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THE BIOLOGICAL EFFICIENCY OF THE PETTEN RESEARCH REACTOR BEAM ON HUMAN LYMPHOCYTES (METHODOLOGICAL APPROACH).

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ABSTRACT

In this paper we present preliminary results of examination of the biological efficiency of the Petten Research Reactor mixed beam with respect to 250 kV X–rays for the induction of DNA damage and chromosomal aberrations in human lymphocytes. Human blood samples or isolated lymphocytes were irradiated by the beam from Research Reactor in ECN Petten, Netherlands and dose response relationships for the level of damage induced were investigated. In order to check any enhancement effect due to the process of boron neutron capture, chemical pretreatment with boric acid or mercaptoborane (containing boron-10 ions) was done. The estimation of the DNA damage was done with the use of a single cell gel-electrophoresis method (SCGE), to asses the frequency of chromosomal aberrations culturing of lymphocytes for the evaluation of cytogenetic damage was performed. Abnormal behavior of blood samples during a culture procedure and abnormally low metaphases frequency was noticed. During the analysis of DNA damage by SCGE assay we have also found the abnormalities in shapes and brightness of investigated comets. Part of the studied lymphocytes was bigger than others and had much bigger fraction of the DNA in tail. Very poor dose response relationship was observed in those results. From this reason, our paper presents the methodological approach and discussion of the results obtained and also studies on the parameters reflecting the level of the DNA in human lymphocytes. In order to eliminate outstanding comets (fluffy) we measured for all our results the relation of the fraction of DNA in tail to the length of the comet tail. The value of this ratio usually fluctuated in range of 0.1 to 0.70. For the fluffy comets mentioned before the tDNA/TL ratio was generally about 0.9, or even more than 1.0 that means that the percentage of fraction of DNA in tail was higher than in usually seen comets with such a tail length. After analysis of distribution of frequency cells with various tDNA/TL ratio, we decided to establish 0.8 as cut off value for the filtration software to eliminate the outstanding results. Elimination of the fluffy comets corrected our results and let observe various dose response relationships for various treatments (X–rays, modified beam of reactor neutrons in normal and boron pretreated cells). Applied correction usually leaded to improvement of statistics. Our additional studies showed that appearance of outstanding comets is also observed as results of disturbance of suspension medium. We have achieved the similar fluffy comets studying with application of the Comet assay the influence of hypothonic solution in cell suspension on comets shape after electrophoresis.

INTRODUCTION:

Ostling and Johanson [1] introduced a model (SCGE) describing the formation of a comet structure from the DNA pieces after electrophoresis. After lysis and unwinding the DNA occupies the cavity in the gel that was formed by its cell, in the electric field the DNA strand breakage is able to migrate towards anode. The shape of a comet and the distribution of

fluorescence reflect the amount of migrated DNA and depend on the frequency of DNA breaks and its size. As a measure of DNA damage level we analyzed the length of the comet tail measured from the edge of the comet head, the fraction of DNA in tail and the third parameter depended from both mentioned above (Tail Moment).

MATERIALS AND METHODS.

Lymphocytes sampling and preparation

Blood sample collected by venepuncture from 28-year-old male donor was placed in sterile tubes with EDTA. The blood was divided to two parts, where in one part of sample the cytogenetic culture procedure was performed. From the other part of blood sample, lymphocytes were isolated for further studies according to the standard procedure [2,3].

In vitro treatment

Blood sample to cytogenetic studies was transferred to eppendorfs and divided into following study groups:

- Referent with BrdU
- Pretreatment with various concentrations of $H_3{}^{10}BO_3$ solution
- Irradiated by neutron beam
- Pretreatment with various concentrations of $H_3{}^{10}BO_3$ solution and irradiated by neutron beam
- Pretreatment with BSH solution and irradiated by neutron beam.

Isolated lymphocytes to DNA damage analysis was divided into following study groups:

- Isolated
- Pretreatment with various concentrations of $H_3{}^{10}BO_3$ solution
- Irradiated by various dose of neutron beam
- Pretreatment with various concentrations of $H_3{}^{10}BO_3$ solution and irradiated by neutron beam
- Irradiated by various doses of X-rays.

Boron treatment

To check any enhancement effect due to the process of boron neutron capture, chemical pretreatment with boric acid (initial $H_3{}^{10}BO_3$ concentration 6.62mg/ml PBS i.e. 100 ppm of boron-10) or mercaptoborane (BSH; initial $Na_2{}^{10}B_{12}H_{11}SH$ concentration 8.75mg/ml in H_2O i.e. 5000 ppm of boron-10) was done to introduce boron-10 ion into cells. Final concentrations of boron-10 in samples with cells were 5, 10, 15, 25 ppm in boric acid and 50 ppm in BSH.

Irradiation

Mixed beam from LRF reactor in Petten

Eppendorf vials containing blood sample or isolated lymphocytes resuspended in PBS (untreated or pretreated earlier with various concentration of boric acid or BSH), were

irradiated with special neutron beam constructed for studies of efficiency of BNC procedure (γ-rays, fast and epithermal neutrons) in the Petten Research LRF Reactor.
The length of irradiation and total dose estimated for lymphocytes without any B-10 pretreatment were as follow:

Time of irradiation (min.)	Total dose (Gy)
12	0.4
18	0.6
30	1.0
60	2.0

For studies an influence of BNC reaction on biological effect blood samples and isolated lymphocytes pretreated with various concentration of ^{10}B were irradiated with the mixed beam dose of 0.4 Gy that was achieved at 6.0 min time of irradiation.

X-rays

The X -ray exposures were carried out in ECN Petten using X-rays machine at 300 kV and 10 mA. Lymphocytes suspended in cold PBS were irradiated at a dose rate of 0.6 Gy/min in room temperature. After irradiation with the total doses 0.5, 1.0, 1.5, 2.0 Gy samples were kept in room temperature for 30 min (adequate to neutron beam irradiation).

Culture procedure

After irradiation the standard culture procedure was performed [4,5]. Blood (1.4 ml) from a donor was incubated at 37°C using the RPMI 1640 medium supplemented with 20% fetal serum and antibiotics. One culture was incubated with addition BrdU (5-bromo-2-deoxyuridine). Lymphocytes were stimulated with phytohemagglutinin (LF-7). Cultures were incubated for 48h. Two hours before the end of culturing, 0,1μl/ml colcemid solution was added to each culture. Fixation and staining were performed by standard cytological methods [4].

DNA damage analysis (SCGE)

To estimate the level of DNA damage, the alkaline version of single cell gel electrophoresis technique also known as a Comet assay was applied. All chemicals and procedure of slide preparation were used according to standard procedures described elsewhere [2,3,6]. The slides were immersed in cold lysing solution (2,5M NaCl, 100mM EDTA, 1%, sodium sarcosinate, 100 mM TRIS) with 10% DMSO and 1% Triton X-100 added immediately before use. After lysis**,** the slides were transferred to the electrophoretic apparatus and immersed in freshly made, cold electrophoretic buffer (1mM EDTA, 300mM NaOH) of pH=13. After 20 minutes of DNA unwinding, electrophoresis was carried out at 8°C at 26V, 300mA, for 30min. Then the slides were washed in 0,4 M buffer Tris of pH 7.5 (3 times for 5 minutes). All the stages, from lysis to the neutralisation of slides, were conducted in the dark in order to avoid any additional damage caused by light. After neutralisation, the slides were placed in a light-proof box and kept moist in PBS and transported to the Laboratory of the Department of Radiation and Environmental Biology, Institute of Nuclear Physics, where they were stained with 60μl of ethidinum bromide (17μg/ml).

DNA damage analysis

The slides were analysed by means of the epifluorescence microscope Olympus BX-50 (100W mercuric lamp, excitation filter 515-560 nm, barrier filter from 590 nm) connected with the CCD camera. For the analysis of the comet pictures, the Komet 3.0 program from the Kinetic Imaging Company (Liverpool, UK) was applied.
To estimate the DNA damage, three parameters from this software were used:

- **TL** - tail length (length of the comet tail),
- **tDNA**- tail DNA (DNA percentage in the comet tail),
- **TM** - tail moment (fraction of DNA in the tail multiplied by the tail length).

Statistics

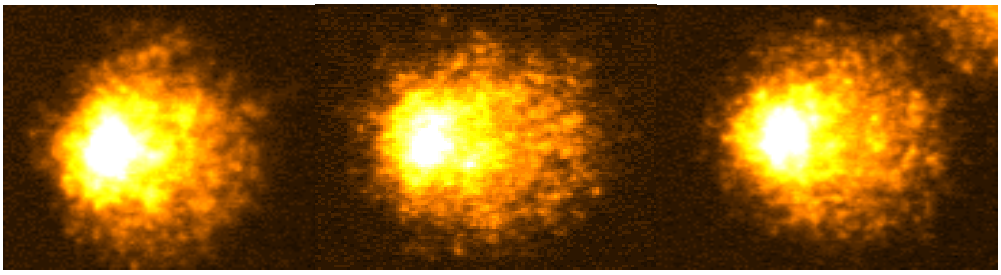
Komet 3.0 Kinetic IMG calculated the mean values of Tail Length, Tail Moment, Tail DNA. Statistical analysis was performed using Microsoft Excel for Windows.

RESULTS

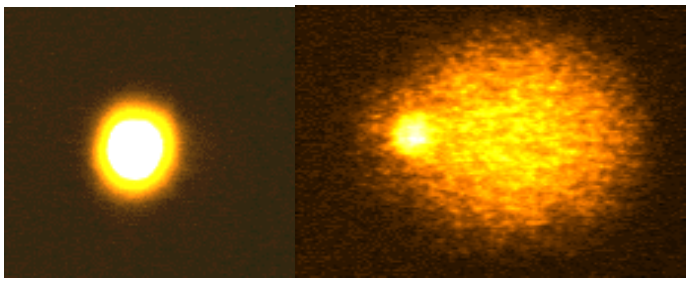
Abnormal behavior of blood samples during the cytogenetic culture procedure and abnormally low metaphases frequency was found. No metaphase were found in 60% of cultures and only 40% of cultures resulted in a very few metaphases spreads, this made analysis of dose response relationship impossible.

During analysis of slides with results of electrophoresis we founded the abnormalities both in comets shapes. Part of the studied lymphocytes was bigger than others and had much bigger fraction of the DNA in tail (picture 1a). From this reason, our paper presents the methodological approach to the results of studies on the parameters reflecting the level of the DNA in human lymphocytes.

(a)



(b)



Picture 1. The fluffy comets (a) with the abnormal distribution of DNA in comet tail and very short tail length comparing to natural shape of non-damaged and high-damaged cell after electrophoresis (b).

In normal, regular comets usually percentage of DNA fraction in the tail is usually increasing with the comet tail length. In order to eliminate fluffy comets we measured for all our results the relation of the fraction of DNA in tail to the length of the comet tail. Table 1 presents the results of the tDNA/TL ratio calculated for X-rays irradiated lymphocytes of various healthy donors in our previous study [7]. The mean values of tDNA/TL ratio for damaged cells were at range of 0.23 to 0.37. In at least 98% of X-rays induced comets their ratio fluctuated at range of 0.06 to 0.7.

Table 1. The mean values and ranges of tDNA/TL ratio for X-rays irradiated lymphocytes of various healthy donors in our previous experiment [7].

Number of cells	Treatment	Mean tDNA/TL ± SD	Range of tDNA/TL ratio	Cell %	Range of tDNA/TL ratio	Cell %
150	X-rays 1.5 Gy	0.23±0.13	0.03 – 0.60	100	-	-
150	X-rays 2 Gy	0.29±0.15	0.06 – 0.70	99.3	0.70-0.73	0.7
150	X-rays 2 Gy	0.32±0.14	0.08 – 0.66	100	-	-
150	X-rays 2 Gy	0.29±0.16	0.06 – 0.70	98	0.70-0.88	2
150	X-rays 2 Gy	0.37±0.12	0.14 –0.68	100	-	-

Table 2 presents the results of the tDNA/TL ratio calculated for cells after various treatments in the Petten experiment. The mean value of this ratio changes between 0.44-0.87. Their ratio fluctuated at range 0.01-1.71. The percentage fraction of cells with the ratio higher than 0.7 was much higher and varied between 19 and 75.

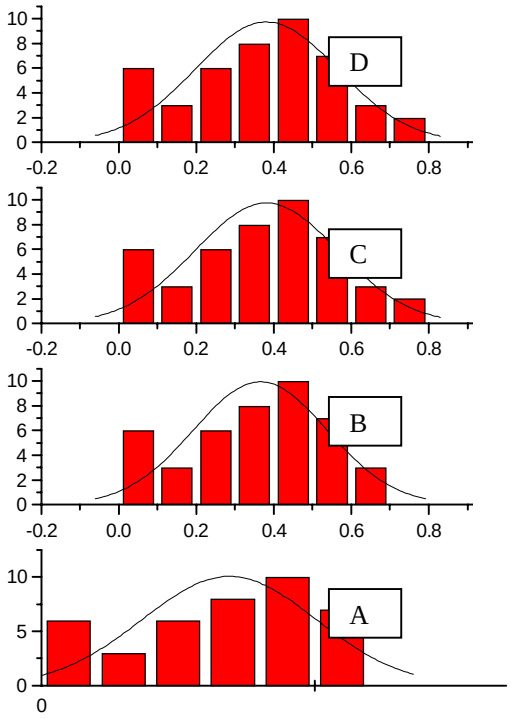
Table 2. The mean values and ranges of tDNA/TL ratio for lymphocytes non-irradiated, X-rays and neutron beam irradiated in the Petten experiment.

Number of cells	Treatment	Mean tDNA/TL ± SD	Range of tDNA/TL ratio	Cell %	Range of tDNA/TL ratio	Cell %
200	Control 5ppm B^{10}	0.44 ± 0.3	0.01-0.70	81	0.70-1.73	19
200	Control 10ppm B^{10}	0.46 ± 0.33	0.03-0.70	79	0.70-1.65	21
200	0.4 Gy Neutrons	0.67 ± 0.29	0.03-0.70	47	0.70-1.41	53
200	0.6 Gy Neutrons	0.55 ± 0.39	0.02-0.70	66	0.70-1.71	36
200	2 Gy Neutrons	0.58 ± 0.35	0.06-0.70	69	0.70-1.65	31
200	1.5 Gy X-rays	0.80 ± 0.26	0.26-0.70	39	0.70-1.61	61
200	2 Gy X-rays	0.87 ± 0.21	0.42-0.70	25	0.70-1.60	75

For the fluffy comets mentioned before the tDNA/TL ratio is about 0.9, or even more than 1.0 which means that the percentage of DNA in tail is about two or three times higher than generally observed in normal or treated cells (Table 1).

The distribution of the frequency of comets with various tDNA/TL ratio for the cut off conditions changing at range of 0.6 to 0.9 presents Figure 1(A-D respectively).

The visible difference for distribution shape can be observed at Figures 1B and 1C (for the ratio cut off established at 0.7 and 0.8 respectively). This is in agreement with distribution observed for normal comets. The range of observed mean value of this ratio ± 3SD is 0.62 to 0.77 (Table 1). For the higher cut off conditions, shape of the curve describing the ratio frequency distribution did not change significantly. We decided to establish 0.8 as cut off value for the filtration software.



Range	A 0.6	B 0.7	C 0.8	D 0.9
0-0.05	6	6	6	6
0.05-0.15	3	3	3	3
0.15-0.25	6	6	6	6
0.25-0.35	8	8	8	8
0.35-0.45	10	10	10	10
0.45-0.55	7	7	7	7
0.55-0.65	0	3	3	3
0.65-0.75	0	0	2	2
0.75-0.85	0	0	0	0

Figure 1. The distribution of the tDNA/TL ratio frequency for the cut off conditions changing at range of 0.6 (A) to 0.9 (D)

Table 3 presents results of dose response relationship for the level of DNA damage induced by mixed reactor beam. The level of the DNA damage is expressed as a comet: tail moment, tail DNA and tail length initially measured (i) and after correction of data by separation of fluffy comets (m). Figure 2 presents the dose-response curves of the tail moment and tail DNA parameters after irradiation by mixed reactor beam.

Chemical pretreatment with boric acid was done to introduce boron-10 ions into cells in order to check any enhancement effect due to the boron neutron capture process. Tables 4 and 5 present the influence of boric acid containing B-10 on the level of the DNA damage in non-irradiated and irradiated by 0.4 Gy of neutron beam, respectively. There is seen an increasing level of DNA damage with increasing concentration of B-10 in non-irradiated cells, however we haven't found any enhancement effect of boron neutron capture process when lymphocytes were irradiated by 0.4 Gy of neutron beam. Results of the initial (i) and modified (m) DNA damage after various doses irradiation of human lymphocytes with X-rays are presented in Table 6.

Table 3. The dose response relationship for the initial (i) and corrected (m) DNA damage level induced by neutron beam (outstanding results are presented in gray fields).

Dose of mixed neutron beam	TM±SE (i)	tDNA±SE (i)	TL±SE (i)	TM ±SE (m)	tDNA±SE (m)	TL±SE (m)
Referent	1.9±0.3	6.6±0.7	23.8±1.2	1.8 ± 0.3	5.9 ± 0.6	24.1 ± 1.3
0.6 Gy	5.7±0.4	16.7±1.0	29.0±0.8	3.8 ± 0.4	10.9 ± 0.7	28.3 ± 1.0
1.0 Gy	11.8±0.6	22.1±0.9	43.7±0.9	10.5 ± 0.6	19.5 ± 0.8	43.4 ± 0.9
2.0 Gy	7.1±0.6	19.1±1.1	30.9±0.9	4.3 ± 0.4	12.5 ± 0.7	28.9 ± 1.0

Table 4. The influence of boric acid pretreatment on the level of the initial (i) and modified (m) DNA damage in non-irradiated lymphocytes.

	TM±SE (i)	tDNA±SE (i)	TL±SE (i)	TM ±SE (m)	tDNA±SE (m)	TL±SE (m)
5ppm B-10	6.6±0.6	15.7±1.0	32.0±1.0	4.4 ± 0.5	11.5 ± 0.7	30.2 ± 1.0
10 ppm B-10	6.5±0.7	15.5±1.1	30.5±1.0	4.4 ± 0.6	11.2 ± 0.8	29.2 ± 1.1
15 ppm B-10	9.6±0.7	20.9±1.2	35.9±1.1	6.8 ± 0.6	15.6 ± 0.9	33.5 ± 1.2
25 ppm B-10	11.3±0.8	24.3±1.3	38.2±1.1	7.7 ± 0.7	17.2 ± 1.0	36.0 ± 1.2

Table 5. The influence of boric acid pretreatment on the level of the initial (i) and modified (m) DNA damage in lymphocytes irradiated with 0.4 Gy of neutron beam .

0.4 Gy+B-10	TM±SE (i)	tDNA±SE (i)	TL±SE (i)	TM ±SE (m)	tDNA±SE (m)	TL±SE (m)
10 ppm B-10	9.1±0.7	21.6±1.1	34.7±1.1	8.4 ± 0.8	20.2 ± 1.2	34.1 ± 1.3
15 ppm B-10	9.1±1.0	23.3±2.0	34.0±1.5	9.1 ± 1.0	23.3 ± 2.0	34.0 ± 1.6
25 ppm B-10	13.6±1.0	24.8±1.2	43.9±1.4	12.2 ± 1.1	21.5 ± 1.2	44.4 ± 1.6

Table 6. The dose response relationship for the initial (i) and modified (m) DNA damage induced by X-rays in human lymphocytes.

X –rays dose	TM±SE (i)	tDNA±SE (i)	TL±SE (i)	TM ±SE (m)	tDNA±SE (m)	TL±SE (m)
0.5 Gy	5.1±0.6	12.5±1.0	28.3±1.1	3.9±0.5	9.8±1.2	27.6±1.2
1.0 Gy	4.7±0.5	12.2±1.0	26.7±1.1	3.4±0.4	9.2±0.7	25.6±1.1
1.5 Gy	28.1±1.0	46.1±1.1	58.5±1.1	24.6±1.5	37.0±1.4	61.6±1.7
2.0 Gy	28.5±0.9	48.4±0.8	57.2±1.0	27.9±1.5	41.7±1.2	63.7±1.8

DISCUSSION:

Based on our previous study [8] after neutron beam treatment we were expecting an increasing DNA damage with a linear dose-response relationship. The analysis of our results revealed disturbances at the DNA damage level after irradiation by 0.4 and 2.0 Gy of neutron beam. After separation of two outstanding results we achieved a liner-polynomial dose response relationship with visible saturation (Fig. 2). This may suggest the changes in the biochemical environment of irradiated cells.

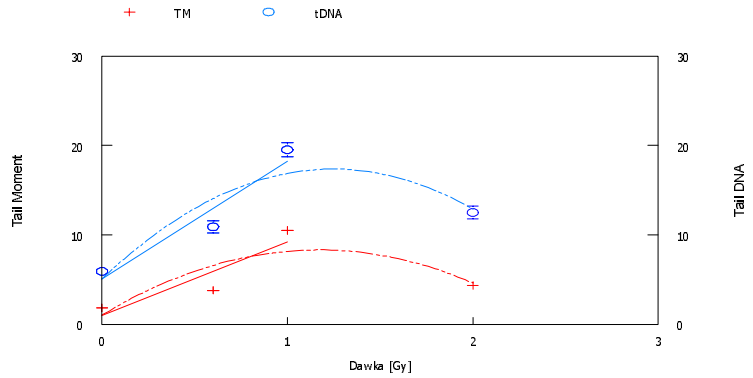


Fig.2 The dose response relationship for the DNA damage level after neutron beam irradiation. The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

Polynomial fit:

$Y(TM)= (-5.33 \pm 4.05) D^2 + (12.47 \pm 8.78) D + (1.02 \pm 3.65)$ $(r^2)=0.67$

$Y(tDNA)= (-7.91 \pm 4.58) D^2 + (19.74 \pm 9.92) D + (5.05 \pm 4.13)$ $(r^2)=0.86$

Linear fit at dose range 0-1Gy:

$Y(TM)= (9.62\pm 2.44) D + (1.34\pm1.64)$ $(r^2)=0.94$

$Y(tDNA)= (15.61\pm 0.91) D + (6.81\pm 0.61)$ $(r^2)=0.997$

Figures 3a and 3b present the dose response relationships for the initial and modified by elimination of fluffy comets DNA damage level after irradiation by X-rays. In agreement with our previous study we achieved a linear-quadratic dose response relationships. Application of tDNA/TL ratio cut off decreased the level of DNA damage and increased correlation coefficients. Figures 4a and 4b present the influence of various concentration of boron-10 in boric acid on the level of initial and modified DNA damage in non-irradiated human lymphocytes. There is seen an increasing level of DNA damage with increasing concentration of B-10. Application of tDNA/TL ratio cut off decreased the level of DNA damage but improved correlation coefficient only in case of tail moment parameter.

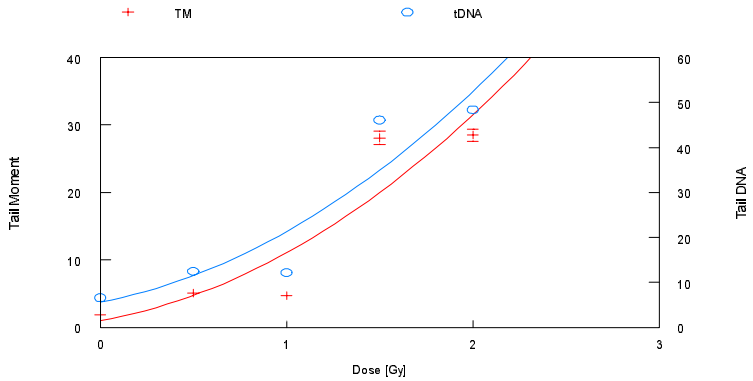


Fig.3a The dose response relationship for the DNA damage level after X-ray irradiation (initial data).

The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

$Y(TM)= (5.2 \pm 8.15)\ D^2 + (4.85 \pm 17.0)\ D + (1.01 \pm 7.15)$ $(r^2)=0.84$

$Y(tDNA)= (7.71 \pm 11.35)\ D^2 + (8.01 \pm 23.68)\ D + (5.58 \pm 10.0)$ $(r^2)=0.86$

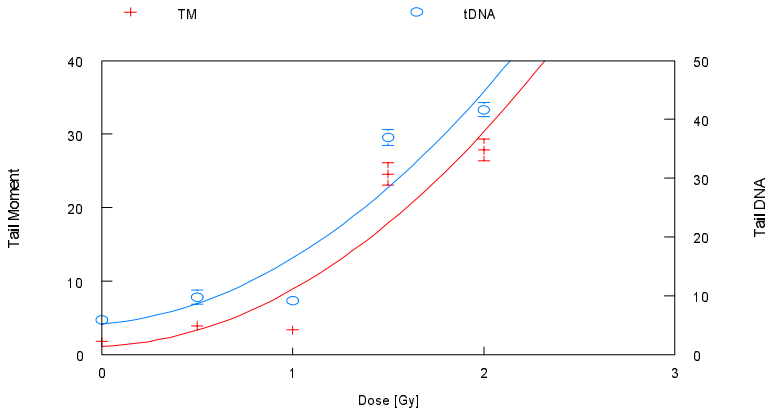


Fig.3b The dose response relationship for the DNA damage level after X-ray irradiation (modified data). The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

$Y(TM)= (6.85 \pm 6.86)\ D^2 + (0.86 \pm 14.31)\ D + (1.18 \pm 6.04)$ $(r^2)=0.87$

$Y(tDNA)=(8.57 \pm 8.82)\ D^2 + (2.59 \pm 18.39)\ D + (5.27 \pm 7.76)$ $(r^2)=0.88$

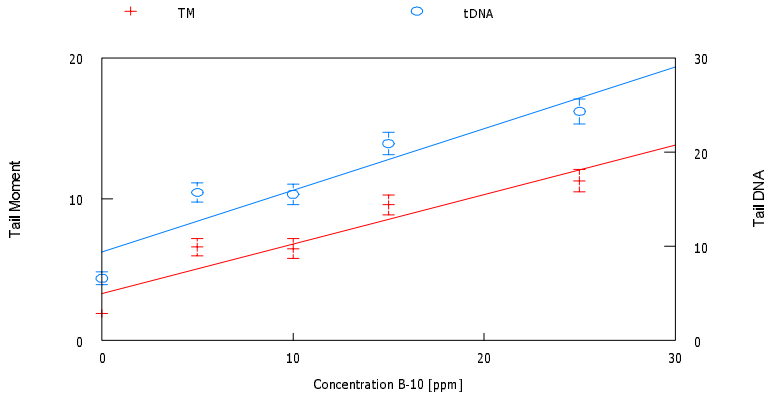


Fig.4a. DNA damage detected in lymphocytes pretreated with various concentration of boron-10 (initial data). The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

$Y(TM)= (0.35 \pm 0.07)\ D + (3.32 \pm 1.04)$ $(r^2)=0.88$

$Y(tDNA)= (0.65 \pm 0.14)\ D + (9.42 \pm 1.97)$ $(r^2)=0.88$

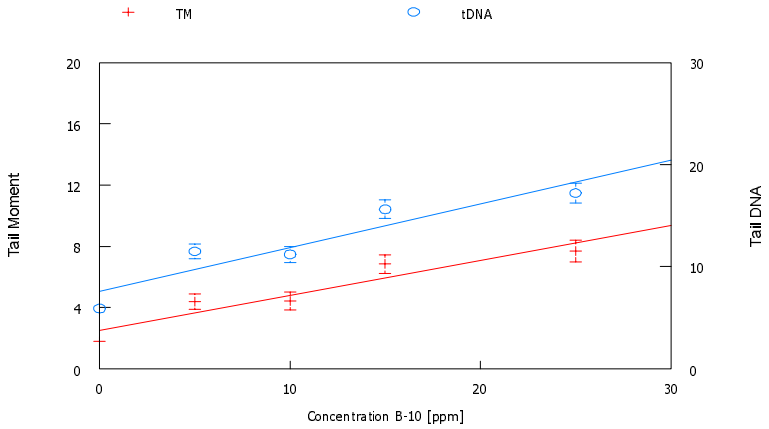


Fig.4b. DNA damage detected in lymphocytes pretreated with various concentration of boron-10 (modified data). The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

$$Y(TM)= (0.23\pm 0.05)\ D + (2.52\pm0.63) \qquad (r^2)=0.90$$
$$Y(tDNA)= (0.43\pm 0.10)\ D + (7.58\pm1.34) \qquad (r^2)=0.87$$

Results presented on Figures 4a and 4b show that, in spite of the problem with fluffy comets, in both cases without and with correction of the data, there is seen increase of the DNA damage detected in cells pretreated with boron containing chemical. The increase observed is proportional to increasing concentration of boron-10. As the beam that are modified for BNC reaction usually contain a lot of γ-rays, and much less fast and epithermal neutrons that normal fission spectrum [9], this increase looks promising.

In order to find the origin of fluffy comets we have performed some explaining experiments. Wondering about the origin of the outstanding comets, we stroke upon an idea that the specific shape of the comet may issue from the various hypothonic solution. In order to confirm that idea we investigated an influence of hypothonic solution in various concentration on RPMI medium on comets shape after electrophoresis. Using the Comet assay, we proved that various hyphotonic conditions (80, 60, 50 % consistence of RPMI 1640 in distillated H_2O) in cell suspension might affect on comets shape. In twice diluted cell suspension we have found the image of fluffy cell after electrophoresis. It suggests that some changes in experimental conditions may affect on comets shape however, that fact still didn't explain the origin of artifacts in our experiment. One of the agents that could be potentially responsible for fluffy comets is activation of immunology system caused by the blood donor's flu. From this reason we performed experiment using X-rays on stimulated human lymphocytes (Figure 5).

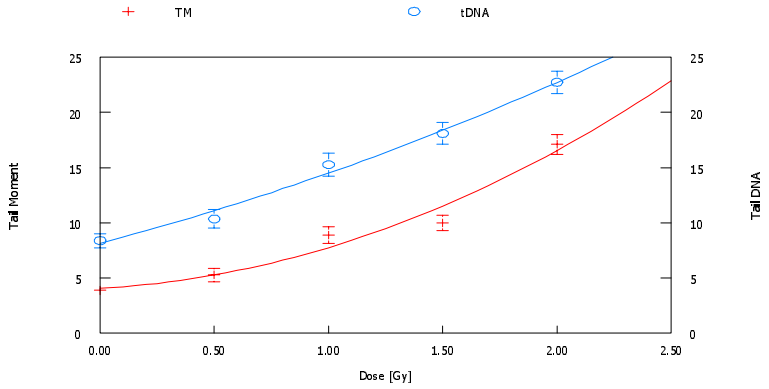


Fig.5 The dose response relationship for the DNA damage level after X-ray irradiation of stimulated human lymphocytes.

The equations of the fitted curves with standard errors and correlation coefficients (r^2) are presented below:

$$Y(TM)= (2.55\pm 1.50)\ D^2 + (1.14\pm3.13)\ D + (4.07\pm1.32) \qquad (r^2)=0.90$$

$$Y(tDNA)= (0.93\pm 0.86)\ D^2 + (5.43\ \pm1.78)\ D + (8.15\pm0.75) \qquad (r^2)=0.99$$

Experiment, which was performed, in order to explain the appearance of fluffy comets didn't show any similar response of X-rays irradiated lymphocytes to those observed in Petten. We tried to find whether differences in pH or ionicity of chemicals used during experimental work leaded to artifacts (fluffy comets, spoiled cultures). We also examined an influence of different temperatures (4, 25, 37^{o}C) during incubation on comets shape. Performed experiments (non published data) showed that neither disturbances in biochemical environment of irradiated cells during experiment nor various temperatures during incubation did not affect on the nucleus structure and arising of artifacts. So, anyhow further studies are needed to find out the mechanism leading to the appearance of fluffy comets. However we think that filtration based on analysis of the ratio distribution improved our results.

Acknowledgments:
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REFERENCES

[1] O. Ostling, K.J. Johanson. Microelectrophoretic study of radiation-induced DNA damage in individual mammalian cells. Biochem Biophys Res Commun 123 (1984) 291-298.

[2] A. Cebulska-Wasilewska, W. Niedzwiedz, D. Nowak, R. Pawlinski. Hydrogen peroxide and radiation induced DNA damage investigated by the Comet assay. Nukleonika 41 (2) (1996) 33-42.

[3]D. Anderson, T.W. Yu, B.J. Philips, P. Schmezer. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the Comet assay. Mutat. Res. 307 (1994) 261-271.

[4] D. Anderson, J. A. Hughes, A. Cebulska-Wasilewska, A. Wierzewska, E. Kasper. Biological Monitoring of Workers Exposed to Emissions from Petroleum Plants. Env. Health Persp. (1996) vol.104; 609-613.

[5] A. Cebulska-Wasilewska, Z. Drąg, E. Kasper, W. Dyga, A. Wierzewska. Monitoring of genotoxic effects in lymphocytes from people exposed to pesticides (polish group). Report INP, No 1831/B (1999) 49-60.
[6] W. Dyga, W. Niedzwiedz, A. Cebulska-Wasilewska. Repair of the DNA damage induced in UV-irradiated human lymphocytes. Polish J. of Med. Phys., vol.5, no.3 (17) (1999) 117-127.
[7] W. Dyga, A. Cebulska-Wasilewska. Variability in repair of the DNA damage induced in human lymphocytes by X-rays (preliminary data). Neoplazma 46(1999) 47-49.
[8] D.Florjan, W. Niedzwiedz, K. Schneider, A. Kreft and A. Cebulska-Wasilewska Application of SCGE assay, classical cytogenetics and FISH for studying *in vitro* californium-252 neutrons irradiations and BSH pretreatment on human lymphocytes. Report INP, No 1831/B (1999) 61-74.
[9] A. Cebulska-Wasilewska, K.Schneider, J. Capała, J. Coderre. Biological efficiency of the Brookhaven Medical Research Reactor mixed neutron beam estimated from gene mutations in Tradescantia stamen hair cells assay. Nukleonika 45 (2000); 115-119