The Effects of Radiation and Alkylating Agents on Chromatin Degradation in Normal and Tumour Lymphoid Cells

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Abstract. The dynamics of chromatin degradation was studied in thymocytes and LS/BL tumour cells. In permeabilised LS/BL cells, the rate of DNA degradation induced by endogenous calcium and magnesium-dependent endonuclease was approx. 25 times slowlier than in thymocytes. In LS/BL cells irradiation does not induce chromatin degradation. The alkylating agent TS 160 induced chromatin degradation in both LS/BL lymphosarcoma cells and thymocytes.

Key words: Chromatin degradation — Radiation — Alkylating agents — Thymocytes — Lymphosarcoma — Cells

Introduction

Radiation (Skalka et al. 1976; Umanskij et al. 1981; Ryabchenko and Ivannik 1987), hormones (Umanskij et al. 1981; Wyllie 1980), and alkylating agents (Matyášová et al. 1979) have been shown to induce during interphase cell death, internucleosomal DNA fragmentation and chromatin degradation. The same picture is found with chromatin degradation by endogenous Ca^{2+}/Mg^{2+} dependent endonuclease (Ryabchenko and Ivannik 1987; Nelipovich et al. 1980; Chodarev et al. 1983). Malignization of lymphoid cells changes the character of their response to the action of damaging factors. In ascites lymphosarcoma radiation did not induce chromatin degradation, while the use of TS 160 induced its degradation in both the tumour cells and thymocytes (Ryabchenko et al. 1985). The present work reports further results of experiments in which the

Table 1. The effects of radiation on the PDN content (in percent) of polymeric DNA in murine thymocytes and in LS/BL cells

	Dose Gy	Postirradiation interval, h		
		3	6	15
Murine thymocytes	Controls		0.63 ± 0.02 %	
	2	—	22.60 ± 0.60 %	3.00 ± 0.90 %
	4	—	26.70 ± 0.80 %	9.10 ± 1.10 %
Rat thymocytes	Controls		2.70 ± 0.60 %	
	10	25.10 ± 1.40 %	44.80 ± 2.00 %	
LS/BL	Controls		0.49 ± 0.10 %	1.80 ± 0.18 %
	2			1.82 ± 0.27 %
	4		0.56 ± 0.03 %	2.48 ± 0.72 %
	8		0.56 ± 0.08 %	—
	12		0.59 ± 0.09 %	—
	24		0.50 ± 0.11 %	—

mechanism were considered determining the differences between normal and malignised lymphoid cells as regards chromatin degradation following radiation and TS 160.

Materials and Methods

LS/BL lymphosarcoma cells were cultured in the peritoneal cavity of F_1 hybrid C57BL × BALB/c mice. The cell suspension was transferred to a new host once weekly, at the rate of 10^6 cells per mouse. On the fifth day after the tumour inoculation the host mice were irradiated with ^{60}Co gamma, or received an i.p. injection of tris-(2-chlorethyl)-amine hydrochloride (SPOFA TS 160), 20 mg per kg. After 6 or 15 hours the mice were sacrificed, and the LS/BL cells were obtained by flushing the peritoneal cavity using Hanks solution without Ca^{2+} and Mg^{2+} ions.

Mice (C57BL, age 3 weeks) and rats (weight 140—200 g) were irradiated or injected TS 160 at 8:00 a.m. and sacrified after 3 and 6 hours. Another group or animals were irradiated or injected TS 160 at 5:00 p.m. and sacrificed after 15 hours. After sacrificing, the thymi were quickly removed, minced with a scalpel in a cold STM buffer (0.25 mol/l sucrose, 3 mmol/l $MgCl_2$, 10 mmol/l Tris-HCl, pH 7.2) and filtered through capron gauze.

Permeabilised and hypotonised LS/BL cells and thymocytes were obtained by centrifugation

Table 2. The effects of the alkylating agent TS 160 on the PDN content (in percent) of polymeric DNA in rat thymocytes and in LS/BL cells

	Hrs after TS 160 administration	
	6	16
Controls		$2.7 \pm 0.6\,\%$
Rat thymocytes	—	$30.2 \pm 3.7\,\%$
Controls		$1.8 \pm 0.2\,\%$
LS/BL	$4.6 \pm 0.8\%$	$19.3 \pm 3.0\,\%$

and were resuspended in TM buffer consisting of: 10 mmol/l Tris-HCl, 3 mmol/l $MgCl_2$, pH 7.2. All cells are stained with trypane blue under these conditions.

Endogenous chromatin degradation was induced by incubation of hypotonised cells in TMC and TME buffers at 37 °C. The TMC buffer contained: 10 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 50 mmol/l Tris-HCl, pH 7.5. The TME buffer contained: 10 mol/l $MgCl_2$, 1 mmol/l EDTA, 50 mmol/l Tris-HCl, pH 7.5.

Chromatin degradation was evaluated by changes in the levels of polydeoxyribonucleotides (PDN) extractable with diluted EDTA solutions and dissolved in 0.15 mol/l NaCl. To determine the PDN levels, the cells were centrifuged and the sediment resuspended in 3—5 ml 0.7 mol/l EDTA, pH 7.0 and incubated on an ice bath for 60 min under constant stirring. Then 4 mol/l NaCl was added to a concentration of 0.15 mol/l and the suspension was centrifuged at 5000 rpm for 30 min. DNA was determined by the fluorometric method with diaminobenzoic acid in the sediment containing undegraded deoxyribonucleoprotein, and in the supernatant containing PDN. Results are given as percentage contents of DNA in the PDN fraction (Ryabchenko and Ivannik 1987).

Results

It follows from the data shown in Table 1 that radiation does not induce chromatin degradation in LS/BL lymphosarcoma cells which is typical of irradiated thymocytes. On the other hand, TS 160 induces chromatin degradation in thymocytes as well as LS/BL cells (Table 2).

Other reports have suggested that Ca^{2+}/Mg^{2+} dependent endonuclease participates in the post-irradiational degradation of chromatin (Umanskij et al. 1981; Ryabchenko and Ivannik 1987). To determine the pattern of endonucleolysis of chromatin in LS/BL cells and thymocytes following irradiation or the action of TS 160, cell suspensions were incubated for various lengths of time in

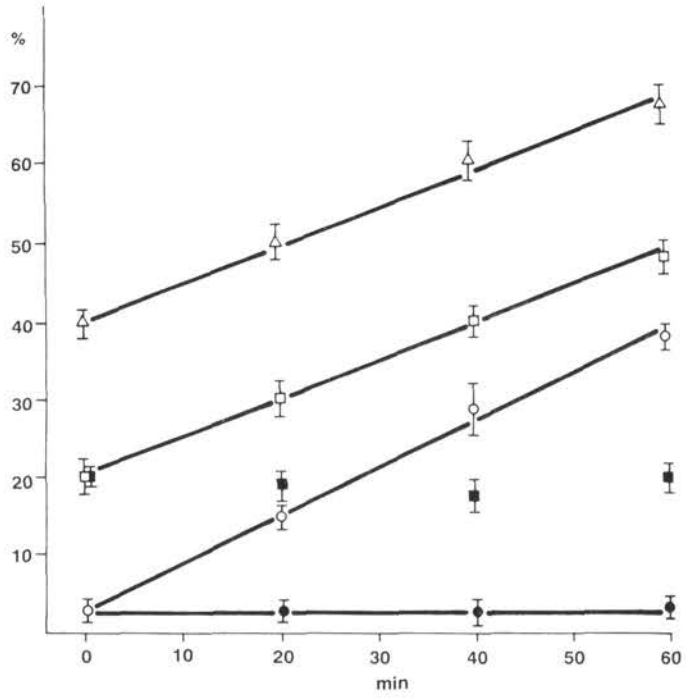

Fig. 1. Chromatin endonucleolysis in hypotonized rat thymocytes: ○ ■ nonirradiated, □ ■ obtained 3 h or △ 6 h after 10 Gy; incubated at 37 °C in ○ □ △ or ● ■ TME buffers. Abscissa: PDN content in per cent in polymeric DNA, Ordinate: time of incubation.

TMC and TME buffers. Fig. 1 shows that the incubation of nonirradiated thymocytes in TMC buffer leads to chromatin degradation, and the percentages of PDN in cells increase with the increasing length of incubation. In thymocytes obtained 3 and 6 hours after irradiation with 10 Gy, endonucleolysis starts at a considerably higher level of PDN (Fig. 1, Table 1). This has, however, no effect on the rate of endogenous chromatin degradation in the TMC buffer. The elimination of Ca^{2+} ions from the incubation medium or their intercellular binding with 1.0 mmol/l EDTA (in TME bufer) completely inhibits nucleolysis in irradiated and nonirradiated thymocytes. (Fig. 1). This suggests the participation of Ca^{2+}/Mg^{2+} dependent nuclease in the process. Fig. 2 shows the results obtained in a study of endonucleolytic chromatin degradation in hypotonised LS/BL cells obtained from control and irradiated hosts (dose 10 Gy, 6 hours after the irradiation). It follows from these results that the rate of endonucleo-

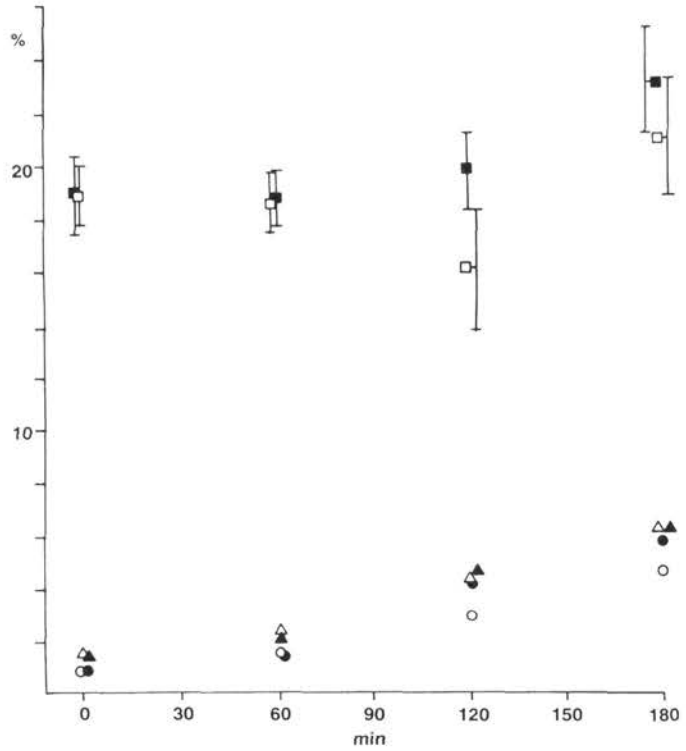

Fig. 2. Chromatin endonucleolysis in hypotonized LS/BL cells: ○ ● nonirradiated, △ ▲ obtained 6 h after 10 Gy or □ ■ 16 h after TS 160; incubated at 37 °C in ○ □ △ TMS or in ● ■ ▲ TME buffers. *Abscissa: PDN content in per cent of polymeric DNA. Ordinate: time of incubation.*

lysis is about 25 times slowlier than observed in control and irradiated thymocytes (Fig. 1). Another specific feature of endogenous chromatin degradation in LS/BL cells is the lack of stimulation by Ca^{2+}ions. Chromatin degradation is almost the same when LS/BL cells are incubated in TMC buffer containing Ca^{2+} as in TME buffer containing EDTA, without calcium.

A decreased activity of Ca^{2+}/Mg^{2+} dependent endonuclease in LS/BL tumour cells seems to be the reason for irradiation being unable to induce in them postirradiational chromatin degradation. At the same time, as follows from the results shown in Table 2, the alkylating agent TS 160 induces chromatin degradation in both thymocytes and LS/BL lymphosarcoma cells.

Figures 2 and 3 illustrate the results obtained 16 hours after TS 160 injec-

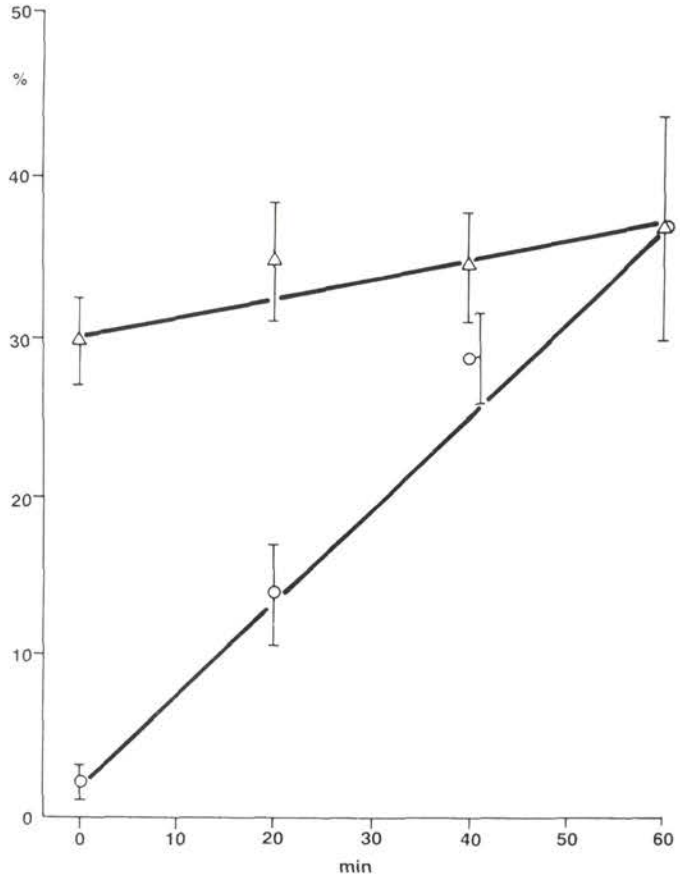

Fig. 3. Effect of TS 160 treatment on endogenous chromatin degradation in rat thymocytes: ○ control, △ obtained 16 h after TS 160; incubated at 37 °C in TMC buffer. Abscissa — PDN content in per cent of polymeric DNA. Ordinate — time of incubation.

tion to mice and rats: chromatin degradation occurred in thymocytes and in LS/BL cells. In contrast to radiation TS 160, although inducing chromatin degradation in thymocytes, considerably reduces the effectiveness of endonuclease degradation in permeabilized cells incubated in TMC buffer.

Discussion

The aim of the present work was to analyse the mechanism of chromatin

degradation in normal an tumour cells. Very low rates of endogenous chromatin degradation were measured for tumour LS/BL cells in TMC buffer containing Ca^{2+} and Mg^{2+} ions in concentrations optimal for the activity of Ca^{2+}/Mg^{2+} dependent endonucleases (Figs. 1 and 2, Ryabchenko and Ivannik 1987; Nelipovich et al. 1980; Chodarev et al. 1983). In LS/BL cells, the removal of Ca^{2+} ions from the medium (Fig. 2) did not inhibit chromatin endonucleolysis, in contrast to thymocytes, in which chromatin degradation occurred under the same conditions (Fig. 1). Our results and those of other authors (Chodarev et al. 1983) suggest that malignant transformation results in a reduction of activity (lack of enzyme?) of Ca^{2+}/Mg^{2+} dependent endonuclease. On the basis of published data (Umanskij et al. 1981; Ryabchenko and Ivannik 1987; Nelipovich et al. 1980) one can suppose that this enzyme participates in postirradiational chromatin degradation in thymocytes. It can thus be assumed that a decreased Ca^{2+}/Mg^{2+} dependent endonuclease activity in LS/BL cells is one of the causes for the absence of internucleosomal fragmentation of DNA in irradiated LS/BL mouse tumour cells (Table 1 Fig. 2; Ryabchenko et al. 1985). However it is not only this endonuclease which participates in chromatin degradation caused by various damaging factors. The ability of TS 160 to induce chromatin degradation (Fig. 2, Table 2) and internucleosomal fragmentation of DNA in tumour cells (Ryabchenko et al. 1985), where the activity of Ca^{2+}/Mg^{2+} dependent endonuclease is very low (Figs. 1 and 2), indicates that under the influence of alkylating agents, enzymatic systems other than Ca^{2+}/Mg^{2+} dependent endonuclease which cause chromatin degradation in irradiated cells are involved in chromatin degradation.

Previous works have reported (Jurášková and Drášil 1978; Kubíček et al. 1981) that irradiated LS/BL cells rapidly disappear from the peritoneal cavity of host mice. Since no pyknosis or rapid chromatin degradation typical of interphase death in non-malignised lymphoid cells, has been observed in irradiated LS/BL cells, one must suppose that the fall in cellularity in irradiated suspensions injected to host animal is due to mechanisms independent of chromatin degradation and of the pyknotic destruction of the nuclear apparatus.

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