



Micronuclei versus Chromosomal Aberrations Induced by X-Ray in Radiosensitive Mammalian Cells

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Abstract

Background: An experimental study was accomplished to compare estimation methods of ionizing radiations genotoxicity in mammalian cell cultures by means of two cytogenetic parameters with focus on aberrant cells characterized by multiple chromosomal damages.

Methods: In vitro study was carried out on the genotoxicity of low-medium doses of 190 kV X-rays absorbed in Chinese hamster ovary cell cultures. Micronuclei and ten types of chromosomal aberrations were identified with Giemsa dyeing and optical microscope screening.

Results: The first parameter consisting in micronuclei relative frequency has led to higher linear correlation coefficient than the second one consistent with chromosomal aberrations relative frequency. However, the latter parameter estimated as the sum of all chromosomal aberrations appeared to be more sensitive to radiation dose increasing in the studied dose range, from 0 to 3 Gy. The number of micronuclei occurring simultaneously in a single cell was not higher than 3, while the number of chromosomal aberrations observed in the same cell reached the value of 5 for doses over 1 Gy.

Conclusion: Polynomial dose-response curves were evidenced for cells with N_i micronuclei ($i=1,3$) while non-monotonic curves were evidenced through detailed analysis of aberrant cells with N_i chromosomal changes ($i = \overline{1,5}$) - in concordance with *in vitro* studies from literature. The investigation could be important for public health issues where micronucleus screening is routinely applied but also for research purposes where various chromosomal aberrations could be of particular interest.

Keywords: X-ray genotoxicity, Mammalian ovary cells, Multiple chromosomal changes

Introduction

Chromosomal aberrations from human lymphocytes are used for decades as cytogenetic biomarkers in the study of genetic risk induced by different genotoxic factors – external constraints representing either physical or chemical gradients (1). The results of epidemiological studies have sustained the suitability of chromosomal aberrations utilization as cytogenetic tool in the investigation of health status of human population lots (2). The array of chromosomal aberrations types is

rather large and requires special attention and diversified techniques. The micronuclei formation is the consequence of some chromosome breaking and encapsulation in membrane like coatings making them easier to identify - which resulted in largely spreading of micronuclei test for genotoxicity evaluation.

In another report (3) the authors have carried out lymphocyte screening for chromosomal aberrations as well as for micronuclei in hospital workers,

professionally exposed to ionizing radiation in comparison with non-exposed people; the frequency ratio was of about 3:2 in radiation-exposed group compared to control one for both chromosomal aberrations and micronuclei test. In (4) chromatid breaks as well as dicentric chromosomes were reported following analysis of peripheral lymphocytes from hospital radiation professionals. Researchers attempted to check if micronucleus-counting method is suitable in cancer incidence detection or in the assessing of consequent lethal effects but this were not successfully since no dose-response correlation was found (1). The human micronucleus (HUMN) project launched in 1997 (5), paid high attention to micronuclei base-line identification in large human populations by careful study of many factors susceptible of influencing that important cytogenetic parameter; the main result was the notification of higher micronuclei frequency in non-exposed women lot compared with men from the same age group. Good statistical correlation was found between radiation survival relationship and the curve of micronuclei increase (6) that sustained micronuclei test suitability in radiosensitivity assessing. In case of radioactive risk estimation in large-scale radiation events automatic rapid method of micronuclei counting was found to have equivalent accuracy with manual microscopic triage (7, 8); dicentric chromosomes, ring chromosomes and micronuclei incidences were found similarly sensitive to the detection of absorbed dose bioeffects (9). Although in public health practice micronuclei are known as specific cellular markers of radiation exposure effects, some putative genotoxic chemicals— such as pesticides - were found to induce undoubtable increase in human lymphocyte micronuclei frequency (10). Circulating lymphocytes with micronuclei in hospital workers manipulating chemiotherapeutic substances were reported in (11) while microwave professional exposure was also identified as source of in vivo micronuclei formation according to (12). Chromosomal aberrations and micronuclei induced by microwaves were discussed in (13) based on in vitro studies of human lymphocytes cul-

tures; UV radiation induced micronuclei and chromosomal aberrations were reported in (14) while capacity of inducing such genetic disorders of chemicals/particulate matter complexes were analyzed in the case of smokers by the authors of (15) and in the case of traffic pollutants that were found responsible for DNA damage in sensitive plants (16).

In the investigation presented below the authors carried out parallel screening of micronuclei and chromosomal aberrations with focus on aberrant cells characterized by more than one cytogenetic modification due to radiation genotoxicity.

Methods

Biological material

Radiosensitive cell cultures chosen for the investigation were developed from ovary tissues of Chinese hamster characterized by relatively small number of chromosomes (i.e. 22 chromosomes) that made them convenient for qualitative and quantitative investigation of DNA changes induced by controlled radiation exposure.

Radiation exposure

Five doses, between 0 and 3 Gy were adjusted for exposure to 190 kV X-ray. For each radiation dose two sample arrays were arranged to identify chromosomal aberrations and micronuclei so that counting was carried out on ten cell culture samples.

Cell culture investigation

Following radiation exposure cell samples planned for micronuclei analysis were treated with 0.14 M KCl hypotonic solutions while 0.075 M KCl solution was used to treat samples intended for chromosomal aberrations study. Fixation of induced cytogenetic changes was accomplished using two reagents respectively 12:13:3 methanol:0.9%NaCl:acetic acid and 4:1 acetic acid:methanol in the case of micronuclei investigation while 3:1 methanol:acetic acid was the fixation reagent used for chromosomal aberrations revealing. Giemsa method of tissue sample dying (17) was applied to

the cell suspensions as prepared by dropping onto glass slides of light microscope device with 400× magnitude.

Over 2,200 metaphase cells were screened for micronuclei presence for each irradiation dose and control (non-irradiated sample), which resulted in the cell sorting according to the counted number of micronuclei per cell: 0; 1; 2; 3. For each dose and control 1,500-2,000 cells were carefully analyzed for ten types of chromosomal aberrations detection.

Results

The results of micronuclei and chromosomal aberrations counting are presented in the next four graphs.

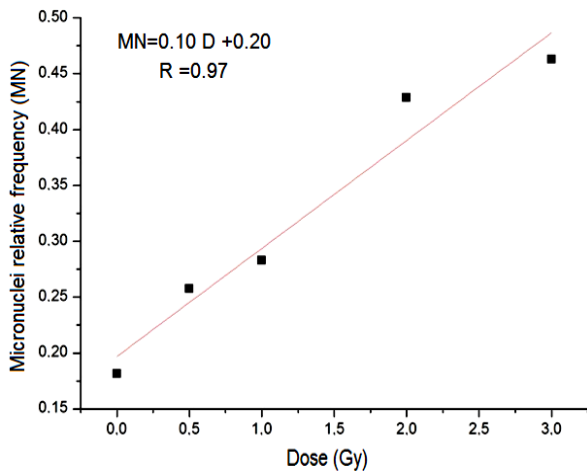


Fig. 1: Dose-response curve for micronuclei

In Fig. 1 the micronuclei relative frequency (MN), calculated with relation [1] was given as function of the radiation dose:

$$MN = \frac{1 \times N_1 + 2 \times N_2 + 3 \times N_3}{N}, \quad [1]$$

where N_1 , N_2 and N_3 are the numbers of cells presenting 1; 2; respectively 3 micronuclei while N is the total number of analyzed cells: $N = N_0 + N_1 + N_2 + N_3$, with N_0 representing the number of cells missing micronuclei.

The dose-response curve of induced micronuclei is a linear one in the range of 0-3Gy absorbed

doses. Compared with the control, non-irradiated samples, about three times increase of micronuclei relative frequency was noticed for largest radiation dose of 3 Gy.

In Fig. 2 it could be seen in more details that the micronuclei incidence increased for each of the three considered cases in contrast with cells missing micronuclei – that decreased correspondingly as expected. One can observe that, indeed, the frequency of cells with single induced micronucleus has increased 2.5 times - from 10 (in control, non-irradiated samples) to 25 (in 3.0 Gy irradiated samples), while the frequency of two micronuclei cells increased from 5.0 to 15 (i.e. 3 times); also the cell number with three micronuclei has increased from 2.3 to 7.0 (i.e 3 times approximately).

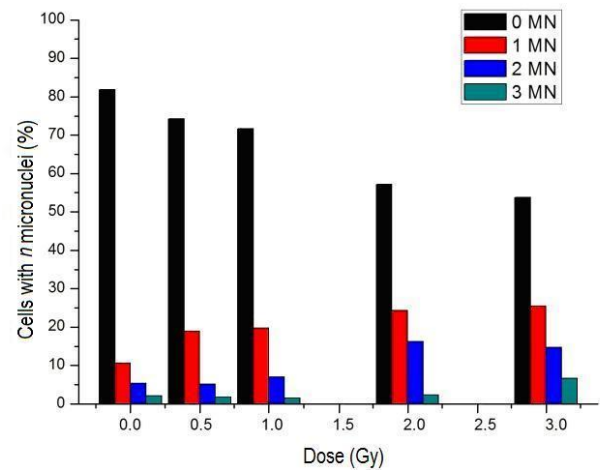


Fig. 2: Frequency of micronuclei, MN (0, 1, 2, respectively 3 micronuclei) versus absorbed dose, D

The chromosomal aberrations screening resulted in two other graphical plots (Figs. 3-4). The relative frequency of chromosomal aberrations was calculated with relation [2]:

$$CA = \frac{B+b+dic+R+M+m+IE+SU+G+g}{N} \quad [2]$$

where N is the sum of all analyzed cells either normal or aberrant ones; the significance of the other symbols – expressing aberrant cell percentages - is as follows:

B - chromosome **B**reak – terminal part of irradiated chromosome is sectioned yielding a chromosomal fragment;
 b - chromatid **b**reak - one of chromosome chromatids experienced a disruption;
 dic- dicentric chromosome – two contact points between sister chromatids appear instead of one;
 R- **R**ing chromosome –chromosome edges are united generating a ring shaped structure;
 M- chromosome **M**inute – extrachromosomal DNA fragments - having „coma” shape (,) – similar to „minute” symbol;
 m- chromatid **m**inute – small fragment of chromatid DNA - a manifestation of gene amplification;
 IE- **I**nterchromosomal **E**xchange – involves the movement of chromosomal segment(s) between chromosomes;
 SU- **S**ister **U**nion - sister chromatids are physically bound and held together;
 G- chromosome **G**ap – disruption of chromosome edge that remains with the main chromosome part;
 g- chromatid **g**ap – disruption of one chromatid edge remaining further in the constitution of the such affected chromosome.

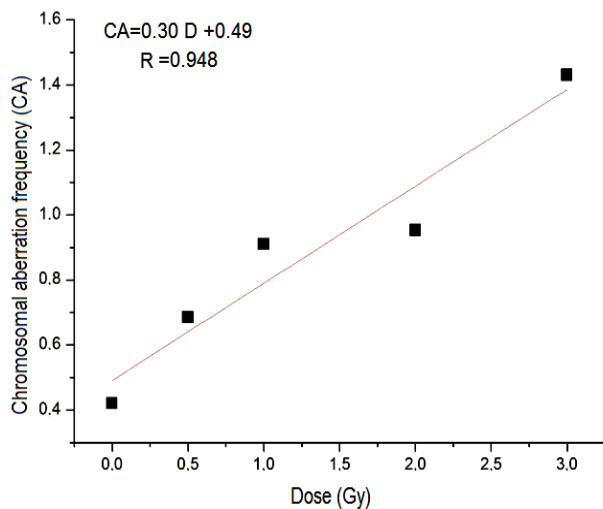


Fig. 3: Dose-response curve for chromosomal aberrations

In Fig. 3 the relative frequency of chromosomal aberrations versus radiation dose is presented. Linear dose-response was evidenced like in Fig. 1.

Compared with the control, four times increase of aberrant cell relative frequency was revealed for largest radiation dose of 3 Gy.

As in the case of micronuclei – where N_n with $n=1; 2; 3$ was represented on three distinct histograms (Fig. 4) also distinct counting was accomplished for abnormal dividing cells with multiple aberrations, i.e. $N_n=1; 2; 3; 4; 5$ since the quantitative aspect was considered to prevail over the qualitative one in this study. Mainly non-monotonous dependences of N_n on the radiation dose could be noticed – except for the normal dividing cells ($N_n=0$) – where monotonous diminution was evidenced to the dose increasing. The cell number with single chromosomal aberration (N_1) was doubled in 2 Gy sample compared to non-irradiated cells; six times increased number of cells with two chromosomal aberrations (N_2) and ten times increased number of cells with three aberrations (N_3) was counted for 3 Gy sample compared to control. For doses of 1 to 3 Gy also cells with four or five chromosomal aberrations were identified but neither N_4 nor N_5 were larger than 3%.

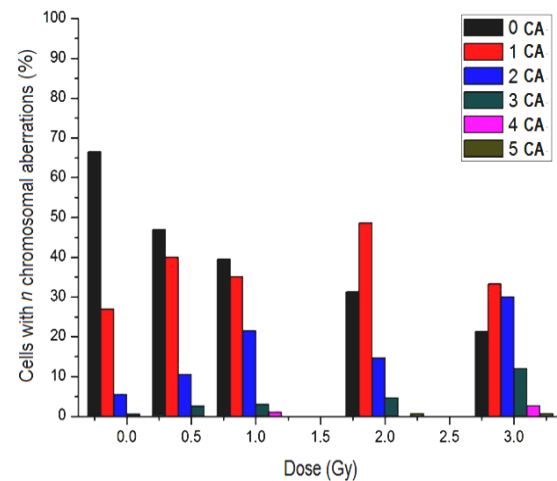


Fig. 4: Incidence of chromosomal aberrations, CA (0, 1, 2, 3, 4 respectively 5) versus the absorbed dose, D

Discussion

Nowadays screening of micronuclei in human lymphocytes was much developed aiming rapid imaging and automated counting for public health

evaluation in case of human professional exposure or nuclear accident. However the animal model remains an available approach especially when tissue samples could be withdrawn avoiding lethal consequences of laboratory study; also optical microscope and human eye accuracy still represents one of the best alternative in the field of radiobiology research due to high ability of searching for specific details in the complex images revealing the behavior of living cells under radiation impact. The radiation dose range that we have chosen was suggested by the results from (18) that have shown that the surviving fraction of human oocytes gave by LD_{50} (lethal dose killing 50%) was estimated to be $<2\text{Gy}$. In addition, the dose range of $0\text{-}3\text{Gy}$ was chosen in a recent study attempting to validate flow-cytometry estimation of micronuclei induced by radiation in the blood cells against standard biodosimetry using a mouse animal model (19).

First comparison between micronuclei and chromosomal aberration frequency could be done based on the linear regression parameters from Fig. 1 and Fig. 3.

Micronuclei dose-response (Fig. 1) has linear correlation coefficient of approximately 0.970 which is higher than that of the linear regression corresponding to chromosomal aberrations (Fig. 3), of 0.948. Higher slope and intercept values for chromosomal aberrations dose-response line than for micronuclei was revealed; higher slope (0.30 for chromosomal aberrations compared to 0.10 for micronuclei) could be related to higher sensitivity for applied radiation doses of the cytogenetic

parameter based on relative frequency of chromosomal aberrations induced in ovary cells compared with the parameter based on micronuclei frequency. Also higher intercept value (0.49 for chromosomal aberrations compared to 0.20 for micronuclei) was emphasized meaning larger number of cytogenetic changes classified as chromosomal aberrations compared to micronuclei occurred in the control, non-irradiated sample. As known external constraints either physical or chemical gradients could be responsible for spontaneous genetic changes mainly when biological factors impede repairs of DNA strand damages.

According to relation [1] and Fig. 2, as the weight of N_2 and N_3 are higher than for N_1 , the increase of MN (micronuclei relative frequency) highlights the fact that more intensive irradiation was able to induce genetically modified cells with 2 or 3 nuclei instead of one. At molecular level it means that in the DNA molecules double strand breakings occurred with higher probability for higher absorbed doses resulting in higher number of micronuclei per damaged cell.

Secondly the comparative study revealed differences between data presented in Fig. 2 and Fig. 4. The attempt to evidence dose-response curves of linear shape for each of the three cases illustrated in Fig 2 – cells with 1; 2 or 3 micronuclei, failed in the favor of different convexity polynomials $N_i(D)$ (D being the radiation dose) with relatively high correlation coefficients as can be seen in Table 1. However, complementary values of normal dividing cell frequency (N_0) depend linearly on the dose with still higher correlation coefficient (0.986).

Table 1: Micronuclei induced as function of the radiation dose

Micronuclei/cell	Regression equation	Correlation coefficient
1	$N_1 = -2.007 D^2 + 10.549 D + 11.608$	0.975
2	$N_2 = -0.650 D^2 + 5.869 D + 3.822$	0.904
3	$N_3 = 1.294 D^2 - 2.416 D + 2.350$	0.975
0	$N_0 = -9.866 D + 80.72$	0.986

As up to five aberrations within the same cell were noticed, in Fig. 4 the n index was taken as $n = \overline{(1,5)}$. All over the sample array screened for chromosomal aberrations (Fig. 4) four or five

chromosomal aberrations appeared only for radiation doses $\geq 1\text{Gy}$, in contrast with micronuclei screening where for all doses we identified cells with 1, 2 and 3 micronuclei.

We also mention that the control samples presented the expected lowest level of chromosomal changes in comparison to irradiated samples. For chromosomal aberrations, mainly non-monotonic dose response dependences (Fig. 4) were observed (in contrast with the case of micronuclei frequency) with no evident mathematical correlation established. Only the complementary parameter representing the cells with normal divisions without chromosomal aberrations (Fig. 4) appears to decrease monotonic: $N_0 = -15.60D + 63.28$ with linear correlation coefficient $R = 0.967$. The decreasing slope (-15.60) indicates that for absorbed dose increasing, the diminution of cells without chromosomal aberrations was more rapid than of cells without micronuclei (where the slope was estimated to -9.86 according to Table 1).

Conclusion

The cytogenetic impact of X-ray doses of 0.5-3Gy on *in vitro* ovary cell cultures - recognized for their radiosensitivity, has revealed higher complexity DNA damages for doses over 1 Gy. From quantitative viewpoint this was suggested by non-linear dependence on the absorbed dose of cell percentages with 1; 2; 3 micronuclei as well as by non-monotonic dependence of cells with 1; 2; 3; 4; 5 chromosomal aberrations while qualitatively - by the presence of complex aberrations including more than 3 distinct types for doses higher than 1 Gy.

The chromosomal aberration frequency appeared to be more sensitive to radiation dose increasing in the range 0-3Gy as denoted by higher linear regression slope (0.4) compared to 0.1 in the case of micronuclei relative frequency, but in the same time, the micronuclei linear regression has highest correlation coefficient (0.970 compared to 0.948). In the future investigation mammalian cells with other radiosensitivity levels will be investigated for micronuclei versus chromosomal aberrations.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgement

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