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Radio-adaptive response in human lymphocytes

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Abstract

The term radio-adaptive response (AR) applies to the phenomenon of protection or enhanced repair induced by a low radiation dose. Blood microcultures from 4 donors were irradiated to test the existence of AR in human lymphocytes using two different irradiation schemes. An adapting dose of 0.01 Gy and a challenging dose of 1.5 Gy of gamma radiation from a 2.5 MeV LINAC was used in all experiments. A cytogenetic analysis of unstable chromosome aberrations was applied as the endpoint. An inter-individual variability response was observed in one of the irradiation schemes: one donor expressed AR, two did not and the last showed an apparent synergistic response. The other irradiation scheme showed low mitotic indices (MI), suggesting a G2 arrest.

Key Words: *radio-adaptive response; human lymphocytes; ionizing radiation; low doses*

INTRODUCTION

The adaptive response (AR), process by which cells or organisms pre-exposed to a low dose of ionizing radiation or other mutagenic factor show less damage caused by a subsequent high dose, was first described by Samson and Cairns (1977) in Escherichia coli for alkylating agents. Since then, it has been studied soundly in human stimulated lymphocytes. However the occurrence of the adaptive response to ionizing radiation is still a matter of controversy, because its mechanism remains unclear and because data on the phenomenon are contradictory.

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Although many have observed it, considerable negative results also exist (for a review see Wojcik and Streffer, 1994, Wojcik and Shadley, 2000). Several studies supporting the existence of an AR to ionizing radiation (IR) revealed that it requires certain minimal dose before the phenomenon becomes active. It occurs only within a relatively small window of doses, it is dose rate dependent; and relies on the genetic constitution of the individuals exposed, with some being unresponsive (Wolff, 1998). Further experiments showed that the expression of the AR was not instantaneous but took 5 to 6 hr to become fully developed (Shadley et al., 1987; Wang, 1991), and that it could be prevented if during this period protein synthesis was inhibited (Wang, 1991). It was also shown that the response was transient and lasted about 3 cell cycles (Shadley et al., 1987). The AR induced by low doses of IR was attributed to the induction of a novel efficient chromosome break repair mechanism, that if it was active when the challenging dose was given, less damage was observed (Wolff, 1998). The existence of such fact indicates that the current risk assessment methods for carcinogens could be exaggerating the effects produced by low doses of ionizing radiation.

The aim of the present work was to determine the existence of an Adaptive Response in human lymphocytes for two different irradiation schemes.

MATERIAL AND METHODS

Donors and Culture Conditions. Experiments were carried out with venous blood from 4 healthy adult donors (2 male and 2 female) whose ages were 22-25 years. 3 drops of whole blood were added to 1 ml of pre-warmed complete medium: 0.9 ml RPMI 1640 (Gibco, with L-glutamine), 0.1 ml of fetal calf serum (Gibco), 100 UI/ml penicillin, 100 µg/ml streptomycin and 2% phytohemagglutinin (PHA) M (Gibco). Final pH was 7.4. Samples were incubated at 37ºC.

Harvest started with the addition of colchicine (Merck, 1mg%) and then one more hour of incubation. After hipotonic treatment with KCl (0.075M), cells were fixed with methanol-acetic acid (3:1), spread on wet clean slides and stained with Giemsa (Merck 2.9%). Cells were fixed 2.5 hours after the initiation of harvest.

Irradiation and Harvest of Samples. Lymphocyte samples were irradiated at room temperature with a 2.5 MeV LINAC (4 µA, 100pps) at a dose rate of 0.26 Gy/min. Gamma rays were obtained by bremsstrahlung. Tubes were constantly agitated during the irradiation.

The adapting dose (0.01 Gy) was given alone, 24hr after PHA stimulation or with a challenging dose (1.5 Gy) given at 30 or 48 hr (24+30 and 24+48 schemes, respectively). Blood samples were harvested 51hr culture after initiation for donors 1 and 2. Some modifications of the original protocol were introduced for donors 3 and 4 (Table 1). Three parallel cultures were set up for the irradiation scheme 24+30 in these donors. Harvesting times for the irradiation scheme 24+48 were extended in donors 3 and 4 because low mitotic indices were obtained.

Aberration Analysis. Where possible, at least 100 metaphases per sample were scored blind. Unstable aberrations were recorded (dicentrics, centric rings and acentric fragments). At least 500 cells per sample were scored for the evaluation of the mitotic index (MI). Cellular distribution of chromosome aberrations (CA) was compared to the Poisson distribution.

Statistic Analysis. The effect of the challenging dose given on its own (Ch) was compared with the effect observed after both doses were given successively by the Mann-Whitney non-parametric test. CA frequency analysis between parallel cultures was carried out with the Mann-Whitney's and the Kruskall-Wallis's tests.

Cellular distribution of chromosome aberrations was compared with the Poisson distribution using the µtest described by Edwards et al. (1979).

Table 1. Mitotic index obtained for the different harvesting times used for the 4 donors in the 24+48 irradiation scheme

Donor	51 hr			54 hr		56 hr		58 hr		72 hr	
	Control	Pr48	AP 24+48	Pr48	AP 24+48	Pr48	AP 24+48	Pr48	AP 24+48	Pr48	AP 24+48
1	3.29 (1)	0 (1)	0 (1)	-	-	-	-	-	-	-	-
2	1.07 (1)	0 (1)	0.06 (1)	-	-	-	-	-	-	-	-
3	3.84 (1)	0.26 (3)	0 (3)	0. 34 (1)	0.43 (1)	-	-	-	-	-	-
4	3.49 (1)	0.1 (1)	0 (1)	0 (1)	0.40 (1)	0.09 (2)	0.15 (2)	**2.43 (2)**	**2.19 (2)**	**3.66 (2)**	**3.35 (2)**

Numbers in bold represent the samples for which frequencies of chromosome aberrations could be obtained. Numbers in brackets are the number of parallel cultures set up for each treatment. When more than 1 culture was set up, the value of the mitotic index is the average value.
-, not determined.

RESULTS

Adapting dose and parallel cultures. CA frequency analysis between identically irradiated samples (parallel cultures) of the same donor showed no significant differences ($P<0.05$) for dicentrics plus rings (D+R) and dicentrics alone (D). Data from parallel cultures were pooled.
No significant differences ($P<0.05$) were found between controls and the samples exposed to the adapting dose (0.01 Gy) alone.

Irradiation Scheme 24+30. The results obtained for the four donors under the irradiation scheme 24+30 are shown in Table 2. An AR was observed only for donor 2 whereas donor 3 showed an apparent synergistic response. A reduction of a 51% was seen in the D+R of adapted cells of donor 2. The increase of CA in pre-treated (adapted) cells of donor 3 was significant ($P<0.05$) only for the total aberrations.

The comparison of the cellular distribution of CA with the expected for Poisson can be observed also in Table 2.

Comparison of the cellular distribution between treatments Ch30 and AC24+30 is shown for donor 2 (Fig. 1). The low number of D+R in the preconditioned cells was due to a reduction of the cells with one aberration.

Irradiation Scheme 24+48. All samples irradiated with the challenging dose at 48 hr (previously adapted or not) showed very low MI. Normal MI were only obtained for donor 4 when the harvesting times were extended to 58 and 72 hr. Results are shown in Table 1.

CA frequencies when harvesting at 58 hr were considerable less than those obtained at 72 hr (Table 3). For the latter an AR could be seen, while not for the former. A 57% reduction in the D+R was observed in AC24+30 (72hr).

Comparison of the cellular distribution between treatments harvested at 72hr is shown in Fig. 2. A Similar distribution between treatments was found. The reduced number of D+R observed in lymphocytes preconditioned was due to a reduction in all types of aberrant cells.

DISCUSSION

Only when acentric fragments were included in the analysis, significant differences between parallel cultures were observed. A reasonably explanation for this is the uncertainty in the scoring of acentrics fragments (Edwards et al., 1979). Therefore we considered the D+R as the most reliable parameter to analyze radio-adaptive response.

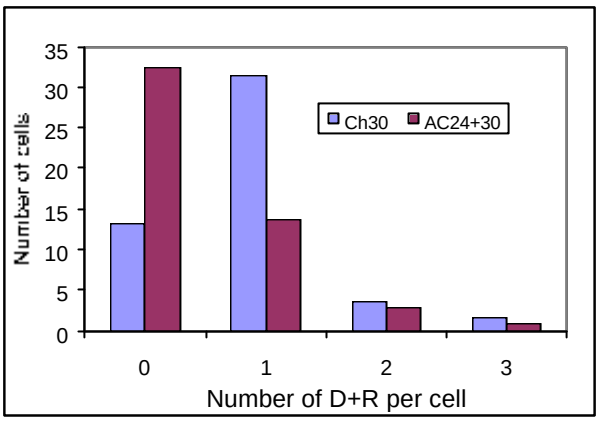

Fig. 1. Comparison of the cellular distribution of D+R in 50 cells for donor 2

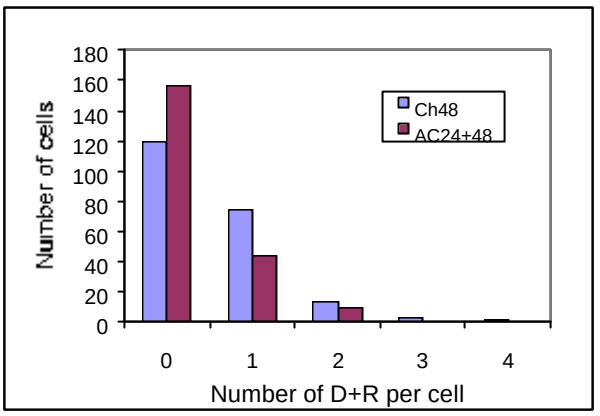

Fig. 2. Comparison of the cellular distribution of D+R in 210 cells harvested at 72 hr for donor 4

Irradiation scheme 24+30. Only one of the four donors tested expressed a clear adaptive response (AR) when their lymphocytes were pre-treated. This indicates that the AR cannot be induced in all donors, as was also observed elsewhere (Bosi and Olivieri, 1989; Khandogina et al., 1991; Hain et al., 1992; Wojcik and Streffer, 1995; Ryabchenko et al., 1998). We have also obtained an apparent synergistic response for one donor and two AR negative results. Similar results were obtained by Ryabchenko et al. (1998), who for the same irradiation scheme observed that out of seven donors, two expressed AR, four did not and one showed a synergistic response.

The heterogeneity of the human population for the induction of the AR could be attributed to three factors: differences in the conditions necessary for the expression of the AR between donors, changes in the physiological state of the donors (Bosi and Olivieri, 1989; Wojcik and Streffer, 1994) and a genetic component required for the induction of the AR that is not present in all donors (Youngblom, 1989; Bosi and Olivieri, 1989, Khandogina et al., 1991; Wolff, 1998). The methodology applied for the detection of the AR in human lymphocytes proved to be important too (Wojcik and Streffer , 1995).

Table 2. Results for the 4 donors irradiated under the 24+30 scheme

Donor	Treatment	Parallel Cultures	Cells analyzed	%Ab	Dicentrics + Rings [a]	Fragments [a]	Total aberrations [a]	DI		MI
								D+R	Total	
1	Control	1	100	10	0.01±0.01	0.14±0.04	0.15±0.05		-	3.29
	Ad24	1	100	15	0.06±0.02	0.16±0.04	0.22±0.06		-	1.89
	Ch30	1	100	89	0.52±0.07	2.03±0.13	2.55±0.16	-0.57	-0.33	2.49
	AC24+30	1	52	97	0.46±0.10	1.81±0.17	2.27±0.18	0.31	-1.47	2.36
2	Control	1	100	7	0	0.1±0.04	0.1±0.04		-	1.07
	Ad24	1	100	6	0.01±0.01	0.08±0.03	0.09±0.04		-	1.15
	Ch30	1	57	98.25	0.88±0.09	3.37±0.19	4.25±0.2	-2.46 [e]	-2.49 [e]	1.05
	AC24+30	1	51	90.2 [d]	0.45±0.10 [b]	1.47±0.13 [b]	1.92±0.16 [b]	0.46	-1.49	0.85
3	Control	1	100	3	0	0.03±0.02	0.03±0.02		-	3.84
	Ad24	1	100	12	0	0.14±0.04	0.14±0.04		-	3.26
	Ch30	3	166	92.8	0.45±0.05	2.14±0.10	2.58±0.11	-1.15	-1.66	1.71
	AC24+30	3	222	91.4	0.59±0.05	2.50±0.11	3.08±0.13 [c]	-0.84	1.59	1.86
4	Control	1	105	1.91	0	0.02±0.01	0.02±0.01		-	3.49
	Ad24	1	105	5.71	0.01±0.01	0.06±0.02	0.07±0.03		-	3.42
	Ch30	3	307	91.86	0.70±0.04	1.93±0.08	2.63±0.10	-1.49	0.59	2.49
	AC24+30	3	315	90.16	0.74±0.04	1.98±0.08	2.72±0.10	-2.51 [e]	1.30	2

D+R: dicentrics plus rings. % Ab: Percentage of aberrant cells (with at least one aberration of any of the analyzed type).
DI: Dispersion index (Poisson μ-test). MI: mitotic index.
a Mean of CA per cell ± SE
b Significantly less (P<0.01) than Ch30 (Mann Whitney's Test).
c Significantly more (P<0.05) than Ch30 (Mann Whitney's Test).
d Significantly less (P<0.05) than Ch30 (proportions difference's test).
e Subdispersion from Poisson distribution (P<0.05).

Table 3. Results of the 24+48 scheme for donor 4

Treatment	Cells analyzed	%Ab	Dicentrics + Rings [a]	Fragments [a]	Total aberrations [a]	DI		MI
						D+R	Total	
Ch48 (58hs)	195	31.8	0.06±0.02	0.41±0.05	0.47±0.06	1.44	3.57 [e]	2.43
AC24+48 (58hs)	178	35.4	0.10±0.02	0.52±0.06	0.62±0.07	-0.87	4.21 [e]	2.19
Ch48 (72hs)	210	79	0.61±0.06	1.58±0.10	2.19±0.12	0.72	4.82 [e]	3.66
AC24+48 (72hs)	210	64.3 [d]	0.35±0.04 [b]	1.29±0.09 [c]	1.64±0.18 [b]	0.82	-0.15	3.35

D+R: dicentrics plus rings. % Ab: Percentage of aberrant cells (with at least one aberration of any of the analyzed type).
DI: Dispersion index (Poisson μ-test). MI: mitotic index.
a Mean of CA per cell ± SE
b Significantly less (P<0.01) than Ch48 (72hr) (Mann Whitney's Test).
c Significantly less (P<0.05) than Ch48 (72hr) (Mann Whitney's Test).
d Significantly less (P<0.05) than Ch48 (72hr) (proportions difference's test).
e Overdispersion from Poisson distribution (P<0.05).

The number of cells scored for the donor who expressed the AR was rather small, but the notorious differences in the frequencies of CA, between adapted cells and cells which received the challenging dose alone, excludes the possibility that they are due to the analysis of small samples.

The results of the comparison of the cellular distribution of CA with the one expected for Poisson distribution showed a good fit. Similar results were obtained by Ryabchenko et al. (1998) when irradiating cells in G1 phase. He argues that the Poisson distribution would be expected if the initial lesions were repaired independently from each other (i.e., the lesion served as a locus of repair). If this is so, the probability of repairing single lesions in cells would be greater than the one for repairing multiple lesions. In the donor who expressed the AR, the reduction in the number of aberrations in adapted cells is the result of a reduction in the number of cells with one aberration. However,

Shadley and Dai (1992) found cytogenetic adaptive responses in phase G1 as a result of reductions in multiply aberrant cells.

It was also seen that the cellular distribution in non-adapted cells of this donor did not fit the Poisson distribution whereas the adapted cells did. Changes in the cellular distributions of CA by the adapting dose were also observed by Wolff (1991).

Irradiation Scheme 24+48. The experiments carried out under this scheme showed low levels of mitotic indices (MI) for the four donors when cells were irradiated at 48 hr and harvested at 51 hr. This was observed independently of the fact that the cells were previously adapted or not. Harvesting times were extended in the last two donors in order to evaluate the possibility that the lack of mitosis obtained was due to a mitotic arrest. A recovery from the mitotic arrest was seen in one donor when harvesting 10 hr after the challenging dose was given. This delay occurs in the G2 stage and it is caused by the impossibility of damaged cells to go through the G2/M checkpoint (Maity et al., 1994). This fact gives the cells more time to repair the DNA damage (Lane, 1992)

Cells harvested at 58 hr showed very low frequencies of D+R probably because they were irradiated in the G2 stage, when the predominant aberration type produced is the chromatidic (Ryabchenko et al., 1998). Cells showing this kind of aberrations would not be distinguished from normal cells by the methodology employed. No significant differences between adapted and non-adapted cells were observed contrary to what Ryabchenko et al. (1998) found for the same irradiation scheme, although they didn't find a mitotic arrest.

No correlation was found between the sensitivity of the G2 checkpoint activation and the presence of AR in human lymphocytes, as it was shown in a recent publication (Pretazzoli et al., 2000).

A clear AR was observed in samples harvested at 72 hrs in the last donor, both for the frequencies of CA and the percentages of aberrant cells. Cellular populations of these samples were probably a mixture of cells which received the challenging dose in different cycle's stages. It is known that the frequency of aberrations observed in the first post-irradiation mitosis varies significantly depending on the cell stage at which the cells were exposed to radiation (Luchnick, 1976). However, it was shown that the AR is not caused by a change in the rate of progression to mitosis after a challenging dose (Olivieri et al., 1984; Wolff, 1996). Cell stage sensitivity is not a determining factor in the adaptive response. It was also shown that the reduced number of CA observed in the AR was not due to a delay of the damaged cells to reach mitosis (Olivieri et al., 1984; Wang, 1991; Shadley and Dai, 1992).

Comparison between schemes. Both ARs obtained for different donors showed similar reduction in the D+R, despite the differences observed in the type of aberrant cells, which were decreased, and the CA cellular distribution between them. Similar levels of AR between different donors and irradiation schemes were also seen elsewhere (Olivieri et al., 1984; Bosi and Olivieri, 1989; Ryabchenko et al., 1998).

CONCLUSIONS

Once more the variability in the expression of the adaptive response in the human population is addressed. We could observe all kind of possible results in a reduced number of donors: adaptive and synergistic responses, as well as the absence of any response. It could be also shown that radiation doses such as 1,5 Gy may induce a mitotic arrest in the G2 phase of human lymphocytes. This phenomenon should be taken in a count for future investigations on the adaptive response.

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Resumen

El termino respuesta radio-adaptativa (AR) se aplica al fenómeno de protección o reparación estimulada inducido por una dosis baja de radiación. Microcultivos sanguíneos de 4 donantes fueron irradiados para provar la ocurrencia de la AR en linfocitos humanos utilizando 2 esquemas de radiación diferentes. En todos los experimentos se utilizó una dosis adaptadora de 0,01 Gy y una de prueba de 1,5 Gy de radiación gamma obtenida a partir de un LINAC (2,5 MeV). Se llevo a cabo un análisis citogenético de aberraciones cromosómicas inestables. Se observaron respuestas variables en uno de los esquemas de irradiación: un donante expresó una AR, 2 no lo hicieron y el último mostró una aparente respuesta sinergística. En el otro esquema de irradiación se obtuvieron bajos índices mitóticos (MI), sugiriendo un retraso en G2.

Palabras clave: *respuesta radio-adaptativa; linfocitos humanos; radiación ionizante; bajas dosis*

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