Evaluation of the Mononuclear Phagocyte System State under the Influence of Dioxin-containing Herbicide Amino Salt 2,4-Dichlorophenoxyacetic acid

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Introduction

Biologic effects of dioxin-containing chemical compounds, in particular 2,4-dichlorophenoxy acetic acid amino salt (2,4-DA), are varied and according to the current literature data they include immunotoxic effect [1, 8]. Chlorophenoxyherbicides are known to cause a decrease in cell-mediated and antibody responses[10, 11].The state of macrophage system exposured to dioxins and compounds containing them has not been studied yet.
The purpose of the present study is to analyse the mononuclear phagocyte system in intoxication with phenoxyherbicide amino salt 2,4-dichlorophenoxy acetic acid.

Materials and Methods

A commercially available 40% herbicide containing dioxins [an average concentration of 30 ng/kg, including 2,3,7,8-tetraclorodibenzo-p-dioxin (TCDD) at a concentration of 1 ng/kg] was used in the assay. Experiments were carried out in 124 white rats weighing 180-200 g. In experimental groups the substance was orally administered at a daily dose of 2 and 20 mg/kg body weight. The dose of 2 mg/kg corresponds to a total of 1/20 LD_{50}, and the dose of 20 mg/kg – to the dose of ½ LD_{50} administered to the animals within 1 month. NaCl isotonic solution was administered to control rats. On day 14 and 28 the animals were scarified and studies of blood and bone marrow were carried out with simultaneously, employing routine method of Romanowsky-Giemza staining assisted with light microscopy [5,6]. We also used the method of cloning precursors of hemopoietic cells in diffusion cameras in vivo [13]. The claster and colony forming (CLF, CF) ability were determined from the number of cluster and colony units.
On day 28 peritoneal macrophages (PM) of rats were obtained by peritoneal lavage with medium 199. Erithrocytes were removed by osmotic shock, then the PM were resuspended with 4% NaCL, clarified by centrifugation with 0,85% NaCL and the suspense in the amount of $6x10^6$ cells/ml solution was prepared [2,6].
Macrophage adhesiveness and plastic properties were evaluated by the method of E.G.Redtchits and O.V.Guzeva [14], phagocytic activation was measured using latex particles in 30 min incubation at 37^0C[4]. The assessment of reduced nitroblue tetrazolium (NBT) was done in a spontaneous NBT-test [12]. Fc-dependent processes [Fc-dependent phagocytosis, expression and affinity of Fc-receptors (Fc-R), Fc-rosetting] were evaluated by the method of R. Bar-Shavit et al[3].
The results obtained were statistically evaluated using a Student t-test[6].

Results and Discussion

The results obtained demonstrated that CLF ability of control rats bone marrow was 16,9±0,05 per 10000 mononuclear cells (MC). The maximum doze of 2,4-DA did not influence CLF ability of the bone marrow and the doze of ½ LD_{50} stimulated it.
The CF ability of common granulocyte-macrophage progenitors was increased in both experimental groups of animals and made up 2,6±,05/10000 MC and 2,7±0,03/10000 MC regarding to ½ and 1/20 LD_{50} versus 2,2±0,01/10000MC in control group ($p<0,05$), respectively.
The CF ability of granulocyte predecessors was reduced and made up 0,7±0,02/10000 MC and 0,3±0,01/10000 MC at dozes of ½ and 1/20 LD_{50} of 2,4-DA versus to 1,0±0,01/10000 MC in control, respectively.
The monocyte predecessors of animals, receiving 2,4-DA at investigated doses, were activated and were higher by 14,6 and 29,2% ($p<0,01$) than the control levels.
The data obtained were consistent with the count of hemopoietic precursor cells of bone marrow. There was a marked increase in the number of common granulocyte-macrophage precursors. The amount of more differentiated granular cells in the bone marrow of rats treated was decreased, that, probably, resulted from their migration to the peripheral blood. The amount monocyte precursors was raised in rats, receiving 1/20 LD_{50}. It is suggested, that 2,4-DA stimulated proliferation and differentiation of cells in bone marrow and their migration to the peripheral blood. The amount of separate populations of white blood cells has shown that an increase in a total number of leukocytes was caused at the expense of amounts of neutrophils, monocytes, and as well as of lymphocytes at 14 day.
The property of effect of 2,4-DA on the peripheral blood was absolute monocytosis, observed in both series of our experiments. Phenoxyherbicide in a dose of 1/2 LD_{50} induced monocytes redistribution and at a dose of ½ LD_{50} - absolute monocytosis.
We then studied functional activity of macrophage system peripheral link. The viability of PM of rats treated within 1 month in our experiment in both groups of animals did not differ from control data.
The phagocytosis activity of PM was decreased: the number of active phagocytes was lower than the control number by 30,9 and 38,2% ($p<0,05$), phagocytosis index - 44 and 11,1% in groups of rats, receiving ½ and 1/20 LD_{50} 2,4-DA, respectively.
Analysis of Fc-depended processes showed, that a maximum dose of 2,4-DA depressed the expression of Fc-R and their affinity. This fact led to suppression of Fc-dependend phagocytosis of PM: the number of active PM was reduced by 28,9 and 20,6%, and phagocytosis index by 23,3 and 20,8% compared the control group (1/2 and 1/20 LD_{50} respectively).
According to the current literature data concerning heterogeneity of PM population it is possible that suppression of its functions may be the result of redistribution of their subpopulation. The macrophage ability to antigenpresentation is known to be typical of Ia^+ -subpopulation of PM, having low Fc-R expression. Our previous studies have shown that antigenpresenting function of mice PM after treatment with 2,4-DA was reduced by 29,3 and 72,8% as compared to the control level, respectively[7]. At the same time the adhesion of cells (the marker of functional and metabolic activity of macrophages) was raised. The NBT-test of PM was also increased.

The current literature data on immune dysfunction in exposure to dioxins and dioxin –containing phenoxyherbicides are characterised by cell-mediated and humoral response depression and confirmed by experimental and clinical findings[1,8,11)]. However, functions of accessor cells are also important for the immune response initiation. Their dysfunction in chemical exposure may be one of mechanisms leading to immune response defects. That gave rise to an experimental analysis of the function of macrophages exposed to dioxin-containing phenoxyherbicide. The results of assays carried out confirm a significant disorder of the macrophage system.
Thus, the applied doses of herbicide increased the amount of PM, decreased their Fc-R expression, antigenpresenting function, i.e. depressed functions of antigenpresenting and non-antigenpresenting subpopulation of PM. Depressive effects of 2,4-DA on functions of PM, were probably the result of metabolic reorganisation in cells. Phenoxyherbicides are known to be highly penetrative and may stimulate oxidising processes within animal cells.
So, we conclude, that herbicide 2,4-DA, widely used in agriculture, in the doses used by us during for 1 month induced changes in various macrophage subpopulations at various levels of their differentiation: an increase in colony forming ability of precursors at the central level, a decrease in the plastic properties of membrane, Fc-dependend processes, phagocytosis activity , antigenpresenting function at the peripheral level. The metabolic activation of cells accompanied by their functional insufficiency, that led to monocytopoiesis activation and migration monocytes in the peripheral blood.

Conclusions

1. Dioxin-containing herbicide 2,4-dichlorophenoxyacetic acid amino salt in daily administration to animals in a dose of 2 and 20 mg/kg body weight within 28 days enhance differentiation predecessors of monocyte-macrophages in the bone marrow, increases the number of monocytes in blood and decreases functions of tissue macrophages.
2.Depressive effects of 2,4-dichlorophenoxyacetic acid amino salt consist of inhibition membrane processes: an expression and affinity of Fc-receptors, absorbing, Fc-depended and Fc-independed phagocytosis, antigenpresenting function of macrophages.
3.The metabolic activation of macrophages exhibited in a high ability to reducing nitroblue tetrazolium, adhesion of cells.

References

1. Ballester, O.F.; Moscinski, L.; Spiers, A.; Baldueci,L. *Am. Geriat.Soc*.**1993**,41,11,1245-1254.
2. Bashner, R.L.; Murrman, S.K.; Davis, J.; Jonston,R.B. *J. Clin.Invest.***1975**,56,571.
3. Bar-Shavit R., Kahn A., Femton J.W. *J. Cell. Biol.* **1983**, 96, 282-285
4. Boyum, A.; *Tissue Antigens,* **1974**,4, 269-274.
5. Dawson, M.A.; Renfo,J.L,; *J.J.Pharmacol. Exp.Ther*.**1993**,266,2,673-677.
6. Fedoseeva, V.N.; Poryadin, G.V.; Kovalchuk, L.V.; Cheredeev, A.N.; Kogan, V.Yu. The Manuel on Immunologic and allergologic methods in hygenic studies; Promedek, Moscow,**1993**;320.
7. Imelbayeva, E.A.; Kamilov, F.Kh.; Teplova, S.N. et al. in *Dioxin`97;* Indianapolis (USA),**1997**, 43,253-258.
8. Hardell, L.; Erisson, M,; Degerman,A. *Cancer.Res.* **1994**, 54, 2386-2389.
9. Knopp, D. *Occup.Environ.Med.***1994**,51(3), 152-159.
10. Morris, D.L.; Gordan,S.D.; Holsapple, M.E. *Immunopharmacology*,**1991**,21(3), 159-169.
11. Neubert,R.; Jacob-Miller,H.; Helge,H. *Arch.Toxicol.***1991**,65 (3), 213-219.
12. Park, B.U.; Fikrig,S.M.; Smithwick,E.M. *Lancet*, **1968**,11,532-534.
13. Popov, G.K.; Krivohizhina, L.V.; Pochinski, A.G. et al. The technique of cultivation of hemopoietic precursor cells in diffusion cameras. Chelyabinsk,**1992**,16.
14. Redtchits, E.G.; Guzeva, O.V. *Lab.Delo*,**1990**, 5,4-8.