ADAPTIVE RESPONSE INDUCED BY OCCUPATIONAL EXPOSURES TO IONIZING RADIATION

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Abstract

We have found a significant decreased sensitivity to the cytogenetic effects of ionizing radiation (IR) and bleomycin (BLM) in lymphocytes from individuals occupationally exposed to IR when compared with a control population. These results suggest that occupational exposures to IR can induce adaptive response that can be detected by a subsequent treatment by IR or by BLM. However, no correlation between the results obtained with both treatments was observed. A great heterogeneity in the frequencies of chromatid aberrations induced by BLM was observed. The study of the influence of different harvesting times showed that there was no correlation with the frequencies of chromatid breaks. Our results indicate that the use of BLM to detect adaptive response has several difficulties at the individual level.

Introduction

In vitro pretreatments with tritiated thymidine or with low doses of X-rays make phytohemagglutinin stimulated human lymphocytes less susceptible to cytogenetic damage by subsequent high acute doses of X-rays [1,2]. This phenomenon has been called

Adaptive Response (AR). The ability to respond to small doses of X-rays has only been detected when the adaptive dose is done on phytohemagglutinin stimulated human lymphocytes [3,4]. On the other hand, Wolff et al [5] reported that human blood lymphocytes adapted by *in vitro* exposure to low doses of ionizing radiation (IR) showed a decrease in the frequency of chromatid and isochromatid lesions induced by a subsequent treatment with bleomycin (BLM). Moreover, individuals exposed to IR accidentally also showed a decreased sensitivity of their lymphocytes to a challenge dose of BLM [6]. Pretreatment with low concentrations of BLM can also induce AR [7].

Wojcik and Streffer [8] showed that the fixation time could influence the frequency of chromosomal abnormalities induced by IR.

The aim of the present study was to compare the effect of the challenge doses of IR and BLM on lymphocytes of an occupationally exposed population and if there is any relationship between the time of fixation and the level of chromosome aberrations observed after *in vitro* treatment of human lymphocytes with BLM.

Materials and Methods

Peripheral blood samples from 12 occupationally exposed individuals to X and/or gamma-rays, that work in radiotherapy and radiodiagnostic services, were used. For comparisons peripheral blood samples from non-exposed individuals were used.

Blood samples were irradiated with a challenge dose of 2 Gy using a cobalt source. Dose rates varied from 96.12 to 91.95 cGy/min. In order to emulate *in vivo* conditions, IAEA recommendations were followed [9]. After irradiation blood samples were cultured for 48h. The chromosomal aberrations considered were dicentrics (dic).

In the study of the effect of BLM, a challenge dose of 0.03U/ml was added 5 hours before harvesting.

In the study of the influence of harvesting time performed in three individuals, a total of 24 cultures were made for each individual. Eight cultures were harvested at 48h, eight at 50h

and the remaining eight at 52h. For each time of harvesting, the eight cultures were treated as follows: two (C) were used as control, two (A) received only the adaptive dose of BLM (0.03mU/ml) 24h after the culture start, two (CH) received only the challenge dose of BLM (0.03U/ml) 5h before harvesting and the other two (T) received both the adaptive and the challenge treatment with BLM.

For BLM sudies, chromatid breaks (ctb) were considered.

In all cases, to select first division metaphases, 12 μg/ml of BrdU was added since the set up of the cultures.

Results and Discussion

Adaptive response for the challenge dose of 2 Gy of γ-rays

In the control study, the frequency of dic did not shows significant differences between the exposed and the non-exposed population, although the frequency of acentric chromosomes was significantly higher in the exposed population ($p<0.001$). Increased frequencies of structural chromosome abnormalities in populations exposed to IR have been described by several authors [10-17].

Table I.- Cytogenetic results after IR and BLM treatments.

	2 Gy of gamma-rays		0.03U/ml of BLM	
	Non exposed	Occupationally exposed	Non exposed	Occupationally exposed
Number of subjects	8	12	11	12
Cells analyzed	2504	4742	2138	2502
ctb/cell (rank)			0.4 (0.21 to 0.66)	0.29 ()0.18 to 0.43
dic/cell (rank)	0.31 (0.26 to 0.37)	0.26 (0.21 to 0.31)		

After 2 Gy irradiation, the frequency of dic was significantly lower in the exposed population when compared with the non-exposed one ($p<0.001$) (table I). These results indicate that occupational exposures to very low doses of IR make human lymphocytes less susceptible to subsequent *in vitro* irradiation at high doses. The individual frequencies of dic ranged from 0.263 to 0.375 in the non-occupationally exposed population and from 0.214 to 0.307 in the occupationally exposed one. In both cases the Pearson's chi-squared test showed homogeneity in the frequencies of dic per cell. A significant negative correlation was observed between the frequency of dic and the doses occupationally received during the last year ($p<0.025$) and the mean dose of the last three years ($p<0.05$). These results could indicate that the differences in the response to subsequent high doses of IR could be related mainly to the occupationally doses received recently [18].

It is interesting to note that these results could have some implications in biological dosimetry. The estimated dose after 2 Gy irradiation was, in general, lower in the occupationally exposed individuals (mean= 1.86 Gy) than in the non-exposed ones (mean= 2.05 Gy). In three occupationally exposed individuals, the 95% confidence interval of the estimated dose did not include the challenge dose of 2 Gy [18].

Adaptive response for the challenge dose of BLM

In the control study the frequencies of ctb were higher in the occupationally exposed population but the difference was not significant. After BLM treatment, the occupationally exposed population showed significantly lower frequencies of ctb than the non-exposed one ($p<0.025$) (table I). This result could indicate that an AR induced by occupational exposures to IR can also be detected after BLM treatment of peripheral blood cultures [19]. This is in agreement with the idea that an AR induced by a low dose of a mutagenic agent can be detected after a challenge dose of the same or similar DNA damaging agent [5,7]. Both, IR and BLM induce double-strand breaks in DNA.

When the individual frequencies of ctb were considered, Pearson's chi-squared test showed heterogeneity in both populations (occupationally exposed $p<0.0005$; non-exposed $p<0.005$). The ctb frequencies ranged from 0.21 to 0.66 in the non-exposed

population and from 0.18 to 0.43 in the occupationally exposed one. It is interesting to note that there is a considerable overlaping between the distribution of the individual frequencies of ctb between both populations. Similar heterogeneity was observed by Tedeschi et al [20] after BLM treatment of blood cultures from children contaminated as a consequence of the Chernobyl accident.

When the IR and BLM results were considered, no correlation was observed between the effect of IR and BLM in both populations. The homogeneity in the frequencies of dic observed after 2 Gy treatment could be due to the irradiation of cells at the G_0 stage. However, the heterogeneity observed after BLM treatment 5h before harvesting could be due to the clastogenic effect on a cell population growing asynchronically.

Influence of different harvesting times on the study of the AR to BLM

Variability for the induction of AR has been suggested to be due to differences in cell cycle kinetics [8]. However other studies suggested that adaptation is not caused by changes in the rate of cell progression to mitosis after a challenge dose, indicating that cell stage sensitivity could not be an important factor in AR [21,22].

In the present study, no correlation was observed between the time of harvesting and the frequencies of ctb induced either on T or CH+A-C cultures. Moreover, the frequencies of ctb per cell in the three individuals studied were, in general, lower in the T cultures than in

Table II.- Frequencies of ctb/cell induced by BLM treatment in the T and CH+A-C cultures of three individuals.

	Individual 1			Individual 2			Individual 3		
Harvesting time	48h	50h	52h	48h	50h	52h	48h	50h	52h
ctb/cell T culture	1.21	0.72	0.87	0.64	0.70	0.52	1.08	0.93	1.23
ctb/cell CH+A-C cultures	0.89	0.98	1.18	0.80	1.03	0.69	1.27	0.98	0.70

T= Adaptive and challenge dose; CH=Challenge dose; A=Adaptive dose; C=Control

the CH+A-C cultures for any time of harvesting although, the differences were not significant (table II). On the contrary, only for individual 3 the frequency of ctb per cell in the T/52h culture (1.23) was significantly increased when compared with the frequency of CH+A-C/52h cultures (0.7)($t=2.003$; $p<0.025$ one-sided test).

The great interindividual variability observed to the sensitivity to BLM, indicate that the use of BLM to detect AR has several difficulties at the individual level and the results obtained must be considered carefully.

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