	F	G_0/G_1 control (48 h)	400	2	0	0·5			0	—			6
		G_0, buty. control (48 h)	200	1	0	0·5			0	—			6
		G_1, buty. control (48/50 h)	Very few mitoses										
		G_0, 1·5 Gy (48 h)	400	166[c]	5	42·8	1·3	⩽ 0·01	50	12·5	1·2	N.S.	3
		G_0, buty.+1·5 Gy (50 h)	200	100[d]	9	54·5			30	15·0			2
		G_1, 1·5 Gy (50 h)	223	93[b]	5	43·9	1·2	N.S.	22	9·9	1·3	N.S.	0
		G_1, buty.+1·5 Gy (50 h)	103	52	2	52·4			13	12·6			0
	G	G_0/G_1 control (48 h)	200	0	0	—			0	—			7
		G_0, buty. control (50 h)	200	0	0	—			4	2·0		5	5
		G_1, buty. control (50 h)	200	0	0	—			1	0·5			5
		G_0, 1·5 Gy (48 h)	382	120	6	32·6	1·2	N.S.	58	15·2	1·1	N.S.	4
		G_0, buty.+1·5 Gy (50 h)	385	139[b]	8	38·2			63	16·4			4
		G_1, 1·5 Gy (48 h)	400	133[d]	14	36·8	1·2	0·03	52	13·0	1·5	0·02	5
		G_1, buty.+1·5 Gy (50 h)	300	126[e]	6	44·0			57	19·0			4

[a] Includes 4 tricentrics each counted as 2 dicentrics; [b] Includes 1 tricentric counted as 2 dicentrics; [c] Includes 3 tricentrics each counted as 2 dicentrics; [d] Includes 2 tricentrics each counted as 2 dicentrics; [e] Includes 5 tricentrics each counted as 2 dicentrics.

the same donors with respect to the modifying effects of butyrate pre-treatment i.e. lymphocytes from donors which respond with a low or high relative increase in chromosomal aberration frequencies after butyrate pre-treatment in G_0 also respond with a low or high relative increase, respectively, in G_1. For instance, with donor B (Table 2) the relative increase in aberration yield (with butyrate pre-treatment) is more than by a factor of 1·5 in both the stages; with donors E, F and G (Table 3) however, the increases are smaller (range: 1·2–1·3x). It is unlikely that these results reflect different proportions of second division cells in the different series because these proportions are approximately the same (last column of Table 3).

(*c*) *Experiments with bleomycin*

The results of a pilot study using butyrate (3 mM)-pretreated G_0 lymphocytes, 2 h bleomycin treatment and with a culture time (after PHA simulation) of 46 h (results not given) showed that such pre-treatment also leads to an increase (1·2–1·6-fold) in the yields of dicentrics and rings. With acentrics and chromatid aberrations, striking increases were noted only at the highest concentration of bleomycin. However, in this experiment, the proportions of second division cells were relatively high in some treatment groups. Consequently additional experiments were done with blood from donors A and B using a fixation time of 44 h. The data are summarized in Table 4 and show that butyrate pre-treatment leads to an enhancement in the frequencies of bleomycin-induced dicentrics and rings (relative increases in the range from 1·4x to 1·8x); the results with acentrics however show much variation (no enhancement to 2·6-fold enhancement).

(*d*) *Comparison of the effects of X-rays and bleomycin in butyrate-pretreated G_0 lymphocytes from the same donors*

These experiments were carried out to inquire whether any parallelism can be discerned between the responses of X-irradiated or bleomycin-treated G_0 cells from the same donor to the modifying effects of butyrate pre-treatment i.e. whether the X-ray response will be predictive of bleomycin response. In order to examine this, we used blood from two donors, one whose lymphocytes responded with a high relative increase in the yield of chromosomal aberrations and one with a relatively low relative increase, in the 'butyrate + X-ray' treatment groups. The data are given in Table 5. As can be seen, with donor B, the relative increases are by factors in the range of 1·4–1·6 in the 'butyrate + X-ray' and in the 'butyrate + bleomycin' groups. With donor J on the other hand, these increases are smaller. These data thus lend credence to the view that there exist inter-individual differences with respect to the modifying effects of butyrate.

(*e*) *Experiments with Down syndrome G_0 lymphocytes*

In these experiments which involved butyrate pre-treatment and X-irradiation of G_0 lymphocytes from Down syndrome patients, the hypotheses and expectations were the following: (i) if the enhancement in the frequencies of X-ray-induced chromosomal aberrations in the butyrate-treated G_0 lymphocytes from normal individuals is essentially a consequence of chromatin structural modification, then, the Down syndrome lymphocytes should also be responsive to this and the relative increase in aberration yield in the latter in the 'butyrate + X-ray' group would be similar to that observed with lymphocytes from normal individuals and (ii) if however, there is an additive or synergistic effect of chromatin structural modification and of the postulated enhanced misrepair of chromosome breaks in Down syndrome lymphocytes, then, in the 'butyrate + X-ray' group, the enhancement in aberration yield would be much higher than in normal lymphocytes under the same treatment conditions.

The results are given in Table 6. It is clear that pre-irradiation treatment of Down syndrome lymphocytes with butyrate leads to an increase (by about 50%) in the frequencies of dicentrics and rings as is the case with lymphocytes from normal individuals. One is tempted to interpret this finding as being consistent with the first expectation outlined in the preceding paragraph but the results of biochemical studies (Meschini *et al.*, in preparation) to be mentioned later, suggest that the situation is more complicated and even the hypotheses tested may not be sound.

The absolute frequencies of these aberrations in the X-ray series (20·7%, 21·5%, 25·0%, 21%) however, are not strikingly different from those recorded for lymphocytes from normal individuals (16–19%; see Tables 1 and 2). In this context, it is worth recalling that in the experiments of Sasaki and Tonomura (1969) in which blood samples from 34 Down patients were used to study the response to radiation (160 rad of gamma rays), overall there was an enhanced radiosensitivity of Down syndrome lymphocytes to the induction of dicentrics and rings; however, the yields of aberrations varied between patients and in some, the frequencies were well within the range recorded for normal individuals. Since lymphocytes from only two Down patients were used in the present work, and the aim was to examine butyrate effects, it does not seem profitable to speculate on the possible reasons for the near-similarity of responses between normal and Down syndrome lymphocytes to the X-ray induction of chromosomal damage.

Possible mechanism of butyrate effect on radiation-induced chromosomal aberrations. In our earlier work with G_0 lymphocytes (Sankaranarayanan *et al.* 1985), we tentatively interpreted the cytogenetic results (enhancement of the frequencies of radiation-induced chromosomal aberrations in butyrate-treated groups)

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Table 4. *Effects of sodium butyrate pre-treatment (5 mM) on the frequencies of chromosomal aberrations induced by bleomycin (BL) in human lymphocytes at the G_0 stage of the cell cycle. All fixations were done at 44 h after PHA stimulation*

Expt no.	Donor	Group	No. cells scored	D	R	D+R/100 cells	RI	*P*	Acentrics *n*	Acentrics %	RI	*P*	% 2nd division cells
6	A	Control	200	0	0	—			1	0·5			1
		Buty. control	200	0	0	—			1	0·5			0
		BL (60 μg/ml)	200	29[a]	0	14·5	1·8	⩽ 0·01	5	2·5	2·6	0·02	4
		Buty.+BL (60 μg/ml)	200	48[a]	3	25·5			13	6·5			0
		BL (120 μg/ml)	300	55	5	20·0	1·4	0·01	17	5·7	1·6	N.S.	2
		Buty.+BL (120 μg/ml)	300	83	3	28·7			28	9·3			0
		BL (180 μg/ml)	300	69[a]	7	25·3	1·4	⩽ 0·01	30	10·0	1·0	N.S.	0
		Buty.+BL (180 μg/ml)	300	100	7	35·7			31	10·3			0
	B	Control	100	0	0	—			0	—			0
		Buty. control	100	0	0	—			1	1·0			0
		BL (60 μg/ml)	100	17[a]	0	17·0	1·7	N.S.	2	2·0	2·3	N.S.	4
		Buty.+BL (60 μg/ml)	42	12[a]	0	28·6			2	4·5			0
		BL (120 μg/ml)	100	20[a]	1	21·0	1·6	0·04	4	4·0	1·3	N.S.	2
		Buty.+BL (120 μg/ml)	100[b]	32[a]	1	33·0			5	5·0			0
		BL (180 μg/ml)	100	26	0	26·0	1·5	0·03	16	16·0	—	—	1
		Buty.+BL (180 μg/ml)	100	35	3	39·0			15	15·0			0

[a] Includes 1 tricentric counted as 2 dicentrics.

[b] Two of these cells were highly abnormal: one contained 4 dicentrics, 3 tricentrics, 3 tetracentrics, 1 pentacentric, 1 ring, 6 acentric fragments (and 3 chromatid breaks and 1 gap); the other abnormal cell contained 26 chromatid breaks and 1 acentric fragment. The aberrations from these cells were excluded from calculations.

Table 5. *Comparison of the effects of sodium butyrate pre-treatment on the frequencies of chromosomal aberrations induced in G_0 lymphocytes by X-irradiation and by bleomycin*

Expt no.	Donor	Group and fixation time	No. cells scored	D	R	D+R/ 100 cells	RI	*P*	Acentrics *n*	Acentrics %	RI	*P*	% 2nd division cells
7	B	Control (48 h)	200	0	0	—			1	0·5			—
		Buty. control (50 h)	200	2	0	1·0			1	0·5			—
		1·5 Gy (48 h)	400	133[a]	14	36·8	1·4	≪ 0·01	52	14·0	1·3	0·03	—
		Buty.+1·5 Gy (50 h)	401	187[a]	16	50·6			73	18·2			—
		BL (60 μg/ml) (44 h)	403	43[b]	3	11·4	1·5	< 0·01	17	4·2	1·9	0·02	—
		Buty.+BL (60 μg/ml) (44 h)	400	66[c]	5	17·8			32	8·0			—
		BL (120 μg/ml) (44 h)	400	76[a]	3	19·8	1·4	≪ 0·01	28	7·0	1·7	0·01	—
		Buty.+BL (120 μg/ml) (44 h)	400	108[c]	5	28·2			48	12·0			—
	J	Control (48 h)	200	0	0	—			1	0·5			5
		Buty. control (50 h)	100	0	0	—			1	0·5			5
		1·5 Gy (48 h)	405	147[a]	4	37·3	1·2	0·02	43	10·6	1·2	N.S.	3
		Buty.+1·5 Gy (50 h)	403	163[c]	16	44·4			49	12·2			2
		BL (60 μg/ml) (44 h)	406	94[d]	5	24·4	1·3	N.S.	63	15·5	1·1	N.S.	5
		Buty.+BL (60 μg/ml) (44 h)	200	58[a]	3	30·5			35	17·5			3
		BL (120 μg/ml) (44 h)	408	117[c]	7	30·4	1·3	0·04	66	16·1	1·2	N.S.	2
		Buty.+BL (120 μg/ml) (44 h)	200	70	6	38·0			40	20·0			1

[a] Includes 1 tricentric counted as 2 dicentrics.
[b] Includes 3 tricentrics each counted as 2 dicentrics.
[c] Includes 2 tricentrics each counted as 2 dicentrics.
[d] Includes 5 tricentrics each counted as 2 dicentrics.

Table 6. *Effects of sodium butyrate-pre-treatment on the frequencies of chromosomal aberrations induced by 1 Gy of X-rays at the G_0 stage of the cell cycle of lymphocytes from 2 Down syndrome (DS1 and DS2) patients*

Expt no.	Group and fixation time	No. cells scored	D	R	D+R/ 100 cells	RI	*P*	Acentrics				Chromatid aberrations per 100 cells	
								n	%	RI	*P*	Breaks	Exchanges
8	DS1, control, 48 h	200	0	0	—			0	—			3·0	0
	DS1, buty. control, 48 h	200	0	0	—			3	1·5			5·0	0
	DS1, 1 Gy, 46 h	300	60	2	20·7	1·5	< 0·01	27	9·0	1·6	0·05	4·7	0
	DS1, buty.+1 Gy, 46 h	200	61	1	31·0			28	14·0			7·0	0
	DS1, 1 Gy, 48 h	200	42	1	21·5	1·5	0·01	19	9·5	1·3	N.S.	8·5	0
	DS1, Buty.+1 Gy, 48 h	200	61	3	32·0			24	12·0			4·5	0
	DS2, control, 48 h	200	0	0	—			1	0·5	—	—	2·0	0
	DS2, buty. control, 48 h	200	1	0	0·5			4	2·0	—	—	8·0	0
	DS2, 1 Gy, 46 h	300	69	6	25·0	1·6	≪ 0·01	37	12·3	1·1	N.S.	6·5	0
	DS2, buty.+1 Gy, 46 h	200	76	3	39·5			28	14·0			5·5	0·5
	DS2, 1 Gy, 48 h	200	41	1	21·0	1·4	0·02	9	4·5	1·9	N.S.	6·0	0·5
	DS2, buty.+1 Gy, 48 h	200	57	3	30·0			17	8·5			6·0	0·5

as a consequence of butyrate-induced conformational change in the chromatin. The basis for this interpretation was two-fold: (i) butyrate is known to induce conformational changes in chromatin and (ii) in our biochemical experiments, the numbers and extent of repair of DNA strand breaks (at 10 Gy; measured using the nucleoid sedimentation technique as described by Mullenders *et al.* 1983) were the same in butyrate-treated and untreated cells. Experiments with lower doses (unpublished results) also did not provide any consistent evidence for increased frequency of strand breaks in butyrate-pretreated cells.

In attempts to quantify induced strand breaks using the alkaline elution technique, again no significant differences were found between the 'X-ray only' and in the 'butyrate+X-ray' groups (Meschini *et al.* under preparation). The results were also negative when the frequency of strand breaks was measured using the competitive immunoassay employing a monoclonal antibody directed against single-stranded DNA as described by Schans *et al.* (1989).

All these results – obtained with different biochemical approaches to study the mechanism of butyrate effect – show that butyrate-induced chromatin modification does not lead to an enhancement of DNA damage in X-irradiated cells: therefore, increased strand breaks cannot be invoked to explain the observed increases in the frequencies of chromosomal aberrations in the 'butyrate+X-ray' groups. Similar conclusions can be drawn from the data published by Smith (1986).

Further experiments were carried out to examine whether the increased aberration frequencies in the 'butyrate+X-ray' groups could be due to *inhibition* of strand-break repair by butyrate. Alkaline elution studies as well as premature chromosome condensation studies indicated (data not shown; Mechini *et al.* under preparation) that there was an impairment of repair of DNA strand breaks: in both types of experiments, the frequency of strand breaks remaining unrepaired was higher at different recovery times in lymphocytes treated with butyrate relative to those which received no butyrate treatment. These results thus suggest that it is the inhibition of repair by butyrate and not differential induction of strand breaks (in the butyrate-treated versus untreated lymphocytes) that is probably responsible for the increased frequencies of chromosomal aberrations in the butyrate pre-treated lymphocytes irradiated with X-rays, relative to those lymphocytes which received X-rays alone.

In conclusion, the results presented in this paper and in the earlier one (Sankaranarayanan *et al.* 1985) demonstrate that *in vitro* butyrate pre-treatment of human lymphocytes either in the G_0 or in the G_1 stage leads to an enhancement of the frequencies of X-ray-induced chromosomal aberrations; such pre-treatment of G_0 lymphocytes from Down syndrome individuals also leads to increased yields of chromosomal aberra-

tions. There are inter-individual differences in sensitivity to the X-ray induction of chromosomal aberrations in G_0 and in G_1 lymphocytes, and the responses to butyrate pre-irradiation treatment follow a similar pattern: the higher the relative increase in aberration yield in G_0, the higher is such an increase in G_1 and vice versa.

In G_0 lymphocytes, butyrate pre-treatment leads to an enhancement of the frequencies of dicentrics and rings induced by the radiomimetic compound, bleomycin. The relative increase in the yield of these aberrations in G_0 lymphocytes in the 'butyrate + X-ray' group provides a reasonably good indication of the magnitude of enhancement expected with butyrate + bleomycin treatment.

The results of biochemical studies (to be published later) lend credence to the view that butyrate pre-treatment inhibits repair of DNA damage in X-irradiated lymphocytes and that this (and not increased strand breaks in the butyrate-treated groups) may be responsible for the increase in aberration yields in X-irradiated lymphocytes pre-treated with butyrate. If this interpretation is correct, it would also mean that the butyrate-induced chromatin conformational changes do not interfere with the factors responsible for the higher yield of chromosomal aberrations recorded in earlier studies with Down syndrome G_0 lymphocytes exposed to X-irradiation.

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