ANALYSIS OF THE BIOLOGICAL RESPONSE IN CHO-K1 CELLS TO HIGH LET RADIATION*

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The impact of irradiated cells and their progeny on non-irradiated cells was investigated. The experimental set up with a horizontal heavy ion beam designed for radiobiological research at the Heavy Ion Laboratory of the University of Warsaw (HIL) was used. Chinese Hamster Ovary (CHO-K1) cells were irradiated in the dose range 0.1 Gy–4 Gy of high Linear Energy Transfer (LET) ^{12}C ions and X rays. To examine the bystander effect, irradiated and non-irradiated cells were co-cultured in special Petri dishes with inserts. The cells shared medium but could not touch each other. To assess the biological response in individual cells a micronucleus assay was performed.

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1. Introduction

The interaction of ionizing radiation with biological material is of great importance in the planning of radiotherapy and space radiation biology. It is essential to understand the relationship between exposure and biological response of cells. For many years a radiation-induced so-called "bystander

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effect" has been described in the literature [1]. It occurs in cells not directly hit by an ionizing track but which are influenced by signals from irradiated cells. The aim of our study was to examine biological response in Chinese Hamster Ovary (CHO-K1) cells co-cultured with cells irradiated with ^{12}C ions and X rays.

2. Materials and methods

2.1. Irradiation

The experimental set-up has been described previously [2] and, therefore, only a short review is presented. A ^{12}C ion beam from the Heavy Ion Laboratory of the University of Warsaw (HIL) was used to irradiate the biological samples under physiological conditions. Special Petri dishes designed for this experimental set-up were fastened to a movable sample holder, mounted on an x–y–z stepping motor with remote control. A silicon detector placed at 20° in the scattering chamber was used for on-line ion beam monitoring. The signals from the detector were counted in a fast programmable scaler. When the number of registered particles reached the preset value (defining the dose), a start signal was created and the stepping motor changed its position according to a planned route. The system allowed for homogeneous irradiation of all biological samples. A schematic view of the facility is shown in figure 1.

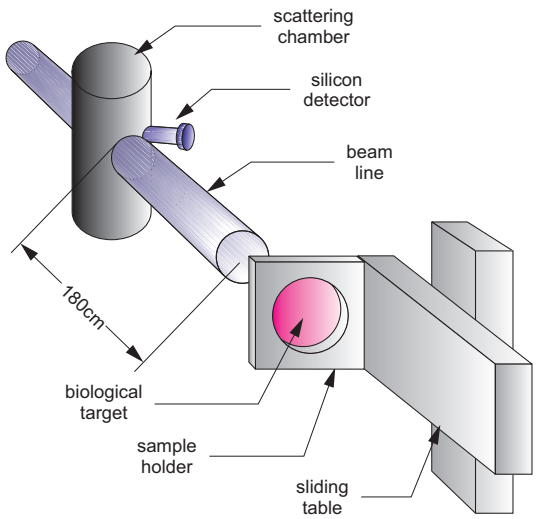

Fig. 1. Schematic view of the set-up for radiobiological studies with the horizontal beam line.

2.2. Cell line and culture conditions

Chinese Hamster Ovary cells (CHO-K1) were exposed to ionizing radiation of high Linear Energy Transfer (LET) ^{12}C ions and X rays. Since the dishes were irradiated in a vertical position to enable adhesion of the cells to the bottom, cells were seeded on the mylar bottom (polyester film) of a special Petri dish one day before irradiation.

In order to investigate the bystander effect in a co-culture of irradiated and non-irradiated cells, the irradiated cells were immediately transferred into transwell culture insert dishes. The scheme of a dish used in our study is shown in figure 2. The diameter of the pores in the insert membrane was 1 μm and, therefore, cells from the membrane and well shared the medium but could not touch each other. The cells were incubated in a humidified atmosphere at 37°C with 5% CO_2.

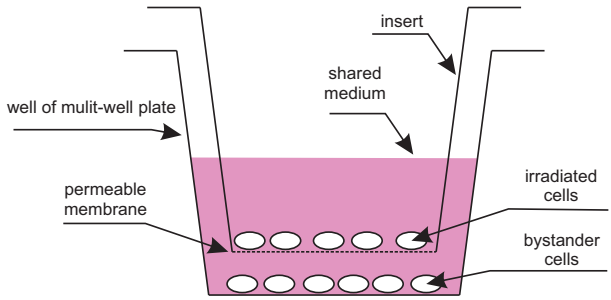

Fig. 2. Scheme of transwell culture insert dish with permeable membrane. In order to maintain clarity, the figure is not drawn in proportion.

2.3. Micronucleus assay

Micronucleus assay is a multi-target genotoxic endpoint, assessing not only clastogenic and aneugenic events but also some epigenetic effects, which is simple to score, accurate and applicable in different cell types [3]. Moreover, this assay is one of the preferred methods for assessing chromosome damage that can be caused by exposure to ionising radiation [4]. Micronuclei (MN) are expressed in dividing cells that either contain acentric chromosome fragments or whole chromosomes that are unable to travel to the spindle poles during mitosis. A special method that identifies cells that complete nuclear division by their binucleate appearance when blocked from performing cytokinesis by cytochalasin-B was used [5]. The scheme of MN formation is shown in figure 3.

Directly irradiated and bystander cells were plated at 125×10^3 cells/insert/well. One hour after irradiation, cytochalasin-B (85 μl/ml) was added into the cell culture medium and the cells were incubated for 24 hours in

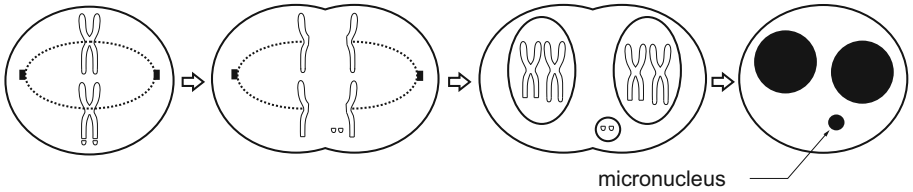

Fig. 3. Scheme of micronuclei formation in a cell to which cytochalasin-B was added.

a humidified atmosphere at 37°C with 5% CO_2. Then, cells were tripsinized and centrifuged at 1000 rpm for 8 min. Supernatant was discarded and 7 ml of cold hypotonic KCl (0.075 M) was added to allow cell swelling. The cells were centrifuged at 1000 rpm for 8 min. Supernatant was again discarded and 7 ml of methanol/acetic acid/ringer solution was added and stored overnight at 4°C. Following this procedure, cells were centrifuged at 1000 rpm for 10 min. Supernatant was again discarded and the fixation steps with cold methanol/acetic acid were repeated twice more. After that the cells were dropped on clean, dry slides. For fluorescence microscopy, cells were mounted and stained in Vectashield mounting medium containing DAPI (4',6-diamidyno-2-fenyloindol). Slides were analyzed with the fully automated image acquisition and analysis system Metafer (Metasystems, Germany). The frequency of micronuclei in binucleated cells (BNC) was scored according to the criteria proposed by Fenech [6]. All counted cells were binucleated and both nuclei were situated within the same cytoplasmic boundary. The two nuclei in BNC were approximately equal in size and could touch but not overlap each other. Micronuclei were morphologically identical to main nuclei but smaller than nuclei. The diameter of MN in the scored cells was less than 1/3 of the mean diameter of the main nuclei. Representative images of bystander CHO-K1 cells scored in this assay are shown in figure 4.

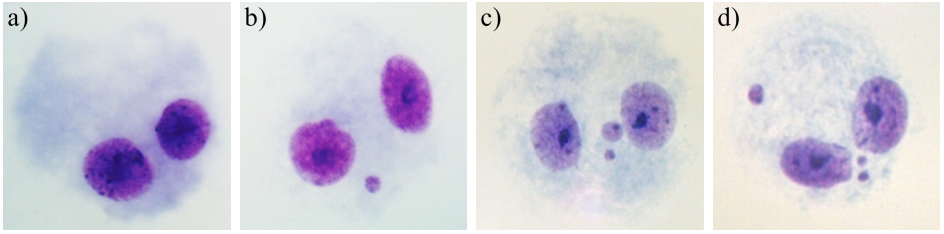

Fig. 4. Representative images of binucleated bystander cells stained with Giemsa (a) without MN, (b) with 1 MN, (c) with 2 MN and (d) with 3 MN.

3. Results

The number of micronuclei in binucleated bystander cells co-cultured with cells irradiated with ions is expressed as the mean ± standard error [7] of four separate experiments in two repetitions per dose. The number of micronuclei in binucleated bystander cells co-cultured with cells irradiated with X rays is expressed as the mean ± standard error of three separate experiments in two repetitions per dose. The experiments in which cells were irradiated with carbon beam were separated in time, the frequency of MN in control cells was scored separately for each experiment/dose (figure 5 (a)). In the case of cells irradiated with X rays, all samples were irradiated on the same day. Therefore, only one column for the control cells is shown (figure 5 (b)).

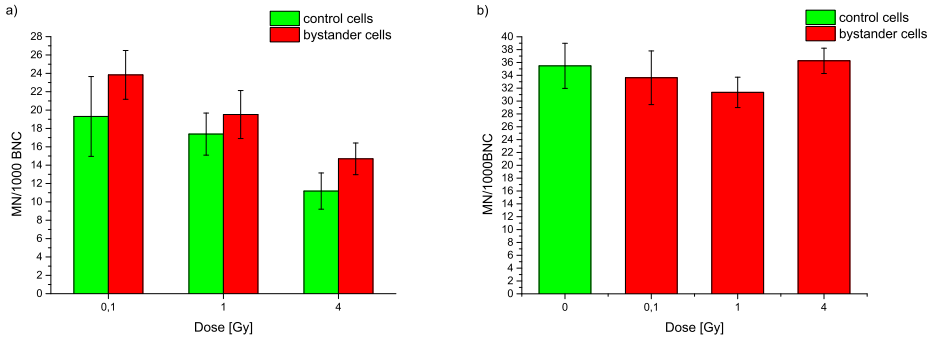

Fig. 5. The frequency of MN in 1000 BNC co-cultured with cells irradiated with ions (a) and X rays (b).

The micronucleus assay results revealed that the frequency of micronuclei in bystander cells co-cultured with cells irradiated with various doses of ions and X rays was not significantly different than in the control cells.

4. Discussion and conclusion

Current models for the interaction between ionising radiation and living cells are based on direct genetic damage produced by energy deposition in cellular DNA. An important observation which has questioned this basic assumption is the radiation-induced bystander response, in which cells which have not been directly targeted respond if their neighbours have been exposed [8]. The bystander effect is mainly observed in *in vitro* experiments using very low doses of alpha particles (range; mGy, cGy), but also after conventional irradiation (X rays, gamma rays) and heavy ions at low as well as conventional doses [9]. In contrast, there are data in the literature

showing no evidence of a bystander effect in a variety of cell lines, including clonogenic survival, induction of chromatid breaks and micronuclei in hamster cells [10, 11]. To reconcile these conflicting data, we studied biological response in Chinese Hamster Ovary (CHO-K1) cells co-cultured with cells irradiated with ^{12}C ions and X rays. Although it was occasionally possible to detect an increase in the frequency of micronuclei in bystander (CHO-K1) cells using carbon ions and X rays in single experiments, the results were not reproducible and statistically significant. It is possible that the specific cell line, experimental design or the medium supplements may be critical for inducing bystander effects. To test our hypothesis, additional experiments with different cell lines are necessary.

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REFERENCES

[1] C. Mothersill, C. Seymour, *Mutat. Res.* **568**, 121 (2004).
[2] J. Czub *et al.*, *Radiat. Prot. Dosim.* **122**, 207 (2006).
[3] M. Kirsch-Volders *et al.*, *Mutagenesis* **26**, 177 (2011).
[4] International Atomic Energy Agency, Technical Report series, 405 (2001).
[5] M. Fenech, *Mutat. Res.* **455**, 81 (2000).
[6] M. Fenech *et al.*, *Mutat. Res.* **534**, 65 (2003).
[7] D.G. Altman, J.M. Bland, *Brit. Med. J.* **331**, 903 (2005).
[8] K.M. Prise *et al.*, *Radiat. Prot. Dosim.* **104**, 347 (2003).
[9] M. Widel *et al.*, *Postępy Higieny i Medycyny Doświadczalnej* **63**, 377 (2009) (in Polish only).
[10] T. Groesser *et al.*, *Radiat. Res.* **170**, 794 (2008).
[11] N.A. Zyuzikov *et al.*, *Radiat. Res.* **175**, 322 (2011).