Suppression of Natural Killer Cells after Acute Intoxication with Alcohols and Cholinotropic Preparations and Their Reactivation with T-Activin

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Acute poisoning with alcohols and cholinotropic preparations carboxyphosphamide and atropine (0.8 LD_{50}) was modeled on male outbred mice weighing 18-24 g. The decrease in activity of natural killer cells was most pronounced after injection of atropine, but insignificant after treatment with ethanol. The inhibitory effect of ethylene glycol, methanol, and methanol on functional activity of natural killer cells *in vitro* directly depended on their concentration. The effects of alcohols in equimolar concentrations of 10, 100, and 500 mM were similar. Therefore, immunotoxicity of alcohols was associated with the action of their metabolites. The ability of products formed after biotransformation of ethylene glycol, methanol, and ethanol in equimolar concentrations to cause damage to natural killer cells decreased in the following order: glyoxylic acid>formic acid>acetaldehyde>glycolaldehyde>glycolic acid. T-Activin injected subcutaneously in doses of 2.5 and 5.0 μg/kg for 3 days normalized activity of natural killer cells suppressed after acute poisoning with alcohols and cholinotropic preparations.

Key Words: *T-activin; alcohols; carboxyphosphamide; atropine; natural killer cells*

Studies of the effects produced by alcohols and cholinotropic preparations (CTP) carboxyphosphamide and atropine on the immune system are an urgent problem of toxicology and pharmacology. The incidence of acute poisoning with these agents constantly increases [2-7]. Various immunostimulators can prevent the development of postintoxication infections and diseases associated with immunodeficiency.

Methanol and ethylene glycol most often produce severe poisoning. The absolute rate of mortality from acute intoxication with ethanol is >60%, which far surpasses the corresponding value for other non-military poisonings [6,7].

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Organophosphorus compounds (OPC) are widely used in agriculture and chemical industry and for domestic needs. These compounds can induce acute poisonings. The mortality rate from these diseases is 20-24% [6]. Acute intoxications with atropine-like compounds are associated with accidental overdosing, consumption of narcotics, and suicidal acts [5]. Destruction of OPC and atropine-like preparations (*e.g.*, 3-quinuclidinyl-3-benzylate) [5] belonging to military chemical agents can be followed by massive acute poisonings.

High mortality rate [4,6] and considerable severity of acute poisoning with alcohols and CTP are associated with infectious complications and diseases, which often results from suppression of natural killer cells (NKC) [2,3].

Here we studied the inhibitory effects of ethylene glycol, methanol, ethanol, their metabolites, carboxy-

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TABLE 1. Reduction of Natural Cytotoxicity (%) in Mice after Acute Poisoning with Alcohols and Cholinotropic Preparations (0.8 LD_{50}) and Recovery of Activity with T-Activin (*M*±*m*, *n*=9-11)

Substance	T-activin, μg/kg			
	0	1.0	2.5	5.0
Control	25.5±3.0			
Ethylene glycol	13.4±2.2*	16.3±2.7*	21.1±2.5	24.3±2.9
Methanol	11.2±2.1*+	14.6±2.4*	19.0±1.9	23.1±2.5
Ethanol	16.7±2.3*	19.5±2.8*	23.7±2.3	27.0±3.1
Carboxyphosphamide	8.2±1.1*+	11.4±1.7*+	18.0±2.6*	20.4±2.7
Atropine	6.7±1.2*+	9.8±1.9*+	16.3±2.1*	18.7±2.5

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to ethanol.

phosphamide, and atropine on NKC. We evaluated whether T-activin can normalize activity of these cells.

MATERIALS AND METHODS

Experiments were performed on male outbred mice weighing 18-24 g (*in vivo*) and mouse splenocytes (*in vitro*). Activity of NKC was assayed after peroral treatment with ethylene glycol, methanol, and ethanol and subcutaneous injection of OPC and atropine (0.8 LD_{50}). In *in vitro* experiments alcohols and their metabolites were used in equimolar concentrations of 10, 100, and 500 mM. The effects of ethylene glycol and its major toxic metabolites glycolaldehyde, glycolic and glyoxylic acids, methanol, and formic acid, ethanol, and acetaldehyde (ICN Pharmaceuticals) were studied *in vitro*. T-Activin was injected subcutaneously in doses of 1.0, 2.5, and 5 μg/kg 30 min after treatment with toxicants. Then this agent was administered daily for 2 days.

Natural cytotoxicity of NKC was determined spectrophotometrically [1]. Mouse splenocytes and chicken erythrocytes served as effector and target cells, respectively. Functional activity of NKC was estimated 3 days after treatment with toxicants. Test cells were incubated at the 10:1 effector/target ratio for 4 h. Natural cytotoxicity was studied *in vitro* after incubation of the effector cells in medium 199 containing alcohols or their metabolites in concentrations of 10, 100, and 1000 mM for 4 h. The index of cytotoxicity was evaluated by optical density of hemoglobin released from NKC undestroyed by effector cells. The precipitate was lysed with 0.25% sodium dodecyl sulfate as described elsewhere [1].

The results were analyzed by Student's *t* test and Mann—Whitney *U* test.

RESULTS

Activity of NKC decreased after acute poisoning with alcohols and CTP in a dose of 0.8 LD_{50} (Table 1). The potency of toxicants in producing damage to NKC decreased in the following order: atropine>carboxyphosphamide>methanol>ethylene glycol>ethanol.

We studied *in vitro* effects of alcohols and their metabolites in equimolar concentrations on activity of NKC (Table 2). The test compounds dose-dependently reduced functional activity of NKC. Ethylene glycol, methanol, and ethanol in concentrations of 100 and 500 mM and their metabolites in various doses significantly decreased activity of NKC. *In vitro* effects of ethylene glycol, methanol, and ethanol on functional activity of NKC were similar. Metabolites more significantly impaired activity of NKC than alcohols. It should be emphasized that alcohol metabolites in high doses produced the most severe damage to NKC. Suppressive activity of products formed after biotransformation of ethylene glycol decreased in the following order: glyoxylic acid>glycolic acid>glycolaldehyde. In maximum doses the immunotoxic effect of glyoxylic acid surpassed that of glycolic acid ($p<0.05$). Formic acid in various doses was more potent than methanol in producing damage to NKC ($p<0.05$). Ethanol and acetaldehyde in concentrations of 100 and 500 mM produced severe damage to NKC. We compared the effects of products formed after biotransformation of ethylene glycol, methanol, and ethanol on functional activity of NKC. Immunotoxicity of these agents increased in the following order: glycolic acid<glycolaldehyde<atropine<formic acid<glyoxylic acid. Formic and glyoxylic acids produced a greater inhibitory effect than glycolic acid in the highest concentration ($p<0.05$). Glycolic acid, glycolaldehyde, atropine, formic acid, and glyoxylic acid similarly decreased functional activity of NKC.

Published data show that *in vivo* damage to NKC is associated with the effects of glycolaldehyde and glycolic acid, but not with the influence of glyoxylic acid. In biological media the concentration of glycolic acid 1300-fold surpasses the content of more toxic glyoxylic acid [10].

TABLE 2. *In Vitro* Effects of Alcohols on Natural Cytotoxicity (%) in Mice ($M \pm m$, n=9-11)

Alcohols and metabolites	Concentration, mM		
	10	100	500
Control		27.8±2.9	
Ethylene glycol	22.5±2.7	17.1±1.2*	12.7±2.9*
Glycolaldehyde	13.5±2.0*	10.2±1.9*	5.6±1.7*
Glycolic acid	16.3±2.5*	8.8±1.2*	6.9±1.1*
Glyoxylic acid	10.1±2.1*	5.8±1.5*	3.6±1.0*
Methanol	19.8±2.8	14.9±2.1*	10.8±1.7*
Formic acid	9.3±2.1*	6.2±1.8*	3.8±0.9*
Ethanol	23.7±2.9	15.3±2.0*	10.1±1.9*
Acetaldehyde	14.3±2.4*	7.0±1.5*	4.3±1.2*

Therefore, the decrease in functional activity of NKC during *in vivo* poisoning with ethylene glycol, methanol, and ethanol is related to the interaction of highly toxic products formed after biotransformation of ethylene glycol (glycolaldehyde, glycolic acid, and glyoxylic acid), methanol (formic acid), and ethanol (acetaldehyde) with enzyme systems in NKC. *In vitro* effects of ethylene glycol, methanol, and ethanol in equimolar concentrations were practically similar. Metabolites of ethylene glycol and methanol can affect sulfhydryl and amino groups of enzymes and inhibit tissue respiration and oxidative phosphorylation in NKC [12,13]. These changes led to inactivation of NKC and suppressed secretion of interleukin-2 (IL-2) and interferon-γ (IFN-γ) by T helper cells and other lymphocytes.

The effects of CTP on NKC are probably realized via blockade of surface muscarinic cholinoceptors with atropine, influence of acetylcholine or carboxyphosphamide on cholinoceptors on NKC during poisoning with carboxyphosphamide [2,14], and inhibition of nonspecific cytosolic esterases in NKC (possible descendant of T cells expressing these enzymes [8]) with carboxyphosphamide [2,11].

T-Activin in doses of 2.5 and 5 μg/kg normalized functional activity of NKC 3 days after poisoning with alcohols and CTP (Table 1).

Functional activity of NKC after poisoning with ethylene glycol, methanol, ethanol, carboxyphosphamide, and atropine is probably impaired due to inhibition of IL-2 and IFN-γ production by T helper cells and other lymphocytes, since IFN-γ acts as the most potent activator of these cells [8]. T-Activin normalizes functional activity of NKC impaired after intoxication, intensifies synthesis of IFN-γ and other interferons stimulating these cells, and induces expression of receptors for IL-2 on the cell surface. Previous studies showed that stimulators of cytokine synthesis increase functional activity of NKC suppressed after physical exercises [9]. In our experiments T-activin stimulated NKC after treatment with toxicants. These data indicate that T-activin normalizes functional activity of NKC after exposure to extreme chemical and physical factors.

Our experiments showed that acute poisoning with alcohols and CTP suppressed NKC activity. *In vitro* experiments demonstrated that immunotoxicity of alcohols is related to the influence of their metabolites with different toxicity. T-activin reactivated NKC suppressed after treatment with various toxicants, including alcohols and CTP.

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