ACTA SCIENTIFIC MICROBIOLOGY

Volume 2 Issue 5 May 2019

Research Article

The Effect of Dichloethane Subacute Intoxication on Cellular, Humoral Immune Reactions and the Content of Cytokines in the Blood

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Received: March 18, 2019; **Published:** April 03, 2019

Abstract

Experiments on random-bred albino rats showed that 1,2-dichloroethane subacute intoxication (0.3 LD_{50} daily for 3 days) decreases parameters cellular immune response, Th1 cell function is more than humoral immune response and Th2 lymphocyte activity; reduces blood content of immunoregulatory cytokines IFN-γ, IL-2, IL-4 and anti-inflammatory cytokine IL-13; increases the proinflammatory cytokine IL-6 concentration, has virtually no effect on the concentration of anti-inflammatory cytokine IL-10.

Keywords: dichloroethane; Th1, Th2 lymphocytes; immune responses; immunotoxicity; cytokines.

Introduction

Dichloroethane (DChE, 1,2-dichloroethane) is widely used in organic synthesis, as well as an extractant, a means of cleaning and degreasing clothes, and a solvent of toxic substances. Acute poisonings are characterized by high mortality (more than 90%) [1,2]. In case of accidents at chemical objects, DChE can cause group and mass acute poisonings. This may be accompanied by a toxicant entering the air, soil, and water and cause subacute and chronic intoxication of people. In case of poisoning with DChE, practically all organs and systems of the body are affected (central nervous system, system, kidneys and liver, gastrointestinal, cardiovascular, coagulation and anticoagulant systems of the blood, etc.) [1,3].

DChE can cause mutagenic and carcinogenic effects [1,4]. Dysfunctions of the immune system, in particular, Th1 and Th2 lymphocytes and the synthesis of cytokines by them and other blood cells, are poisoned with DChE for the purpose of their targeted correction to prevent infectious complications and disease, as well as to reduce the mortality of patients [1,2,5-10].

Aim of the study

The aim of the study was DChE subacute poisoning (0.3 LD_{50} daily for 3 days) on immune responses, as well as on blood levels of immunoregulatory, proinflammatory and anti-inflammatory cytokines (γ-interferon - IFN-γ, IL-2, IL-4, IL-6 and IL-10 and IL-13).

Materials and Methods

The experiments were performed on random-bred albino rats of both sexes weighing 180-240 g. DChE was administered intragastrically (per os) in an olive oil solution for 3 days, the daily dose was 0.3 LD_{50}. (LD_{50} DChE by oral administration - 0.75 ± 0.10 g/kg). Control animals received per os equal volume of olive oil. Immunity system indicators were evaluated by generally accepted methods in experimental immunotoxicology and immunology [1,2,6,7,11]. The humoral immune response to T-dependent antigen (red sheep blood cells - RSBC)), which characterizes the ability of Th1 cells to participate in the production of IgM by B cells (plasma cells), was determined by the number of antibody-forming cells (AFC) in the spleen 4 days after immunization (peak IgM production) which was administered intraperitoneally at dose of 2×10^8 RSBC. Similarly, we evaluated the humoral immune response to a T-independent typhoid Vi-antigen (Vi-Ag), reflecting the function of B cells and the synthesis of IgM by plasma cells (B cells) of the rat spleen. In this case, rats were immunized of Vi-Ag at a dose of 8 μg/kg [1,8]. The function of Th1 lymphocytes was determined by delayed-type hypersensitivity (DTH) reaction. The DTH was studied in animals by weight gain of the hind paw foot in %. The resolving dose of RSBC (5×10^8) was administered under the aponeurosis of the foot of the hind paw 4 days after immunization, which was performed intraperitoneally almost simultaneously with the first administered of DChE. The reaction of DTH was evaluated after 24 h [1,2].

The function of Th2 lymphocytes was investigated by the number of AFC, synthesizing IgG to RSBC, in the spleen after 7 days after immunization (2×10^8 RSBC; almost simultaneously with the first administered of DChE) by indirect local hemolysis in the gel [1,7,11]. Evaluation of the activity of natural killer cells (NK) and antibody-dependent cellular cytotoxicity (ADCC) was performed by the spectrophotometric method 4 days after the first injection of DChE [1,2]. In the control and in the experiments, rats were immunized (10^8 RSBC) intraperitoneally 15 to 30 minutes after the first administration of DChE.

The concentration of immunoregulatory cytokines IFN-γ (#MBS824935), IL-2 (# MBS2885949), IL-4 (# MBS2883072), proinflammatory cytokine IL-6 (# MBS2885203)) and anti-inflammatory cytokines IL-10 (#MBS2087187), IL-13 (#MBS495243) [1] was examined in rat blood plasma 4 days after injection of DChE using kits (ELISA Kits MyBioSoure) in accordance with the manufacturer's instructions. Blood for research was taken from the retroorbital venous sinus. The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p<0.05$.

Results

The parameters of T-dependent - AFC to RSBC (IgM), AFC to RSBC (IgG) after DChE subacute intoxication as well as the T-independent humoral immune response - AFC to Vi-Ag (IgM) decreased after 4 days, respectively, in 2.06; 2.02 and 1.66 times ($p<0.05$) [table 1]. This suggests that DChE subacute intoxication affects T cells to greater extent than the B lymphocytes.

Lymphocytes	Parameters	Control	DChE
Th1	AFC to RSBC (IgM), 10^3	33,3 ± 4,0	16,2 ± 1,9*
	DTH, %	20,0 ± 1,9	9,9 ± 1,0*
Th2	AFC to RSBC (IgG), 10^3	30,1 ± 3,1	18,1 ± 2,3*
B cells	AFC to Vi-Ag (IgM), 10^3	31,3 ± 3,3	11,9 ± 1,6*
NK	NK activity, %	14,0 ± 1,5	6,3 ± 0,9*
Cells of ADCC	ADCC, %	37,5 ± 3,6	16,4 ± 1,8*

Table 1: The effect of dichloethane subacute intoxication (0.3 LD_{50} daily for 3 days) on rats immunity parameters (M ± m; n=8-9)

* -p <0,05 as compared to control

The parameters of cellular immune reactions — the activity of NK, ADCC, DTH — after DChE subacute intoxication decreased in 2.63; 2.22 and 2.28 times ($p<0.05$).

The obtained data show that the humoral immune response after DChE subacute intoxication decreases on average in 1.91 ± 0.15 times, and the cellular immune reactions - in 2.38 ± 0.14 times. The decrease in the average activity of Th1 cells (AFC to Vi-Ag - IgM), DTH) and Th2 lymphocytes (AFC to RSBC - IgG) [1] after DChE subacute intoxication was recorded in 2.23 and 1.61 times, respectively.

After DChE subacute intoxication (Table 2) the blood levels of immunoregulatory cytokines IFN-γ, IL-2, IL-4 and anti-inflammatory cytokine IL-13 decreased, respectively, in 2.72; 2.29; 1.57 and 1.61 times ($p < 0.05$). The blood concentration of proinflammatory cytokine IL-6 increased in 1.43 times ($p < 0.05$), and the content of anti-inflammatory cytokine IL-10 did not significantly differ from the control, increasing in 1.26 times ($p > 0.05$).

Cytokines	Control	DChE
ИФН-γ	987 ± 102	363 ± 37 [a]
ИЛ-2	1168 ± 121	510 ± 46 [a]
ИЛ-4	149 ± 17	95 ± 11 [a]
ИФНγ/ИЛ-4	6,6 ± 0,7	3,8 ± 0,6 [a]
ИЛ-6	63 ± 8	90 ± 9 [a]
ИЛ-10	356 ± 43	447 ± 44
ИЛ- 13	134 ± 14	83 ± 8 [a]

Table 2: The effect of dichloethane subacute intoxication (0.3 LD_{50} daily for 3 days) on concentration of cytokines in the rats blood, pg/ml (M ± m; n= 8)

* -p <0,05 as compared to control

The IFNγ/IL-4 ratio in the control was 6.6 ± 0.7, and after DChE subacute intoxication - 3.8 ± 0.6 ($p < 0.05$). This confirms the data obtained indicating that under the influence of DChE Th1 lymphocytes are affected to greater extent than Th2 cells [1,11].

Discussion

The reduction of DChE intoxication of the humoral and cellular immune response is due to the effect on the lymphocytes of both the toxicant molecule and its more toxic than DChE metabolites (2-chloroethanol, chloroacetic aldehyde, monochloroacetic acid) [1,2,5,9,10].

The decrease of activity of Th1 cells may be due to significant increase in blood due to DChE subacute intoxication the concentration of corticosterone [1,12], to which Th1 lymphocytes are more sensitive than Th2 cells [11]. In addition, this is probably due to the anticholinesterase effect of DChE and its metabolites (2-chloroethanol, chloroacetic aldehyde, monochloroacetic acid) [6,9,10] and probably a high content of esterase on the outer membrane and in the cytosol of Th1 lymphocytes [1].

The suppression of concentration of immunoregulatory cytokine IFN-γ in the blood is associated with the action of DChE, mainly on Th1 lymphocytes, as well as on NK, ADCC, cytotoxic T lymphocytes [13]. The decrease in blood plasma after chronic intoxication of DChE of the immunoregulatory cytokine IL-2 indicates the suppression of its production by T cells, including Th0 and Th1 lymphocytes, decrease proliferation of T cell and B lymphocytes and activity of NK, ADCC [14].

The decrease synthesis of IL-4 (immunoregulatory and, in certain cases, anti-inflammatory cytokine) [15] is due to the destruction of DChE of Th2 lymphocytes [1,15].

The increase of proinflammatory cytokine IL-6 in blood is associated with the action of DChE and its metabolic products [1,2,9,10] on macrophages and lymphoid dendritic cells [11,16] due to inflammatory changes in the liver [3].

A slight increase of the antiinflammatory cytokine IL-10 in the blood under the influence of DChE appears to be due to the compensatory response of CD4+CD25+Foxp3+ regulatory T cells to lesion of lymphocytes, monocytes and macrophages by toxicant [15,17,18].

Thus, the immunotoxic effect of DChE is accompanied by decrease of humoral and cellular immune responses, impaired cytokine production by lymphoid dendritic cells, lymphocytes and other blood cells. These changes are mainly caused by the products of biotransformation DChE (2-chloroethanol, chloroacetic aldehyde, monochloroacetic acid), which are several times more toxic than DChE [1,2,9,10]. The mechanisms of dysfunction of the cells of the immune system under the influence of DChE can be neuroendocrine changes (in particular, increased production of corticosteroids), lipid peroxidation initiation of the immune system cells membranes, damage to the immunocyte genome, inhibition of their various enzymes, in particular, cytochrome P-450, tissue respiratory enzymes and oxidative phosphorylation, etc. [1,2,12].

Conclusion

1. DChE subacute intoxication (0,3 LD_{50} daily for 3 days) causes a decrease in the cellular immune response, Th1 cell function to a greater extent than humoral immune response and Th2 lymphocyte activity.
2. The subacute intoxication of DChE reduces the content of immunoregulatory cytokines IFN-γ, IL-2, IL-4 and anti-inflammatory cytokine IL-13 in the blood, practically does not change the concentration of anti-inflammatory cytokine IL-10, and increases the content of proinflammatory cytokine IL-6.

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