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BENZ(a)ANTHRACENE AND 2,3,7,8-TETRACHLORODIBENZO(*p*)DIOXIN MODULATE MITOGEN-STIMULATED LYMPHOCYTE PROLIFERATION

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UDC 615.916:661.715.6].017:615.277.4] .015.44:616.155.32-018.15

KEY WORDS: lymphocytes; proliferation; xenobiotics; benzpyrene hydroxylase

Polycyclic aromatic hydrocarbons and polyhalogenated aromatic hydrocarbons are the xenobiotics most dangerous for human health [5]. In this investigation we studied the functional properties of human peripheral blood mononuclear cells (MC) exposed to the action of benz(a)anthracene (BA) and 2,3,7,8-tetrachlorodibenzo(*p*)dioxin (TCDD) under different conditions of incubation of lymphocytes.

EXPERIMENTAL METHOD

MC were isolated by centrifugation of heparinized blood from healthy blood donors in a Verografin–Ficoll density gradient [4]. MC harvested from the interphase were washed three times with buffered physiological saline, after which the cell suspension was transferred into RPMI-1640 culture medium. Populations of T lymphocytes were identified by methods of rosette formation with different species of test erythrocytes. The total number of T cells was determined by the method of rosette formation with sheep's erythrocytes (E-RFC [1]). The number of active (early) E-rosette-forming cells ($E\text{-}RFC_{ac}$) was determined by the method [8]. To determine the relative number of lymphocytes forming rosettes with autologous erythrocytes the method in [7] was used. A lymphocyte population enriched with T-cells was obtained by sedimentation of E-RFC in a Verografin–Ficoll gradient, followed by lysis of the erythrocytes by hypoosmolar shock. To assess the effect of xenobiotics on the subpopulation structure and functional properties of the lymphocytes, the MC were preincubated for 24 h in culture medium consisting of medium RPMI-1640, 20% of serum from group IV blood donors heated to 56°C, 2 mM glutamine, and 40 μg/ml gentamicin. The cells were incubated in the presence of 10 μM BA or 10 nM TCDD, generously provided by Dr. D. Nebert (National Institutes of Health, USA). After washing three times to remove the xenobiotics, the functional properties of the treated lymphocytes were estimated. Activity of natural killer (NK) cells was determined by their ability to lyse the target cell (K-562 [3]). To estimate the proliferative activity of MC, lymphocytes numbering 10^6 per well were stimulated with concanavalin A (con A) in a concentration of 25 μg/ml in round-bottomed planchets for immunologic research. The intensity from incorporation of ^{3}H-thymidine.

In some experiments, as regulators of lymphocyte proliferation we used MC (10^6 per well), treated for 45 min at 37°C with mitomycin C (mit C) in a concentration of 35 μg/ml. To assess benzpyrene hydroxylase activity, freshly isolated cells or cells stimulated for 72 h with phytohemagglutinin (PHA), in a concentration of 10 μg/ml, were treated with BA, as inducer of the monooxygenase system, in a concentration of 10 μA. Activity of benzpyrene hydroxylase was determined from the quantity of

Laboratory of Xenobiochemistry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 569-571, June, 1990. Original article submitted November 10, 1989.

TABLE 1. Activity of Benzpyrene Hydroxylase in Culture of Intact and PHA-Stimulated Human Lymphocytes

MC	Without BA	With BA	Index of inducibility
	10^{-12} M 3-hydroxybenzpyrene/million cells/min		
PHA-stimulated (incubation for 96 h, addition of BA 24 h before end of incubation) (n = 58)	0,52±0,08	1,24±0,14	3,6±0,52
Freshly isolated (incubation without mitogen for 24 h in presence of BA) (n = 12)	0,016±0,004	0,07±0,02	5,4±1.70
	<0,01	<0,01	>0,05
	Student's t test		

TABLE 2. Proliferative Response of MC in the Presence of Lymphocytes Preincubated with BA or with TCDD

№	MC_{fr} (I)	MC_{fr} + MC_c (II)	MC_{fr} + MC_{BA} (III)	MC_{fr} + MC_{TCDD} (IV)
1[a]	2,4[b]	3,0	4,1	3,8
2	2,9	3,5	5,4	4,2
3	2,3	3,2	6,5	3,6
4	1,4	2,8	4,0	3,5
M	2,3	3,1	5,0	3,8

Legend. a) Freshly isolated MC (MC_{fr}) stimulated by con A for 96 h, separately or in the presence of mit C-treated MC, preincubated for 24 h with BA (MC_{BA}) or with TCDD (MC_{TCDD}). Cells preincubated without xenobiotics were added as control to the stimulated lymphocytes. [b]) Number of counts per minute $\times 10^4$. I-II) $p = 0.05$, I-III and I-IV) $p < 0.05$, II-III) $p < 0.05$, II-IV $p = 0.05$ (Wilcoxon–Mann–Whitney test).

enzyme reaction product (3-hydroxybenzpyrene) per million cells per minute, by our modified method [2]. The index of inducibility was determined as the ratio of activity of the enzyme in induced MC and its activity in intact (control) lymphocytes.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that mitogenic stimulation led to a marked increase in benzpyrene hydroxylase activity, in both control and BA-induced mononuclear cells, but it did not affect the index of inducibility of the enzyme. In intact MC, preincubated for 24 h with BA, a significant change was observed in the subpopulation structure of the T cells. The number of lymphocytes with markers of adult active E-RFC among MC preincubated with BA was almost twice the number of $E\text{-}RFC_{ac}$ among cells not treated with the xenobiotic, and amounted to 79 ± 7.3 and 48.6 ± 9.8% respectively ($n = 6$, $p = 0.05$ — Wilcoxon's paired test). After incubation with BA, the less mature T cells with receptors for autologous arythrocytes (auto-RFC) virtually disappeared from the culture of MC. For instance, the initial number of auto-RFC among the freshly isolated T cells averaged 28.4 ± 3.3%. After incubation with BA for 24 h this parameter decreased to 2.7 ± 1.4%. Among the control lymphocytes preincubated without the xenobiotic, the number of auto-RFC was 8.8 ± 1.0% ($n = 5$, $p = 0.05$ — Wilcoxon's paired test). No increase was observed in the relative percentage of dying cells or no increase in the total number of MC compared with the control lymphocytes, untreated with xenobiotics in the concentrations indicated above.

The increase in the relative number of mature T-lymphocytes was accompanied by a change in the functional properties of MC. Preincubation of the cells with BA led to lowering of their NK activity. The mean percent of lysis of the target cells (K-562 lymphocytes), treated for 24 h with BA, was 22.0%. The same parameter for cells preincubated without the xenobiotic was 29% ($n = 5$, $p = 0.5$ — Wilcoxon's paired test).

Treatment of the resting cells for 24 h with BA or TCDD before mitogenic stimulation led to more intensive blast-transformation of the lymphocytes. For instance, the proliferative response of MC preincubated with BA or TCDD was increased by 21% ($n = 11, p = 0.068$, Fisher's exact method) and 38.5% ($n = 7, p = 0.01$ — Wilcoxon's paired test) respectively compared with control cells, preincubated without the xenobiotics. It is interesting to note that MC preincubated for 24 h with BA or TCDD and then stimulated by con A, after treatment with mit C possessed significantly greater ability to intensify blast transformation of the freshly isolated allogeneic lymphocytes than control cells, not treated previously with xenobiotics (Table 2). The constant presence of BA in the culture of MC throughout the period of culture of the cells with con A, however, inhibits the proliferative response of the lymphocytes on average by 20% ($n = 6, p = 0.01$ — Wilcoxon's paired test).

It must be pointed out that existing views on xenobiotics which are inducers of the monooxygenase system as substances possessing an exceptionally toxic and suppressor activity are correct only for certain conditions of exposure of lymphocytes to these compounds. Our own results are evidence that lowering of proliferative activity is observed in cell cultures stimulated by mitogen against the background of high activity of benzpyrene hydroxylase, one form of cytochrome P-450. Low activity of the enzyme in resting cells evidently does not favor the formation of a sufficient amount of metabolites, which are known to possess genotoxic properties [6]. Under these conditions of incubation of the lymphocytes the relative number of mature T cells increases, mitogen-stimulated proliferation and helper activity of the preincubated MC are intensified, and their natural cytotoxicity is depressed.

Thus the same concentration of xenobiotics can modify the functional activity of immunocompetent cells. This property of these widely distributed compounds may, in our view, have a significant influence on the working of the immune system, and accordingly they require further study.

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