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Research Article

Effect of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin on Nonspecific Body Resistance, Immune System and Lipid Peroxidation Parameters

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Abstract

In experiments on random-bred albino rats and CBA mice it was found that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TChDBD) induced a dose of 1.0; 4.0 and 8.0 μg/kg when administered single time. The main parameters of nonspecific body resistance (NBR), antibody formation mainly to the T-independent antigen, delayed-type hypersensitivity reactions (DTH) were dose-dependently reduced. TChDBD at the doses used had almost no effect on NK activity. Reduction of immune system parameters was directly related to the initiation of lipid peroxidation.

Keywords: 2,3,7,8-tetrachlorodibenzo-p-dioxin; Nonspecific Body Resistance; Immune System; Lipid Peroxidation

Introduction

The group of polychlorinated dibenzodioxins (PChDBD) includes more than 75 compounds. The most studied of them is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TChDBD, dioxin). The main sources of PChDBD emissions into the environment are metallurgical plants, pulp and paper mills, motor transport, organic synthesis production, industrial and household waste incineration processes, forest fires [16-18]. Studies in Germany, Canada, Japan have shown that with basic foodstuffs (meat, milk) a person receives on average 98 pg/day of these substances. Calculations show that from 1 litre of milk the body receives 12 times the dose of PChDBD than from inhaled air in 1 day. The food chain is the main route by which dioxins enter the body, which must be taken into account when developing standards for their content in the environment. With food 98% of PChDBD enters the body, with air - 2%, drinking water - less than 0.01% of the total intake. From food, 50% of PChDBD comes from meat, 27% from milk, 10% from fish and 11% from other food. The average intake of dioxins ranges from 0.03-0.05 ng/day. Emissions from incinerators ensure approximately 160 times less dioxins than intake of dioxins through food and drinking water [4,10].

Massive acute PChDBD poisonings are possible. After the world-famous accident in Seveso in 1976 with release of significant amounts of TChDBD, its content in the blood of people living near the plant exceeded the average levels in industrial areas by 2000-4000 times. Epidemiological studies of the long-term effects of exposure to TChDBD have shown some increase in cases of soft tissue sarcoma, liver cancer, lymphatic and hematopoietic tumours [3,4].

The acute effects of TChDBD on nonspecific body resistance (NBR) and immunocyte populations and subpopulations involved in the formation of immune responses have not been sufficiently investigated, and the literature on the acute immunotropic effects of TChDBD is highly inconsistent [3-6].

Aim of the Study

The aim of the study was to evaluate nonspecific body resistance parameters, changes in basic humoral and cellular immune reactions during acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in random-bred albino rats and CBA mice.

Materials and Methods

The experiments were performed on random-bred albino rats and CBA mice of both sexes weighing 180-240 g and 18-22 g respectively. 2,3,7,8-tetrachlorodibenzo-p-dioxin was administered intragastrically (per os) a single doses of 1.0; 4.0 and 8.0 μg/kg in an olive oil solution. (LD_{50} of TChDBD for rats and CBA mice at intragastric administration was 35 ± 3 and 56 ± 5 μg/kg respectively). Nonspecific body resistance (in combination with the integral immunological resistance of the organism - IIRO) was assessed after 5 days by the average lethal dose of *E. coli* and the average life time of rats (Et_{50}) in experimental infection caused by intraperitoneal injection of *E. coli* diurnal culture at the doses of 5.0; 6.5 and 8.0 ·10^9 CFUs after preliminary immunization with *E. coli* (10^6 CFUs) for 36h [2]. NBR rat parameters - serum bactericidal activity (SBA), serum lysozyme, platelet cationic protein (PCP), neutrophil functional activity in the nitroblue tetrazolium (NBT) test - were determined by conventional methods [3] 5 days after intoxication.

The activity of natural killer cells (NK) was examined in rats spectrophotometrically by the number of target cells remaining undestroyed in the cytotoxic test 5 days after TChDD application according to the method [1]. The humoral immune response to T-dependent (sheep red blood cells - SRBC)) and T-independent typhoid antigens (Vi-Ag) was assessed in rats 5 days later by the number of antibody-forming cells (AFC) in the spleen by conventional methods [3] after administration of TChDBD. The humoral immune response to SRBC injection describes the ability of T helper type 1 (Th1) cells to participate in the production of B-lymphocytes (plasmacytes) by IgM. The formation of delayed-type hypersensitivity reactions (DTH) reflecting the function of the cellular immune response (in particular Th1 activity) was determined by the increase (in %) in hind paw foot weight. The rats were immunised by intraperitoneal injection of 10^8 SRBC. A permissive dose of SRBC ($5x10^8$) was injected under the aponeurosis of the hind foot after 5 days. DTH was determined after 24h.

The ability of macrophages to induce a humoral immune response (AMIHIR) was assessed after 5 days by the number of AFC to SRBC in recipient mice (CBA) after administration of TChDBD to syngeneic donor mice. Macrophages from donor mice were transferred to recipients 1 day after intoxication. 1.5h before peritoneal macrophage transfer into the abdominal cavity, mice were injected with $2,5x10^8$ SRBC in 0.1 ml of isotonic sodium chloride solution (saline). Lipid peroxidation (LPO) was assessed in rats by total radical production (TRP) using phorbol ester-activated luminoluminescence, malondialdehyde (MDA), catalase and peroxidase activities in blood spectrophotometrically [11] 3 days after TChDBD administration. The activity of catalase and peroxidase was an indicator of the function of the antioxidant system (AOS).

The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p < 0.05$. The correlation coefficient (r) of immune status, AOS and LPO parameters was determined.

Results

After the action of TChDBD (Table 1) at the doses of 1.0; 4.0 and 8.0 μg/kg (there was a dose-dependent decrease of *E. coli* LD_{50} and Et_{50} in the test with simulated experimental infection after *E. coli* immunization, indicating the reduction of NBR and IIRO. After TChDBD intoxication of mice, a direct dose-related decrease in SBA, serum lysozyme content, PCP and neutrophil activity index in the NST test compared to the control level was observed. Statistically significant differences in parameters were observed at doses of 4.0 and 8.0 μg/kg ($p < 0.05$). Thus, under the action of TChDBD at a dose of 8.0 μg/kg, the SBA, lysozyme, phagocytic-metabolic activity of neutrophils in the NBT test decreased by 1.84; 3.21; 1.74 and 3.00 times, respectively.

It was experimentally established (Table 2) that TChDBD, when acted upon (1.0; 4.0 and 8.0 μg/kg), caused a dose-dependent reduction of the humoral immune response to T-dependent (SRBC), T-independent antigens and a suppression of the DTH. Significant changes in T-dependent humoral immune response and DTH compared to controls were observed at doses of 4.0 and 8.0 μg/kg ($p < 0.05$). A statistically significant reduction of antibody production to T-independent antigen (Vi-Ag) was detected upon the action of TChDBD at the doses making up 1.0; 4.0 and 8.0 μg/kg ($p < 0.05$). Thus, upon the action of 2,3,7,8-tetrachlorodibenzo-p-dioxin at a

Parameter	Control	Dose, μg/kg		
		1,0	4,0	8,0
LD_{50} *E. coli,* 10^9 CFUs	5,52 ± 0,31	4,65 ± 0,32	3,95 ± 0,30*	3,02 ± 0,28*
Et_{50}, h	20,0 ± 1,8	16,2 ± 1,4	14,4 ± 1,3*	8,5 ± 1,2*
SBA, %	76,3 ± ,0	67,2 ± 4,1	53,3 ± 3,8*	40,4 ± 3,1*
Lysozyme, mg/l	10,6 ± 1,3	8,2 ± 0,9	5,0 ± 0,6*	3,3 ± 0,5*
PCP, %	64,3 ± 2,7	56,1 ± 3,0	45,2 ± 3,4*	37,0 ± 3,2*
Neutrophilic activity index (NBT test)	0,24 ± 0,02	0,18 ± 0,03	0,14 ± 0,02*	0,08 ± 0,02*

Table 1: Effect of 2,3,7,8-tetrachlyorodibenzo-p-dioxin on IIRO and NBR in rats (M+m, n = 15-25).

* -p < 0,05 as compared to control.

Parameter	Control	Dose, μg/kg		
		1,0	4,0	8,0
AFC to SRBC, 10^3	43,5 ± 4,3	34,5 ± 3,3	25,2 ± 3,1*	15,1 ± 02,5*
AFC to Vi-Ag, 10^3	35,1 ± 3,4	25,1 ± 3,0*	13,5 ± 2,6*	9,3 ± 01,5*
NK activity,%	25,4 ± 03,2	20,2 ± 02,3	22,0 ± 03,7	28,9 ± 04,0
DTH, %	27,4 ± 02,6	21,9 ± 02,2	17,4 ± 02,2*	13,5 ± 02,0*
AMIHIR (AFC to SRBC 10^3)	9,3 ± 01,5	5,6 ± 00,6*	4,0 ± 00,5*	2,7 ± 00,3*

Table 2: Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on immune system parameters of rats and the ability of macrophages to induce a humoral immune response (AMIHIR) of CBA mice (M+m, n=7-9)

dose of 4.0 μg/kg, the number of AFC to SRBC, T-independent antibody formation (number of AFC to Vi-Ag), the DTH reaction decreased by 1.73; 2,60 and 1,57 times, but under the action of TChDBD at a dose of 8,0 μg/kg these parameters decreased respectively by 2,88; 3,77 and 2,03 times (p < 0,05). There was no statistically significant change in NK activity under the influence of TChDBD at the doses of 1.0; 4.0 and 8.0 μg/kg.

The data indicate that the T-independent humoral immune response was reduced under the influence of TChDBD to a greater extent than the T-dependent humoral immune response (p < 0.05). This is due to the ability of TChDBD to phosphorylate proteins with molecular weights of 29, 45, 52 and 63 kD only in B-lymphocytes [13]. Dose-dependent decrease in AMIHIR was detected in CBA mice under the influence of dioxin.

It was established (Table 3) that in acute intoxication with TChDBD in dose the activity of catalase and peroxidase, characterizing AOS, decreased respectively by 1.83 and 1.98 times (p < 0.05). The main LPO product of MDA increased 1.30-fold (p < 0.05) and TRP increased 1.73-fold (p < 0.05) in acute TChDBD poisoning. Changes in LPO indices in blood undoubtedly reflect the process of free-radical oxidation of lipids, both of all cells of different organs in general, and of the organs of the immune system and, in particular, of lymphocytes [3,5].

When calculating the correlation coefficients between the number of AFC to SRBC in acute poisoning with TChDBD and the content of catalase and peroxidase in the blood of rats, they were found to be respectively 0.771 (p < 0.05) and 0.760 (p < 0.05). The correlation coefficients between the number of AFC to SRBC and the MDA content in blood were -0.766 (p < 0.05) and -0.725 (p < 0.05), respectively, under the action of a toxicant. The "r" values between the other immune system parameters during the acute action of TChDBD and the AOS indices ranged from 0,690 to 0,789 (p < 0,05), and the correlation coefficients between the blood MDA content and the immune status indices during the action of TChDBD were -0,672 to -0,788 (p < 0,05).

Discussion

The changes in IIRO, NBR parameters and immune status under the action of TChDBD are due to the expression of the genes responsible for the synthesis of cytochrome P-450-dependent monooxygenases. This process is realized by the formation of a complex consisting of xenobiotic molecule and specific Ah-receptor located in the cell cytoplasm [4]. The observed decrease of NBR, IIRO and immune system indices under the action of TChDBD is due to the disruption of nucleic exchange in blood cells, including immuno-

Experiment series	Catalase, mmol/min/l	Peroxidase, µmol/min/l	Total radical production, conditional units	Malone dialdehyde, nmol/ml
Control	275,8 ± 21,5	49,7 ± 3,9	35,8 ± 3,7	6,17 ± 0,31
Intoxication with TChDBD	150,7 ± 15,2*	25,1 ± 3,0*	62,0 ± 5,0*	8,03 ± 0,30*

Table 3: Effect of acute intoxication with 2,3,7,8-tetrachlorodibenzo-p-dioxin (8 µg/kg) on LPO parameters of rats (M+m, n = 7-10).

* -p < 0,05 as compared to control

cytes; the effect of dioxin on the differentiation of B-lymphocytes [15]; reduction in the ability of T cells to activate B-lymphocytes in the immune response [12]; reduction of IL-2 production by T-cells and proliferation of thymocytes at a very early stage of their development; disruption of the process of interaction between thymocytes and stroma [9], inhibition of T-helper function (suppressor cells are not involved in the immunosuppressive effect of dioxin) [14]. It is known that the disruption of thymocyte maturation caused by TChDBD is detected through the Ah-receptor of rat (mouse) thymic epithelial cells. In humans, TChDBD, when acting directly on the Ah-receptor of thymic epithelial cells, causes a similar effect [7]. There is evidence that the immunosuppression induced by TChDBD can be achieved by altering the regulatory processes controlled by tyrosine kinases [8].

Probably, LPO initiation under the influence of TChDBD may be, along with the described mechanisms, one of the factors contributing to the post-toxicant immunodeficiency state.

Thus, 2,3,7,8-tetrachlorodibenzo-p-dioxin at the doses of 1.0; 4.0 and 8.0 µkg/kg caused a dose-dependent reduction of IIRO and NBR parameters, suppression of the predominantly T-independent antibody production, decrease of DTH in rats and reduction ability of macrophages to induce a humoral immune response in CBA mice. The suppression of immune system parameters was directly related to the initiation of lipid peroxidation.

Conclusion

- The 2,3,7,8-tetrachlorodibenzo-p-dioxin (1,0; 4,0 and 8,0 µg/kg) induced a dose-dependent decrease in the basic parameters of the nonspecific body resistance and the integral immunological resistance of the organism in rats.
- The action of 2,3,7,8-tetrachlorodibenzo-p-dioxin at the doses 1.0; 4.0 and 8.0 µg/kg leads to a dose-dependent reduction of the predominantly T-independent humoral immune response, delayed-type hypersensitivity reactions and the ability of macrophages to induce a humoral immune response.
- Acute intoxication with 2,3,7,8-tetrachlorodibenzo-p-dioxin at doses of 1.0; 4.0 and 8.0 µg/kg had no effect on NK activity in rats.
- Reduction in immune system parameters has been directly linked to the initiation of lipid peroxidation.

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