Evaluation of a Macrophage antigenpresenting Function in Exposure to Dioxin-containing Herbicide

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Abstract

2,4-dichlorophenoxyacetic acid amino salt in daily administration to the animals in doses of 4,9 and 49 mg/kg during 10 days induced depressive influence on macrophage absorption and antigenpresenting function.

An increase in the activity of neutroblue tetrazolium (NBT) reduction of macrophages under the influence of 2,4-dichlorophenoxyacetic amino salt may be one of the mechanisms of phenoxyherbicides destructive effects on phagocytic cells membrane functions.

Introduction

Biologic effects of dioxin-containing chemical compounds, phenoxyherbicides in particular, are varied and according to the current literature data they include immunotoxic effect[8,17]. Chlorophenoxyherbicides are known to cause a decrease in cell mediated and antibody responses [16]. The antigenpresenting function of macrofages exposured to dioxins and compounds containing them has not been studied yet.

The purpose of the present study is to analyze the macrophage antigenpresenting function change in intoxication with phenoxyherbicide 2,4-dichlorophenoxyacetic acid amino salt (2,4-DA).

Experimental methods

A commercially available 40% herbiside containing dioxins (an average concentration of 30 mg/kg, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at a concentration of 1 ng/kg) was used in the assay.

Experiments were carried out in 48 "Black" male mice weighing 18-20 g. The assay was conducted according to the V.G.Galaktionov and G.V.Anfalova [6] method (1974) in a model of syngenic transfer of peritoneal macrophages (PM) from mice-donors given a substance (physiological salt solution in control) to intact host. In experimental groups the substance was orally administered in a daily dose of 4,9 and 49 mg/kg body weight. The dose of 4,9 mg/kg corresponds to a cumulative dose $^1/_{20}$ LD_{50} , and the dose of 49 mg/kg - to the substance dose of $^1/_2$ LD_{50}, administered to animals within 10 experimental days. Control mice-donors were administered NaCl isotonic solution.

On day 10 the animals were "immunized" by introgastric administration of sheep red blood cells (SRBC) in a dose of $2 \cdot 10^7$ per 1 kg body weight. Two hours later "primed" PM were obtained by peritoneal lavage with NaCl isotonic solution. 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 $6 \cdot 10^6$ cells in a 1 ml solution was prepared. The 1,0 ml PM suspense was adoptively transfered to intact syngenic mice. On day 5 the spleen of mice-recipients was removed and weighed. The number of nuclear cells (NC) in it as well as anti-SRBC plaque-forming cells (anti-SRBC PFC) in the spleen using A.J.Cunningham and A.S.Zenberg[4] method were measured. The number of hemolysis spots in the chamber after 1,5 h incubation at 37^0 C, anti-SRBC PFC number per 10^6 NC and the spleen were countered.

Along with the assessment of macrophage antigenpresenting capacity in mice-donors exposed to herbicide, phagocytic and metabolic activity of phagocytizing cells until immunizing by SRBC was done. Macrophage phagocytic activation was measured using latex particles in 30 min incubation at 37^0 C[2,5]. The assessment of reduced NBT was done in a spontaneous NBT-test[15].

The results obtained were statistically evaluated using Student t-criterion[5].

Results and discussion

The results of macrophage antigenpresenting capacity assays are presented in Table 1. According to it PM transfer from control animals to the donor group gave anti-SRBC PFC level equal to 249,7±10,6 per 10^6 NC. PM transfer from 2,4-DA-exposed animals-donors was accompanied by a lower level of anti-SRBC PFC as compared to the control one. Similar regularity was revealed anti-SRBC PFC number per the organ mass : in control it was $30,2±9,7·10^2$; in experiment - $3,05±0,2·10^2$ and $4,25±1,02·10^2$ (unit) in the group of mice given PM from donors, exposed to 2,4-DA in a dose of 4,9 and 49 mg/kg, respectively ($P<0,05$).

In addition to the macrophage antigenpresenting function in donors given herbicide, phagocytosis state and reduced NBT level of PM activity was studied. Macrophage absorbing activity after herbicide administration reduced and on day 10 it was 0,7±0,2 in administration of 2,4-DA in a dose of 4,9 mg/kg ($p>0,05$) and 0,3±0,14 in a dose of 49 mg/kg ($p<0,05$) compared to 0,81±0,08 (unit) in control.

At the same time NBT-positive cells number in peritoneal exudate under the phenoxyherbicide exposure greatly increased.

The current literature data on immune dysfunctions in exposure to dioxin-containing phenoxyherbicides which are characterized by cell-mediated and humoral immune response depression are confirmed by experimental and clinical findings [7,8,10]. So, in human exposed to phenoxyherbicides a moderate immune deficient state, leading to the appearence of non-Hogkin's lymphoms is determined[3,10]. Dioxins have similar 2,4-D immunosuppressive effects and capable to act in vitro similar to polyclonal B-cell activator. St.aureus cowan Strain I suppressing humoral immune response to T-dependent antigens[12]. It is also well known that $CD4^+CDw29^+$ (helper-inducer cells) are the main targets of TCDD effects[12]. However, functions of accessor cells are also important in the immune response initiation. Their dysfunction in dioxin exposure may be one of mechanisms leading to immune response defects. That gave rise to an experimental analysis of this function of macrophages exposed to dioxin-containing phenoxyherbicide. The results of assays carried out confirm a significant disorder of the macrophage antigenpresenting function under the influence of 2,4 - DA established in the system of syngenic transfer of accessor cells

primed by T-dependent antigen, to intact recipients from animal-donors, given the substance in a dose of 4,9 and 49 mg/kg. Parallel study of PM phagocytic activity made it possible to detect a decrease in the absorbing capture of phagocytosis objects. This may reflect one of the mechanisms causing a reduction in accessor cells antigenpresenting function, the first stage of which is the capture of foreign antigens with their subsequent processing. An increase in PM NBT-reduction shows "oxidative burst", products of which expose to phagocyte killing function and xenobiotic metabolism[11].

The NBT-test is widely used to measure superoxid anions released by polymorphs and macrophages during an oxidative burst[1,13]. So, oxygen aggressive forms accumulation may bring about cellular membrane destruction, this in its turn may reduce macrophage absorbing activity. NBT-test is a marker of NADP·H oxidase activity though it is also reduced by other "diaphorase" enzymes[11]. The NADP·H-oxidase is membrane enzyme and its activity may also alter membrane processes, particularly antigen presentation. Thus, a reduction in anti-SRBC PFC number in exposure to dioxin containing herbicide may be result of defects both of T-helpers and accessor cells taking part in immune response initiation. A reduction in absorbing activity, intensification of "oxidative burst" may become critical in damage of antigen processing by accessor cells and suppression of their antigenpresenting function, immune response initiation under the conditions of phenoxyherbicide exposure.

Conclusions

1. Dioxincontaining herbicide 2,4-dichlorophenoxyacetic acid amino salt in daily administration to animals in doses of 4,9 and 49 mg/kg within 10 days depresses absorbing, phagocytic and antigenpresenting macrophage function.

2. An icrease in macrophage "oxidative burst" under the influence of 2,4-DA may lead to intensification of degradation foreign antigen material and destructive effects of phenoxyherbicides regarding membranes of phagocytic cells which reduce absorbing activity and may be one of basic disorders in antigenpresenting functions of accessor immune cells.

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Table 1

Anti-SRBC PFC number in the spleen of mice-recipients given macrophages of syngenic donors, intact (control) and exposed to 2,4-DA in doses of 4,9 and 49 mg/kg for 10 days within.

Group, substance dose	Number of NC in the spleen	Anti-SRBC PFC per 10^6 NC	Spleen mass (g)	Anti-SRBC PFC per spleen mass ($\cdot 10^2$)
1. Control	901,3±21,1	249,7±10,6	0,11±0,013	30,2±9,7
2. 4,9 mg/kg	934,8±3,5	67,98±7,7	0,16±0,042	3,05±0,2
p	>0,05	<0,05	>0,05	<0,05
3. 49 mg/kg	706,6±2,85	177,0±53,4	0,11±0,02	4,25±1,02
p	<0,05	>0,05	>0,05	<0,05