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BIOLOGICAL EFFICIENCY OF CALIFORNIUM-252 SOURCE EVALUATED BY COMET ASSAY, CLASSICAL CYTOGENETICS AND FISH IN HUMAN LYMPHOCYTES IRRADIATED WITHOUT AND WITH BSH PRETREATMENT

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ABSTRACT

Biological effectiveness of californium-252 source was evaluated after irradiations *in vitro* of normal or pre-treated with compound enriched in B-10 ion cells. Peripheral blood lymphocytes were used as a model for human cells. DNA and chromosomal damage were studied to compare biological effectiveness of irradiation. Human blood samples or isolated lymphocytes were irradiated with the isotopic source of ^{252}Cf, at the Faculty of Physics and Nuclear Techniques at the University of Mining and Metallurgy (both neutron source and samples were placed in „infinite" polyethylene block). Chemical pretreatment with $Na_2{}^{10}B_{12}H_{11}SH$ (BSH) was performed to introduce boron-10 ion into cells in order to check any enhancement effect due to the process of boron neutron capture. Single cell gel electrophoresis also known as the Comet assay was done to investigate the DNA damage. Classical cytogenetic analysis was applied to assess the frequencies of unstable aberrations (dicentrics, rings and a centric fragments). To evaluate the frequencies of stable aberrations the fluorescence *in situ* hybridisation (FISH) with probes for chromosomes 1, 4 (14.3% of the whole genome) was performed. Linear (or close to linear) increase with radiation dose were observed for the DNA damage and aberration frequencies in lymphocytes both untreated or pre-treated with BSH. Levels of translocations evaluated for the whole genome were comparable with the frequencies of dicentrics and rings. No significant differences were detected due to radiation dose in the frequencies of sister chromatid exchanges (SCE) detected in the second mitosis. No statistically significant differences were observed in various biological end-points between normal or boron pre-treated cells.

INTRODUCTION

Neutrons were applied for tumour therapy almost as soon as the neutron was discovered, and in spite of the long period since the first patient was treated, there is still no general agreement on the place of neutrons in radiotherapy [1].

Neutrons lose energy either by elastic or inelastic collision with the nuclei of the absorbing material. In elastic scattering, the greatest energy loss occurs in the case of collisions with nuclei of similar mass. Therefore, in biological tissue during elastic collision neutrons transfer most of their energy to hydrogen nuclei; recoil protons, which result from this interaction, produce ionisation like any heavy, charged particles. Most inelastic interactions occur with nuclei other than hydrogen.

Energetic charged particles (e.g., proton or α particles) often are ejected from nuclei excited by inelastic interaction [1]. Clinical trials are underway for used low energy neutrons for

cancer therapy based on the boron neutron capture reaction (BNC). In the boron neutron capture therapy generally is used the reaction $^{10}B(n, \alpha)\ ^{7}Li$. Cross section of this reaction with thermal neutrons is one of the highest known, so that why fission neutrons are the most frequently applied beams for BNCT [1]. A similar spectrum of neutron energy to fission neutrons has Californium-252 source [1].

In this study peripheral blood lymphocytes were used to evaluate *in vitro* the effectiveness of radiation from ^{252}Cf source in normal cells or pre-treated with compound enriched in B-10 ion. Three various methods were applied on the molecular and cellular level to detect any damage induced. The alkaline version of SCGE assay was performed to detect DNA strand breakage. Classic cytogenetic and fluorescence *in situ* hybridisation (FISH) analyses were carried out to evaluate chromosome aberration frequencies (dicentrics, rings, translocations). Sister chromatid exchange (SCE) frequencies were scored to evaluate biological effects during the second division and any potential influence of radiation on cell cycle kinetics.

MATERIALS AND METHODS

1. Lymphocytes preparation and BSH pretreatment

Blood was collected by venepuncture from healthy male donor. Samples of heparinized whole blood to study cytogenetic damage or isolated lymphocytes for the DNA damage analysis were used for irradiation.

Lymphocytes were separated using Histopaque separation medium. The cells were washed in RPMI 1640 supplemented with 15% foetal calf serum, and suspended in the same medium at room temperature.

To study the influence of BNC reaction, half of the samples blood or isolated lymphocytes were pre-treated with mercaptoborane $Na_2B_{12}H_{11}SH$ (BSH; final BSH concentration 31.8 μg/ml, i.e. 18.9 ppm of boron-10) supplied by Centronics Limited, Croydon, UK. The treatment with BSH was done at 37°C one hour before the irradiation. Samples 2 ml of blood or 0.5 ml of lymphocytes (approx. 100 000 cells) were irradiated at room temperature in eppendorfs; one sample untreated or pre-treated with BSH at the same time.

2. Irradiation

^{252}Cf neutrons

Irradiated was done with ^{252}Cf source (4.5 mCi - May 1998) of the Faculty of Physics and Nuclear Techniques of the University of Mining and Metallurgy. For irradiation eppendorfs were placed in the polyethylene chamber filled with distilled water to obtain a uniform distribution of fast and low energy neutrons. The distance of the samples from the radiation source was 2 cm what gave the dose-rates of the fast neutrons: 37.24 and 70.1 mGy/h for whole blood and isolated lymphocytes, respectively. Thermal neutrons dose-rate was 10 times lower. Basic parameters taken for the evaluation of the dose rates of fast and thermal neutrons from Cf-252 source calculated with the application of the MCNP software [2] are presented in the Tables 1a and 1b.

Table 1a.
Dose rates estimated for samples placed at the point 2 cm from ^{252}Cf source yielding $1x10^7$ neutrons (both source and samples placed in „infinite" polyethylene block).

Quantity / Type of radiation	Flux density $cm^{-1}\ s^{-1}$	Convertion factor k_n $(mSv \times cm^2 \times s)/h$	$H = k_n\Phi$ MSv/h	Q Sv/Gy	D = H/Q mGy/h	
					whole blood	isolated lymphocytes
Fast neutrons	4×10^5	1.26×10^{-3}	500	25	22.28	41.95
Thermal neutrons	2×10^5	0.04×10^{-3}	8	4	2.2	4.1
Gamma rays					14.96	28.91

Table 1b.
Basic parameters taken for the evaluation of the dose rates of fast and thermal neutrons from Cf-252 source calculated with the application of the MCNP software. Dose rates estimated for human lymphocytes (volume ≈ 0.0622 cm^3) and whole blood samples (volume ≈ 1.2 cm^3) placed at the point 2 cm from ^{252}Cf source yielding $1x10^7$ neutrons (both source and samples placed in „infinite" polyethylene block).

Type of radiation / Sample	Neutrons [mGy/h ± SD]	Gamma rays [mGy/h ± SD]	Induced gamma rays [mGy/h ± SD]	Total [mGy/h ± SD]
Isolated lymphocytes	41.95 ± 1.2	27.8 ± 1.0	1.15 ± 0.13	70.1 ± 1.6
Whole blood	22.28 ± 1.0	14.09 ± 0.8	0.87 ± 0.06	37.25 ± 1.3

To obtain the required doses in the range, from 0.12 to 0.92 Gy samples were irradiated for 3.3 –13 hours.

To check an influence of the prolonged time of exposure on the level of the DNA damage, samples of isolated and unexposed lymphocyte were kept at the room temperature, for the same period of time as irradiated cells.

To evaluate biological efficiency of the source on the molecular and mitotic level appropriate biological samples were irradiated per each dose:

* for the DNA damage analysis - isolated lymphocytes one sample of untreated and one sample of pre-treated with BSH.
* for chromosomal damage analysis - four whole blood samples untreated or pre-treated with BSH.

As soon as irradiation procedure was completed, the samples were put into thermal cube to the temperature ~37^oC and transported to laboratories of the Department of Radiation and Environmental Biology at the Institute of Nuclear Physics where appropriate culturing and tests were performed immediately.

X-rays irradiation

Irradiation was performed at the Institute of Nuclear Physics using Philips X-rays machine with an average dose of 1.72 Gy/h. Isolated lymphocytes resuspended in RPMI 1640 at the finale concentration of 100 000 cells per ml were exposed to following doses of X-rays 0, 0.6, 1.2, 1.8 Gy (0, 21, 42, and 63 min., respectively). The irradiation was done at room temperature.

3. DNA damage analysis by single cell gel electrophoresis assay

3.1. Slide preparation

Fully frosted microscope slides (two replicate slides for each dose) were prepared according to the standard procedure described elsewhere [3,4]. Slides were immersed for 1 h at 4°C in freshly prepared cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 1% sodium sarcosinate), with 1% Triton X-100 and 10% DMSO added just before use. Slides were then washed with distilled H_2O (4°C) and placed in a horizontal gel electrophoresis tank filled with fresh electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. After 20 min of unwinding the DNA, electrophoresis was conducted at 4°C for 30 min at 25 V, 300 mA (0.72 V/cm). All of the steps after lysis were conducted under yellow light to prevent any induction of additional DNA damage. After electrophoresis slides were gently washed 3 times for 5 min with Tris buffer (0.4 M Tris pH 7.5) and than stained with 60 µl of 17 µg/ml ethidium bromide in distilled water and covered with a coverslip. Before analysis slides were stored in a light proof box containing moist PBS at 4°C.

3.2. Slide analysis

For evaluation of the damage slides were examined at 200x magnification using an epifluorescence microscope (Olympus BX-50) equipped with a 100 W mercury lamp, an excitation filter of 515-560 nm, a barrier filter of 590 nm and a CCD camera. Automatic evaluation of the comet parameters was performed with Komet 3.0 software from Kinetic Imaging. One hundred fifty cells were evaluated per control or irradiation dose (75 cells from each of two replicate slides). Following parameters were used as a measure of the DNA damage:

- Tail length (extension of the comet),
- Tail DNA (percent of the DNA in the comet tail),
- Tail moment (fraction of the DNA in the comet tail multiplied by the tail length).

To evaluate the DNA damage induced by irradiation with each dose, the influence of time of prolonged exposure was excluded by subtraction, of the damages detected in the cells non-irradiated but only incubated for the period of exposures, from the values of the DNA damages detected in irradiated cells.

4. Chromosome damage analysis

4.1. Lymphocyte irradiation and cultures

Four samples (2ml each) of heparinized blood untreated or pre-treated with BSH were irradiated per each dose in eppendorfs. Irradiated blood was divided into 5 groups and appropriate medium was added to start various culturing procedures. Whole blood samples of 1.4 ml were added to 20 ml RPMI 1640 medium with 20% foetal calf serum and antibiotics. An addition of 0.075 µM of 5-bromo-2-deoxyuridine to the cultures for the detection of sister chromatid exchange and unstable chromosome aberrations was done to distinguish between the first and subsequent mitosis. Lymphocytes were stimulated with LF-7, the Polish substitute of phytohemaglutinin [5]. Four cultures were incubated at 37°C for 50 h for chromosome aberration induction (one culture with BrdU - for analysis of unstable aberration frequencies, and three cultures without it - for fluorescence *in situ* hybridisation). Fifth culture was incubated for 72 h for SCE analysis. Two hours before the end of the culture, colcemid (0.1 µl/ml) was added to each culture to arrest mitosis. The cells were fixed with Carnoy's solution according to standard procedures described elsewhere [5,6]. The slides were stained using the Hoechst-Giemsa technique to distinguish between the first and subsequent mitosis; additionally, one slide from the cultures without BrdU per each dose was stained with Giemsa solution for unstable aberration frequency analysis.

4.2. Classical cytogenetic analysis

The analysis of chromosome damage was performed in the first mitosis for the presence of chromosome aberrations (CA) and in the second mitosis for sister chromatid exchanges

(SCE). Each acceptable good spread of the metaphase was analysed for CA and 50 metaphases were scored for SCE. The percentage of aberrant cells (AbC), dicentric and ring frequencies (CAbF), total aberration frequencies including gaps (TAbF), aberration frequencies excluding gaps (AbF) and SCE frequencies per cell were used as a measure of cytogenetic damage. Potential influence on cell cycle kinetics, reported as proliferative rate index, was evaluated according to the following formula:

$(PRI) = (M_1 + 2 \times M_2 + 3 \times M_3)/(M_1 + M_2 + M_3)$,
where:
M_1, M_2, M_3 were the numbers of the first, second and third metaphases, detected respectively in one hundred of analysed metaphases [7].

4.3. Chromosomal damage by fluorescence in situ hybridisation (FISH)

The following chemicals were used for the hybridisation procedure; SSC (sodium chloride and sodium citrate solution) and Tween 20 from Sigma-Aldrich, UK, DAPI/PI (4',6-diamidino-2-phenylindole dihydrochloride hydrate) from Cytocell ltd., "Chromoprobe-M" kit containing directly labelled whole chromosome painting probes for chromosome 1 and 4 labelled with Cy3 and pancentromeric probe labelled with fluorescein isothiocyanate FITC from Cytocell ltd. Somerville Court, Oxfordshire, OX 17 3SN, UK.

4.3.1. Preparation of slides and probes

Slides were made prior to hybridisation from the fixed metaphases stored at -20°C. The density of the metaphases and the chromosome spreading were checked under a phase contrast microscope and the hybridisation area was marked underside of slide with a diamond pen. All procedures of the hybridisation were performed according to the protocol from the "Chromoprobe –M" kit by Cytocell ltd. The slides were washed in 2 x SSC for 2 min. at room temperature were dehydrated through an ethanol series (2 min. each in 70%, 85% and absolute ethanol) and prewarmed for approx. 5 min at 37°C. Simultaneously, chromoprobe coverslips covered with probes and 15 µl of hybridisation fluid per slide were prewarmed and afterwards they were placed onto the slides and the edges of the coverslips were sealed by application of rubber solution.

4.3.2. Hybridisation

Probes and target DNA were denatured for 5 min on a hotplate with a surface temperature of 75°C. Hybridisation was performed in a humidified chamber at 37°C overnight. The next day the stringent washes were done for 5 min. each at following solutions (pH 7.0):

- three times at 50% formamide/1 x SSC, at 45°C,
- at 1 x SSC, at 45°C,
- at 1 x ST (4 x SSC and 0.05% Tween 20), at 45°C,
- at 1 x ST at room temperature.

DAPI/PI -Antifade solution was pipetted onto the slides (10 µl per slide) for counter staining.

4.3.3. Slide analysis

For observation and photography, a fluorescent microscope (Olympus BX-50) equipped with triple wavelength filter (DAPI+FITC+TRITC) was used to detect fluorescence. Fujicolor 400 film or CCD camera was used to photograph the translocations detected.

Translocations involving chromosomes 1 and 4 were scored and F_p measured, on the

basis of frequencies measured, values of the translocation frequencies per the whole genome (F_G) were evaluated according to Lucas *et al*. [8]:

$$F_G = F_p / 2.05 \times f_p \times (1 - f_p),$$

where: F_p means observed frequency of translocations measured with FISH and f_p is a fraction of a genome labelled with FISH. For chromosomes 1 and 4 the value of f_p is 0.143.

5. Statistics

To evaluate a cut-off for HFC distributions of the SCE in a control populations were studied with a "Frequency" procedure from SPSS statistical package. The cut-off for HFC determined as percent of cells containing number of exchanges above 95% of total in control distribution was 13 ≥ SCE/cell for the normal cells. The least square best fit for the dose-response curves and correlation coefficients were calculated with a use of Excel 5.0. Student's t or ANOVA analysis from SPSS program were applied to determine statistical significance differences between DNA damages due to irradiation or BSH pretreatment.

RESULTS

Results of the DNA damage estimated with the SCGE assay after californium-252 source irradiation of human lymphocytes without and with BSH pretreatment are presented in Table 2a and Table 2b, respectively.

Table 2a.
Influence of the irradiation on the DNA damage induced in human lymphocytes untreated or pretreated with BSH.

Irradiation time (h)	Dose [Gy]	Chemical pretreatment	Tail DNA (%) ± SD	Tail length (μM) ± SD	Tail moment ± SD
3.3	0.23	-	8.04 ±1.93	12.78 ± 2.35	1.75 ± 0.71
6.6	0.46	-	12.74 ± 2.08	45.87 ± 5.18	7.37 ± 1.95
13.2	0.92	-	16.56 ± 3.33	49.80 ± 4.99	9.46 ± 1.68
3.3	0.23	18.9	8.23 ± 1.84	14.94 ± 3.28	2.50 ± 1.65
6.6	0.46	18.9	10.52 ± 1.30	46.20 ± 6.64	6.06 ± 1.14
13.2	0.92	18.9	15.33 ± 2.09	53.34 ±2.87	9.52 ± 1.39

Tail length (extension of the comet), % tail DNA (percent of the DNA in the comet tail), and Tail moment (fraction of the DNA in the comet tail multiplied by the tail length)

Table 2b.
Influence of the time of incubation on DNA damage in human lymphocytes without and with BSH pretreatment.

Incubation time (h)	Chemical treatment	Tail DNA (%) ± SD	Tail length (μm) ± SD	Tail moment ± SD
3.3	-	5.46 ± 1.18	11.67 ± 2.12	0.92 ± 0.43
6.6	-	7.21 ± 1.08	32.79 ± 2.77	2.54 ± 0.23

13.2	-	7.43 ± 1.00	35.60 ± 3.23	3.14 ± 0.70
3.3	18.9	6.66 ± 1.89	12.47 ± 3.10	1.19 ± 0.76
6.6	18.9	6.49 ± 0.81	33.10 ± 10.39	2.42 ± 0.62
13.2	18.9	9.39 ± 1.43	36.90 ± 5.50	3.76 ± 0.81

Table 2c presented the mean values of the DNA damage evaluated in human lymphocytes irradiated with low dose rate of X-rays (1.72 Gy/h).

Table 2c.
DNA damage evaluated in human lymphocytes irradiated with low dose rate of X-rays (1.72 Gy/h).

Irradiation time (min)	Dose [Gy]	Tail DNA (%) ± SD	Tail length (μM) ± SD	Tail moment ± SD
0	0	3.42 ±1.46	17.82 ± 5.02	0.88 ± 0.47
21	0.6	5.09 ± 2.04	20.23 ± 6.24	1.19 ± 0.58
43	1.2	6.86 ± 1.97	36.33 ± 5.25	2.84 ± 0.57
63	1.8	9.82 ± 1.95	43.83 ± 4.67	4.65 ± 1.36

Figure 1 presents the dose-response curves for the biological effect measured as Tail DNA and Tail moment after subtracting control values of results obtained for each dose of radiation. Open markers present results obtained in normal lymphocytes, and filled markers present the results obtained in pre-treated with BSH cells. There is a close to linear dose-dependent response throughout the dose range under the study. Dose response relationship shows at lower dose range linear shape, with a tendency to saturation at higher doses. The better approximation of the dose response relationship is given by polynomial fit. No significant difference between boron pre-treated and normal cells was observed for the levels of the DNA damage in the comet. However, after irradiation without BSH pre-treatment the length of the comet tail is slightly lower compared to its value in BSH pre-treated lymphocytes. Finally, the Tail moment measures do not show statistically significant difference between pre-treated and normal cells.

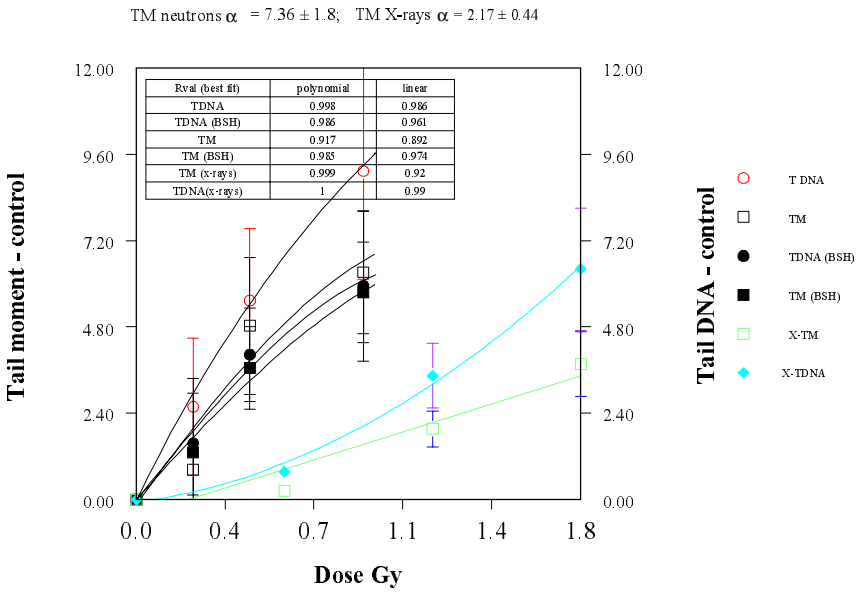

Fig.1. Tail DNA and Tail moment after Cf-252 source irradiation of lymphocytes untreated (open markers) or pre-treated with BSH (filled markers).

Results from the measurements of unstable chromosomal aberrations induced by californium-252 source without and with BSH pretreatment are presented in Table 3a and Table 3b, respectively. The analysis for the presence of chromosomal damage, performed in the first mitosis, revealed a dose-dependent increase of unstable aberration frequencies reported as dicentrics and rings frequency (CAbF), aberration frequency excluding gaps (AbF) and including gaps (TAbF), and percent of aberrant cells (AbC). Dose-dependent increase was observed after irradiation both without and with BSH pretreatment of the cells.

Table 3a.
Chromosomal damages detected by classical cytogenetics after 252-Cf source irradiation.

Total dose (Gy)	NM	CAbF ± SE	AbF ± SE	TAbF ± SE	AbC ± SE
0.0	896	0.002 ± 0.001	0.044 ± 0.007	0.057 ± 0.008	3.68 ± 0.06
0.12	179	0.060 ± 0.018	0.117 ± 0.026	0.134 ± 0.027	10.61 ± 0.24
0.24	1366	0.102 ± 0.009	0.218 ± 0.013	0.237 ± 0.013	15.96 ± 0.11
0.49	647	0.230 ± 0.019	0.423 ± 0.026	0.450 ± 0.026	28.75 ± 0.21

NM - number of metaphases ; CAbF - dicentrics and ring frequency; AbF - aberration frequency (excluding gaps); AbF - total aberration frequency (including gaps);AbC - percent of aberrant cells

Table 3b.
Chromosomal damages detected by classical cytogenetics after 252-Cf source irradiations in BSH pre-treated cells.

Initial dose (Gy)	NM	CAbF ± SE	AbF± SE	TAbF± SE	AbC± SE
0.0	449	0.000± 0.000	0.036± 0.009	0.038± 0.009	3.34± 0.09

0.12	760	0.042± 0.007	0.120± 0.020	0.137± 0.013	9.74± 0.11
0.24	357	0.081± 0.015	0.224± 0.025	0.241± 0.026	17.09 ± 0.29
0.49	586	0.152± 0.016	0.326± 0.024	0.336± 0.024	22.36± 0.19

Abbreviations as in Table 3.

Figure 2a and Figure 2b show chromosomal damage reported as CAbF and AbF respectively after Cf-252 irradiation of the lymphocytes without or with BSH pretreatment. There is a close to linear dose-dependence observed after irradiation of untreated cells and a non-linear one, in case of irradiation of BSH pre-treated lymphocytes. No significant difference between the frequencies of chromosomal aberrations was observed in not treated or pre-treated with BSH cells, at a whole dose range under the study (p-value – 0.17 and 0.4, respectively). Dose response relationship shows at lower dose range linear shape, with a tendency to saturation at higher doses. Finally, the better approximation of the dose response relationship is given by polynomial fit.

a/ b/

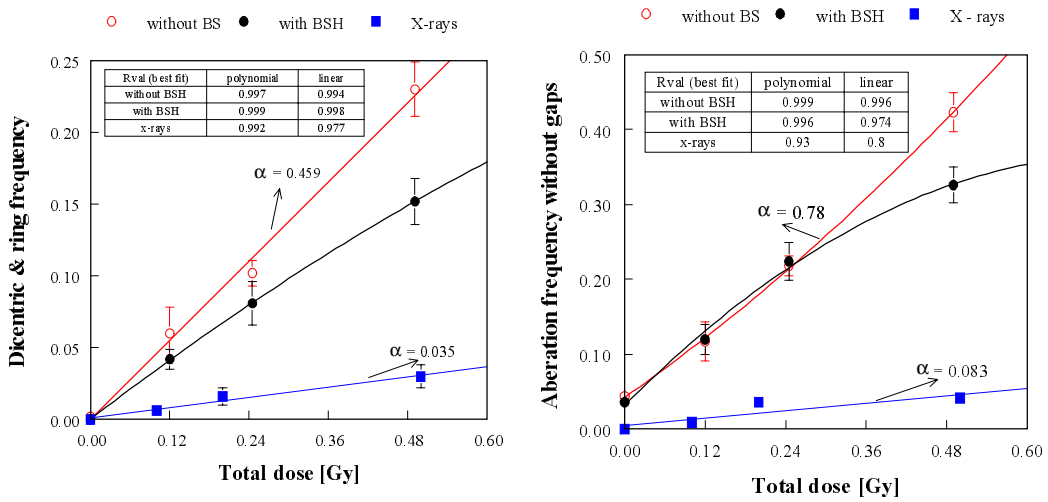

Fig.2. Frequencies of chromosome (dicentric and ring) Fig. 2a, and aberration without gaps (AbF) Fig. 2b, after lymphocytes irradiation with Cf-252 source at presence and absence of BSH.

Results of studies of the induction of stable chromosomal aberrations (translocations) by californium-252 source without and with BSH pre-treatment by fluorescence *in situ* hybridisation method (FISH) with an application of whole painting probes for chromosomes 1 and 4, are shown in Table 4a and 4b, respectively. There is an increase in the frequencies of translocations with the dose for lymphocytes pre-treated or not with BSH.

Table 4a.
Translocation frequencies induced in human lymphocytes by irradiation with ^{252}Cf source.

Total dose (Gy)	NM	MT	T1,T4	$F_p \pm SE$	$F_G \pm SE$
0.0	119	0	0	0.000 ± 0.000	0.000 ± 0.000
0.12	168	2	2	0.011 ± 0.008	0.044 ± 0.016

0.24	260	6	6	0.023 ± 0.009	0.092 ± 0.019
0.49	264	9	10	0.038 ± 0.012	0.151 ± 0.023

NM - number of metaphases; MT - metaphases with translocations; T1,T4 - number of translocations involving chromosome 1 or 4; F_G - frequency of translocations per the whole genome acc. Lucas *et al.* (1992): $F_G = F_p/2.05f_p(1-f_p)$, where F_p - observed frequency of translocations, f_p - fraction of genome covered with FISH probes (for chromosomes 1 and 4 $f_p = 0.143$);

Table 4b.
Translocation frequencies after 252-Cf source irradiation of BSH pretreated cells.

Initial dose	**with BSH**				
(Gy)	NM	MT	T1,T4	$F_p \pm SE$	$F_G \pm SE$
0.0	215	0	0	0.000 ± 0.000	0.000 ± 0.000
0.12	180	2	2	0.011 ± 0.008	0.044 ± 0.016
0.24	164	3	3	0.018 ± 0.010	0.072 ± 0.021
0.49	185	7	8	0.043 ± 0.015	0.171 ± 0.030

Abbreviations as in Table 4a.

Figure 3 present dose-response curves conducted for the frequencies of translocations detected and evaluated for the whole genome in presence and absence of BSH pretreatment. There is close to linear dependence of translocation induction on dose and no difference is observed for lymphocytes pre-treated or not treated with BSH.

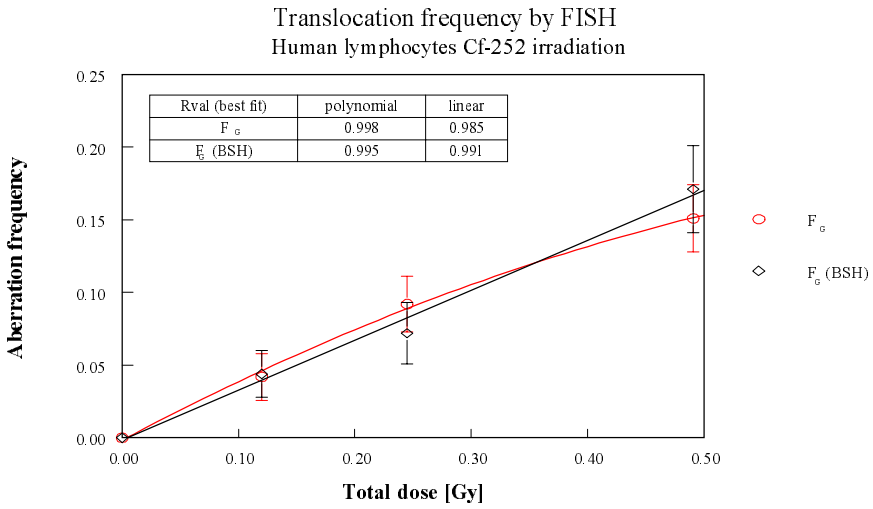

Fig.3. Translocations frequencies evaluated with FISH technique for the whole genome (from the frequencies of translocation detected in the chromosome 1 and 4) after Cf-252 source irradiation of lymphocytes without or with BSH pretreatment.

On the Figures 4a and 4b are presented the dose response curves for the frequencies of translocations assessed for the whole genome, and the dose response curves conducted for dicentric frequencies in the case of untreated lymphocytes (Fig. 4a) and pretreated with BSH

(Fig.4b). Comparison of the frequencies of dicentrics measured with classical method and translocations measured with FISH, revealed in normal cells a higher frequency rate of the detected unstable chromosomal aberrations. Due to lower efficiency of unstable aberration in pre-treated cells similar efficiency in the induction of both types of chromosomal damage in the whole dose range is observed in case of irradiation with BSH pre-treatment (Figures 4a and 4b).

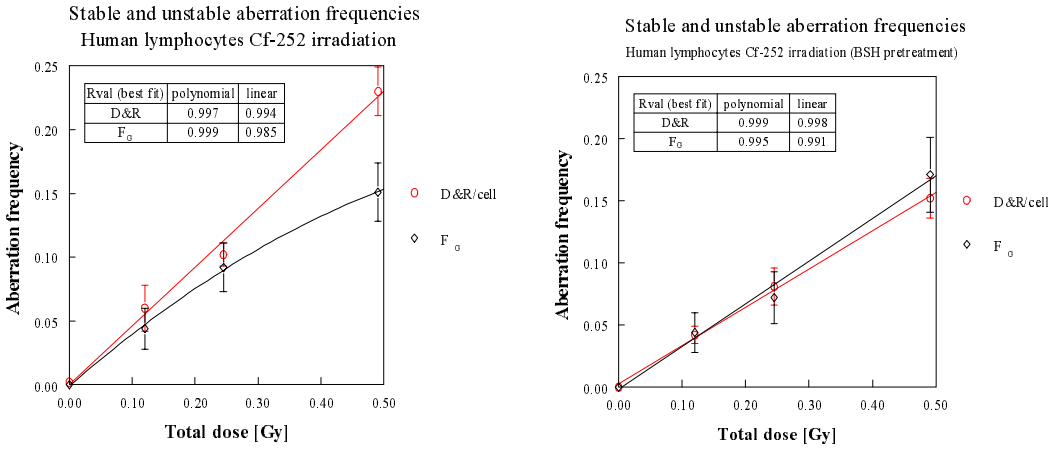

Fig.4. Comparison of stable and unstable chromosomal aberration frequencies after Cf-252 source irradiation of lymphocytes without (Fig.4a) and with (Fig.4b) BSH pretreatment.

There is a slight influence of the Cf-252 irradiation on the SCE frequencies induced in pretreated cells (Table 5, Fig.5) and none on high frequency cells (HFC) analysed in the second cell division (Table 4). Again, on the average there is no significant difference observed in the response of cells untreated or pre-treated with BSH. Proliferating rate index analysis showed no evidence of the irradiation influence on cell cycle kinetics, as well (Table 5).

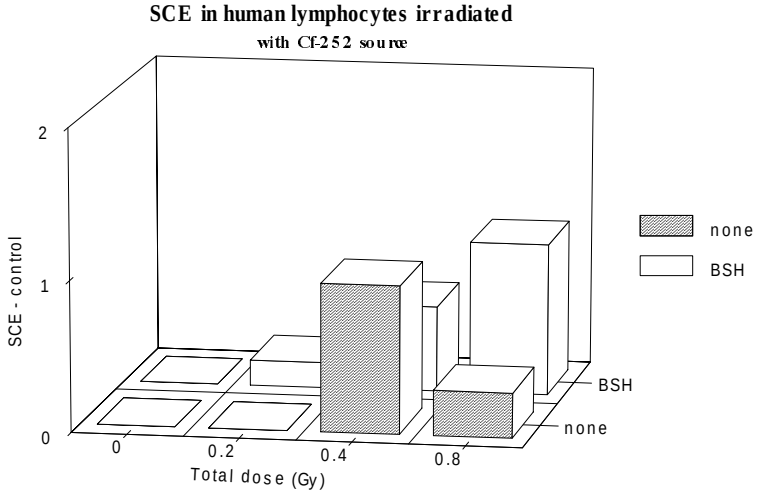

Fig.5. SCEs frequency observed in lymphocytes with and without BSH pre-treatment after Cf-252 source irradiations.

Table 5.
SCE, HFC frequencies and proliferating index (PRI) measured after 252-Cf source irradiation of the cells without and with BSH pre-treatment.

Dose (Gy)	without BSH			with BSH		
	SCE ± SE	HFC (%)	PRI	SCE ± SE	HFC (%)	PRI
0.0	7.96 ± 0.49	4	2.45	7.35 ± 0.51	4	2.71
0.12	7.76 ± 0.40	4	2.56	7.52 ± 0.39	2	2.62
0.24	8.94 ± 0.39	4	2.33	7.92 ± 0.37	4	2.55
0.49	8.26 ± 0.39	4.3	2.45	8.36 ± 0.41	6	2.63

SCE - sister chromatid exchanges per cell, scored in 50 cells; HFC - high frequency cells (percent of cells displaying number of exchanges per cell higher than the 95% of the control population distribution); PRI - proliferating rate index = $(M_1+2M_2+3M_3)/(M_1+M_2+M_3)$.

Table 6 presents values of the α coefficients from the dose response curves for chromosomal aberrations evaluated after irradiation of the lymphocytes with Cf-252 source and X-ray source in our previous studies [9].

Table 6.
The α coefficients values and maximal RBE evaluated from chromosomal aberrations.

	Radiation	$\alpha \pm SE$	RBE_{max}
Chromosomal	X- rays	0.035 ± 0.010	-
Aberrations	^{252}Cf	0.46 ± 0.05	13.14
Tail moment	X-rays	2.17 ± 0.44	-
Tail moment	^{252}Cf	7.36 ± 1.8	3.4

DISCUSSION

The objective of this study was to evaluate the effectiveness of the DNA and chromosomal damage induction in human lymphocytes exposed *in vitro* to Californium-252 isotopic source at very low dose rate, i.e. 37.25 – 70.9 mGy/h, at a dose range of 0 - 0.92 Gy. A special attention was paid to check whether there is any enhancement effect due to the presence of the boron-10 ions inside the cells. There were three methods applied to investigate these problems: SCGE assay, classical cytogenetics and fluorescence in situ hybridisation with whole painting probes for chromosomes 1 and 4.

The analysis of the results revealed a close to linear dose-dependent response throughout the dose range under the study, both for DNA damage and for chromosomal aberrations, respectively. A close to linear dependence was observed for all measured endpoints after irradiation without chemical pre-treatment as well as for DNA damage and translocations after irradiation with BSH pre-treatment. Non-linear dependence was observed for unstable aberrations analysed in not treated with BSH cells, as well as in cells pre-treated with BSH. This might suggest that different energy deposition path influence on type of the damage.

The maximal relative biological efficiency (RBE) evaluated from the α coefficients are

presented in the Table 6. The maximal value of relative biological effectiveness for cytogenetic endpoints (RBE) proceeding californium irradiation of untreated lymphocytes was calculated as the ratio of the fitted linear coefficients of dose response curves for dicentrics and rings induced with californium source ($\alpha = 0.287 \pm 0.014$), and X-rays ($\alpha = 0.035 \pm 0.010$) from our previous studies [9]. The RBE value estimated for chromosomal aberrations was 13.14, whereas the value of the RBE calculated for 5.6MeV fast neutrons and fission neutrons from the previous studies were about 10 and 20 respectively [9]. The RBE value estimated for DNA damage (Tail moment parameter) was 3.4, whereas the value of the RBE calculated for 5.6MeV fast neutrons from the previous study was about 4.6 [4]. RBE value obtained by Tanaka et al. for unfiltered Cf-252 neutrons from chromosomal aberrations was 12.3 [10]. The higher RBE value evaluated from chromosomal aberrations in our study (13.14) comparing to RBE value obtained by Tanaka at al. (12.3) could be a results of irradiation of the cells in different condition (lymphocytes were irradiated in the infinite block and in polyethylene chamber filled with distilled water, while those by Tanaka at al. [10] in the air). Moreover, taking in to account that in our exposure condition gamma rays impact was about 40%, and in Tanaka condition [10] he could neglected the gamma impact, our RBE is in a good agreement with his results.

Fluorescence *in situ* hybridisation with whole chromosome painting probes appears to be especially useful for analysis of stable cytogenetic damage (translocations) as a very rapid and sensitive test, although, quite expensive.. Comparison of the dose-response curves for dicentrics analysed after conventional staining and translocations visualized by chromosome painting revealed similar frequencies of radiation-induced translocations and dicentrics. Other authors have also reported equal frequencies of dicentrics and translocations [11-13].

To investigate an association between molecular and mitotic damages detected after irradiation with californium source, we determined the Pearson Product Moment correlation coefficients (r^2). Results from the studies in the case of the percentage of aberrant cells and tail DNA expressed the $r^2 = 0.99$. The percentage of aberrant cells and SCGE Tail moment expressed the value of $r^2 = 0.95$. The dicentrics and ring frequency and tail DNA expressed the $r^2 = 0.98$, and the dicentrics and ring frequency and the tail moment expressed an $r^2 = 0.98$. In our previous [14] studies the comparison of the DNA damage in the Comet assay and cytogenetic damage induced by irradiation without chemical pretreatment revealed also very high correlation for the two types of damage as it was presented.

Results of statistical analysis of our data have elicited that pretreatment, with the BSH for 1 hour before the irradiation with Californium-252 source at a dose range 0 - 0.49 Gy, didn't altered significantly neither the DNA strand breakage, reported as Tail moment in SCGE assay, nor cytogenetic damages detected in the first and second cell divisions.

The effectiveness of boron neutron capture depends on the number of ^{10}B atoms delivered to the cells, the subcellular distribution of ^{10}B and the thermal neutron flux. According to Pöller et al. [15] the presence of 600ppm ^{10}B (boric acid) in the cell medium during irradiation with fast d(14)+Be neutrons (mean energy 5.8MeV) in a phantom enhances the DNA damage measured with SCGE assay by 20% compared with neutron irradiation alone. The lack or unclear effects of boron enhancement in our study might be due to the low concentration of ^{10}B in the lymphocytes (~ 20ppm) and too low impact of the thermal neutrons or too short time of incubation with boron enriched compound [16]. The further studies are needed to explain which factor is responsible for the lack or unclear effects of boron enhancement in our results.

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