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Effect of 3-aminobenzamide on chromosome damage in human blood lymphocytes adapted to bleomycin

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Human blood lymphocytes, pre-treated with very low (adaptation) concentrations of bleomycin for 48 h and then exposed to a high (challenge) dose of the same agent or X-rays, became significantly less sensitive to the induction of chromosome damage than those which did not receive the pre-treatment, indicating an induction of 'adaptive repair' process. This repair process was negated when 3-aminobenzamide, an inhibitor of poly (ADP) polymerase, was added to the cultures immediately after the challenge treatment. The magnitude of negation in the adaptation response was greater in the case of lymphocytes challenged with X-rays as compared with those challenged with bleomycin.

Introduction

There is growing evidence for the existence of an adaptive response in human blood lymphocytes, similar to that found in *Escherichia coli* (Samson and Cairns, 1977) and rodent cells (Samson and Schwartz, 1980). Olivieri *et al.* (1984) first reported that prior exposure of human lymphocytes to low levels of radioactive thymidine (^{3}[H]dThd) led to a significant reduction in the chromosome damage induced by a subsequent high dose of X-rays. Shadley and Wolff (1987) also observed that an X-ray dose, as low as 0.01 Gy, can protect the lymphocytes against the cytogenetic damage induced by a subsequent exposure to 1.5 Gy X-rays. The radiation dose used for adaptation seemed to have an effect; so also the time-interval between the adaptation and challenge doses (Shadley *et al.*, 1987), the dose rate and the quality of radiation (Wiencke *et al.*, 1987). Sankaranarayanan *et al.* (1989) have confirmed the original results of Olivieri *et al.* (1984). More recently, Wolff *et al.* (1988) reported that human blood lymphocytes adapted to low doses of ionizing radiation became refractory to chromosome damage, not only by a high dose of radiation but also by chemical mutagens that induce similar kinds of lesions in DNA.

Bleomycin (BLM) is an anti-tumour antibiotic drug, widely used in the treatment of a variety of human malignancies (Bonadonna *et al.*, 1972). Its action on DNA and chromosomes has been shown to be similar to that of ionizing radiation (Tamura *et al.*, 1974; Dresp *et al.*, 1978). These similarities prompted us to investigate whether or not an adaptation response can be elicited by BLM in human blood lymphocytes. We observed that prior exposure of the lymphocytes to low doses of BLM made them significantly less sensitive to the induction of chromosome damage by subsequent exposure to a high dose of not only BLM but also X-rays (Vijayalaxmi and Burkart, 1989). These results lend further support to the operation of an adaptive repair process in human lymphocytes which offers resistance and cross-resistance to chromosome damage by the same or similar DNA damaging agents.

All these studies point to an inducible resistance to DNA damage and the possible role of the enzymes involved in the repair of damaged DNA. The activity of poly (ADP-ribose) polymerase (Skidmore *et al.*, 1979; Durkacz *et al.*, 1980) has been implicated in lymphocytes adapted to ^{3}[H]dThd and X-rays (Wiencke *et al.*, 1986; Shadley *et al.*, 1987). A similar study has been conducted to examine the role of 3-aminobenzamide (3AB), a potent inhibitor of poly (ADP-ribose) polymerase, on the chromosome damage in human blood lymphocytes adapted to BLM. The results are reported here.

Materials and methods

Heparinized blood samples were obtained from two healthy, non-smoking females, aged between 25 and 30 years. From each sample, separate cultures were set up using 1 ml blood in 10 ml RPMI 1640 medium containing 15% fetal calf serum, 1% PHA (Gibco), 1% glutamine, 10 U/ml penicillin, 10 μg/ml streptomycin and kept at 37°C in 5% carbon dioxide incubator. Four hours later, the adaptation doses of BLM (Lundbeck, Denmark), freshly prepared in sterile distilled water, were added to give final concentrations of 0.01 and 0.05 μg/ml and the cultures returned to the incubator. At 48 h, the cells were exposed to a challenge dose of 1.5 μg/ml BLM or 1.5 Gy X-rays (Philips X-ray machine, model MCN 321, 240 kV, 7mA, 1-mm aluminium filter; cultures were kept at a distance of 50 cm from the radiation source and the dose rate of 1.5 Gy was checked using Farmer dosemeter 2570). 3AB (Sigma), freshly made up in medium to give a final concentration of 2 mM, was added immediately after the challenge dose. The incubation continued for a further period of 6 h. For the last 2 h, the cells were also treated with 1 μg/ml colcemid (Gibco). Lymphocytes were collected, treated with 75 mM potassium chloride for 7 min and then fixed in 3:1 methanol:acetic acid mixture. Fixed cells were dropped onto clean slides, air-dried and stained with Giemsa. Coded slides were examined for chromosome damage. Two-hundred metaphases were analysed from each culture. Gaps and achromatic lesions less than the width of a chromatid were not included in the scoring. Very few chromatid exchanges and dicentric chromosomes were recorded but not included in the analysis. The data on the incidence of chromatid and isochromatid breaks (observed versus expected) were subjected to statistical analysis using one-tailed *t*-test.

Results and discussion

The overall response of the lymphocytes from two blood samples studied is similar and the pooled data on chromosome (chromatid + isochromatid) breaks are given in Table I. As can be seen, the adaptation doses of 0.01 and 0.05 μg/ml BLM induces a small increase (2–5%) in the frequency of chromosome breaks, whereas the challenge dose of 1.5 μg/ml BLM alone, given 6 h before fixing the cells, induces a significant number of chromosome breaks (65%). In lymphocytes which received both adaptation and challenge doses of BLM, the incidence of chromosome breaks is significantly lower than those expected on the basis of additive effects of two individual treatments. The reduction observed with the adaptation doses of 0.01 and 0.05 μg/ml BLM is 61 and 54% respectively. The challenge dose of 1.5 Gy X-rays alone, given to the lymphocytes 6 h before fixation, induces 30% chromosome breaks. When the cells adapted to BLM are subsequently challenged with 1.5 Gy X-rays, the yield of chromosome breaks is significantly lower than the sum of the effects induced by two treatments separately. The reduction observed with the adaptation doses of 0.01 and 0.05 μg/ml BLM is 59 and 52%, respectively. These data confirm the results of our previous experiments (Vijayalaxmi and

Table I. Effect of 3AB on chromosome damage in human blood lymphocytes adapted to BLM

Adaptive treatment BLM (μg/ml)	Challenge treatment	Chromatid and isochromatid breaks in 200 cells		Total breaks observed in 400 cells	Expected	Percent decrease
		Blood sample 1	Blood sample 2			
–	–	3	1	4		
–	3AB	3	1	4		
–	BLM	124	136	260		
–	X-rays	60	66	126		
0.01	–	5	4	9		
0.05	–	10	12	22		
0.01	BLM	50	53	103[a]	265	61
0.01	BLM + 3AB	91	107	198[b]	265	25
0.05	BLM	55	72	127[a]	278	54
0.05	BLM + 3AB	99	111	210[b]	278	24
0.01	X-rays	26	28	54[a]	131	59
0.01	X-rays + 3AB	53	60	113[c]	131	14
0.05	X-rays	30	39	69[a]	144	52
0.05	X-rays + 3AB	56	67	123[c]	144	15

The treatment schedule is described in Materials and methods. Expected values are the sum of two individual treatments minus the control. Difference between observed and expected values:
[a]$P < 0.001$; [b]$P < 0.025$; [c]not significant.
Difference between ± 3AB cultures: $P < 0.001$.

Burkart, 1989) and are in agreement with other published reports on the induction of adaptation response in human blood lymphocytes.

The data presented in Table I also indicate that the addition of 3AB alone, 6 h before fixing the lymphocytes, has no effect on the frequency of chromosome breaks over the control value. When 3AB is added to the adapted lymphocytes, immediately after the challenge treatment with 1.5 μg/ml BLM, the reduction in the incidence of chromosome damage is 25–24%, as compared with 61–54% (without 3AB treatment). A similar difference, with and without 3AB treatment, is also observed in the case of challenge with 1.5 Gy X-rays: 15–14% as compared with 59–52%. These results suggest that the addition of 3AB negates the adaptation response in human lymphocytes and support the observations made by Wiencke *et al.* (1986) and Shadley *et al.* (1987). However, the magnitude of reduction in the adaptation response by 3AB is greater in the case of challenge with X-rays as compared with BLM.

Chinese hamster ovary cells treated with BLM have been shown to synthesize poly (ADP-ribose) in a reaction which is dose- and time-dependent and the addition of inhibitors like 3AB inhibited this reaction resulting in increased cell killing (Huet and Laval, 1985) and chromosome damage (Zwanenburg *et al.*, 1985). Enhancement of anti-tumour activity of BLM by inhibitors of poly (ADP-ribose) polymerase has also been demonstrated *in vivo* and *in vitro* (Kawamitsu *et al.*, 1982; Sakamoto *et al.*, 1983). These reports are interesting in the context of the present study and implicate that poly (ADP-ribose) polymerase is involved not only in the repair of BLM-induced DNA damage but also in negating the adaptation response in human lymphocytes. It would be interesting to study the effect of 3AB on the rate of poly (ADP-ribose) synthesis and DNA repair kinetics in BLM-adapted lymphocytes.

The magnitude of negation in the adaptation response is greater in the case of challenge with 1.5 Gy X-rays as compared with 1.5 μg/ml BLM. It may be relevant to refer to the data which suggest that BLM is inactivated by an enzyme, BLM-hydroxylase (Umezawa, 1973) and that the therapeutic effects of BLM could be related to the distribution of BLM-hydroxylase in the tissues (Ichikawa, 1969; Clinical screening group of EORTC, 1970). Transplantable tumour cell lines which have higher BLM-hydroxylase are shown to be comparatively more resistant to BLM (Yoshioka *et al.*, 1978). Whether the adaptation doses of BLM stimulate the activity of BLM-hydroxylase, which offers the cells an increased resistance to BLM challenge dose, and whether the enzyme also plays a role in negating the adaptation response is not known. Experiments are in progress along these lines.

BLM is widely used as a therapeutic agent for a variety of human cancers; depending upon the fractionation of the doses employed and the duration of treatment, the possibility of an adaptation response of the tumour tissues/cells should be carefully assessed. Recently, Sakamoto *et al.* (1983) have demonstrated that daily treatment with various doses of BLM + benzamide, given to mice with Ehrlich ascites tumours, has increased the survival of the animals as compared with those mice on treatment with BLM alone. The usefulness of the administration of inhibitors of poly (ADP-ribose) polymerase/BLM-hydroxylase along with BLM in the treatment of human cancers remains to be elucidated.

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