Structure of Nuclear Chromatin in Cells of Lymphoid Organs and Blood from Mice Maintained under Abnormal Light-Dark Cycle and Treated with Benz(a)pyrene

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We studied the effects of benzo(a)pyrene on lymphocyte chromatin in mice with experimental desynchronosis. Desynchronosis altered chromatin structure in immune system cells and suppressed the immune response. In mice with experimental desynchronosis, benzo(a)pyrene produced more pronounced changes in the chromatin structure compared to the control. It was inferred that light-dark cycle disturbances impair the resistance of organs and systems to damaging environmental factors.

Key Words: *lymphocytes; chromatin; desynchronosis; benzo(a)pyrene*

Immune system, one of the most important homeostatic defense mechanisms, usually responds to endogenous and exogenous factors [2]. At the same time, the immune system complies the principle of rhythmycity of all biological processes [8,10]. It can be hypothesized that the degree or the direction of immunotrophic influence of negative environmental agents can alter under conditions of desynchronosis (DS), *i.e.* phase desynchronization of circadian rhythms of various functions. However, the sensitivity/resistance ratio of the immune system to damaging factors under conditions of desynchronization of biological rhythms was poorly investigated. Chemical pollution responsible for increased incidence of various pathologies in industrially loaded territories is now one of the main ecological problems [1,4,5]. According to WHO criteria, 3,4-benzo(a)pyrene (BP) is the most dangerous and widespread chemical substance [4]. BP is a carcinogen, its metabolites intercalate into DNA, damage the genetic apparatus, induce mutations [4], and enhance the action of oncogenes [11]. Immunosuppressive properties of BP are also known: it affects processes of B- and T-cell differentiation and suppresses their response to IL-2 [4,13].

Here we studied the effect of BP on the structure of nuclear chromatin in mouse lymphocytes under conditions of experimental DS.

MATERIALS AND METHODS

For DS modeling, 3-4-month-old male (CBA×C57Bl) F_1 mice (n=35) obtained from nursery of Institute of Clinical Immunology were kept at constant illumination (CI) for 2 weeks [7]. Control groups were kept under normal light/dark regimen. BP (20 mg/kg in 0.2 ml of olive oil) was administered intraperitoneally at 10.00 for 3 days starting from day 15 of the experiment. The same volume of olive oil was used as active control. The animals were decapitated at 10.00 on the next day after the 3rd injection of BP or olive oil. The structure of nuclear chromatin was studied on Feulgen-stained blood smears and suspension of lymphoid organs by means of optical structural com-

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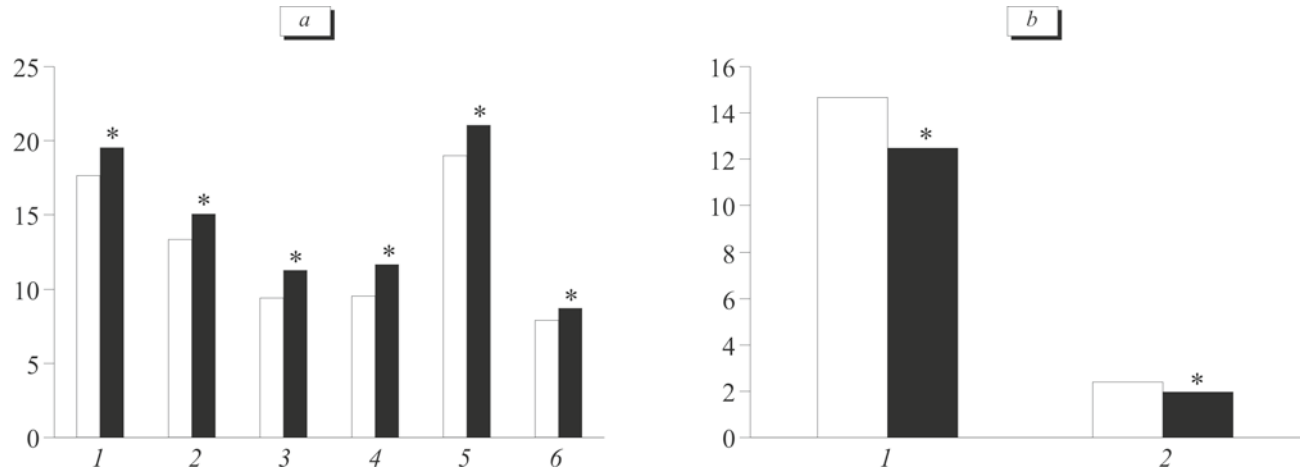

Fig. 1. Densitogeometric properties of nuclear chromatin in lymphocytes from the blood (*a*) and mesenterial lymph nodes (*b*) of mice kept under conditions of normal light regimen (light bars) and CI (dark bars). *a*: *1*) perimeter of euchromatin; *2*) area of euchromatin distribution; *3*) perimeter of heterochromatin; *4*) area of heterochromatin distribution; *5*) total chromatin distribution area, *6*) integral optical density of chromatin; *b*: *1*) area of euchromatin distribution; *2*) integral optical density of euchromatin. Ordinate: μ and μ² for geometric indices and arb. units for optical density. **p*<0.05 compared to the control.

puter analysis using Russian microstructure analyzer (Lumam PM-11 scanning light microscope connected to an IBM PC computer) and special software created in Laboratory for Immunomorphology of Institute of Clinical and Experimental Lymphology. The following parameters were recorded: perimeter and area of

TABLE 1. Densitogeometric Properties of Nuclear Chromatin in Lymphocytes from the Blood and Lymphoid Organs after BP Administration to Mice Kept under Normal Light Conditions (*M*±*SE*)

Group/Parameter	Blood	Thymus	Spleen
Euchromatin perimeter, μ			
Control	17.64±0.272	20.028±0.293	18.768±0.484
Active control (olive oil)	20.43±0.60*	19.024±0.486	18.646±0.353
BP	19.56±0.384*	20.892±0.589+	20.353±0.47*+
Euchromatin distribution area, μ²			
Control	9.428±0.245	13.063±0.397	10.968±0.600
Active control (olive oil)	10.17±0.77	10.165±0.543*	9.399±0.503
BP	9.418±0.417	11.971±0.890	11.232±0.789+
Total chromatin distribution area, μ²			
Control	18.978±0.422	23.842±0.788	21.085±1.102
Active control (olive oil)	23.561±1.350	20.565±0.951*	19.872±0.865
BP	21.253±0.610	24.094±1.033+	22.875±1.018
Mean optical density of euchromain, arb. units			
Control	0.212±0.009	0.165±0.013	0.192±0.004
Active control (olive oil)	0.152±0.007*	0.098±0.005*	0.101±0.005*
BP	0.175±0.003*+	0.087±0.008*	0.094±0.005*
Mean optical density of heterochromatin, arb. units			
Active control (olive oil)	0.327±0.029	0.176±0.011*	0.181±0.009*
BP	0.411±0.016+	0.160±0.016*	0.180±0.013*

Note. Here and in Table 2: $p<0.05$ compared to: *control, +active control.

structures occupied by eu- and heterochromatin, total chromatin area (area of nucleus), integral and mean optical density of eu- and heterochromatin (reflects the amount and the degree of chromatin compaction), and integral optical density of the whole chromatin (reflects the amount of nuclear DNA). Humoral immune response to T-dependent antigen (sheep erythrocytes) was evaluated by the number of antibody-producing cells in the spleen on day 4 after intraperitoneal administration of the antigen by Cunningham method [3]. Statistical analysis was performed using Statistica 5.0 software. Significance of difference was evaluated using nonparametric Mann–Whitney test.

RESULTS

The structure of nuclear chromatin in lymphocytes from the blood and mesenteric lymph nodes was changed under conditions of CI. In blood lymphocytes, the total nuclei area and perimeter and area of structures occupied by eu- and heterochromatin increased. In the nuclei of lymph node cells, the area occupied by euchromatin and the content of heterochromatin decreased, which can attest to inhibition of synthetic processes involving DNA (Fig. 1). These changes were followed by a decrease in humoral immune response to T-dependent antigen (Fig. 2).

TABLE 2. Densitogeometric Properties of Nuclear Chromatin of Cells from Lymphoid Organs after BP Administration to Mice Kept under Conditions of CI (*M*±*SE*)

Group/Parameter	Lymph nodes	Thymus	Spleen
Heterochromatin perimeter, μ²			
Control	14.72±0.33	14.57±0.47	13.50±0.19
Active control (olive oil)	16.71±0.58*	14.38±0.59	13.42±0.41
BP	14.10±0.32+	12.95±0.34*+	12.15±0.31*+
Euchromatin perimeter, μ			
Control	20.05±0.24	20.087±0.472	18.78±0.25
Active control (olive oil)	21.00±0.56	19.510±0.434	18.24±0.31
BP	19.06±0.36*+	18.320±0.409*	16.80±0.23*+
Euchromatin distribution area, μ²			
Control	12.475±0.356	12.978±0.687	10.59±0.21
Active control (olive oil)	11.985±0.775	11.140±0.371*	9.80±0.24*
BP	11.210±0.514	10.897±0.415*	8.94±0.26*+
Heterochromatin distribution area, μ²			
Control	11.40±0.35	11.71±0.65	10.13±0.29
Active control (olive oil)	12.02±0.74	10.31±0.65	9.44±0.54
BP	9.85±0.39*+	8.62±0.47*	7.87±0.31*+
Total chromatin distribution area, μ²			
Control	23.883±0.701	24.688±1.298	20.73±0.46
Active control (olive oil)	24.001±1.410	21.455±0.880	19.24±0.63
BP	21.060±0.843*	19.524±0.755*	16.82±0.41*+
Mean heterochromatin optical density, arb. units			
Control	0.328±0.016	0.35±0.01	0.398±0.039
Active control (olive oil)	0.205±0.004*	0.21±0.01*	0.249±0.010*
BP	0.203±0.011*	0.18±0.01*+	0.253±0.013*
Integral chromatin optical density, arb. units			
Control	7.45±0.13	8.16±0.36	7.85±0.23
Olive oil administration	5.02±0.11*	4.66±0.24*	4.76±0.21*
BP	4.27±0.21*+	3.56±0.24*+	4.20±0.13*+

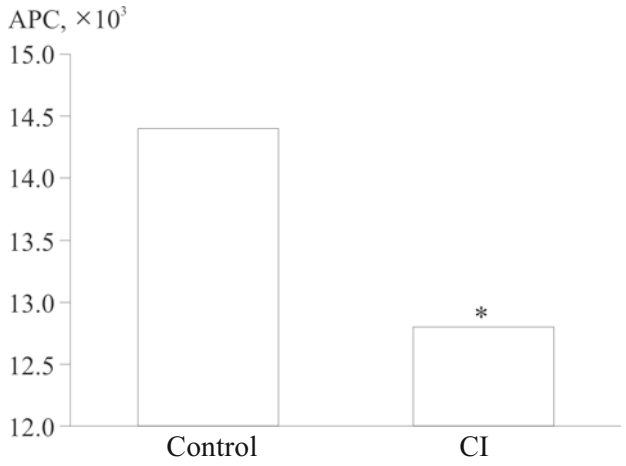

Fig. 2. Amount of antibody-producing cells (APC) in mouse spleen on day 4 after immunization with sheep erythrocytes. *p<0.05 compared to the control.

Administration of BP to control mice increased the mean optical density of nuclear chromatin in blood lymphocytes, perimeter of structures occupied by euchromatin, total area of thymocyte nuclei, and perimeter and area of structures occupied by euchromatin in splenocyte nuclei (Table 1). Thus, under normal light conditions BP administration increased the volume occupied by euchromatin in the nucleus, which can reflect increased intensity of synthetic processes related to BP metabolism or development of the immune response to autoantigens of damaged cells.

BP administration to mice kept under conditions of CI reduced the perimeter, area, and mean optical density of heterochromatin and total DNA content in thymus cells. In splenocytes, the perimeter and area of both types of chromatin, DNA content and area of nuclei decreased. In lymph nodes, we observed a decrease in the perimeter of structures occupied by eu- and heterochromatin, area of heterochromatin, and total DNA content (Table 2).

Thus, BP produced a more pronounced damaging effect on nuclear apparatus of lymphoid cells in animals kept under conditions of CI. This can be associated with altered state of chromatin organization under abnormal light regimen, because BP and its metabolites bind proteins of active and inactive chromatin in different ways [12]. It is also possible that CI enhances negative effect of BP at the expense of metabolic dysfunctions resulting from impairment of microsomal mitochondrial apparatus of hepatocytes [7]. This can lead to imbalance of pro- and antioxidant system components, modulation of glutathione metabolism, and inhibition of the synthesis of the corresponding glutathione-S-transferases mainly functioning as cell protectors against lipid peroxidation products and xenobiotics, *e.g.* against carcinogenic action of BP [6,14]. This hypothesis is supported by the data on altered circadian cycle of glutathione metabolism in the liver of mice maintained under inverted light/dark regimen [9]. It can be concluded that disturbances in circadian temporal organization lead to structural and metabolic deviations impairing the resistance of organs and systems to damaging environmental factors.

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