A Comparison of Chromatin Degradation in Irradiated Normal and Tumorous Lymphoid Cells

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Abstract. Irradiation of mice with doses of 2 and 4 Gy induced extensive chromatin degradation in the thymocytes within 6 hours accompanied by an increase in polydeoxynucleotide (PDN) content (36 and 42 times, respectively). Fifteen hours after irradiation the PDN level was considerably lower, however, still being 4.7 and 14 times the control values after doses of 2 and 4 Gy. The PDN content in control LS/BL lymphosarcoma cells was similar as that in the thymocytes of non-irradiated mice. Unlike in the thymocytes, irradiation of lymphosarcoma cells did induce no statistically significant increase in the PDN level 6 and 15 hours after the irradiation, respectively. It has been reported previously (Matyášová et al. 1973) that chromatin of LS/BL cells degraded similarly as that in the irradiated thymocytes. The results of the present experiments thus provide additional evidence for changes of LS/BL cell properties due to long term cultivation. These cells, however, are still able to react by chromatin fragmentation to nitrogen mustard treatment.

Key words: Chromatin degradation — Thymocytes — Lymphosarcoma cells

Introduction

Irradiation induces a decrease in the cellularity of the lymphoid tissue resulting from the interphase deaths of lymphocytes (Okada 1974; Fedorova et al. 1972). Irradiated mouse lymphosarcoma LS/BL cells disappear rapidly from the peritoneal cavity of mice; this may indicate that interphase death contributes to the extinction of these malignized lymphoid cells (Jurášková and Drášil 1978; Kubíček et al. 1981).

The interphase cell death is associated with an increased permeability of cell

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membranes for vital stains, pyknosis of the nuclei, chromatin fragmentation, secondary postirradiational degradation of DNA and the activation of nucleases (Okada 1974; Fedorova et al. 1972; Ryabchenko 1979; Ivannik et al. 1976; Ivannik et al. 1978). Though to a smaller extent, degradation of chromatin DNA in internucleosomal spacer segments (Matyášová et al. 1973) of lymphosarcoma cells has regularly been found within 6 h after irradiation, similarly as in the thymic and splenic lymphocytes. Morphological studies had, however, failed to show pyknotic changes in the nuclei of irradiated LS/BL cells (Kubíček et al. 1981).

Experiments carried out so far have not allowed any clear conclusion as for the existence of interphase deaths in malignized lymphatic LS/BL cells. A comparative analysis of chromatin degradation in irradiated thymus and LS/BL cells was therefore undertaken by estimating polydeoxyribonucleotides soluble in physiological salt solutions. In addition, an electrophoretic analysis of chromatin DNA fragments of irradiated thymocytes and LS/BL lymphosarcoma cells was also performed.

Materials and Methods

LS/BL lymphosarcoma cell suspension was cultured in the peritoneal cavity of C57BL mice (10^7 cells/animal). Five days after the tumour transfer the host animals were exposed to ^{60}Co gamma radiation and killed 6 or 15 h after the irradiation. Tumour cells were obtained by flushing the peritoneal cavity with Hanks' solution (Ca^{2+}- and Mg^{2+}-free).

The effect of irradiation on chromatin degradation in the thymocytes was studied in three-week-old mice. Six and fifteen hours after irradiation, the mice were killed and the thymus rapidly removed. A cell suspension was prepared from the thymus by cutting and crushing it with a scalpel at 0 °C with subsequent filtering through a capron sieve.

A previously described (Ivannik et al. 1978) method was used to fractionate chromatin of irradiated and non-irradiated cells into the deoxyribonucleoprotein complex (insoluble in 0.15 NaCl) and soluble polydeoxyribonucleotides (PDN). After centrifugation 5×10^7 cells were resuspended in 2 ml cold 0.01 mol . l^{-1} Na_2EDTA, pH 7.3, and kept at 0 °C (LS/BL for 60 min and thymocytes for 20 min under constant stirring). Then 2 ml of cold 0.3 mol . l^{-1} NaCl were added to the samples and after additional 3—5 min the suspension was centrifuged at 2500 rpm for 20 min. The sediment contained chromatin insoluble in 0.15 mol . l^{-1} NaCl, the supernatant contained PDN soluble in 0.15 mol . l^{-1} NaCl. It has been shown in a previous work (Ivannik et al. 1978) that 0.01 mol . l^{-1} EDTA disrupts the cells and allows complete extraction of both PDN and chromatin.

The determination of polymeric DNA in the chromatin and PDN fractions was performed fluorometrically with diaminobenzoic acid (DABA) using a method described previously (Thomas and Farquhar 1978). Five millilitres of 5% trichloracetic acid (TCA) were added to the chromatin sediment. TCA 20% (1.33 ml) were added to the samples containing PDN. After staining for 1—2 h at 0 °C and centrifugation sediments containing polymeric DNA were hydrolysed in 5% TCA at 90 °C for 20 min. To 0.5 ml, hydrolysate 0.1 ml DABA (200 mg/ml) was added and the mixture was incubated at for 45 min 60 °C. The fluorescence of samples was measured at 400 and 520 nm on a mfr. Aminco spectrofluorimeter. Fluorimetric data were used to calculate the relative amount (%) of polymeric DNA in PDN fractions.

Samples for gel electrophoresis of DNA were processed as follows: DNA from LS/BL and thymus cells of irradiated and control mice was isolated (Matyášová et al. 1973) 6 and 16 h after the irradiation

Table 1. Radiation effects on the PDN content (in percent) in polymeric DNA in murine thymocytes.

Dose, Gy	Post-irradiation interval, h	
	6	15
Controls	0.63 ± 0.02 % ($n = 5$)	
2 Gy	22.6 ± 0.6 % ($n = 3$)	3.0 ± 0.9 % ($n = 3$)
4 Gy	26.7 ± 0.8 % ($n = 3$)	9.1 ± 1.1 % ($n = 3$)

n — number of animals

with doses of 6; 12; and 18 Gy (^{60}Co). For comparison, DNA was also isolated from LS/BL cells 16 h after in vivo treatment of mice with tris-(2-chlorethyl)-amine hydrochloride (TS 160 Spofa, i.p., 0.2 mg/10 g body mass). Samples of DNA from LS/BL cells were incubated with RNase (100 μg RNase/1 mg DNA). Isolated DNA was applied on gels for electrophoresis in 3.5% polyacrylamide gels under native conditions (Loening 1967), or denatured with formamide for electrophoresis in 6% polyacrylamide gels with urea (Simpson and Whitlock 1976). The gels were stained with Stains-all and scanned on a Pye-Unicam SP 1809 densitometer.

Results and Discussion

The PDN content in the thymocytes of non-irradiated mice was 0.63% (Table 1). Irradiation of mice with doses of 2 and 4 Gy induced, within 6 h, extensive chromatin degradation accompanied by a 36 and 42 — fold increases in the PDN content, respectively. Fifteen hours after the irradiation, the PDN level was considerably lower than after 6 h the former still being 4.7 and 14 times the 2 Gy and 4 Gy control values, respectively.

Table 2 summarizes results of the PDN content in control and irradiated LS/BL lymphosarcoma cells. Five days after the transplantation, the PDN level in control cells was the same as in the thymocytes of non-irradiated mice. On the 6 th posttransplantation day, the PDN content in the LS/BL cells increased; this seemed to be associated with the transition to the stationary phase of growth. Unlike in the thymocytes, the irradiation of lymphosarcoma cells induced no statistically significant increase in the PDN level after 6 and 15 h (Table 2).

The degradation of chromatin due to DNA fragmentation in the spacer DNA segments is manifested by an increase in the PDN level. The increase in PDN levels is a sign typical of the interphase deaths of lymphocytes (Okada 1974; Fedorova et all. 1972; Ryabchenko 1979). Our results (Table 1) suggest that in mouse thymocytes the maximum increase in PDN level occurs 6 h after the irradiation.

Table 2. Percentual values of PDN content in polymeric DNA in controls and irradiated LS/BL cells.

Dose, Gy	Post-irradiation interval, h	
	6*	15**
Controls	0.49 ± 0.10 ($n = 4$)	1.8 ± 0.18 ($n = 3$)
2 Gy	—	1.82 ± 0.27 ($n = 3$)
4 Gy	0.56 ± 0.03 ($n = 4$)	2.48 ± 0.72 ($n = 3$)
8 Gy	0.56 ± 0.08 ($n = 4$)	—
12 Gy	0.59 ± 0.09 ($n = 4$)	—
24 Gy	0.50 ± 0.11 ($n = 4$)	—

* — cells 5 days after transplantation
** — cells 6 days after transplantation
n — number of animals

Fifteen hours after the irradiation with 2 and 4 Gy the PDN levels decline. The return to the original low PDN levels was however slower in mice irradiated with the higher dose. The difference can be explained by a slower degradation of the damaged chromatin to final low-molecular products and their removal from the tissue, as well as by a slower recovery of the thymus cell population carrying intact chromatin after the higher radiation dose. It has been shown earlier (Pechenina et al. 1981) that four hours after irradiation with 1.0 Gy, PDN levels of rat thymocytes were increased to 8.6% and that 24 h after irradiation they fell to 1.7%. Following higher radiation doses (≥ 3 Gy) there was no such rapid fall of the PDN levels at later intervals. Dose dependent-differences in the recovery of thymus cell populations with undamaged chromatin probably reflect factors such as age and animal species.

It has previously been found (Matyášová et al. 1973) that the chromatin of LS/BL lymphosarcoma cells similarly as in the thymocytes, degrades to nucleosomes and their oligomers within 6 h after irradiation with a dose of 6 Gy. Results of the present experiments however show that properties of LS/BL cells change after prolonged cultivation: following irradiation with doses of 6; 12; and 18 Gy, at intervals 6 and 16 h, fragmentation of chromatin into regular fragments-monosomes and their multiples — such as can be found in thymus chromatin after irradiation in vivo with a similar dose no longer occurs. Only peaks corresponding to intact macromolecular DNA can be seen (Fig. 1). Electrophoresis of the same DNA samples under denatured conditions revealed that the majority of breaks

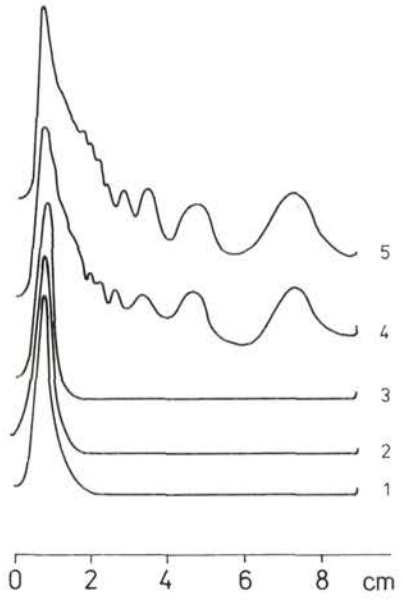

Fig. 1. Densitometric scans of electrophoresis of native DNA samples isolated from LS/BL cells: 1 — control cells, 2 — cells 6 h after whole-body irradiation with 6 Gy, 3 — cells 16 h after whole-body irradiation with 18 Gy, 4 — cells 16 h after in vivo treatment of mice with nitrogen-mustard (0.2 mg/10 g). Curve 5. represents DNA isolated from thymus 6 h after whole-body irradiation with 6 Gy.

were of the double-strand type. No single-strand breaks occurred within the nucleosomal core DNA (not shown).

Results shown in Table 2 also suggest that chromatin degradation, which is characteristic of the interphase deaths in irradiated thymocytes, does not occur in irradiated LS/BL cells. Neither, nuclear pyknotic degeneration as a sign of the interphase deaths, was observed in irradiated LS/BL cells (Kubíček et al. 1981). According to the results of Jurášková and Drášil (1978) the radiobiological characteristics of LS/BL lymphosarcomas change in the course of prolonged in vivo cultivation. Long-term cultivation of LS/BL cells thus resulted in a loss of their ability to react, after in vivo irradiation, by fragmentation of chromatin into regular fragments, corresponding to nucleosomes. It is, however, interesting that these cells remained capable of reacting in this way to nitrogen-mustard treatment in vivo (Fig. 1, curve 5). Other unfavourable conditions (hyperthermia, short cultivation of cells in vitro (Čejková and Skalka, unpublished results) also trigger in these cells, similarly as in thymocytes, processes resulting in the enzymic degradation of chromatin into nucleosomes.

References

Fedorova T. A., Tereshchenko O. Ya., Mazurik V. K. (1972): Nucleic Acids and Proteins in the Radiation-Damaged Organism. Meditsina, Moscow (in Russian)

Ivannik B. P., Proskuryakov S. Ya., Golubeva R. V., Ryabchenko N. I. (1976): Repair and degradation of DNA in radiosensitive tissues of irradiated rats. Radiobiologiya **16,** 803—809 (in Russian)

Ivannik B. P., Golubeva R. V., Murzaev V. N., Ryabchenko N. I. (1978): Role of protein synthesis in postirradiation degradation of DNP and DNA in rat thymocytes. Radiobiologiya **18,** 803—809 (in Russian)

Jurášková V., Drášil V. (1978): Behaviour of murine lymphosarcoma cells during prolonged cultivation *in vivo*. Fol. Biol. (Prague) **24,** 253—263

Kubíček O., Drášil V., Tkadleček L. (1981): On the question of intermitotic death of LS/BL lymphosarcoma cells after irradiation. Stud. Biophys. **83,** 233—238

Loening U. E. (1967): The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide-gel-electrophoresis. Biochemistry **102,** 251

Matyášová J., Skalka M., Čejková M. (1973): Regular character of chromatin degradation in lymphoid tissues after treatment with biological alkylating agents in vivo. Fol. Biol. (Prague) **25,** 380—388

Okada M. (1974): Radiation Biochemistry of the Cell. Mir, Moscow (in Russian)

Pechenina N. A., Ryabchenko N. I., Britun A. I. (1981): Influence of traumas on DNP degradation in irradiated rat thymus. Radiobiologiya **21,** 298—299 (in Russian)

Ryabchenko N. I. (1979): Radiation and DNA. Atomizdat, Moscow (in Russian)

Simpson R. T., Whitlock J. P. (1976): Chemical evidence that chromatin DNA exists as 160 base pair beads interspersed with 40 base pair bridges. Nucl. Acid Res. **3,** 117

Thomas P. S., Farquhar M. N. (1978): Specific measurement of DNA in nuclei and nucleic acids using diaminobenzoic acid. Anal. Biochem. **89,** 35—44

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