In Vitro Effects of Alcohols and Their Metabolites on Antibody-Forming Activity of T and B Lymphocytes

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In vitro experiments on splenocytes from noninbred mice showed that ethylene glycol, methanol, and ethanol dose-dependently suppressed functional activity of T and B cells. These compounds in equimolar concentrations (10, 100, and 500 mM) produced similar effects, hence their immunotoxicity is determined by their metabolites. The suppressive effects of alcohol biotransformation products to antibody-forming activity of T and B cells decreased in the following order: glyoxylic acid — formic acid — glycolaldehyde — glycolic acid — acetaldehyde. The effects of these substances were dose-dependent. Suppression of T cells was more pronounced.

Key Words: *ethylene glycol; methanol; ethanol; T and B cells*

The incidence of acute poisoning with alcohols (primarily, ethanol, methanol, and ethylene glycol) markedly increased during the last decade [4]. These alcohols are widely used as lacquer and paint solvents and components of brake fluids, antifreeze mixtures, and fuels. Intake of toxic alcohols, in particular ethylene glycol and methanol, can be the cause of group or massive poisonings. Acute poisoning with ethylene glycol, methanol, and ethanol is characterized by high mortality [3] determined among other factors by infectious complications and impaired nonspecific resistance and immune state of the organisms. The effects of various alcohols on the immune system are poorly studied [1,2]. The immunotropic effects of alcohols and products of biotransformation of ethylene glycol (glycolaldehyde and glycolic, glyoxylic, and oxalic acids), methanol (formaldehyde and formic acid), and ethanol (acetaldehyde) were not compared.

Understanding of the immunopathogenetic mechanisms underlying the acute effect of alcohols and their metabolites is necessary for pharmacological treatment of post-intoxication immune disturbances and prevention of infectious diseases with immunostimulators [5,8].

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Here we compared *in vitro* effects of ethylene glycol, methanol, and ethanol and their metabolites on antibody-forming activity of T and B cells.

MATERIALS AND METHODS

Experiments were performed on male noninbred mice weighing 20-24 g. Ethylene glycol, its toxic metabolites, methanol, formic acid, ethanol, and acetaldehyde (ICN Pharmaceuticals) were used in equimolar concentrations of 10, 100, and 500 mM. According to published data, blood ethanol concentration of 100 mM corresponds to severe or lethal poisoning [7]. This dose of ethylene glycol and methanol is lethal. We did not evaluate the effects of oxalic acid, since recent studies showed that only 3% ethylene glycol are metabolized to this compound. Therefore, oxalic acid plays minor role in the toxic effect of ethylene glycol during acute poisoning [3,13]. We studied only one methanol metabolite, formic acid, because its precursor formaldehyde plays only a minor role in the toxic effect of methanol. This compound is characterized by short half-life (1.5 min) and is rapidly degraded by NAD-dependent formaldehyde dehydrogenase (EC 1.2.1.1) and aldehyde dehydrogenase (EC 1.2.1.3) [14].

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T cells were isolated by filtering splenocyte suspension through a nylon filter (Nitron) [9]. The reaction of complement-dependent cytolysis was used to isolate B cells. Monoclonal antibodies against Thy 1.2 antigens of mouse T cells were used as the cytotoxic serum (Cedarlane Laboratories Limited). Macrophages were isolated from splenocyte suspension by the method of negative selection (adhesion to a glass surface) [9,15]. Cell viability assessed by trypan blue exclusion test was 95-98%. The incubation mixture contained 10^6, 5×10^5, and 10^7 B cells, T cells, and sheep erythrocytes, respectively [15]. T and B cells for each experiment were isolated from the same mouse (syngeneic cells). Antibody-forming cells (AFC) were counted after 4 days [15]. This test reflects Th-1 cell-mediated synthesis of IgM by spleen B cells.

The results were analyzed by Student's *t* test.

RESULTS

Ethylene glycol dose-dependently suppressed antibody formation. This compound produced similar effects on T and B cells (Table 1), but antibody-forming activity of T cells treated with ethylene glycol in the highest concentration was 22.4% lower than activity of B cells. Methanol in doses of 100 and 500 mM and ethylene glycol in a concentration of 500 mM caused most severe damages to T cells ($p<0.05$, Table 1). Glycolaldehyde, methanol, ethanol, and their metabolites dose-dependently inhibited antibody-forming capacity of T and B cells. Metabolites of ethylene glycol produced the most pronounced effects on T cells. It should be emphasized that the effects of some metabolites in concentrations of 100 and 500 mM on T and B cells differed significantly (Table 1). The function of T cells considerably decreased compared to that of B cells only after incubation with methanol in concentrations of 100 and 500 mM ($p<0.05$, Table 1). Formic acid in all studied concentration produced a more pronounced effect on T cells ($p<0.05$, Table 1). Ethanol produced a selective effect on T cells only in the highest concentration ($p<0.05$). Acetaldehyde in concentrations of 10, 100, and 500 mM produced a more pronounced suppressive effect on T cells ($p<0.05$, Table 1).

The ability of ethylene glycol and its metabolites in equimolar concentrations to suppress *in vitro* antibody-forming activity of T and B cells increased in the following order: glyoxylic acid — glycolaldehyde — glycolic acid — ethylene glycol. Formic acid was more potent than methanol in inhibiting antibody-forming activity of T and B cells. Acetaldehyde in concentrations of 500 and 100-500 mM produced more severe damages to T and B cells, respectively ($p<0.05$ compared to ethanol). Alcohols in equimolar concentrations produced similar inhibitory effects on T and B cells, which indicates that their immunotoxicity is determined by the influence of their more toxic metabolites. We compared the effects of alcohol biotransformation products on T and B cells. Immunotoxicity of these compounds decreased in the following order: glyoxylic acid — formic acid — glycolaldehyde — glycolic acid — acetaldehyde. The inhibitory effect of glyoxylic acid was more pronounced than the effects of glycolic acid and acetaldehyde ($p<0.05$). Glycolaldehyde was more potent than glycolic acid ($p<0.05$). Formic acid surpassed acetaldehyde in the ability to suppress antibody-forming activity of T and B cells ($p<0.05$).

Suppression of T and B cells is probably associated with their functional disturbances. This is related to a direct membranotoxic effect of alcohols, interaction between highly toxic products of their biotransformation and sulfhydryl and amine groups of

TABLE 1. *In Vitro* Effects of Alcohols and Their Metabolites on APC Formation by Mouse Immunocytes (per 10^6 B Cells, *M*±*m*)

Alcohols and their metabolites	T cells+B cells incubated with alcohols and their metabolites, concentrations, mM			B cells+T cells incubated with alcohols and their metabolites, concentrations, mM		
	10	100	500	10	100	500
Ethylene glycol	265±2	201±25*	175±23*	246±20*	182±19*	143±15*
Glycolaldehyde	174±17*	135±13*	111±10*	152±14*	117±11*	76±7*
Glycolic acid	226±19*	178±18*	145±14*	202±20*	126±12*+	98±9*+
Glyoxylic acid	144±16*	104±9*	74±7*	122±13*	81±7*+	66±7*
Methanol	277±26	208±21*	172±16*	230±23*	152±17*+	125±13*+
Formic acid	150±15*	123±10*	99±8*	113±10*+	92±8*+	72±6*+
Ethanol	275±32	235±23*	189±19*	257±24*	187±18*	127±12*+
Acetaldehyde	262±23*	189±18*	133±15*	193±19*+	153±14*	95±10*+

Note. Control, B and T cells incubated with sheep erythrocytes without preincubation with alcohols and their metabolites: 334±32. B cells: 58±8. $p<0.05$: *compared to the control, +compared to the effect of alcohol or metabolite in the same concentration on B cells.

enzymes, and inhibition of tissue respiration, oxidative phosphorylation, and protein synthesis [12,13]. Under these conditions activation of T and B cells is disturbed due to inhibition of cGMP, cAMP, and IL-2 synthesis by T cells [6,11].

Thus, ethylene glycol metabolites glycolaldehyde and glyoxylic acid are most toxic to T and B cells. Glyoxylic acid even in low concentrations uncouples tissue respiration and oxidative phosphorylation [12]. However, *in vivo* damages to cells are probably associated with the effects of glycolaldehyde and glycolic acid. The concentration of glycolic acid in biological media 1300-fold surpassed that of more toxic glyoxylic acid [10]. Our results indicate that *in vivo* immunotoxic activity of alcohol metabolites increases in the following order: acetaldehyde — glycolaldehyde — glycolic acid — formic acid.

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