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INHIBITION OF RADIATION-INDUCED PYCNOSIS OF CELL NUCLEI BY CADMIUM IONS

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KEY WORDS: thymocytes; cadmium; gamma-irradiation; nuclear pycnosis; supercoiled DNA.

Ions of many heavy metals possess marked toxicity. For instance, if cadmium salts enter the body of man and other mammals they induce the development of pathological changes in the kidneys, lungs, and other organs and systems [10]. The problem of the concrete mechanisms of these biological effects has not been finally solved. It can be tentatively suggested that Cd^{++} ions, competing with ions of other trace elements, inhibit activity of certain metabolic processes [12]. The corresponding shifts at the cellular level can be studied by complex anlysis, for it is possible to modify nuclear structures and to produce changes in cell membranes. The lymphoid cells of the thymus are very convenient for such investigations, for they are highly sensitive *in vivo* to the action of Cd^{++} ions [13].

This paper describes a study of the effect of cadmium chloride ($CdCl_2$) on the frequency of nuclear pycnosis in a thymocyte population *in vitro* and the results are compared with data on the supercoiled structure of DNA and integrity of the cell membranes.

EXPERIMENTAL METHOD

Noninbred female albino rats weighing 130-150 g were used. Thymocytes were isolated and counted and suspensions ($5 \cdot 10^6$ cells in 1 ml) prepared in medium 199 with 10% homologous serum by the method described previously [4]. A 4 mM solution of $CdCl_2$ and its dilutions were made up in 0.15 M NaCl solution and kept at −10°C. To each sample of 2 ml, 0.05 ml of $CdCl_2$ solution was added immediately before irradiation. In the control, 0.15 M NaCl was added.

Gamma-irradiation of the cells (^{60}Co) was carried out at 20°C in an atmosphere with 5% CO_2 on a "Luch-1" apparatus with a dose rate of 0.9 Gy/min. The cells were subsequently incubated for 5 h at 37°C.

Pycnotic forms of thymocytes were counted by staining with acridine orange and the fractions of cells carrying receptors for autologous erythrocytes (ARFC) were determined as described previously [14]. Cells in contact with two or more erythrocytes were taken to be ARFC. The thymocytes were washed once for supravital staining. The cells were stained with alcian blue (from Gee Lawson, England) or erythrosin [5], with a final concentration of the dye of 0.02%. Conformational changes in the supercoiled structure of DNA (scDNA) of the thymocytes were assessed as changes in relative viscosity of the cell lysate (nucleoids), by means of an Ostwald viscometer [2].

The results were subjected to statistical analysis by Student's t test [1].

EXPERIMENTAL RESULTS

After incubation of unirradiated thymocytes for 5 h moderate pycnotization of the cell nuclei, characteristic of the model used [14], was observed (Table 1). The presence of Cd^{++} ions (10-100 μM) did not reduce the fraction of ARFC or change the number of cells which

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TABLE 1. Fraction of Cells (in %) Inctact Relative to Different Criteria after Incubation for 5 h in Control or in Presence of Cd^{++} Ions (M ± m)

Concentration of Cd^{++} ions, μM	Nonpycnotic cells	ARFC	Supravital staining	
			erythrosin	alician blue
0	75,3±1,9	20,1±3,7	85,0±1,8	81,6±1,3
10	73,8±2,1	23,7±9,4	—	—
100	81,9±1,6*	27,0±7,6	84,3±0,9	73,3±2,7*

Legend. Here and in Table 2 mean results of 4-8 experiments are given: *P < 0.05.

TABLE 2. Fraction of Stained Cell Forms (in %) on Supravital Staining of Irradiated (4 Gy) Thymocytes after Incubation for 5 h (M ± m, n = 4)

Experimental conditions	Fraction of unstained cells	
	erythrosin	alician blue
Irradiated (4 Gy) control	65,4±2,2	59,5±4,8
4 Gy, 10 μM Cd^{++}	66,7±4,0	58,7±3,1
Fraction of unstained cells	82,5±1,3*	75,2±0,3*

stained with erythrosin. With Cd^{++} present in the medium in a concentration of 100 μM some increase was found in the fraction of nonpycnotic forms and a decrease in the number of unstained thymocytes in the alcian blue test, which could reflect a general toxic effect of Cd^{++}.

When irradiated (4 Gy) thymocytes were investigated marked pycnosis of the nuclei and loss of ARFC receptors were observed, evidence of interphase cell death. However, in samples containing Cd^{++} (10 μM or more) considerable weakening of the pycnotic process and normalization of the ARFC fraction (with Cd^{++} in a concentration of 100 μM in the medium) were observed. Additional data on modification of processes of thymocyte death were obtained by supravital staining of the irradiated cells. The fraction of unstained forms (by tests with erythrosin and alcian blue) amounted to about 75% of the unirradiated control (Table 1, Table 2). The presence of Cd^{++} (100 μM) in the medium prevented the development of these membrane changes virtually completely (Table 2).

Consequently, Cd^{++} ions can inhibit the principal manifestations of postradiation death of thymus cells: pycnosis of the nuclei, loss of ARFC receptors, and a pathological increase in membrane permeability. In the light of this complex effect of Cd^{++} it is unlikely that it should have an isolated effect on the process of DNA fragmentation (and pycnotization of nuclei) under the influence of Ca, Mg-dependent endonuclease similar to that which has been demonstrated for zinc ions [8].

Meanwhile there is evidence of effects of Cd^{++} at the chromosomal level. For instance, shortening of the average length of lymphocyte chromosomes has been demonstrated under the influence of Cd^{++} ions; this was observed in the early period after addition of Cd to the cell culture [7]. Arising from these data, and also in view of the fact that compactization of the "nucleoid" reflects changes in the supramolecular organization of DNA, conformational changes in scDNA were analyzed by titration of thymocyte "nucleoids" with ethidium bromide, with different concentrations of this intercalating agent. In all versions of the experiments scDNA relaxed in the same concentration of ethidium bromide, i.e., the number of supercoils on the "averaged" DNA domain was unchanged in the presence of Cd^{++}. However, after incubation of irradiated (4 Gy) cells in medium containing cadmium (10-100 μM) the relative viscosity of the "nucleoids" was considerably reduced compared with the control (without irradiation). Such changes in viscosity have been interpreted as compactization of scDNA [11]. Consequently, Cd^{++} ions promote compactization of "nucleoids" of irradiated thymocytes.

It can be tentatively suggested that these changes are conformational in character and are caused by disturbances in the system maintaining stability of the DNA structure [9]. Together with other proteins, this role may be played in thymocytes by enzymes known as topoisomerases [3]. The possibility cannot be ruled out that Cd^{++}, by modifying topoisomerase function, modifies the postradiation repair of higher levels of DNA.

In these experiments the toxicity of Cd^{++} *in vitro* relative to unirradiated thymocytes was thus only weak. At the same time, it can be tentatively suggested that the significant antipycnotic effect and the enhanced preservation of the membranes of irradiated thymus cells are the result of inhibition of the endogenous interphase death program by Cd^{++} ions [8].

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EFFECT OF AMINOPYRINE ON THE DEGRADATION RATE OF CYTOCHROME P-450 ISOFORMS

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KEY WORDS: cytochrome P-450; degradation rate; electrophoresis.

Cytochrome P-450 is a key enzyme of the hepatic microsomal chain for oxidation of hydrophobic substances [1]. This group includes many xenobiotics which may enter the body, including some widely used drugs, such as aminopyrine, codeine, morphine, and so on, and also substances of endogenous nature, such as steroids and fatty acids. Introduction of certain xenobiotics into the body causes induction of cytochrome P-450, with an increase in its concentration in the liver and the appearance of new isoforms of the enzyme [10]. Phenobarbital is one of the most extensively studied and widely used inducers of cytochrome P-450. We know that a two-threefold increase in the cytochrome P-450 concentration in the liver is due mainly to more rapid biosynthesis of this enzyme [4, 5]. Until recently, it was held that the rate of degradation of microsomal proteins, including components of the microsomal oxidation chain, on induction by phenobarbital is delayed or remains unchanged [7, 11]. However, evidence has recently been obtained [12] to show that more rapid degradation of cytochrome P-450 and of other enzymes of the microsomal oxidation chain may take place under the influence of phenobarbital.

The aim of this investigation was to study the effect of aminopyrine, a substrate of the mono-oxygenase system of the liver, on the degradation rate of cytochrome P-450 isoforms. Da-

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