



The Effect of Acetonitrile Chronic Intoxication on Parameters of the Immunity System, Blood Concentration Cytokines and T Cell Acetylcholinesterase Activity

Pavel Franzevich Zabrodskii*

Saratov Medical University "REAVIZ", Saratov, Russia

*Corresponding Author: Pavel Franzevich Zabrodskii, Saratov Medical University "REAVIZ", Saratov, Russia.

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Abstract

Experiments on random-bred albino rats showed that acetonitrile chronic intoxication (0.05 LD₅₀ daily for 30 days) equally reduces the immune responses associated with the function of Th1, Th2 and B lymphocytes, decreases the activity of natural killer cells, antibody-dependent cellular cytotoxicity, blood concentrations of immunoregulatory, proinflammatory and anti-inflammatory cytokines (IFN- γ , IL-2, IL-4, IL-6, IL-10 and IL-13), reduces the acetylcholinesterase activity in T lymphocytes.

Keywords: Acetonitrile; Immunotoxicity; Th1, Th2 Lymphocytes; Cytokines; T Cell Acetylcholinesterase

Introduction

Acetonitrile (AN, ethanenitrile, cyanomethane ethyl nitrile methanecarbonitrile, cyanomethane) is a volatile, colorless, flammable liquid with a smell of ether. MC is widely used in the chemical industry in organic synthesis, the production of aromatic substances, pharmaceutical and perfume preparations, as a selective solvent of hydrocarbons, oils [1-3]. AN is one of the most common ligands in coordination chemistry [4]. High-purity acetonitrile is widely used in high-performance liquid chromatography, gas chromatography, and mass spectrometry, as eluent [5]. Due to the possible chronic intoxication, AN in the process of its production and work with it, in case of accidents at chemical plants and contamination of the area can form secondary immunodeficiency states [1,2,6,7]. At the same time, the study of violations of the parameters of the immune system, in particular, the production of cytokines by lymphocytes and other blood cells, is of both theoretical and practical interest for the prevention and treatment of various infectious complications and diseases arising from AN chronic intoxication [1,2,6,7].

Aim of the Study

The aim of the study was to evaluate acetonitrile chronic intoxication (0.05 LD₅₀ daily for 30 days) on immune responses, acetylcholinesterase (AChE) activity in T lymphocytes, and also on blood levels of immunoregulatory, proinflammatory and anti-inflammatory cytokines (IFN- γ , IL-2, IL-4, IL-6, IL-10 and IL-13).

Materials and Methods

The experiments were performed on random-bred albino rats of both sexes weighing 180-240 g. AN was administered subcutaneously daily for 30 days at a dose of 0.05 LD₅₀ (total dose 1.5

LD₅₀). LD₅₀ of AN for rats after subcutaneous administration was 1750 \pm 95 mg/kg. Immunity system indicators were evaluated by generally accepted methods in experimental immunotoxicology and immunology [1,2,8] after chronic intoxication of AN, 30 days after the first injection of the toxicant. 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 \times 10⁸ 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 μ g/kg [1,2,7].

The function of Th1 lymphocytes also 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 \times 10⁸) was administered under the aponeurosis of 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 [1,2,7].

The function of Th2 lymphocytes was investigated by the number of AFC, synthesizing IgG to RSBC, in the spleen after 13 days (peak IgG production [8]) after immunization 2 \times 10⁸ RSBC 16 days after the first injection of AN; almost simultaneously with the first administered of AN) by indirect local hemolysis in the gel [1,2,7,8]. Evaluation of the activity of natural killer cells (NK) and antibody-dependent cellular cytotoxicity (ADCC) was performed by the

spectrophotometric method 30 days after the first injection of AN [1,2,7]. In the control and in the experiments, rats were immunized (108 RSBC) intraperitoneally 15 to 30 minutes after the first administration of AN.

AChE activity in T lymphocytes was determined 10, 20, 30 days after intoxication. The cells were isolated by filtering splenic suspension through Nylon cotton (Nitron) [9]. The tests and estimations were carried out as described previously [9]. The amount of acetylcholine (in μmol) hydrolyzed per 1 min in 1 ml of suspension containing 10^9 T lymphocytes was taken for 1 Unit of AChE activity.

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) [2,10] was examined in rat blood plasma 30 days after the first injection of AN using kits (ELISA Kits MyBioSource) in accordance with the manufacturer's instructions. At the same time, immunization of 2×10^8 RSBC was performed on day 26 after the first injection of toxicant. 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 AN chronic intoxication as well as the T-independent humoral immune response - AFC to Vi-Ag (IgM) decreased after 30 days, respectively, in 1.38; 1.39 and 1.42 times ($p < 0.05$). This suggests that AN chronic intoxication affects T cells and B lymphocytes equally [Table 1].

Parameters	Control	AN
AFC to RSBC (IgM), 10^3	40,5 \pm 4,1	29,4 \pm 2,9*
AFC to RSBC (IgG), 10^3	56,7 \pm 5,5	40,8 \pm 4,3*
AFC to Vi-Ag (IgM), 10^3	29,8 \pm 3,1	21,0 \pm 2,3*
NK activity, %	27,9 \pm 2,9	20,0 \pm 2,1*
ADCC, %	12,8 \pm 1,3	8,9 \pm 1,0*
DTH reaction, %	35,9 \pm 3,7	24,7 \pm 3,0*

Table 1: The effect of acetonitrile chronic intoxication (total dose - 1.5 LD₅₀, 30 days) on the parameters of the immune system of white rats (M \pm m, n = 9-11)

* -p <0,05 as compared to control

The parameters of cellular immune reactions after exposure to the AN for 30 days - the activity of NK, ADCC, the reaction of DTH - decreased respectively in 1.40; 1.44 and 1.45 times ($p < 0.05$). The data obtained suggest that humoral and cellular immunity after AN chronic intoxication are affected almost equally.

The reduction of Th1 cell function (AFC to RSBC - IgM, the DTH reaction) and Th2 lymphocytes (AFC to RSBC - (IgG) occurs equally after AN chronic intoxication.

The activity of NK and ADCC to a certain extent depends on the function of T lymphocytes (helpers) of the first type (Th1). The reduction of these parameters, as evidenced by the data table. 1, approximately the same as the Th2 cell-dependent reaction, as well as the humoral immune response, which characterizes the activity of B lymphocytes (AFC to Vi-Ag - IgM).

There was a decrease equally levels of IFN- γ , IL-2, IL-4 (immunoregulatory cytokines), IL-6 (proinflammatory cytokine), IL-10 and IL-13 (anti-inflammatory cytokines) in blood after AN chronic intoxication (table. 2). So, IFN- γ , IL-2, IL-4, IL-6, IL-10 and IL-13 decreased in 1.34; 1.33; 1.43; 1.51; 1.35 and 1.41 times ($p < 0.05$), respectively. It should be noted that IL-4 and IL-6 can also perform anti-inflammatory functions [10].

After AN chronic intoxication the AChE activity in splenic T lymphocytes in rats 10, 20 and 30 days after the first toxicant administration decreased in 1.16 ($p > 0.05$); 1.38 and 1.64 times ($p < 0.05$), respectively (Table 2,3).

Cytokines	Control	AN
IFN- γ	1016 \pm 85	760 \pm 80*
IL-2	1245 \pm 110	933 \pm 94*
IL-4	93 \pm 9	65 \pm 7*
IFN- γ / IL-4	10,9 \pm 1,1	11,7 \pm 1,2
IL-6	127 \pm 13	84 \pm 10*
IL-10	983 \pm 87	730 \pm 75*
IL-13	110 \pm 12	78 \pm 8*

Table 2: The effect of acetonitrile chronic intoxication (total dose - 1.5 LD₅₀, 30 days) on concentration of cytokines in the rats blood, pg/ml (M \pm m; n= 8-10).

* -p <0,05 as compared to control

Duration of intoxication, days	Control	AN
10	64,7 \pm 6,5	55,8 \pm 5,6
20		46,9 \pm 4,7*
30		39,5 \pm 4,0**

Table 3: The effect of acetonitrile chronic intoxication on acetylcholinesterase activity in splenic T lymphocytes in rats, mUnits/10⁹ cells (M \pm m; n= 9-11)

* -p <0,05 as compared to control; ** p<0,05 compared with the parameter after 10 days.

Discussion

Was established decrease the IFN- γ concentration in 1.34 times ($p < 0.05$) and IL-4 concentration - in 1.43 times ($p < 0.05$) after AN chronic intoxication. The ratio of IFN γ / IL-4 in the control was 10.9 \pm 1.1, and after AN chronic intoxication - 11.7 \pm 1.2. This indicates that the concentrations of IFN- γ and IL-4 in the blood are equally

reduced under the influence of the AN, therefore the function of Th1 and Th2 lymphocytes is affected equally [2,11].

The data obtained suggest that the same decrease of Th1 and Th2 lymphocytes activity equally can lead to development, both microbial (the main protective role is played by Th2 lymphocytes and their associated plasma cells, that synthesize immunoglobulins), and viral infection (the main protective role belongs to Th1 lymphocytes along with other T cells, NK and ADCC) [2,8,11,12].

The decrease the IFN- γ blood level (immunoregulatory cytokine) is associated with AN damage, mainly Th1-lymphocytes, as well as NK, ADCC, cytotoxic T lymphocytes [13].

The reduction in blood plasma after AN chronic intoxication of IL-2 (immunoregulatory cytokine) indicates the suppression of its production by T-cells (including Th0 and Th1 type lymphocytes), a decrease in the proliferation of T and B cells, the activity of NK and ACCD [14]. The decrease of IL-6 in the blood (proinflammatory interleukin and in certain cases - the anti-inflammatory cytokine) characterizes the reduction of its synthesis by macrophages, monocytes and lymphoid dendritic cells as a result of their damage of AN and its metabolic products. The decrease the IL-4 concentration in blood (immunoregulatory and, in certain cases, anti-inflammatory cytokine) [10] is due to AN lesion of Th1 lymphocytes [2,8,10,11].

The suppression of IL-10 synthesis (anti-inflammatory cytokine) under the influence of AN is associated with the defeat of the Th0, Th2 lymphocytes, monocytes, macrophages and B cells by the toxicant [2,8,11,15]. The decrease in the blood level of IL-10 to the same extent as IFN- γ confirms the established damaging effect of AN on Th1 and Th2 lymphocytes.

The reduction of IL-13 (anti-inflammatory cytokine) is due to the AN damage of Th2 lymphocytes, as well as other blood cells. In this case, the modulation of allergic reactions by this cytokine, as well as apoptosis or growth of tumor cells, may be violated [11,16].

AN is metabolized to form cyanide (generally about 2–12 hours), which is mainly due to its immunotoxic effect due to inhibition of the a3 cytochrome-c-oxidase tissue respiration component in cells of the immune system, as well as more than 40 iron, copper, zinc-containing enzymes. In addition, during the biotransformation of AN, rhodanides, formic acid and ammonia are formed, which, with the exception of rhodanides, have a pronounced immunotoxic effect [1,2,7].

We have established the anticholinesterase effect of AN, which is associated with decrease of the AChE activity in splenic T lymphocytes after AN chronic intoxication in rats (after 10, 20 and 30 days after the first toxicant administration). The anticholinesterase

effect (decrease by AChE activity T lymphocytes) causes the immunotoxic effect of many toxic chemicals, primarily organophosphorus compounds [2,6,9], and to a certain extent acrylonitrile [17].

Conclusion

- Acetonitrile chronic intoxication (0.05 LD₅₀ daily for 30 days) equally reduces the activity of Th1 and Th2 lymphocytes, humoral and cellular immune responses.
- The concentration of immunoregulatory, proinflammatory and anti-inflammatory cytokines (IFN- γ , IL-2, IL-4, IL-6, IL-10, IL-13) in the blood decreases equally after acetonitrile chronic intoxication.
- Acetonitrile chronic intoxication reduces the acetylcholinesterase activity in T lymphocytes.

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Zabrodskii.