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SHORT COMMUNICATION

Reciprocal Adaptive Response of Human Peripheral Lymphocytes Induced by Bleomycine or Gamma Rays

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Summary

The adaptive response and reciprocal adaptive response induced *in vitro* by exposure to low doses of gamma rays (0.05 Gy) or bleomycin (0.05 μg/ml) in human peripheral blood lymphocytes were assessed by the frequency of chromosome aberrations. Gamma rays (1.5 Gy) or bleomycin (1.5 μg/ml) were used as the challenge doses. In the experiments, blood samples from 5 healthy donors were investigated. It has been found that low doses of bleomycin and gamma rays induced a reciprocal adaptive response to high doses of gamma rays or bleomycin. Moreover, the results confirmed that the adaptive response did not correlate with the radiosensitivity of the peripheral blood lymphocytes.

Key words

Adaptive response • Reciprocal adaptive response • Gamma rays • Bleomycin • Chromosome aberration

The ability of cells to become more resistant to high doses of mutagens after a preceding exposure to low doses has been termed the adaptive response (AR). For evaluating this adaptive response various indicators have been employed, e.g. the frequency of chromosomal aberrations, sister chromatid exchanges, micronuclei, gene mutations and survival of cells. Olivieri *et al.* (1984) first reported that the pre-treatment of stimulated human lymphocytes with low level of radioactive thymidine (^{3}H-TdR) might cause a significant reduction in chromosomal aberrations after subsequent high doses of X-ray irradiation. Later, it was found that some chemomutagens and carcinogens have a similar ability to induce AR (Wolff 1996, Wolff *et al.* 1988, Vijayalaxmi and Burkart 1989, Cortes *et al.* 1990). The phenomenon of reciprocal AR was observed by Wolff *et al.* (1988) when the adaptive agent differs from challenging one. As regards to mechanisms of the AR some authors (Wiencke *et al.* 1986, Wolff *et al.* 1990, Ikushima *et al.* 1996) have attributed this to a repair process that might be controlled by enzyme poly(ADP-ribose)polymerase. The present paper deals with the induction of reciprocal AR measured by the frequency of chromosome aberrations in human peripheral blood lymphocytes after *in vitro* exposure to low doses of bleomycin (BLM) or gamma irradiation.

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Table 1. Frequency of chromosome aberrations in human peripheral lymphocytes adapted by bleomycin or gamma rays

Donor	Treatment group	No. Of metaphases scored	No. of chromosome aabberations Total	%	Expected [%]	AR
D1	Control	200	2	1.0		
	1.5 μg BLM/ml	190	70	36.8		
	1.5 Gy	200	40	20.0		
	0.05 μg BLM/ml	200	4	2.0		
	0.05 μg BLM/ml + 1.5 Gy	198	29	14.5	21.0	31.00
	0.05 μg BLM/ml + 1.5 μg BLM/ml	200	41	20.5	37.8	45.80
	0.05 Gy	200	2	1.0		
	0.05 Gy + 1.5 Gy	200	49	24.5	36.6	33.08
	0.05 Gy + 1.5 μg BLM./ml	200	22	11.0	18.5	40.50
D2	Control	200	3	1.5		
	1.5 μg BLM/ml	200	72	36.0		
	1.5 Gy	200	33	16.5		
	0.05 μg BLM/ml	190	3	1.6		
	0.05 μg BLM/ml + 1.5 Gy	200	20	10.0	16.6	39.70
	0.05 μg BLM/ml + 1.5 μg BLM/ml	200	42	21.0	36.1	41.80
	0.05 Gy	200	3	1.5		
	0.05 Gy + 1.5 Gy	200	43	21.5	34.5	37.70
	0.05 Gy + 1.5 μg BLM/ml	200	25	12.5	22.5	44.50
D3	Control	200	2	1.0		
	1.5 μg BLM/ml	197	36	18.3		
	1.5 Gy	200	41	20.5		
	0.05 μg BLM/ml	200	3	1.5		
	0.05 μg BLM/ml + 1.5 Gy	200	38	19.0	21.0	9.50 NS
	0.05 μg BLM/ml + 1.5 μg BLM/ml	200	35	17.5	18.8	6.90 NS
	0.05 Gy	200	2	1.0		
	0.05 Gy + 1.5 Gy	200	33	16.5	30.3	45.50
	0.05 Gy + 1.5 μg BLM/ml	192	32	16.0	23.5	31.20
D4	Control	200	3	1.5		
	1.5 μg BLM/ml	200	47	23.5		
	1.5 Gy	200	33	16.5		
	0.05 μg BLM/ml	196	3	1.5		
	0.05 μg BLM/ml + 1.5 Gy	200	20	10.0	16.5	39.44
	0.05 μg BLM/ml + 1.5 μg BLM/ml	200	32	16.0	23.5	31.90
	0.05 Gy	200	3	1.5		
	0.05 Gy + 1.5 Gy	200	24	12.0	29.2	58.90
	0.05 Gy + 1.5 μg BLM/ml	200	40	20.0	26.5	24.50
D5	Control	200	4	2.0		
	1.5 μg BLM/ml	200	64	32.0		
	1.5 Gy	200	45	22.5		
	0.05 μg BLM/ml	200	4	2.0		
	0.05 μg BLM/ml + 1.5 Gy	200	37	18.5	22.5	17.80 NS
	0.05 μg BLM/ml + 1.5 μg BLM/ml	200	52	26.0	32.0	18.80 NS
	0.05 Gy	200	5	2.5		
	0.05 Gy + 1.5 Gy	198	28	14.1	26.5	46.80
	0.05 Gy + 1.5 μg BLM/ml	200	32	16.0	35.3	54.70

NS - non-significant adaptive response

Whole blood cultures in duplicates from five normal healthy donors (25-40 years old) were set up in 5 ml of RPMI 1640 medium (SEVAC, Prague, Czech Republic) supplemented with 20 % calf serum, antibiotics (penicillin and streptomycin), 2 % phytohemagglutinine (HA15, Welcome, UK). Two hours before harvesting and fixation, Colcemid (2.10^{-7} M) was added to the cultures. Fixation was performed six hours after the challenge doses in both series of experiments.

Bleomycin (BLM, Nippon Kayaku) served as the adaptive dose in a low concentration (0.05 $\mu g.ml^{-1}$) in the first series experiments and was used for 24 h after lymphocyte stimulation. The challenge dose of BLM (1.50 $\mu g.ml^{-1}$) was delivered after 44 h of cultivation.

In the second series of experiments, gamma radiation (0.05 Gy) was used as adaptive dose applied 32 h after stimulation. The challenge dose of gamma rays (1.50 Gy) was delivered after 44 h of cultivation. A Chisostat ^{60}Co apparatus was used as a source of gamma rays. The dose rate was 0.5 Gy per minute.

Chromatid and isochromatid breaks were recorded in both series of experiments. The statistical significance of the reduction of chromosome aberrations was determined using χ^2-test with Yates's correction. The expected value of chromosome aberrations was calculated as the sum of individual doses (adaptive + challenge) minus the control value. The adaptive response was expressed as a percentage of the decreased chromosome aberration frequency.

Experimental data concerning the AR induced by low doses of BLM or gamma rays are summarized in Table 1. It can be seen that pre-treatment with the low dose of BLM induced the AR and reciprocal AR in donors D1, D2 and D4, but not in D3 and D5. When the low dose of gamma rays was used as the adaptive dose, the AR was observed in all five donors. The results are in good agreement with the results published earlier on reciprocal AR (Wolff *et al.* 1988, Vijayalaxmi and Burkart 1989). They indicate that the process of induction of the repair system by low adaptive doses of BLM and gamma rays is probably the same. The absence of AR in donors D3 and D5 after low dose of BLM may be the result of some physiological mechanisms that do not allow the induction of adaptive repair mechanisms in their lymphocytes. The presented results demonstrate an apparent heterogeneity in the AR in the cells of investigated subjects that might partly be determined genetically (Bosi and Olivieri 1989, Kalina and Némethová 1996). The heterogeneity of the AR may also be related to its magnitude and to adaptive factors. Further results have confirmed that the AR did not correlate with the radiosensitivity of peripheral blood lymphocytes.

Recently, it was suggested that the evaluation of AR should be performed in cells of individuals exposed *in vivo* to low doses of environmental and occupational mutagens (Šrám 1996). Barquinero *et al.* (1996) observed that occupational exposure to very low doses of ionizing radiation induce the AR which could be detected by a challenge dose of BLM. Similar results on the inducibility of AR *in vivo* by ionizing radiation in case of the Chernobyl catastrophe have been reported by Tedeschi *et al.* (1996). An open question, which still remains to be answered, concerns the problem how the AR resulting in refractoriness of cells to mutagens is related to individual radioresistance of patients, e.g. to chemotherapeutic drugs. More information in this direction might also be of importance for clinical medicine.

References

BARQUINERO JF, BARRIOS L, CABALLIN MR, RIBAS M, SUBIAS A, EGOZCUE J: Decreased sensitivity to the cytogenetic effects of bleomycin in individuals occupationally exposed to ionizing radiation. *Mutat Res* **354:** 81-86, 1996.

BOSI A, OLIVIERI G.: Variability of the adaptive response to ionizing radiation in humans. *Mutat Res* **211:** 13-17, 1989.

CORTES F, DOMINGUEZ I, PINERO J, MATEOS JC: Adaptive response in human lymphocytes conditioned with hydrogen peroxide before irradiation with X-rays. *Mutagenesis* **5:** 555-557, 1990.

IKUSHIMA T, ARITOMI H, MORISITA J: Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells. *Mutat Res* **358:** 193-198, 1996.

KALINA I, NÉMETHOVÁ G: Variability of the adaptive response to low dose radiation in peripheral blood lymphocytes of twins and unrelated donors. *Folia Biol (Prague)* **43:** 91-95, 1997.

OLIVIERI G, BODYCOTE J, WOLFF S: Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* **223:** 594-597, 1984.

ŠRÁM RJ: Future research directions to characterize environmental mutagens in highly polluted areas. *Environ Health Perspect* **104** (Suppl 3)**:** 603-607, 1996.

TEDESCHI B, CAPOROSSI D, VERNOLE P, PADOVANI L, MAURO F: Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? III. Challenge with bleomycin in lymphocytes from children hit by the initial acute dose of ionizing radiation. *Mutat Res* **354:** 77-80, 1996.

VIJAYALAXMI, BURKART W: Resistance and cross-resistance to chromosome damage in human blood lymphocytes adapted to bleomycin. *Mutat Res* **211:** 1-51, 1989.

WIENCZKE JK, AFZAL V, OLIVIERI G, WOLFF S: Evidence that the [^{3}H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-rays involves induction of a chromosomal repair mechanism. *Mutagenesis* **1:** 375-380, 1986.

WOLFF S: Aspects of the adaptive response to very low doses of radiation and other agents. *Mutat Res* **358:** 135-142, 1996.

WOLFF S, AFZAL V, OLIVIERI G: Inducible repair of cytogenetic damage to human lymphocytes adaptation to low-level exposures to DNAS-damaging agents. *Progr Clin Biol Res* **340B**: 397-405, 1990.

WOLFF S, AFZAL V, WIENCKE JK, OLIVIERI G, MICHAELI A: Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation as well as to chemical mutagens that induce double-strand breaks in DNA. *Int J Radiat Biol* **53:** 39-48, 1988.

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