Effect of Tetrachloromethane on the Immune System

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Acute poisoning with tetrachloromethane in a dose of 0.75 LD_{50} suppressed humoral and cell immune reactions in Wistar rats. Immunotoxicity of tetrachloromethane is realized via initiation of lipid peroxidation and inhibition of acetylcholine esterase in T lymphocytes and α-naphthyl AS-acetate esterase and α-naphthyl butyrate esterase in splenocytes.

Key Words: *tetrachloromethane; immunotoxicity; esterases; lipid peroxidation*

Tetrachloromethane (TCM, carbon tetrachloride) is extensively used as an industrial solvent of oils, resins, bitumens, polymers, and rubber, agent for extraction of fats and alkaloids, and decontaminant to treat leather and clean or degrease clothes for domestic and industrial purposes. TCM can enter the organism through the digestive tract, airways, or skin. This compound is an extreme health hazard during emergency industrial situations, when a considerable number of people suffer from inhalation poisoning. Acute poisoning with TCM is one of the most common intoxications with toxic industrial fluids [7,9]. Poisoning with TCM can result from its peroral consumption to achieve alcohol-related drunkenness. Acute poisoning with TCM is accompanied by disturbances in various systems of the organism. High mortality rate from inhalation and peroral poisoning with TCM (20-30%) [9] is related to the development of secondary immunodeficiency [7]. The mechanisms underlying the immunotoxic effects of TCM should be evaluated to justify the treatment of postintoxication immune dysfunction and prevention of infectious complications with immunostimulators [5].

Here we studied the effects of acute poisoning with TCM on the immune system, lipid peroxidation (LPO), and esterase activity in immune cells. The relationships between general immune indexes, intensity of LPO, and acetylcholine esterase (ACE) in T cells were evaluated.

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MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 180-240 g. TCM wag given perorally in a dose of 0.75 LD_{50}. The humoral immune response was determined by the number of antibody-producing cells in the spleen 4 days after TCM administration and intraperitoneal immunization with thymus-dependent (sheep erythrocytes) and T cell-independent antigens (Vi-Ag) in doses of 2×10^8 cells and 8 μg/kg, respectively [11]. Activity of natural killer cells (NKC) was estimated by the natural cytotoxicity index. The number of target cells that remained undamaged in the cytotoxic test was evaluated spectrophotometrically 72 h after TCM administration [3]. Antibody-dependent cytotoxicity was assayed spectrophotometrically 4 days after immunization with sheep erythrocytes (10^8 cells) using rat splenocytes [6]. Delayed-type hypersensitivity (DTH) reflecting the cell-mediated immune response (*e.g.*, Th1 lymphocyte activity) was evaluated by an increase in the weight of mouse hindlimb pad (%). The animals were intraperitoneally immunized with sheep erythrocytes (10^8 cells). Sheep erythrocytes in a provoking dose (5×10^8 cells) were administered under hindlimb aponeurosis 4 days later. DTH was recorded 24 h post-injection. The rats were immunized during administration of TCM to study humoral and cell-mediated immune reactions. The intensity of LPO and the state

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of the antioxidant system (AOS) were determined by catalase and peroxidase activities [2]. The content of malonic dialdehyde (MDA) in the blood was measured photometrically (against butanol) in a 1-cm cuvette on a KFK-2 device at 540 nm 4 days after treatment with TCM [8]. ACE activity in T lymphocytes was estimated 4 days after intoxication. Cells were isolated by filtration of the splenocyte suspension through nylon cotton wool (Nitron) [10]. The reaction and calculations were performed as described elsewhere [4]. The amount of acetylcholine (μmol) hydrolyzed in 1 ml suspension with 10^9 T lymphocytes over 1 min was taken as a unit of ACE activity. Activities of α-naphthyl AS-acetate esterase and α-naphthyl butyrate esterase in T cells of the spleen were assayed histochemically 4 days after TCM administration [5].

The results were analyzed by Student's *t* test. We calculated coefficients of correlation between immune indexes, ACE activity in T cells, and intensity of LPO.

RESULTS

Acute poisoning with TCM attenuated the immune response to T cell-dependent and T cell-independent antigens by 2.51 and 1.48 times, respectively ($p<0.05$, Table 1). Moreover, acute poisoning with TCM decreased antibody-dependent cytotoxicity, natural cytotoxicity, and DTH by 1.92, 1.73, and 1.84 times, respectively ($p<0.05$).

Suppression of the immune system was probably related to the effects of TCM, its metabolites carbon trichloride and carbon dichloride, chlorine, phosgene, hydrogen chloride, carbon oxide, and O—O—CCl, HO—O—CCl_3, and HO—CCl_3 radicals.

TCM not only suppressed the immune system, but also initiated LPO (Table 2). TCM decreased activities of catalase and peroxidase by 1.55 and 1.60 times, respectively, which reflected changes in the state of AOS ($p<0.05$). The content of MDA (main LPO product) increased by 1.44 times after acute poisoning with TCM ($p<0.05$). Changes in LPO in the blood characterize the process of free radical oxidation in various immune cells and organs (*e.g.*, lymphocytes) [5]. The development of postintoxication immunodeficiency is probably associated with initiation of LPO by TCM.

Activation of LPO in immune cell membranes can be directly related to oxidation of SH groups in active sites of membrane-bound enzymes and formation of intra- and intermolecular bonds. Lipid hydroperoxides modulate activity of various enzymes. For example, activity of monoamine oxidase is "switched" from monoamines to other amines. Moreover, MDA can form covalent bonds with amides in immunocompetent cells [1].

TABLE 1. Effect of Acute Poisoning with TCM (0.75 LD_{50}) on the Immune System in Rats (*M*±*m*, *n*=11)

Parameter	Control	TCM
APC to sheep erythrocytes, 10^3	35.1±3.6	14.0±2.2*
APC to Vi-Ag, 10^3	21.0±2.1	14.2±2.5*
ADCC, %	12.5±1.3	6.5±1.5*
NC, %	25.1±3.8	14.5±3.1*
DTH, %	31.2±3.2	17.1±2.3*

Note. APC, antibody-producing cells; ADCC, antibody-dependent cytotoxicity; NC, natural cytotoxicity. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

Suppression of the immune system was accompanied by a decrease in activity of ACE in T lymphocytes and count of α-naphthyl AS-acetate esterase-positive and α-naphthyl butyrate esterase-positive cells in rat spleen (Table 3).

Acute poisoning with TCM decreased activities of ACE in T cells and α-naphthyl AS-acetate esterase and α-naphthyl butyrate esterase in splenocytes by 33.9, 23.1, and 29.1%, respectively ($p<0.05$). Therefore, immunotoxicity of TCM is associated with inhibition of ACE in T lymphocytes and other esterases in macrophages, neutrophils, and monocytes [5].

It should be emphasized that immunotoxicity of TCM can be related not only to the observed changes in LPO and esterase activity of immunocompetent cells. Hypofunction of immunocompetent cells caused by TCM can result from damage to the genome in immune cells, functional inactivation of various enzymes (*e.g.*, enzymes of tissue respiration and oxidative phosphorylation), and neuroendocrine disorders [5].

After acute poisoning with TCM the coefficients of correlation between the number of cells producing antibodies against sheep erythrocytes and activities of catalase and peroxidase in the blood were 0.751±0.131 ($p<0.05$) and 0.792±0.112 ($p<0.05$), respectively. The number of cells producing antibodies against sheep erythrocytes correlated with blood MDA content

TABLE 2. LPO in Rats 4 Days after Acute Poisoning with TCM (*M*±*m*, *n*=11)

Series	Catalase, mmol/liter/min	Peroxidase, μmol/liter/min	MDA, nmol/liter
Control	704.3±71.5	48.7±5.3	5.30±0.28
TCM	453.5±46.4*	30.5±4.1*	7.64±0.31*

TABLE 3. Esterase Activity in Rat Splenocytes 4 Days after Acute Poisoning with TCM (0.75 LD_{50}, $M\pm m$, n=11)

Parameter	Control	TCM
ACE activity in T cells, mU/10^9 cells	61.3±6.8	40.5±4.1*
Count of α-naphthyl AS-acetate esterase-positive cells, %	47.1±3.7	36.2±3.3*
Count of α-naphthyl butyrate esterase-positive cells, %	38.5±4.0	27.3±3.2*

(r=-0.701±0.153, p<0.05). The coefficient of correlation between DTH and peroxidase activity was 0.698± 0.154 (p<0.05). After treatment with TCM a correlation was revealed between blood MDA content and DTH (-0.685±0.160, p<0.05). The count of cells producing antibodies against sheep erythrocytes correlated with ACE activity in T cells (-0.675±0.164, p<0.05).

Our results indicate that acute poisoning with TCM suppressed humoral and cell-mediated immune reactions. These changes are related to initiation of LPO and inhibition of ACE in T lymphocytes and α-naphthyl AS-acetate esterase and α-naphthyl butyrate esterase in splenocytes.

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