Physiol. Res. 53: 335-342, 2004

Cell Cycle Alteration, Apoptosis and Response of Leukemic Cell Lines to Gamma Radiation with High- and Low-Dose Rate

J. VÁVROVÁ[1], M. ŘEZÁČOVÁ[2], D. VOKURKOVÁ[3], J. PSUTKA[4]

[1]Institute of Radiobiology and Immunology, Purkyně Military Medical Academy, Hradec Králové, [2]Department of Medical Biochemistry, Faculty of Medicine Hradec Králové, Charles University Prague, [3]Institute of Clinical Immunology and Allergology, University Hospital, Hradec Králové, [4]Department of Military Medical Service Organization, Purkyně Military Medical Academy, Hradec Králové, Czech Republic

Received April 29, 2003
Accepted July 15, 2003

Summary

The aim of this work was to compare the effect of gamma radiation with sub-low dose-rate 1.8 mGy/min (SLDR), low dose-rate 3.9 mGy/min (LDR) and high dose-rate 0.6 Gy/min (HDR) on human leukemic cell lines with differing p53 status (HL-60, p53 deficient and MOLT-4, p53 wild) and to elucidate the importance of G2/M phase cell cycle arrest during irradiation. Radiosensitivity of HL-60 and MOLT-4 cells was determined by test of clonogenity. Decrease of dose-rate had no effect on radiosensitivity of MOLT-4 cells (D_0 for HDR 0.87 Gy, for LDR 0.78 Gy and for SLDR 0.70 Gy). In contrast, a significant increase of radioresistance after LDR irradiation was observed for p53 negative HL-60 cells (D_0 for HDR 2.20 Gy and for LDR 3.74 Gy). After an additional decrease of dose-rate (SLDR) D_0 value (2.92 Gy) was not significantly different from HDR irradiation. Considering the fact that during HDR the cells are irradiated in all phases of the cell cycle and during LDR mainly in the G2 phase, we have been unable to prove that the G2 phase is the most radiosensitive phase of the cell cycle of HL-60 cells. On the contrary, irradiation of cells in this phase induced damage reparation and increased radioresistance. When the dose-rate was lowered, approximately to 1.8 mGy/min, an opposite effect was detected, i.e. D_0 value decreased to 2.9 Gy. We have proved that during SLDR at first (dose up to 2.5 Gy) the cells accumulated in G2 phase, but then they entered mitosis or, if the cell damage was not sufficiently repaired, the cells entered apoptosis. The entry into mitosis has a radiosensibilizing effect.

Key words
Leukemia cell line • Ionizing radiation • Apoptosis • G2 arrest

Introduction

Recent trials (Press *et al.* 2000) with radio-labeled monoclonal antibodies targeting lymphoid surface membrane antigens have shown high response rates and tolerable toxicity. Radiolabeled antibodies emit continuous, exponentially decreasing, low-dose-rate radiation, whereas conventional external-beam

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

radiotherapy delivers intermittent, fractionated radiation at a higher dose rate. Apoptosis has been suggested to be a major cause of cell death from continuous low-dose rate radiation from radioimmunotherapy (Ning and Knox 1999, Kroger *et al.* 2001).

In our previous study (Mareková *et al.* 2003a) we have demonstrated two different types of apoptotic cell death after exposure of HL-60 cells to ionizing radiation: premitotic and postmitotic apoptosis. Dose of 20 Gy induced a quick apoptosis (occurring 4-6 h after the irradiation), which can be proved by DNA fragmentation and positivity of APO2.7 after permeabilization. It is evident that the cells die by apoptosis from the phase of cell cycle, in which they were irradiated. Moreover, the cells irradiated in G1 phase are less sensitive to this type of apoptosis. Similar results were obtained by Shinomiya *et al.* (2000) on monoblastoid cell line U937. Syljuasen and McBride (1999) evaluated apoptosis induction in Jurkat cells (TP53 mutant) irradiated with a dose of 20 Gy. Jurkat cells were sorted into different populations by centrifugal elutriation. Regardless of their cell cycle position at the time of irradiation, cells with sub-G1-phase DNA content had accumulated in all populations at 6 h after irradiation by dose of 20 Gy, indicating that apoptosis is most likely independent of cell cycle arrests. Cells irradiated in G1 phase underwent apoptosis more slowly than cells irradiated in other phases. This type of death could be characterized as interphase death. Our previous study has proved that mitochondrial membrane protein specific monoclonal antibody APO2.7 (after permeabilization) identifies an early apoptotic response after irradiation with a dose of 20 Gy.

Lower doses (up to 10 Gy) killed the cells by postmitotic apoptosis after G2 phase arrest. Syljuasen and McBride (1999) detected that after irradiation with dose of 2 Gy the onset of apoptosis appeared earlier in cells irradiated in the G2/M phase than in cells irradiated in other phases. Twenty-four hours after irradiation with a dose of 8 Gy, HL-60 cells were accumulated in the G2 phase and part of the cells was apoptotic (subG1 phase, APO2.7 positivity after permeabilization). However, 72 h after irradiation, the majority of cells was late apoptotic, with small cell size (intensity of light scattered in the forward direction correlates with cell size) and APO2.7 positive without permeabilization (Mareková *et al.* 2003a).

The results of Shinohara and Nakano (1993) suggest that X-irradiated human leukemia T-lymphoid cell line MOLT-4 (TP53 wild) suffer from an interphase death as well as a reproductive death. It is probable that apoptosis is the mechanism which is responsible for both modes of the death in these cells. Irradiation with a dose of 3 Gy (HDR) causes only inexpressive accumulation of cells in S and G2 phases of cell cycle at 8 h after irradiation and maximum of apoptosis is detected 12-16 h after irradiation (Vávrová *et al.* 2002).

The eukaryotic strategy to deal with damaged DNA can be divided into three components: the recognition of the injured DNA, the period of damage assessment, and DNA repair or cell death (Rich *et al.* 2000). Cell cycle checkpoints play a critical role in the damaged response system. The radiation-induced cell cycle arrest provides an opportunity for cells to repair DNA damage before entering the mitotic phase. Increased radioresistance of cells without p53 is related to the ability of these cells to repair ionizing radiation-caused damage during G2 phase arrest. Radford (2002) linked the ability to repair complex double strand breaks (DSB) of DNA, which occurred after irradiation, with the function of poly (ADP-ribose) polymerase. In the presence of p53 (wild type) and topoisomerase I, the ability of the cell to repair complex DNA DSB was decreased and cells were preferentially committed to apoptosis.

It still remains to be elucidated whether G2 phase is one of the most radiosensitive phases of the cell cycle and whether the LDR irradiation of cells in this phase of the cell cycle has a radiosensibilizing effect. In this study we compare the apoptosis induction and clonogenity of HL-60 cells after high (0.6 Gy/min), low (3.9 mGy/min) and sub-low (1.8 mGy/min) dose-rate irradiation.

Methods

Cell culture and culture conditions

Human leukemia HL-60 cells were obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK) and human T lymphocyte leukemia MOLT-4 cells were obtained from the American Type Culture Collection (University Blvd., Manassas, USA). The cells were cultured in Iscove's modified Dulbecco's medium (Sigma) supplemented with 20 % fetal calf serum in a humidified incubator at 37 °C and controlled 5 % CO_2 atmosphere. The cultures were divided every 2nd day by dilution to a concentration of $2x10^5$ cells/ml. Cell counts were performed with a

hemocytometer, cell membrane integrity was determined by using the Trypan blue exclusion technique. The cell lines in the maximal range of up to 20 passages were used for this study.

Gamma irradiation

Exponentially growing HL-60 and MOLT-4 cells were suspended at a concentration of $2x10^5$ cells/ml in a complete medium. Ten ml aliquots were plated into 25 cm^2 flasks (Nunc) and irradiated at room temperature using ^{60}Co ɣ-ray source with a dose-rate 0.6 Gy/min (HDR) at a distance 1 m from the source. Dose-rate 3.9 mGy/min for LDR irradiation and 1.8 mGy/min for SLDR irradiation were used. Cells were irradiated in a thermostat at 37 °C. The dose-rate was decreased by insertion of 7 or 8 cm of lead and by adjustment of the distance between the irradiated flask and the source. Thermoluminescent dosimeters were used for determination of the delivered dose.

After irradiation, the flasks have been placed in a 37 °C incubator with 5 % CO_2 for up to 161 h and aliquots of cells were removed at various times after irradiation for analysis. The cells were counted and cell viability was determined with the Trypan blue exclusion assay.

Cell cycle analysis

Untreated control and irradiated cells were collected and washed with cold PBS, fixed in 70 % ethanol. For fixation of low molecular fragments of DNA the cells were incubated for 5 min at room temperature in a phosphate buffer and then stained with propidium iodide (PI) in solution of Vindelov (1977) for 30 min at the temperature of 37 °C. Fluorescence (DNA content) was measured with Coulter Electronic apparatus (Hialeah, FL, USA). A minimum of 10 000 cells analyzed in each sample served to determine the percentages of cells in each phase of the cell cycle, using Multicycle AV software. Three independent experiments were performed.

In vitro clonogenic assay

A radiation survival curve was generated using an *in vitro* clonogenic assay. Untreated control (10^2/ml) and irradiated (10^2-10^5/ml) HL-60 and MOLT-4 cells were grown in 0.9 % methylcelulose in Iscove's modified Dulbecco's medium with 30 % fetal calf serum. All semi-solid cultures were performed in duplicates. HL-60 cells were stimulated by 10 % conditioned medium from the 5637 human bladder carcinoma cell line and 4-units/ml erythropoietin. Colonies (containing 40 or more cells) were counted after 14 days of incubation in 5 % CO_2 and 5 % O_2 at 37 °C and the survival curve was obtained. Two independent experiments were performed with cells irradiated with an increasing dose 0.5-12 Gy.

Statistical analysis

The results have been statistically evaluated with Student's t-test. The values represent means ± S.D. Statistical significance of the difference between means in comparable sets is indicated. Statistical evaluation of clonogenity curves has been performed by bilateral t-test of difference of corresponding (from the polynomial degree point of view statistically optimized) polynomial regression functions for dense net (1000 points) of independent variables, i.e. dose in Gy. For testing the differences of polynomial regression functions in the whole dose range, values of mean integral probability through the whole observed range of independent variable (i.e. dose in Gy) have been used (Knížek *et al.* 2001).

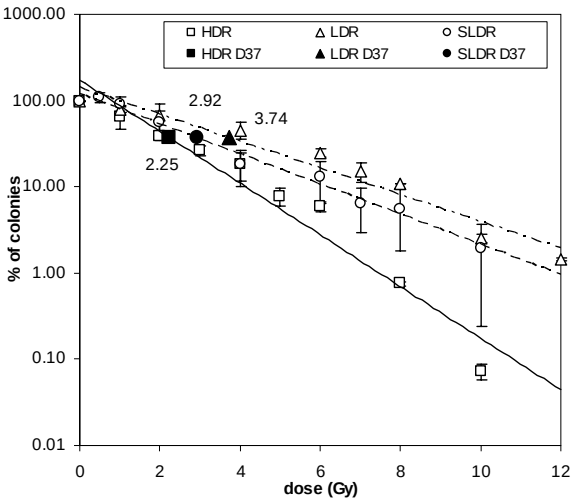

Fig. 1. Effect of different dose-rates on D_0 value of human promyelocyte leukemia cells HL-60. D_0 for HDR = 2.25 Gy, for LDR = 3.74 Gy and for SLDR = 2.92 Gy. For the clonogenic survival data, each point is mean for 4 measurements from two experiments ± S.D.

Results

To evaluate the sensitivity of HL-60 and MOLT-4 cell lines to killing by ionizing radiation with different dose-rate, we used the clonogenity test. Figure 1 compares D_0 values (the dose that results in 37 %

survival of irradiated cells) for HL-60 cells irradiated by different dose-rates of gamma irradiation. After high dose-rate (HDR) irradiation, D_0 was found to be 2.2 Gy. The low dose-rate irradiation (LDR) had protective effect and D_0 was 3.7 Gy. Additional reduction of dose-rate have caused increased radiosensitivity in comparison to LDR – determined D_0 value for sub-low dose-rate (SLDR) irradiation was 2.9 Gy. Dose response curves have been statistically evaluated by bilateral t-test of difference of polynomial regression functions. Mean integral probability (p_{mid}) was 0.06 for HDR and LDR curves, which means that the curves are significantly different in the whole observed dose range. When comparing complete curves for HDP and SLDR, no significant difference was found (p_{mid} = 0.33). However, when complete curves for LDR and SLDR were compared, there was a marginal significance of the difference (p_{mid} = 0.17). When we evaluated the statistical difference of individual segments of the curves, significant difference was found in dose range of 1-7 Gy, but no significant difference was detected for doses above 7 Gy.

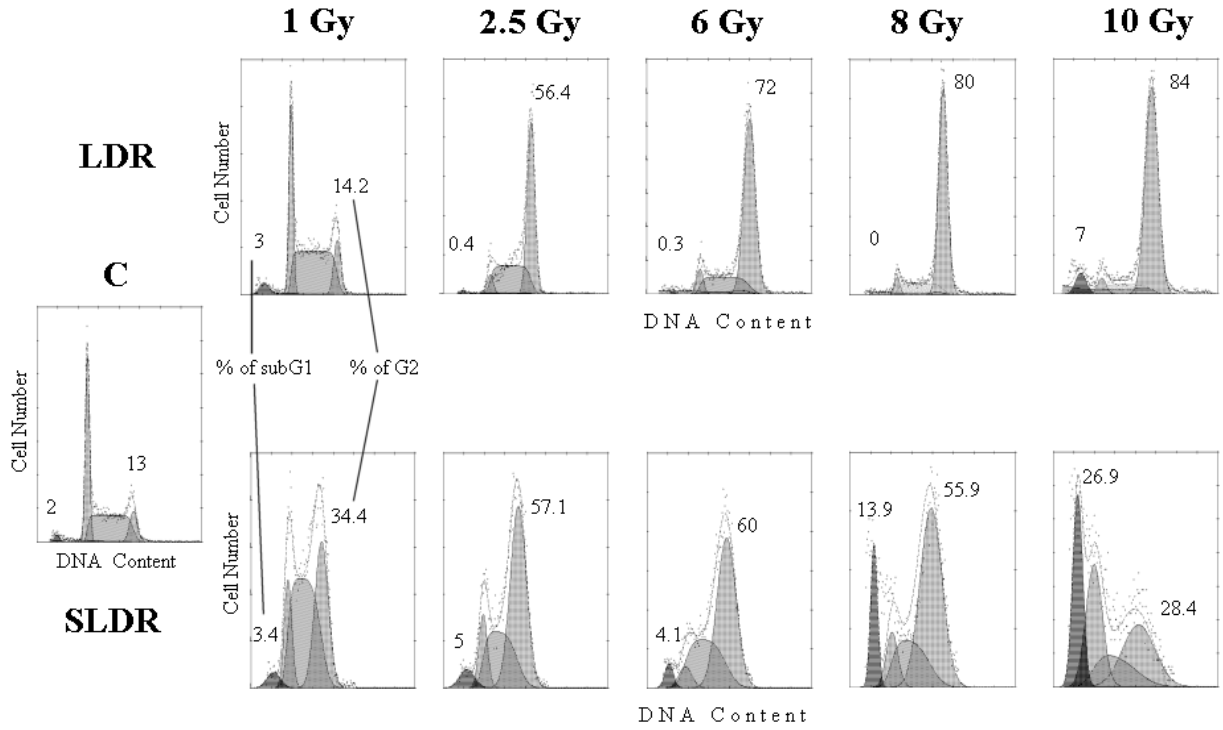

Fig. 2. Effect of different dose-rates on D_0 value of human T-lymphocyte leukemia cells MOLT-4. D_0 for HDR = 0.87 Gy, for LDR = 0.78 Gy and for SLDR = 0.70 Gy. For the clonogenic survival data, each point is mean for 4 measurements from two experiments ± S.D.

Figure 2 shows the clonogenity dose response curves for MOLT-4 cells. It can be seen that D_0 value is 0.87 Gy for HDR, 0.78 Gy for LDR, and 0.70 Gy for SLDR. P_{mid} for comparison of HDR and LDR is 0.48, for HDR and SLDR 0.32 and for LDR and SLDR 0.41. It means that all three curves are not significantly different.

Figure 3 shows the cell cycle course of HL-60 cells after irradiation with increasing dose of ionizing radiation 1-10 Gy with LDR and SLDR. In our cultivation conditions, control non-irradiated cells had 42.9±1.6 % of cells in G1 phase, 45.7±2.4 % in the S phase and 11.4±2.0 % in the G2 phase (average from five experiments). During HDR irradiation, the cells were irradiated in all phases of the cell cycle and the distribution of cells in phases of the cell cycle did not significantly change during the short time of irradiation with the dose 10 Gy (16.6 min). During LDR after the dose of 1 Gy, moderate increase of cells in S and G2 phase could be observed, after the dose of 2.5 Gy 56 % of cells are in G2 phase – it means that most of the cells were irradiated in G2 phase of cell cycle during LDR irradiation. At the end of LDR irradiation by the dose of 10 Gy (time of irradiation was 41 h), 84 % of the cells were in G2 phase and 7 % of cells were apoptotic. During SLDR irradiation after the dose of 1 Gy, moderate increase of cells in S and G2 phase could be observed, after the dose of 2.5 Gy 57 % of cells were in G2 phase – similarly to LDR irradiation. However, during further

irradiation, the cells were not accumulated in G2 phase, but they entered the next cell cycle or died by apoptosis. At the end of SLDR irradiation 10 Gy (time of irradiation was 89 h), 27 % of cells were apoptotic and most of the cells were in G1 phase of cell cycle (53 %).

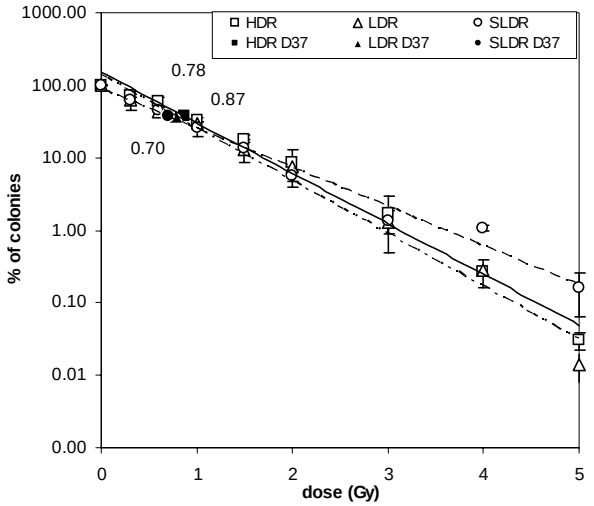

Fig 3. Cell-cycle progression (G2 phase arrest) and apoptosis induction in the end of gamma-irradiation of HL-60 cells by dose 1-10 Gy with LDR and SLDR. Representative results from one of three independent experiments are shown.

Figure 4 compares the distribution of HL-60 cells in the cell cycle after irradiation with the dose of 10 Gy using three different dose-rates. A dose of 10 Gy delivered by HDR was absolutely lethal; 41 h after irradiation, most of the cells became apoptotic. When HL-60 cells were irradiated with the dose of 10 Gy, delivered by LDR for 41 h, at the end of irradiation the majority of the cells was in G2 phase; after 89 h, 61 % of cells were apoptotic, but cells in S phase were also detected. After 161 h, apoptotic cells almost disappeared and the cell cycle distribution was the same as in the control sample – the dose was sublethal. In the case of SLDR irradiation, behavior of cells was different (Fig. 3). The cells entered the next cell cycle or apoptosis during irradiation. After irradiation with the dose of 6 Gy (53.4 h), the cells did not proliferate and 7 % of cells were dead (stained by Trypan blue). After the dose of 8 Gy (72 h), the number of live cells slightly increased ($6.5x10^5$/ml), 13.9 % of cells became apoptotic and 5 % were stained by Trypan blue. At the end of irradiation with the dose of 10 Gy (89 h), 26.9 % of cells became apoptotic and 14 % of cells were dead. During further incubation (161 h after the beginning of irradiation), the number of live cells decreased significantly; 30 % of cells became apoptotic and 30 % were dead.

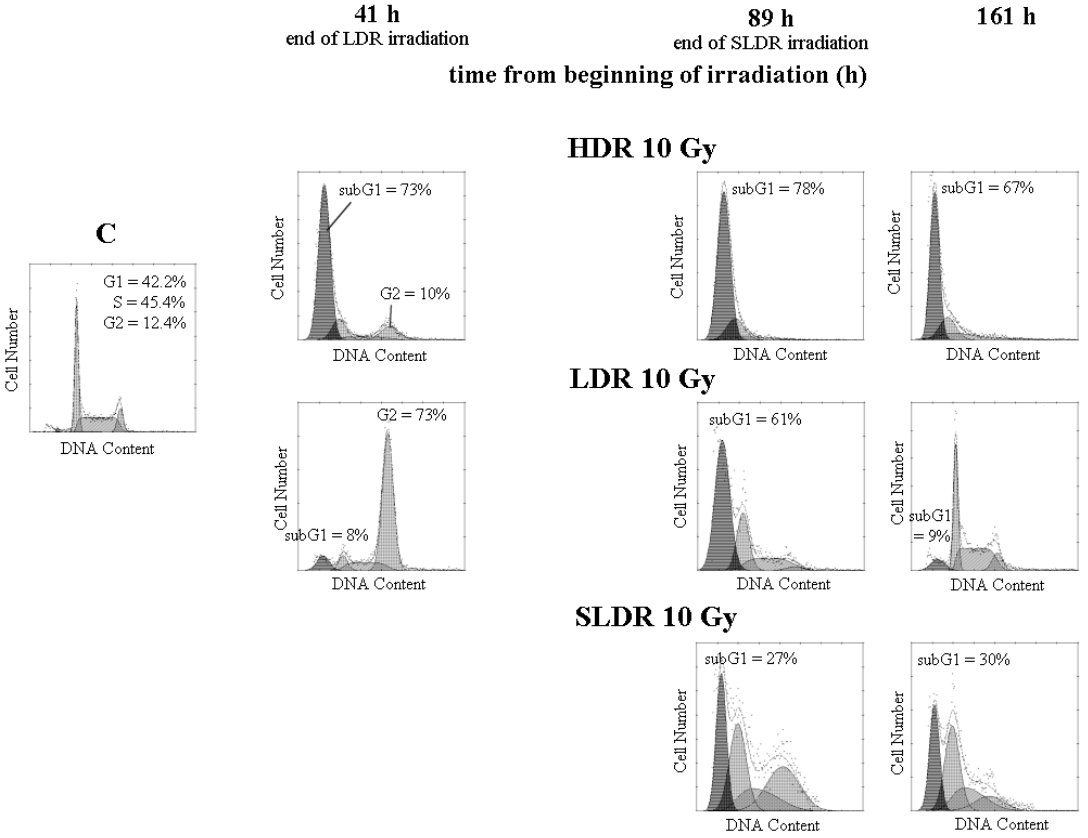

Fig. 4. Cell-cycle progression and apoptosis induction after 10 Gy gamma-irradiation of HL-60 cells with HDR, LDR and SLDR. Cell cycle was studied for 161 hours after beginning of irradiation. Representative results from one of three independent experiments are shown.

Figure 5 shows the number of live cells (not stained by Trypan blue) after irradiation with the dose of 10 Gy. Number of live cells was minimal 161 h after 10 Gy HDR irradiation. After LDR irradiation, the cells retained their proliferative ability – 161 h after beginning of LDR irradiation, significant increase of live cells was apparent when compared with the number of live cells 89 h after beginning of LDR irradiation, and with the number of cells 161 h after both HDR and SLDR irradiation. SLDR caused persistent cell damage and number of live cells decreased significantly between 89 and 161 h after irradiation.

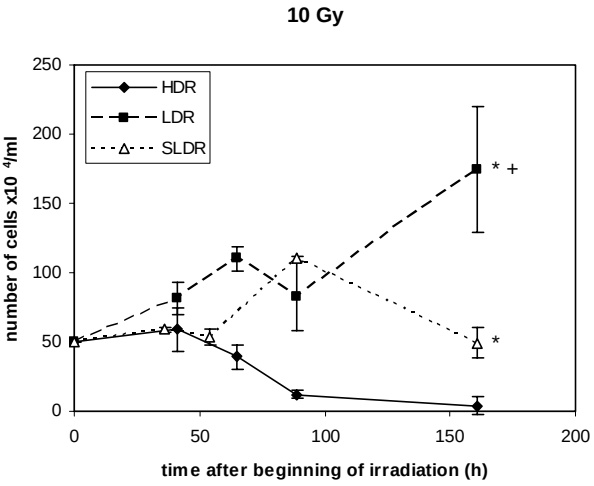

Fig. 5. Kinetics of the effect of 10 Gy gamma-irradiation with HDR, LDR and SLDR on growth of HL-60 cells. The number of viable cells was determined 41-161 hours after beginning of radiation. Each point represents the average of three independent experiments ± S.D. * significantly different from the value 89 hours after the beginning of radiation, $^{+}$ significantly different from HDR and SLDR value.

Discussion

We confirm the results of Ning and Knox (1999) that during a low dose-rate (3.9 mGy/min) irradiation of HL-60 cells, the cells are irradiated mainly in G2 phase of the cell cycle. At the end of irradiation with the dose of 10 Gy, nearly all cells were in the G2 phase and 7-8 % of cells were apoptotic. However, when compared with HDR irradiation, the effect of irradiation was smaller, probably due to the DNA damage reparation already during irradiation. We observed highly significant increase of D_0 value for LDR irradiation (D_0 = 3.7 Gy) in comparison to HDR (D_0 = 2.2 Gy). Considering the fact that during HDR the cells are irradiated in all phases of cell cycle and during LDR mainly in G2 phase, we have been unable to prove that G2 phase is the most radiosensitive phase of the cell cycle of HL-60 cells. On the contrary, irradiation of cells in this phase induced damage reparation and increased radioresistance. When the dose-rate was lowered, approximately to 1.8 mGy/min, opposite effect was detected: D_0 value decreased to 2.9 Gy. We have proved that during SLDR at first (dose up to 2.5 Gy) the cells accumulated in G2 phase, but then they entered mitosis or, if the cell damage was not sufficiently repaired, the cells entered apoptosis. The entry into mitosis has a radiosensibilizing effect. This observation is in good agreement with the findings of Joiner *et al.* (2001) and Mitchell *et al.* (2002) that in some cell lines, which are highly sensitive to low doses (under 0.1 Gy/h), the increase of the dose to 0.3 Gy/h increases radioresistance. Joiner *et al.* (2001) suggested that this process is related to the adaptation of the cells to the irradiation. They observed a radiosensibilizing effect and compared high and very low (<0.3 Gy/h) dose-rate irradiation of human glioblastoma T-98C cells. The irradiation of T-98C cells by low dose-rate 0.3-0.6 Gy/h has shown protective effect in comparison to HDR.

The time of DNA damage repair is also very important. If the dose-rate is very low (<0.1Gy/h), the cells enter mitosis during the irradiation and therefore they are irradiated in the most sensitive phase of cell cycle. The preincubation of the cells with all-trans retinoic acid decreases number of cells accumulation in G2 phase after irradiation and facilitates the apoptosis induction (Mareková *et al.* 2003b). The importance of DNA damage repair is confirmed by the experiments with caffeine. Caffeine in a concentration of 2 mM inhibits G2 checkpoint delay and therefore decreases the reparation time and increases the radiosensitivity of the cells. We proved a radio-sensibilizing effect of caffeine in HL-60 cells after all dose-rates (Mareková *et al.* 2002). Ning and Knox (1999) have reported that irradiation of HL-60 cells by exponentially decreasing dose-rate in presence of caffeine caused a decrease of number of cells in G2 phase and the apoptosis induction was also decreased during the first 24 h. Although we confirmed their results (LDR), we observed a second wave of apoptosis 6-8 days after the beginning of irradiation (Vávrová *et al.* 2004). Apoptosis induction was detected using three methods (subG1 peak, APO2.7, and lamine B cleavage). The clonogenity assay also proved the radiosensibilizing effect of caffeine. It seems that period, during which the cells have become arrested in G2 phase before first mitosis attempt, is critical mainly from the

point of view of DNA damage reparation. In two human sarcoma cell lines with p53 mutant, Bache *et al.* (2001) have proved radiosensibilizing effect of caffeine linked with G2/M phase arrest shortage and inhibition of DNA damage repair. It has been shown that caffeine inhibits the activity of ATM kinase, which plays a crucial role in apoptosis induction by ionizing radiation. This explains the caffeine inhibition of G2/M arrest - ATM kinase phosphorylates Chk1 and 2 kinases and inhibits function of cdc25c and cdc25a phosphatase, disabling the activation of cycline B1/cdc2 complex and the cells are arrested in G2/M phase. Caffeine gives a false signal about the completeness of DNA reparation by ATM kinase inhibition, therefore cycline B1/cdc2 complex is activated and the cell is ready for mitosis. Szumiel *et al.* (2001) studied the effect of irradiation in the presence of caffeine in two murine lymphoblast cell lines LZ-R and LZ-S, which have different ability to repair radiation-induced damage. More radioresistant line LY-R has repaired double strand breaks by both homologous recombination (HR) and non-homologous end-joining (NHEJ), radiosensitive line LY-S is NHEJ deficient. The radiosensibilizing effect of caffeine has been observed in LY-S cells but not in LY-R cells, despite the fact that caffeine has shortened G2 phase arrest in both lines. This indicates that ATM kinase controls predominantly HR repair (working at LY-S cells). LZ-R cells can repair damage by NHEJ repair, which is not affected by caffeine. Radiosensibilizing effect of caffeine at LY-S cells is caused by HR disabling by caffeine. Theron *et al.* (2000) also reported that not only G2 phase arrest suppression, but also inhibition of DSBs reparation by pentoxifylline at melanoma cells is responsible for radiosensibilizing effect of caffeine.

The irradiation with dose-rate 0.2 Gy/h has not significantly changed D_0 value for MOLT-4 cells (D_0=0.9 Gy for HDR and 0.8 Gy for LDR). MOLT-4 cells (unlike HL-60 cells) are p53 wild. Nakano *et al.* (2001) have studied the effects of the expression of various levels of transfected murine mutant p53 cDNA on the induction of apoptosis in X-irradiated or heated MOLT-4 cells. When survival has been determined by the dye exclusion test at 24 h after irradiation, the percentage of X-ray or heat-induced dead cells has decreased markedly, corresponding to the expression level of mutant p53 protein in transfected clones. These results suggest that murine mutant p53 protein has a dominant-negative effect against normal p53 in MOLT-4, and that X-ray-induced apoptosis in MOLT-4 is fully p53-dependent. Kroger *et al.* (2001) found that radioimmunotherapy (low dose-rate) has proven to be particularly effective in the treatment of malignancies such as lymphoma.

It can be concluded from our results that for the determination of dose-rate to be used in the treatment strategy, two main points should be considered – i) the type of the cells (mainly their p53 status), and ii) G2 phase arrest length, i.e. the time which the cells have for damage repair. While the effect of irradiation of human T-lymphocyte leukemia cells MOLT-4 (p53 wild/mutant) is not significantly affected by dose-rate, the dose-rate has significant effect on HL-60 cells. Finally, it can be said that the presence of cells in G2 phase during irradiation increases radioresistance of these cells.

Acknowledgements

The authors would like to thank J. Prokešová and M. Šafářová for excellent technical assistance. This work was supported by grant no. 202/01/0016 of Grant Agency of the Czech Republic.

References

BACHE M, PIGORSCH S, DUNST J, WURL P, MEYE A, BARTE F, SCHMIDT H, RATH FW, TAUBERT H: Loss of G2/M arrest correlates with radiosensitization in two human sarcoma cell lines with mutant p53. *Int J Cancer* **96**: 110-117, 2001.

JOINER MC, MARPLES B, LAMBIN P, SHORT SC, TURESSON I: Low-dose hypersensitivity: current status and possible mechanisms. *Int J Radiat Oncol Biol Phys* **49**: 379-389, 2001.

KNÍŽEK J, ŠINDELÁŘ J, ÖSTERREICHER J, NAVRÁTIL L. Bilateral t-test (in Czech). *Acta Radiobiologica* **1**: 40-44, 2001.

KROGER LA, DENARDO GL, GUMERLOCK PH, XIONG CY, WINTHROP MD, SHI XB, MARK PC, LESHCHINSKY T, DENARDO SJ. Apoptosis-related gene and protein expression in human lymphoma xenografts (Raji) after low dose rate radiation using ^{67}Cu-2IT-BAT-Lym-1. *Cancer Biother Radiopharm* **16**: 213-225, 2001.

MAREKOVÁ M, VÁVROVÁ J, VOKURKOVÁ D: Caffeine-increased radiosensitivity is dependent on a loss of G2/M arrest in human promyelocyte HL-60 cell line. *Radiother Oncol* **64**: 307, 2002.

MAREKOVÁ M, VÁVROVÁ J, VOKURKOVÁ D: Monitoring of premitotic and postmitotic apoptosis in gamma irradiated HL-60 cells by the mitochondrial membrane protein-specific monoclonal antibody APO2.7. *Gen Physiol Biophys* **22**: 191-200, 2003a.

MAREKOVÁ M, VÁVROVÁ J, VOKURKOVÁ D, PSUTKA J: Modulation of ionizing radiation-induced apoptosis and cell cycle arrest by all-trans retinoic acid in promyelocytic leukemia cells (HL-60). *Physiol Res* **52**: 599-606, 2003b.

MITCHELL CR, FOLKARD M, JOINER MC: Effect of exposure to low-dose-rate ^{60}Co gamma rays on tumor cells in vitro. *Radiat Res* **158**: 311-318, 2002.

NAKANO H, KOHARA M, SHINOHARA K: Evaluation of the relative contribution of p53-mediated pathway in X-ray-induced apoptosis in human leukemic MOLT-4 cells by transfection with mutant p53 gene at different expression levels. *Cell Tissue Res* **306**: 101-106, 2001.

NING S, KNOX SJ: G2/M-phase arrest and death by apoptosis of HL-60 cells irradiated with exponentially decreasing low-dose-rate gamma radiation. *Radiat Res* **151**: 659-669, 1999.

PRESS OW, RASEY J: Principles of radioimmunotherapy for hematologists and oncologists. *Semin Oncol* **27**: 62-73, 2000.

RADFORD IR. Model for the initiation of ionizing radiation-induced apoptosis in lymphoid cells by complex DNA double-strand break. *Int J Radiat Biol* **78**: 467-474, 2002.

RICH T, ALLEN RL, WYLLIE AN: Defying death after DNA damage. *Nature* 407: 777-783, 2000.

SHINOMIYA N, KUNO Y, YAMAMOTO F, FUKASAWA M, OKUMURA A, UEFUJI M, ROKUTANDA M: Different mechanisms between premitotic apoptosis and postmitotic apoptosis in X-irradiated U937 cells. *Int J Radiat Oncol Biol Phys* **47**: 767-777, 2000.

SHINOHARA K, NAKANO H. Interphase death and reproductive death in X-irradiated MOLT-4 cells. *Radiat Res* **135**: 197-205, 1993.

SYLJUASEN RG, MCBRIDE WH: Radiation-induced apoptosis and cell cycle progression in Jurkat T cells. *Radiat Res* **152**: 328-331, 1999.

SZUMIEL I, KAPISZEWSKA M, JOHN A, GRADSKA I, KOWALCZYK D, JANIK P: Caffeine-inhabitable control of the radiation-induced G2 arrest in L5178Y-S cells deficient in non-homologous end-joining: *Radiat Environ Biophys* **40**: 137-143, 2001.

THERON T, BINDER A, VERHEYE-DUA F, BOHM L: The role of G2-block abrogation, DNA double-strand break repair and apoptosis in the radiosensitization of melanoma and squamous cell carcinoma cell lines by pentoxifylline. *Int J Radiat Res* **76**: 1197-1208, 2000.

VÁVROVÁ J, MAREKOVÁ M, SZKANDEROVÁ S, VOKURKOVÁ D, STULÍK J, BLÁHA M, JEBAVÝ L, FILIP S: Ionizing radiation-induced apoptosis and degradation of the nuclear matrix in hematopoietic cells. In: *Biophysics of the Genome and Its Interaction.* S KOZUBEK, M KOZUBEK (eds), Masaryk University, Brno, 2002, pp. 86-91.

VÁVROVÁ J, MAREKOVÁ-ŘEZÁČOVÁ M, VOKURKOVÁ D, SZKANDEROVÁ S, PSUTKA J: Caffeine induces a second wave of apoptosis after low dose-rate gamma radiation of HL-620 cells. *Radiat Environ Biophys* **42**: 193-199, 2003.

VINDELOV LL: Microfluorometric analysis of nuclear DNA in cells from tumors and cell suspension. *Virchows Arch Cell Pathol* **173**: 227-242, 1977.

Reprint requests

Assoc. Prof. Jiřina Vávrová, PhD., Institute of Radiobiology and Imunology, Purkyně Military Medical Academy, Třebešská 1575, 50001 Hradec Králové, Czech Republic. E-mail: Vavrova@pmfhk.cz