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X-RAY IRRADIATION OF CULTURE MEDIUM WITH OR WITHOUT CELLS

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The present study was designed to evaluate the radio-induced effects in culture medium with or without cells. Two leukemia cell lines (Jurkat and K562) were used in this study. The culture medium and cell cultures were irradiated with 0 Gy, 0.5 Gy, and 2.5 Gy. We investigated the radio-induced effects for unirradiated cells incubated in separately irradiated culture medium and radio-induced effects for irradiated cells incubated in fresh culture medium, immediately after irradiation. The process of programmed cell death, apoptosis, has been analyzed using the flow cytometry technique and the DNA ladder technique at 48 hours and 72 hours from irradiation. The irradiated culture medium doesn't affect the viability of Jurkat and K562 cells. The irradiated cells incubated in fresh culture medium after X-irradiation suffered significant functional changes.

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1. Introduction

Ionizing radiation is a well-known physical factor that can induce leukemia or other cancers [1, 2]. Leukemia is a type of cancer of the blood or bone marrow. The first time when radiation-induced leukemia was reported in humans was in the 20th century [2-4]. A consequent discovery was therefore that the leukemia cells are very radiosensitive [5].

Apoptosis or the programmed cell death is a mechanism of cellular self-destruction and plays an important role in tumor curability [6, 7]. The radiation absorption in cells can induce DNA aberrations and cell death [8]. In an apoptosis cell there are evident morphologic changes visible with microscope: aggregation of chromatin, condensation of nucleus and cytoplasm and apoptotic bodies [7, 9]. The biochemical hallmark of apoptosis is the formation of DNA fragments [7, 10]. The absence of these DNA fragments shows that the cells aren't apoptotic [7]. Any change in culture medium may influence cell death. After culture cells irradiation, cytokines or other soluble factors are removed from the culture medium [11-13]. In order to eliminate the additional effects we irradiated the culture medium with or without cells in them.

In the present study we investigated the viability of unirradiated cells incubated in irradiated RPMI 1640 and the viability of irradiated cells incubated in fresh RPMI 1640 culture medium using flow cytometry and DNA ladder assay.

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2. Material and methods

2.1. Cell culture and culture conditions.

Human leukemia cell lines used: Jurkat (Disease: acute T cell leukemia; Morphology: lymphoblast) and K562 (Disease: chronic myelogenous leukemia (CML); Morphology: lymphoblast). Jurkat and K562 cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS, GIBCO), 1 % antibiotic antimycotic solution (Sigma) at 37°C in a humidified atmosphere of 5% CO2. The culture medium was changed every 2nd day and the cells at 100% confluence were passaged at a 1:3 split ratio [14]. Cell membrane integrity (viability) was determined by using Trypan blue exclusion technique.

2.2. X- ray irradiation.

Irradiations were performed at room temperature with an X-ray SRT100 belonging to the Department of Radiology in "St. Spiridon" Hospital, Iasi, operated with a locator with a diameter of 10 cm, 70 kV, 10 mA, a depth of 3 cm and the dose rate was 2.27 Gy/min [15].

Jurkat and K562 cells were directly irradiated and after irradiation a part of cells were incubated in fresh RPMI 1640 culture medium. We also irradiated complete culture medium without cells and after irradiation we incubated the unirradiated cells in this medium.

2.3. Assessment of apoptosis induced by X-ray and irradiated culture medium

Determination of apoptosis was done using the Annexin V Binding Buffer, 10X concentrate (BD PharMingen Technical Data Sheet). Cells ($1x10^6$) were assessed at 48 hours and 72 hours after irradiation or after incubation in irradiated culture medium, using a BD FACSCanto II and BD FACSDiva Software. The Jurkat and K562 cells were washed twice in cold TFS (PBS) by centrifugation at 300g, for 5 min. Pellet obtained after the second centrifugation was resuspended in binding buffer. We added 5 μL of annexin V and 5 μL of 7AAD to the cells, vortexed, and incubated for 15 minutes in the dark. Finally, 400 μL of Binding Buffer were added, and samples were evaluated by flow cytometry [16-18].

2.4. DNA ladder assay

After 72 hours from incubation of cells in irradiated culture medium and incubation of irradiated cells in fresh culture medium, the DNA was extracted from Jurkat and K562 cells.

The DNA was mixed with loading dye and electrophoresed in 2% agarose gel. Ethidium bromide was added in 2% agarose gel and the running was made in TBE 1X buffer at 150 Volts for 90 minutes. The digital image of the gel was captured under UV light in the transilluminator.

3. Results

We report two series of experiments. Each experiment was made in triplicate. In the first experiment, we irradiated complete culture medium without cells and immediately after irradiation we incubated unirradiated leukemia cells in this medium irradiated with different doses. In the second experiment we irradiated leukemia cells and after irradiation we eliminated the culture medium and we added fresh culture medium. We wanted to know if the radiation influenced the culture medium and in order to determine this aspect we irradiated the complete culture medium without cells, and then cells were incubated in it. To exclude any influence of medium as a variable, the cells grew in the same medium and same conditions.

RPMI-1640 containing L-glutamine, HEPES, supplemented with 10% fetal bovine serum and 1% antibiotics (10000 U/mL Penicillin G, 10000ug/mL Streptomycin and 25ug/mL Amphotericin B). All culture medium has been in the same flasks.

The difference between directly irradiated cells, irradiated cells incubated in fresh culture medium and cells incubated in irradiated RPMI 1640 medium was determined using flow cytometry technique at 48 and 72 hours and DNA ladder assay at 72 hours from irradiation and cells incubation in irradiated RPMI 1640 medium. We used Annexin V Binding Buffer monitoring cell viability, early apoptosis, late apoptosis and cell death.

Figure 1 shows the results obtained by flow cytometry of Jurkat cells incubated in irradiated RPMI 1640 medium at 48 hours from irradiation of culture medium.

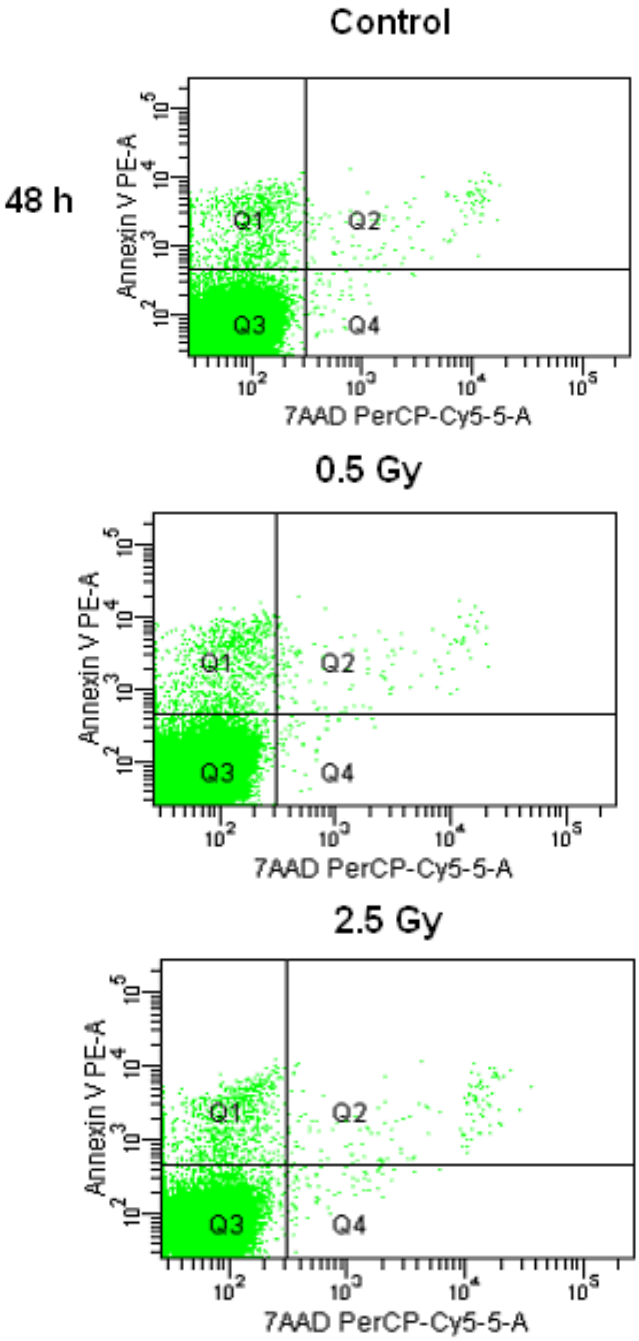

Fig. 1. Dot plots of Jurkat cells incubated in RPMI 1640 medium irradiated with different doses. Flow analysis was performed 48 hours after cells incubation in irradiated RPMI 1640 medium. Lower left quadrants (Q3) are the living cells, the Q1 quadrants show the cells in early apoptosis, and the Q2 quadrants show the cells in late apoptosis whereas the Q4 quadrants show the dead cells.

The response of leukemia cells incubated in irradiation RPMI 1640 culture medium was illustrated in Figs. 2 and 3. 48 and 72 hours after incubation of leukemia cells in irradiated culture medium, the cells have the same evolution like cells incubated in unirradiated culture medium.

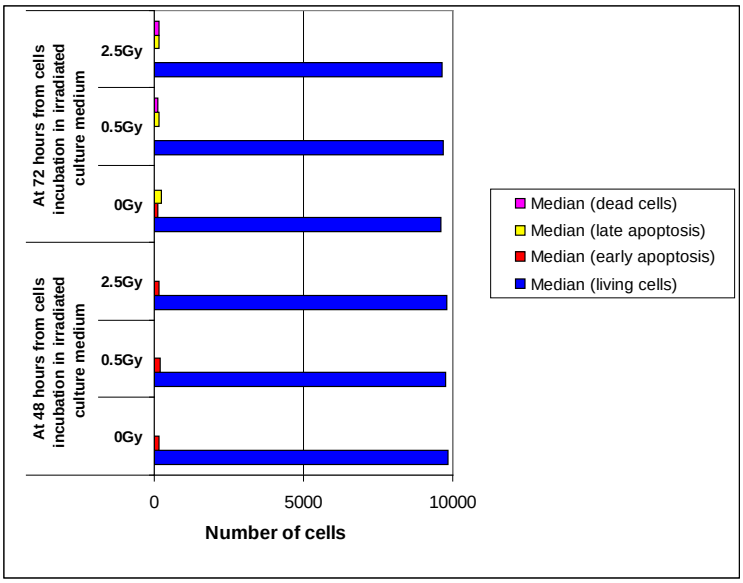

Fig. 2. Analysis of apoptosis in Jurkat cells after incubation in X-irradiation RPMI 1640 culture medium with different doses, i.e. 0 Gy, 0.5 Gy, and 2.5 Gy at various times, i.e. 48 and 72 hours. The variation of number cells is represented as follows: living cells with blue, early apoptosis with red, late apoptosis with yellow and cell death with purple.

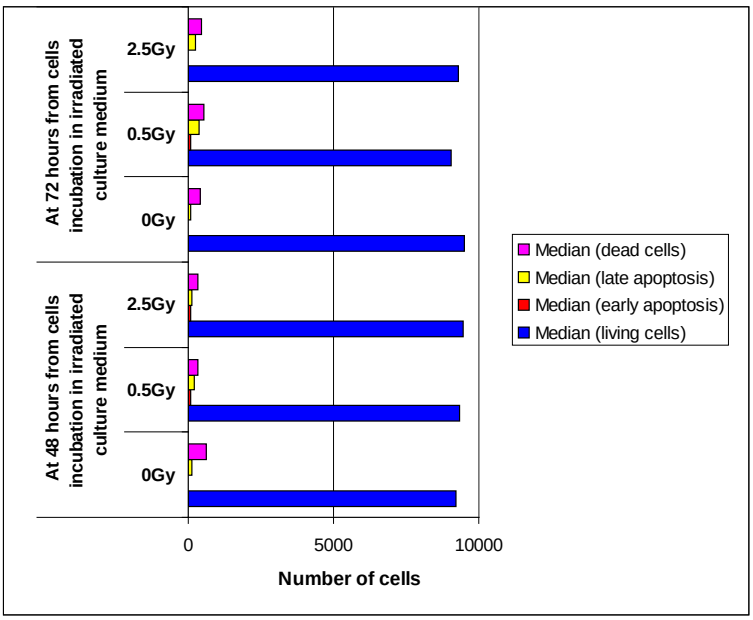

Fig. 3. Analysis of apoptosis in K562 cells after incubation in X-irradiation RPMI 1640 culture medium with different doses, i.e. 0 Gy, 0.5 Gy, and 2.5 Gy, at various times, i.e. 48 and 72 hours. The variation of number cells is represented as follows: living cells with blue, early apoptosis with red, late apoptosis with yellow and cell death with purple.

The integrity of DNA was analyzed with DNA ladder by using the gel electrophoresis technique.

72 hours after incubation of tumor cells in irradiated culture medium, the DNA was integer, there wasn't any DNA fragmentation.

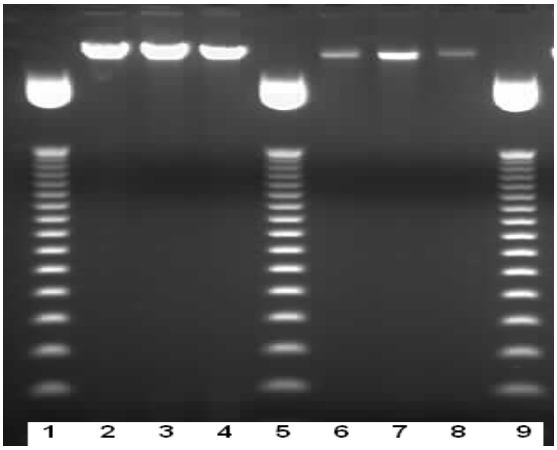

Fig. 4. Gel migration of DNA extracted 72 hours after incubation of K562 and Jurkat cells in X-irradiated RPMI1640 culture medium (without cells). Lines 2-4 and 6-8: DNA extracted from K562, respectively Jurkat, incubated in RPMI 1640 irradiated with 0 Gy, 0.5 Gy and 2.5 Gy. Lines 1, 5, and 9 are 50 bp ladder marker.

In the second experiment we irradiated K562 and Jurkat cells with 0.5 Gy and 2.5 Gy. After irradiation the cells were incubated in fresh culture medium. Figure 5 illustrates the flow cytometry analysis of Jurkat cells irradiated with 0.5 Gy and 2.5 Gy incubated in fresh culture medium, immediately after irradiation. Some of the irradiated cells were incubated in the same culture medium where they were irradiated.

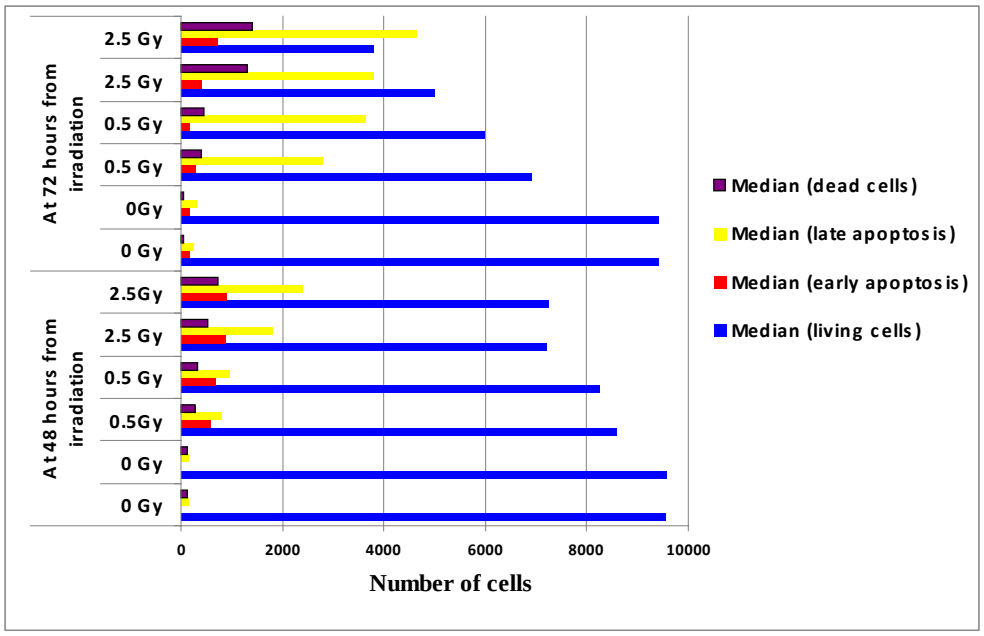

Fig. 5. Analysis of apoptosis in Jurkat cells after X irradiation with different doses: 0 Gy, 0.5 Gy and 2.5 Gy at various times, i.e. 48 and 72 hours. The variation of number cells is represented as follows: living cells with blue, early apoptosis with red, late apoptosis with yellow and cell death with purple.

The response of K562 cells was illustrated in figure 6.

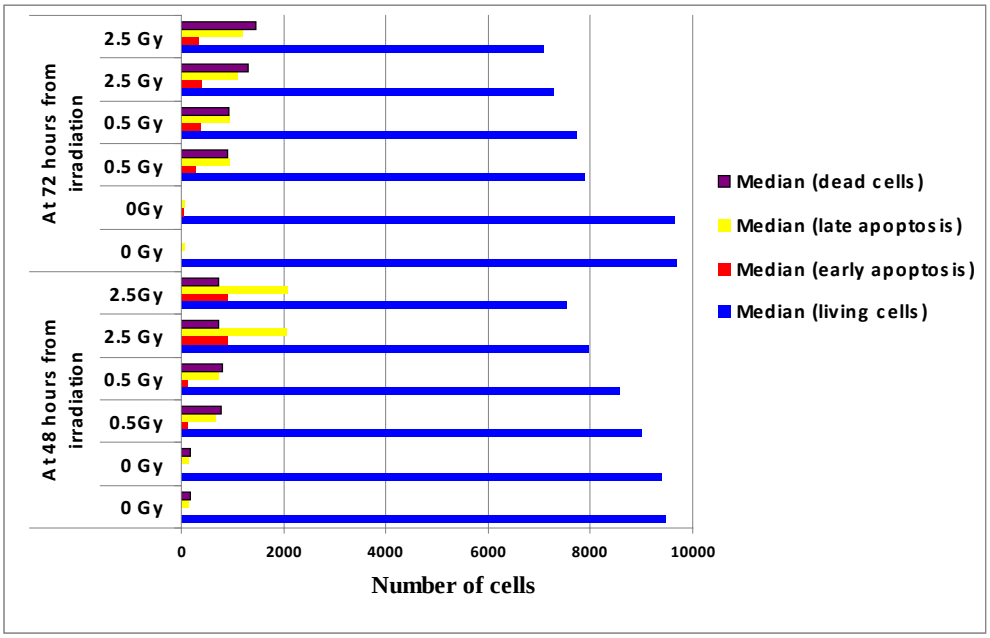

Fig. 6. Analysis of apoptosis in K562 cells after X irradiation with different doses: 0 Gy, 0.5 Gy and 2.5 Gy at various times, i.e. 48 and 72 hours. The variation of number cells is represented as follows: living cells with blue, early apoptosis with red, late apoptosis with yellow and cell death with purple.

This experiment has demonstrated that after 72 hours the DNA wasn't intact, it was fragmented.

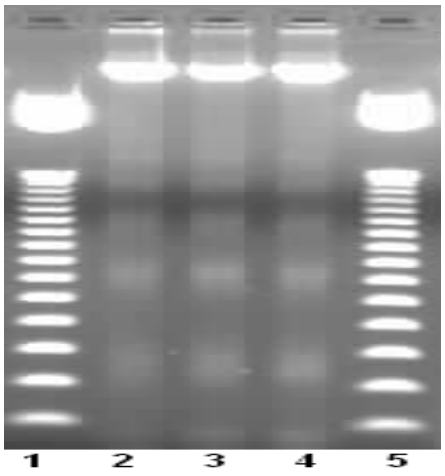

Fig. 7. Gel migration of DNA extracted 72 hours after incubation of irradiated Jurkat cells in fresh RPMI1640 culture medium or incubation in the same culture medium. Lines 2, 3: DNA extracted from Jurkat, irradiated with 0.5 Gy and 2.5 Gy incubated in fresh RPMI 1640. Line 4: DNA extracted from Jurkat, irradiated with 2.5 Gy and lines 1 and 5: 50 bp ladder marker.

Even when irradiated cells were incubated in fresh culture medium, their DNA has suffered injuries as a result of interaction with the radiation X.

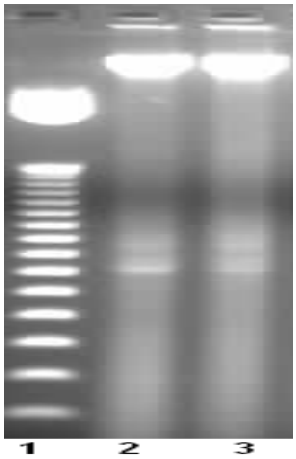

Fig. 8. Gel migration of DNA extracted 72 hours after incubation of irradiated K562 cells in fresh RPMI1640 culture medium or incubation in the same culture medium. Lines 2: DNA extracted from K562, irradiated with 2.5 Gy incubated in fresh RPMI 1640. Line 3: DNA extracted from K562, irradiated with 2.5 Gy and line 1: 50 bp ladder marker.

4. Discussion

Jurkat and K562 cells incubated in irradiated RPMI1640 with different doses didn't suffer alterations. The number of apoptotic and dead cells was much smaller than the number of living cells. After flow cytometry analysis, between Jurkat and K562 cells incubated in irradiated culture medium and the control there was no difference.

It is possible that changes induced by radiation in culture medium may stimulate cell growth [11]. Our study confirms the results obtained from literature, according to which there weren't significant differences between cells incubated in irradiated culture medium and cells incubated in unirradiated culture medium, even for doses 10-100 Gy [11].

The cells incubated in irradiated culture medium do not present alterations, their DNA appearing intact.

Irradiated Jurkat and K562 cells incubated in fresh culture medium show the same evolution as cells incubated in the same culture medium. Cells communication plays a more important role [19] than release soluble factors by irradiated cells [11].

The DNA ladder technique shows that in Jurkat cells the apoptotic process is evident. 72 hours after irradiation the number of apoptotic cells increased compared with living cells. In this period, the number of apoptotic cells from Jurkat culture registered a significant increase compared with the number of K562 cells [20].

The DNA ladder technique shows that the number of apoptotic K562 cells was lower then dead cells. The DNA fragmentations weren't so evident like in the case of Jurkat cells.

5. Conclusions

Complete culture medium (with 10% fetal calf serum) didn't affect the cells incubated in them. There aren't any differences between these cells and control cells. Since the DNA of leukemia cells incubated in irradiated culture medium appeared intact, there weren't any fragmentations.

The cells exposed to ionizing radiation were affected. Even when the cells were incubated in fresh culture medium, directly irradiated cells showed alteration, the same as the cells incubated in the same culture medium.

Acknowledgements

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