Reserve Possibilities of Erythropoiesis in Rats Affected by 2,4 - DA Herbicide

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Introduction

The compounds of 2,4 - D (2,4 - dichlorphenoxiacetic acid), its salts and ethers are extensively used as herbicides. Anemic state was discovered to occur widely among the workers coming into contact with 2,4 - D group herbicides [1, 6, 7]. 2, 4, 5 - trichlorphenol and 2, 3, 7, 8 - TXDD poisoning causes similar impairment of the blood system. These impairments persist for a long period of time and are revealed in the victims after 20 - 30 years following acute poisoning. However, the mechanism of these blood system impairments remains unclear.

We have failed to obtain recorded data characterizing the compensatory possibilities of erythron in man and animals affected by herbicide poisoning. Specifically, the data on blood regeneration following profuse blood loss are not available. The state of erythropoiesis in morphofunctional erythron units i. e. bone marrow erythroblastic islands (EI) has not been studied in vitro, nor has the involvement rate and CFUe complementation with regard to EI central macrophag (CMPh), the character of erythroblast amplification wave movement in the « crown » EICMPh, the peculiarities of macrophags repeated envolvement of maturing EI into erythropoiesis been investigated. The data on erythropoiesis preservation in and out EI in animals affected by 2,4 - DA are not available either.

Experimental Methods

To access the compensatory possibilities of blood loss in rats poisoned with different herbicide doses 1 ml of 40 % amino salt 2,4 - D (2,4 - DA), chemically pure preparation, added into distilled water was intragastrically infused daily.

The subacute length of the experiment made 28 - 30 days. The animals were divided into 3 groups depending on herbicide 2,4 - DA dose : I - during the experiment 2,4 - DA total dose amounted to 1,2 g / kg which was equal to LD_{50} ; II - 0,6 g / kg (1 /2 LD_{50}) ; III - 0,06 g / kg (1 / 20 LD_{50}). The control animals were intragastrically infused. 1 ml of distilled water daily.

The erythropoiesis compensatory possibilities in the animals of these groups were assessed according to its response to acute blood loss (2 % of blood of the total rat's body weight was drawn from the retroorbital space under ether anaesthesia). Posthemorrhagic anemia outcomes were studied after 24 hours on 3, 7, 14, 18, 21 and 25 days following the blood loss. Erythrocytes count, hemoglobine concentration, hematocrit index, reticulocytes count were estimated by conventional hematologic methods. Femoral bone EI count was carried out according to Yu. M. Zakharov et al. (1984) [3].

To estimate the erythroid cells mitotic activity in and out EI, EI cells mitoses cessation of the bone marrow was carried out using colchicine according to O. K. Gavrilov et. al. (1986) [2].

The division of bone marrow EI into maturity classes was determined according to Yu. M. Zakharov et. al. (1990) [4]. The erythropoiesis functional values in bone marrow EI were estimated according to L. V. Vorgovaya, Yu. M. Zakharov (1990) [5]. CFUe total count which entered the erythroid differentiation in EI, CFUe involvement index in differentiation in proerythroblasts in EI, the index of the repeated EI macrophags involvement in erythropoiesis were calculated.

Results and Discussion

The increase in reticulocytes count in ‰ and 1 μl of blood on 1 - 7 days following the blood loss failed to reach the control values in either of the experimental groups of rats. The reaction was less pronounced on the following days of the study. It proves the decreased

central erythrone chain compensatory possibilities in these rats and explains the cause of erythrocytes, hemoglobin and hematocrit slow recovery in the animals following the acute blood loss.

To clear up the cause of reduced compensatory bone marrow response in rats poisoned with 2, 4 - DA following the severe blood loss we studied the character of erythropoietic intercellular relationship and EI erythropoiesis kinetics in rats poisoned with LD_{50} and 1 / 20 LD_{50} doses. The rats intact after the blood loss served as controls.

The estimation of the absolute EI number demonstrated that slowed - up EI formation in the bone marrow of rats poisoned with 2,4 - DA occurred following the blood loss as compared to the control ones.

Statokinetic index assessment of erythroblasts contained in the EI « crown » and dissociated with EI showed that 2,4 - DA infusion failed to impair regular bone marrow erythropoiesis organization i. e. in poisoned rats erythropoiesis occurred in EI as the increased of dividing erythroblasts count outside EI failed to occur in these rats. 2,4 - DA infusion increased EI erythroblasts sensitivity to blood loss : the dose infused intensified erythroblasts mitosis in EI «crown » in comparison with the control group.

The calculation values (table 1) made it possible to analyse in detail the erythropoiesis changes in EI after the blood loss.

Table 1 shows that CFUe involvement kinetics in EI differentiation in response to the blood loss depended on the poison dosage.

LD_{50} dose caused the inhibition of this process after the blood loss at any time of the study, while 1 / 20 LD_{50} dose at an early time following the blood loss failed to inhibit compensatory increased EI formation, but at later time the process of EI bone marrow formation was reduced.

The analysis of EI distribution into maturity classes (table 2) showed that after the blood loss EI content in class 1 was reduced and till the third day in class 2 as well in the groups poisoned with LD_{50}, 1 / 2 LD_{50}. However, both on the 1^{st} and 2^{nd} days after the blood loss EI content in class 3 and EI involuting failed to differ from the values registered in the control animals. On the 7^{th} to 14^{th} days EI content in the bone marrow in class 3 animals poisoned with LD_{50} and 1 / 2 LD_{50} was higher than in the control group, EI values being simultaneously similar in all 3 groups. Thus, normoblasts maturity inhibition developed in LD_{50} and 1 / 2 LD_{50} animals in the area of EI class 3 transfer into EI involuting. Re-

ticulocytosis being less pronounced on the first days after the blood loss in 2, 4 - DA poisoned rats compared to the control group, EI involutings maturity which are the main source of reticulocytes getting into blood may be considered to be inhibited in these animals. EI reconstructing content more than in the control in LD_{50} and 1 / 2 LD_{50} rats on 1, 3, 7 days following the blood loss suggests that the pool replenishment of the newly formed EI in the bone marrow at this period of time is fulfilled by way of greater CFUe joining to EI inv. macrophags rather than residual bone marrow macrophags of these animals. In the later case it would lead to EI class 1 number increase. However, the absolute EI count formed on the basis of erythropoiesis in EI 1 in the femoral hemopoietic tissue of poisoned rats was reduced compared to the control.

Thus, 2, 4 - DA poisoning of rats producers different effect on erythropoiesis : EI erythroblasts mitotic activity becomes increased while CFUe and macrophags contact intensity decreases which reduces EI formation, erythroblasts maturity in EI « crown » and reticulocytes release is slowed down. As a result of the indicated reorganization in the erythron , erythrocytes regeneration slowing down occurs after the blood loss.

Literature Cited

(1) Burkatskaya E. N., Lycina G. G., Karpenko V. N. (1978) Laboratory diagnostics of pesticides, M, Meditsina, 125 p.

(2) Gavrilov O. K., Kozinets G. I., Chernyak N. B. (1985) Bone marrow and peripheral blood cells. M. Meditsina, 288 p.

(3) Zakharov Yu. M., Melnikov I. Yu., Rassokhin A. G. (1984) Hematology and Transfusiology., 29, N 4, 52 - 54.

(4) Zakharov Yu. M., Melnikov I. Yu., Rassokhin A. G. (1990) Archive of anatomy, histology and embryology., 98, N 5, 20 - 22.

(5) Zakharov Yu. M., Vorgova L. V. (1990) Journal of Physiology of the USSR., 76, 200 - 207.

(6) Karpenko V. N. (1981). Col. « Labour Hygiene », Kiev, Issue 17, 75 - 80.

(7) Rumak V. S. (1993) Author's abstract of thesis, Doctor of Sciences (Medicine), St. Petersburg , 35 p.

Table 1.

Functional indexes of erythropoiesis in EI in rats in different groups (1-control, 2-LD50; 3-1/20 LD50) in dynamics after 2% blood loss (M±m; p).

Indexes	1st day			2nd day			3rd day			7th day			14th day		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CFUe count, participating in differentiation in EI ($x10^3$/femur)	446,4 ±72,3	385,0 ±41,9	542,6 ±60,4	398,6 ±39,7	346,7 ±26,2	402,3 ±66,0	522,0 ±62,4	470,0 ±44,7	364,5 ±8,33	670,8 ±41,4	431,7 ±66,5	671,0 ±70,7	532,6± 49,3	493,0± 105,5	442,4± 20,8
P		-	-		-	-		-	-		<0,05	-		-	-
	(5)	(4)	(5)	(5)	(4)	(4)	(5)	(4)	(4)	(5)	(4)	(5)	(5)	(4)	(5)
CFUe involvement amound in erythroid differentiation ($x10^3$/femur)	154,5 ±22,2	115,0 ±18,2	155,8 ±19,4	110,6 ±6,04	83,0± 9,75	101,5 ±15,7	124,0 ±8,04	101,0 ±11,6	80,3 ±5,39	163,8 ±23,4	59,7 ±10,9	129,2 ±11,4	90,2 ±10,1	30,5 ±2,90	68,4 ± 7,44
P		-	-	-	=0,05	-		-	<0,01		<0,05	-		<0,01	-
	(4)	(4)	(5)	(5)	(4)	(4)	(4)	(4)	(4)	(5)	(4)	(5)	(5)	(4)	(5)
MFEI repeated involvement in erythropoiesis (conventional unit)	1,04 ±0,14	1,09 ±0,21	1,05 ±0,08	0,83 ±0,17	0,61 ±0,13	0,87 ±1,36	0,68 ±0,06	0,71 ±0,12	0,56 ±0,05	0,56 ±0,07	0,32 ±0,06	0,39 ±0,04	0,23 ± 0,009	0,11 ±0,03	0,25 ± 0,03
P		-	-		-	-		-	-		-	-		<0,02	-
	(5)	(4)	(5)	(4)	(4)	(3)	(5)	(4)	(5)	(5)	(4)	(5)	(5)	(4)	(5)

Note : P is calculated with regard to the control indexes at each period of study; the number of animals is indicated in brackets.

Table 2.

Distribution of erythroblastic islands of different bone marrow maturity class (%)
in different groups of rats (1 - control; 2 - LD_{50}; 3 - 1/20 LD_{50}) in dynamics after 2% blood loss (M±m, p)

Maturity class	1st day			2nd day			3rd day			7th day			14th day		
EI	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
EI_1	15,0±	6,8±	5,0±	12,5±	7,5±	3,0±	9,6±	4,0±	3,0±	8,0±	4,4±	1,01±	4,0±	2,4±	1,01±
	0,89	1,02	0,58	1,26	0,5	0,58	1,17	0,82	1,29	0,63	0,75	0,57	0,0	0,75	0,57
p		<0,01	<0,01		<0,05	<0,01		<0,05	<0,02		<0,02	<0,01		-	<0,01
	(5)	(4)	(5)	(4)	(4)	(4)	(5)	(4)	(4)	(5)	(5)	(4)	(5)	(5)	(4)
EI_2	21,4±	12,0±	10,5±	18,0±	13,0±	7,0±	11,6±	8,5±	11,5±	14,4±	9,2±	5,79±	8,0±	8,0±	6,0±
	2,56	1,41	0,5	0,89	1,29	0,58	0,98	1,26	3,3	2,93	1,62	1,63	1,41	1,55	1,83
p		<0,05	<0,05		<0,05	<0,01		-	-		-	-		-	-
	(5)	(5)	(4)	(5)	(4)	(4)	(5)	(4)	(4)	(5)	(5)	(4)	(5)	(5)	(4)
EI_3	18,4±	20,8±	13,5±	26,8±	27,0±	22,5±	38,4±	21,0±	26,0±	17,6±	18,4±	29,5±	4,0±	9,2±	14,0±
	2,32	2,58	1,5	3,49	4,51	5,79	3,87	2,89	4,97	1,6	2,93	1,26	0,63	0,49	2,58
p		-	-		-	-		<0,05	-		-	<0,01		<0,01	<0,02
	(5)	(5)	(4)	(5)	(4)	(4)	(5)	(4)	(4)	(5)	(5)	(4)	(5)	(5)	(4)
$EI_{rec.}$	22,4±	30,8±	35,5±	18,0±	23,5±	22,2±	16,4±	23,5±	23,5±	21,6±	18,8±	15,0±	15,6±	16,0±	9,5±
	0,93	2,15	4,03	0,89	2,63	5,48	1,72	1,5	2,75	2,79	1,02	2,65	0,75	1,67	1,71
p		<0,05	<0,05		-	-		<0,05	-		-	-		-	<0,05
	(5)	(5)	(4)	(5)	(4)	(4)		(4)	(4)	(5)	(5)	(4)	(5)	(5)	(4)
$EI_{inv.}$	22,8±	29,6±	35,5±	24,0±	29,0±	40,0±	24,0±	43,0±	36,0±	38,4±	49,2±	38,5±	67,6±	64,4±	56,0±
	2,33	2,23	4,99	4,76	4,65	5,29	1,09	3,0	6,48	0,75	4,36	9,65	2,04	1,17	13,6
p		-	-		-	-		<0,01	-		-	-		-	-
	(5)	(5)	(4)	(4)	(4)	(4)	(5)	(4)	(4)	(5)	(5)	(4)	(5)	(5)	(4)

Note : **p** is calculated with regard to control indexes at each period of study;
the number of animals in each group is indicated in brackets.