XA9745567

EFFECT OF LOW DOSE RADIATION IN LYMPHOCYTES FROM CHILDREN EXPOSED TO IONIZING RADIATION AFTER THE CHERNOBYL ACCIDENT. CYTOGENETIC, CHROMOSOME PAINTING, GPA AND ADAPTIVE RESPONSE STUDIES

Padovani L., Appolloni M., Anzidei P., Spanò M., Stronati L., Testa A. and Mauro F.

Environmental Department, ENEA Casaccia, Rome, Italy

Abstract

The present study concerns the monitoring of some children coming from Byelorussian, Ukrainian and Russian republics, exposed to the fall-out, or to the initial acute dose of radiation with the aim of assessing the effects of ionizing radiation on human health and of verifying the persisting of chromosomal damage several years after the accident. Both structural chromosomes damage (conventional cytogenetic and chromosome painting) and molecular mutation (GPA) have been investigated, moreover the possible induction of an adaptive response has been tested.

INTRODUCTION

Several studies have demonstrated the occurrence of chromosomal aberrations in peripheral lymphocytes from individuals exposed to low doses of ionizing radiation. Following the explosion of the Chernobyl nuclear power station some studies reported an increased frequency of chromosome aberration, both for individuals exposed to a high acute dose and to a chronic low dose [1,2,3,4].
Since 1991 with the coordination of some humanitarian organizations more than 1000 children have been coming in Italy for a 1 month stay; they all underwent medical examinations and analyses, carried out in the laboratories of collaborating hospitals.
During the 1st week of some children stay, the internal contamination from ^{137}Cs and ^{134}Cs was measured by WBC (Whole Body Counter) and urine toxicological analyses in the laboratories of ENEA (National Agency for New Technology Energy and the Environment). Besides on 83 children, selected for their WBC values and the area of origin, conventional cytogenetic analyses have been performed.
Other studies have been carried out on random subsets of subjects in order to reveal stable chromosome damage:

- Chromosome painting by FISH, Fluorescence in Situ Hybridization, has been performed on 13 children in order to focus our attention on long term stable markers of exposure (reciprocal translocation), which are difficult to investigate with the conventional methods.

- Mutations on the gene for glycophorin A (GPA) have been investigated by the flow cytometry approach. GPA is expressed on the RBC membrane and occurs in two major allelic forms that define the M and N types of the MN blood group. In a somatic cell a mutation affecting this gene remains remarkably stable even long time after the exposure to the mutagenic agent. Thus we adopted this test for bio-monitoring the stable chromosome damage in a somatic cell line. One variant cell type, the N/O variant has an hemizygous phenotype, such cells may arise by single base changes, deletion or inactivation of the GPA^M allele, or loss of the chromosome carrying the allele. The variant N/N has not only lost the expression of GPA^M allele but also expresses the GPA^N allele twice the heterozygous level; these variant cells might be generated by chromosomal loss and duplication, gene conversion, or mitotic recombination in erytroid precursor cells.

Several studies report that cells appear to become less susceptible to the induction of radiation damage if a challenge exposure to ionizing radiation is preceded by a low «adaptive» dose [5,6,7]. As contradictory results have been reported about the conditions under which the phenomenon can be evidenced, in our work we have analyzed 13 children subjected to a chronic low «adaptive dose» due to the intake of contaminated foods, to assess if the phenomenon really exists out of laboratory conditions.

MATERIAL AND METHODS

SUBJECTS

A total of 83 children, with age ranging between 8 and 12 years, and without evident signs of pathology due to radiation exposure, arrived in Italy during the last six years. Most of them were coming from areas exposed to the fall-out of the Chernobyl accident, while a small number of children was living near the power station during the explosion and had been exposed to the initial acute dose. 11 subjects came from an area (Smolensk) which is considered to be not contaminated, they showed internal contamination values quite normal (<70 Bq), so that we used them as control group.

CULTURE CONDITION

Blood sample were collected from all children at the time of the WBC analyses. Whole blood was cultured at 37 °C, 5% CO2 for 48 hrs, in RPMI 1640 medium (Flow), supplemented with 10% fetal calf serum (Gibco), 2% phytohoemoagglutinin (Murex) and penicillin-streptomycin. For each subject, three different cultures were set up for independent cytogenetic scoring. Metaphases were obtained according to standard procedures.

CYTOGENETIC

The slides were coded and blindly analyzed by three different observers, for most of the subjects a total of 600 metaphases were scored (a total of 52,217 metaphases have been scored) and the frequency of chromosome aberrations typically induced by ionizing radiation (dicentrics, acentrics, fragments and rings) was evaluated.

CHROMOSOME PAINTING

On 13 subjects (4 contaminated, 6 subjected to a single acute dose and 3 controls) chromosome painting was achieved, DNA probes specific for whole human chromosomes 2, 3 and 4 (Cambio) were used. Bound probes were detected with avidin-FITC and biotinylated goat anti-avidin antibody (Vector).

When possible 900 painted metaphases per subject were analyzed by different observers.

The total genomic translocation frequency per cell (FG) can be calculated from the frequency of translocations per cell detected by chromosome painting (FP) according to the equation:

$$FP = 2.05\ fp(1 - fp)\ FG$$

where fp is the fraction of human genome investigated (fp = 0.22)

GPA

After blood collecting 33 MN sample were typed using the Ortho Diagnostic Systems (Raritan, NJ, USA) blood grouping reagent kit (rabbit anti-M and anti-N) according to the manufacturer protocol. Fixation was carried out overnight at room temperature in PBS solution containing 3.4% formaldehyde and 8.4 μg/ml SDS, successively cells were stored in GPA staining mixture. Monoclonal antibodies anti-GPA(M) 6A7-PE and anti-GPA(N) BRIC-FITC were added and incubated for 1 hr. The day after samples were processed with Facstar Research Plus flow cytometer using standard band-pass filter for FITC and PE fluorescence. As far as the FCM GPA A analysis is concerned, we have analyzed 21 subjects (17 contaminated and 4 controls) and 5 million events were accumulated per each sample at a rate of about 2,000 cells/sec.

ADAPTIVE RESPONSE

13 children with internal contamination ranging from 664 to 26617 Bq were investigated to assess if the chronic exposure they have been subjected to, after Chernobyl accident, could induce a major cell

resistance to a subsequent acute high dose. Each sample was treated with a challenge dose of 1.5 Gy of gamma rays (dose rate 0.10 Gy/min) 48 hours after PHA stimulation and incubated for other 6 hours; furthermore a parallel cell culture, soon after the exposure, was treated with 3AB (3-aminobenzamide, 2mM), an inhibitor of the poly(ADP-ribose)polymerase function affecting mammalian cell radiosensitivity during the last stage of chromosome repair.
The same protocol was utilized in the lymphocytes cultures (4 subjects) of the control group. For the evaluation of chromosome damage, chromosome and chromatid aberrations were scored.

STATISTICS
Data of each type of study we carried out have been analyzed by the one tailed t-student probability test.

RESULTS

CONVENTIONAL CYTOGENETIC
Results obtained by the conventional cytogenetic and the WBC measures are given in table 1.
As a general trend, data show that contaminated and impacted children exhibit higher frequencies of chromosomal aberrations than the control group. Statistic reveals a significant difference for the total chromosomal aberrations between the control and the contaminated children ($p<0.05$). While no significant difference has been found for the impacted group.

Table I - Conventional cytogenetic results

Group	Number of subjects	Number of metaphases	Aberration/100 cells			
			Ace	Dic	Trl	Total
Contaminated (WBC<100)	41	22,463	0.37	0.08	0.12	0.57*
Impacted (WBC 400-32,343)	31	23,864	0.02	0.03	0.04	0.28
Control (WBC <70)	11	6301	0,22	0,04	0,06	0,33

Ace: acentric fragments; Dic: dicentrics; Trl: translocations
*One tailed t-student probability test : statistically significant ($p<0,05$) compared to control group.

CHROMOSOME PAINTING
Chromosome painting results are shown in table 2. It is evident that most of the subjects shows a high yield of translocations.
Because of the small number of samples we analyzed, it was not possible to perform a reliable statistic test. So, in this pilot study, as far as the FISH is concerned the aim is to point out the advantage of chromosome painting analyses when stable markers of radiation damage have to be investigated long time after the exposure.

Table II - Chromosome painting and cytogenetic results

Groups	Conventional cytogenetic			Chromosome painting		
	Cells scored	Trl/1000cells	Trl/cell	Cells scored	Trl/1000 cells	Fg
Contaminated	2,300	1.08	0.0018	4,087	0.92	0.0035
Impacted	3,244	1.36	0.0013	5,293	1.96	0.0056
Control	1,850	0.5	0.0005	2,921	0.59	0.0023

Trl: translocation
Fg: genomic frequency of translocation/cell calculated as in Materials and Methods section

GPA
The results of the FCM GPA A assay are given in table 3, no statistically significant differences have been found between the contaminated and the Smolensk control group.

Table III - Glycophorin A			
	N/O frequency	N/N frequency	Total variant cell frequency
Exposed	3.38 ± 3.31	8.52 ± 5.42	8.52 ± 5.41
Control	3.74 ± 2.68	3.06 ± 2,22	6.81 ± 4.39

ADAPTIVE RESPONSE

Data are shown in table 4, the results show that in the present instance no statistically significant effect of the Chernobyl accident on these end-points can be demonstrated.

The challenge treatment with gamma-rays does not yield a statistically significant difference between control and contaminated children. The trend of the data is in the direction of a higher in vitro radiation effect for the samples from contaminated individuals. Similarly, 3AB does not affect the radiation response.

Table IV - Adaptive response					
treatment	Cell scored	% Damaged Cell	%Chromosome aberrations	% Chromatid aberrations	% Total
Controls: 4 subjects, ground contamination 0,(WBC<40 Bq)					
None	800	1	0	1.7	1.7
γ-Rays	400	21.5	0.5	23	23.5
3AB	400	1.7	0.3	1.7	2.0
γ-Rays + 3AB	400	21.7	0.3	26.2	26.5
Contaminated subjects: 13 individuals, 12-30 Ci/Km^2 (4.4-11.1x10^{11} Bq/Kme2) (WBC range: 664-26617)					
None	2600	3	0.3	3	3.3
γ-Rays	1300	27.5	1.2	31	32.2
3AB	1300	4.5	0.6	4.3	4.9
γ-Rays + 3AB	1300	27	0.8	32.7	33.5

DISCUSSION

CONVENTIONAL CYTOGENETIC

Our data show a very low frequency of chromosome rearrangements related to a damage continuously inflicted by ionizing radiation and a relatively elevated frequency of acentric fragments compared to dicentrics and translocations. Acentric fragments are known to be induced by ionizing radiation but they should not be persistent aberrations, however some studies indicate that more acentrics than dicentrics are induced at very low dose range [4]; furthermore a longer half time of disappearance of lymphocytes containing acentric fragments in excess that lymphocytes with dicentrics and centric rings has also been reported [8]; our study is in line with previous report and confirm that the frequency of acentric fragments could be related with the continuous exposure to very low doses.

Even if the frequency of aberrations is very low, the cytogenetic picture is not back to normal, and this abnormal situation is clearly related to the effects of the Chernobyl accident.

Not clear relation has been revealed between the WBC evaluated internal contamination and the frequency of radio induced aberrations. This is not surprising considering that it is not possible to evaluate the pattern of the internal contamination value for the elapsed time from the accident.

Moreover deserve specific comments that our data show an elevated abnormal frequency of aberrations in unexposed Russian subjects used as control, this is not easy to explain and suggest further investigations.

CHROMOSOME PAINTING

Even if the number of the subjects studied is to small to see a statistical difference the data indicate an increased translocation frequency in both contaminated and impacted groups compared to the controls. Our study confirms the advantage of chromosome painting analysis but it should be noted that because the number of translocation for the whole genome is deduced from the number of observed translocations detected with chromosome-specific probes, the reliability of extrapolated results is low when translocations are not found.

GPA

The GPA A test results do not evidence statistically significant differences, indicating that within the limits of the assay the total accumulated radiation dose has not produced a relevant number of mutated cells in the bone marrow. The variant cell frequency is remarkably within the range of values reported in literature for control groups even though this is the only group with such a young average age.

ADAPTIVE RESPONSE

The result of the present work show that an effect, , on circulating lymphocytes, that could be described as an adaptive response cannot be observed in children exposed to very small doses of ionizing radiation (using as end point chromosome and chromatid aberrations). Our observation is not in agreement as far as ionizing radiation is concerned , with previous studies carried out on sample pre-exposed in vivo and challenge with in vitro treatment [9,10,11], however it must be pointed out that the exposure modality and the end-points investigated were different. Furthermore some in vitro studies [6] report that the adaptive response cannot be induced in the G_0 phase of the cell cycle, while, in vivo, the exposure of the most of the circulating lymphocytes take place just in the G_0 phase.
More generally, the detection and even the occurrence of the adaptive response appear to depend upon a variety of factors such as the radiation, the condition, the end-points, the cell cycle phase, the dose and the dose rate etc.

REFERENCES

[1] Pilinskaya, M.A., Shemetum A.M., Bondar'a Yu and Dybskii S.S., Cytogenetic effect in the somatic cells of persons exposed to radiation after the Chernobyl accident (in Russian), Vestn. Akad. Med. Nauk. SSSR, 8 (1991a) 40-43.

[2] Pilinskaya, M.A., Shemetum A.M., Eremeeva M.N., Red'ko D.V., Fedorenko V.G. and Shepelev S.E., Effect on peripheral blood lymphocytes of persons with acute radiation sickness as a result of the accident at the Chernobyl atomic power plant (in Russian), Tsitol. Genet., 25 (1991b) 17-21.

[3] Pilinskaya, M.A., Shemetum A.M., Red'ko D.V. and Shepelev S.E., Dynamics of the cytogenetic effect noted during repeated examinations of people who participated in neutralizing the accident at the Chernobyl atomic power plant in various period after irradiation (in Russian), Tsitol. Genet., 25 (1991c) 3-9.

[4] Stephan G., Oestreicher U., An increased frequency of structural chromosome aberrations in persons present in the vicinity of Chernobyl during and after the reactor accident. Is this effect caused by radiation exposure?, Mutation Res., 223 (1989) 7-12.

[5] Olivieri G. and Bosi A., Possible cases of variability of the adaptive response in human lymphocytes. In G. Obe and A.T. Natarajan (Eds), Chromosomal aberration, Basic and Applied Aspects, Berlin, Hidelberg, New York: Springer-Verlag, (1990) pp.130.

[6] Shadley J.D., Afzal V. and Wolff S. Characterization of the adaptive response to ionizing radiation induced by low doses of X-rays to human lymphocytes, Radiat. Res. 111 (1987) 511-517.

[7] Wolff S., Afzal V., Wiencke J.K., Olivieri G. and Michaeli A. Human lymphocytes exposed to low doses of ionizing radiation become refractory to high doses of ionizing radiation as well as to chemical agents that induced double strand breaks in DNA. Int. J. Biol., 53 (1988) 39-48.

[8] Ramalho A.T. and Nascimento A.C.H. The fate of chromosomal aberrations in ^{137}Cs exposed individuals in the Goiana radiation accident, Health Phys., 60 (1991) 67-70.

[9] Tuschl H., Altmann H., Kovac R., Topaloglou A., Egg G. and Gunther R., Effects of low dose radiation on repair processes in human lymphocytes, Radiat. Res., 81 (1980) 1-9.

[10] Tuschl H., Kovac R. and Wottawa A. T-lymphocytes subsets in occupationally exposed persons, Int. J. Radiat. Biol., 58 (1990) 651-659.

[11] Cai L. and Liu S.Z., Induction of cytogenetic adaptive response of somatic and germ cells in vivo and in vitro by low dose X-irradiation, Int. J. Radiat. Biol. 58 (1990) 187-194.