Effect of Dioxin Derivatives on Erythropoiesis in Erythroblastic Islands of Bone Marrow.

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1. INTRODUTION

Herbicides, derivatives of chlorophenoxi compounds (2,4 - D) produce immune depression, neutropenia and anemia [6] when they penetrate human and animal organism. Anemia is accompanied by a reduced osmotic and acid erythrocytes stability, their hypochromia, delayed erythroblast maturing in bone marrow[7]. However, the mechanism of erythron impairment is not clear. In this connection we have investignated the effect of 2,4 - DA on precursor cells of bone marrow erythroid group, on intercellular interaction maintaining erythropoiesis in erythroblastic islands (EI) of bone marrow that is association of erythroid cells and bone marrow macrophages providing favourable condition for erythroblasts development, on amplification character and erythroblasts maturing in EI, as well as the function of erythropoietically active bone marrow macrophages subpopulations.

2. MATERIALS AND METHODS.

This study was carried out on 450 white male and female rats weighting 180 - 200 gr.. The experimental animals have been administered 40 % amino salt 2,4 - D in doses LD50 , 1/2LD50 and 1/4LD50 for 30 days to induce subacute course of the disease. The rats were killed in 30 days. Control rats were administered distilled water in similar doses. Peripheral blood analyses were carried out using routine technique after 24 hours to 30-th day following the drug administration. Differentiation, estimation and classification of erythroblastic islands into 5 maturing classes were performed after Yu. M. Zacharov et. al. [4].

I EI class contains in the "crown" 2-8 erythoid cells after CFU - E differentiation into proerythroblast and subsequent division; II class - 9-16; III class - more than 16 nuclear - containing erythroid cells; involuted EI contain in the "crown" normoblasts which are unable to divide and reticulocytes; the class of EI reconstructing contains in crown cells (pro- and basophilic erythroblasts) capable and uncapable of dividing. The estimation of CFU - E involvement in differentiation into proerythroblasts, the possibility of the EI central macrophage (CM) to be reinvolved in new erythropoiesis process was carried out after Yu. M. Zakharov et

al. [3] technique. Simultaneosly, osmotic and acidic erythrocytes resistance as well as their maloni dialdehyde content was determined.

3. *RESULTS AND DISCUSSION.*

The results of study are consistent with those of other researchers who found derivatives of 2,4 - D to cause anemia. The number of erythrocytes and hemoglobin in all groups of rats was found to considerable decrease during the experiment, but it was most pronounced following LD50 administration on day 21 - 4, 37 + 12 12
+ 0,08*10 /l compared to control 5,14 + 0,03*10 /l ($p < 0,01$) and hemoglobin content after LD50, I/2LD50 and I/4LD50 administration as well as at lowest LD50 by 1,0 - 1,7 gr% compared to control value. The compound caused early erythrocyte destruction in the peripheral blood channel (which is demoustrated by their acid and osmotic resistance changes) possibly, as we have discovered, due to increased lipid peroxidate oxydation in erythrocyte membrane. Alongside with accelerated erythrocyte destruction their reproduction in the bone marrow is damaged which is confirmed by decreased absolute EI number/ femur, sharply inhibited CFU - E differentiation into proerythroblasts both in I EI class and nervly EI reconstructing, amplification wave inhibition and erythroblasts maturing in proliferating EI classes. Reduced erythropoiesis maturing in proliferating EI classes. Reduced erythropoiesis reconstrution in EI was associated with decrease in erythropoietinactive macrophages of the bone marrow; their decrease is caused by repeated association with CFU - E as well as their lysosomal - plate complex functional properties. The mentioned changes were most marked in the group LD50 (table 1,2,3).

Dioxin effect inhibiting EI erythropoiesis appears to reflect toxins feature to inhibit intercellular interaction mechanisms [11,13]. The disturbance of intercellular interaction in EI as a results of dioxin effect is likely to be due to increasing proteolytic enzyme activity, free radical oxydation and lipid peroxides which damage erythropoiesis formation in EI [10]. At the same time reduction of CFU - E involvement in differentiation into EI proerythroblasts (in normoxy condition) in animals administered 2,4 - D suggests that the compound is able to alter cell receptors sensitivity to erythropoietin and possibly other hormones activating differentiation and erythroid cells proliferation. Dioxins appear to modulate Ah - receptors by changing cell response to hormone stimuli [14,16]. Dioxins were also able to reduce serum thyroid hormones concentration [12,15], reproduction of hemopoietic growth factors [IL - 3, KCF - GM0 due to early thymus tissue atrophy [15] which could cause erythropoiesis disturbance as well [5]. However, these suggestions require further investigation.

4. REFERENCES.

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Table 1. Indexes of erythropoiesis kinetics in EI in rats.

Indexes: \ \ \ \ Dose	Total CFU - E number involved in EI differentiation: EI1 + EI2 + EI3+ EIinv + 2EIrec., 3 *10 / femur	Index of CFU - E differentiation in EI: EI1 + + EIrec., 3 *10 /femur	Index of repeated EICM involvment in erythropoiesis EI rec. / EI inv.
Control n = 10	702,3 + 56,6	134,6 + 12,41	0,41 + 0,04
I/4LD50 n = 10	518,0 + 34,6 p < 0,05	63,64 + 24,4 p < 0,01	0,21 + 0,04 p < 0,01
I/2LD50 n = 10	588,4 + 63,5 p > 0,05	76,29 + 14,3 p < 0,01	0,26 + 0,04 p < 0,02
LD50(healthy) n = 4	310,8 + 63,2 p < 0,001	10,02 + 3,49 p < 0,001	0,04 + 0,009 p < 0,001
LD50(diseased) n = 5	459,5 + 33,8 p < 0,01	24,13 + 9,57 p < 0,001	0,10 + 0,04 p < 0,001

Table 2. Indexes of Hemopoiesis in EI in Subacute Intoxication.

Group of rats	EI/thousand per femur	EI distribution of different maturing classes (%)				
		EI1	EI2	EI3	EIrec	EIinv
Control (n = 10)	593,9 + 47,7	4,4 + 0,3	16,6 + 1,1	14,6 + 1,9	16,6 + 1,8	46,4 + 2,5
1/4LD50 (n = 10)	643,1 + 31,2 -	2,0 + 0,5 p < 0,01	12,8 + 1,2 p < 0,05	13,6 + 1,7 -	12,0 + 1,6 -	59,8 + 2,9 p < 0,01
1/2LD50 (n = 10)	524,0 + 55,6 -	2,2 + 0,5 p < 0,01	15,8 + 0,9 -	18,2 + 3,7 -	12,4 + 2,0 -	51,4 + 3,8 -
LD50 "Healthy" (n = 4)	300,8 + 59,8 -	0,0 + 0	11,5 + 0,5 p < 0,02	6,5 + 3,3 -	3,0 + 0,6 p < 0,001	79,0 + 3,8 p < 0,01
"Diseased" (n = 5)	435,4 + 26,5 p < 0,05	0,0 + 0	13,6 + 2,9 -	15,2 + 5,4 -	5,6 + 1,9 p < 0,01	65,6 + 8,7 -

Table 3. Number of lysosomes in EI macrophage cytoplasm

Group of rats	Control (n = 10)	1/4LD50 (n = 10)	1/2LD50 (n = 10)	LD50 "Healthy" (n = 4)	LD50 "Diseased" (n = 5)
	5,42 + 0,17 (n = 250)	5,36 + 0,16 (n = 250) -	5,13 + 0,15 (n = 250) -	4,46 + 0,20 p < 0,02	4,83 + 0,20 -