Immunotoxic Effects during Acute Ethylene Glycol Poisoning

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Experiments on outbred and inbred male CBA mice showed that acute poisoning with ethylene glycol (0.8 LD_{50}) increased mortality from infections, decreased the number of spleen colony-forming units, inhibited antibody formation (mainly to thymus-dependent antigens, and suppressed natural and antibody-dependent cytotoxicity and delayed-type hypersensitivity. Ethylene glycol in concentrations of 10 and 100 mM *in vitro* inhibited the formation of antibody-producing cells by acting on T and B lymphocytes.

Key Words: *ethylene glycol; nonspecific body resistance; natural killer cells; immunotoxicity*

Ethylene glycol (EG) is a dihydric alcohol used in dielectric fluids and antifreeze mixtures. Acute poisoning with EG usually occurs in subjects consumed it as an alcohol surrogate or subjects committed suicides. In some cases, EG possessing a sweet taste was presented as a liqueur and caused massive poisoning. The effects of various alcohols on the immune system are poorly understood. In addition, there are no data on the influence of acute EG poisoning on the immune system and nonspecific resistance of the body (NRB) [3]. Understanding of the mechanisms underlying EG effects is valuable for pharmacological treatment of postintoxication immune disturbances and prevention of various infectious diseases and complications, in particular, with regard to current approaches to the use of immunostimulators [7,8].

Here we evaluated NRB and changes in humoral and cell immune reactions after acute poisoning with EG.

MATERIALS AND METHODS

Experiments were performed on outbred and inbred CBA mice weighing 18-24 g. EG was administered perorally in a dose of 0.8 LD_{50} (LD_{50}=15.0±3.5 g/kg). Antiinfectious NRB of outbred CBA mice was evaluated by mortality rate from peritonitis 36 h after intraperitoneal injection of 1-day-old *E. coli* culture (2×10^8 microbial cells). Natural cytotoxicity (activity of natural killer cells) was assayed 48 h after EG administration [2]. The effects of EG on spleen colony-forming units (CFU_S) were evaluated by the method of endogenous colony formation [14]. The number of CFU_S was calculated 8 days after lethal irradiation in a dose of 8 Gy; the bone marrow in hindlimbs was shielded to a level of leg height [8]. EG was administered 30 min postirradiation. The humoral immune response to thymus-dependent (sheep erythrocytes) and T cell-independent (Vi-Ag) antigens was evaluated by the number of antibody-producing cells (APC) in the spleen 5 days after EG administration and intraperitoneal immunization with these antigens in doses of 2×10^8 cells and 8 μg/kg, respectively [1,11]. Antibody-dependent cytotoxicity of splenocytes was assayed spectrophotometrically 5 days after immunization with sheep erythrocytes (10^8 cells) [4]. Delayed-type hypersensitivity (DTH) reaction was evaluated by the weight of mouse hindlimbs. The mice were intravenously immunized with sheep erythrocytes (10^8 cells) 30 min after EG administration. Sheep erythrocytes in a provoking dose (5×10^8 cells) were administered subaponeurotically into hindlimb pads 4 days later. DTH was evaluated 24 h postinjection. The role of T and B cells in the EG-induced formation of APC was studied *in vitro* [13]. Intact T and B cells were incubated with sheep erythrocytes; in some experiments, one lymphocyte population was preincubated with 10

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or 100 mM EG for 1 h. The results were analyzed by Student's *t* test.

RESULTS

Acute EG poisoning increased mortality from *E. coli*-induced peritonitis (75.0±9.6 *vs.* 40.0±10.9% in the control, n=20, p<0.05), which attested to impairement of the antiinfectious NRB. EG decreased the number of CFU_S and attenuated the humoral immune response to T cell-dependent and T cell-independent antigens (by 2 and 1.5 times, respectively, Table 1). Acute poisoning with EG markedly suppressed DTH and natural and antibody-dependent cytotoxicity. EG in concentrations of 10 and 100 mM *in vitro* inhibited functions of T (by 1.33 and 1.81 times, respectively) and B lymphocytes (by 1.26 and 1.70 times, respectively, Table 2). There were no significant differences in *in vitro* effects of EG on T and B cells (p>0.05).

This decrease in the content of CFU_S is probably associated with EG-induced death of stem hemopoietic cells, suppressed migration of CFU_S from the bone marrow to the spleen, and low number of T helper cells necessary for normal hemopoiesis. *In vivo* suppression of humoral and cell immune reactions probably results from dysfunction of immunocytes due to interaction of highly toxic EG biotransformation products (glycolic aldehyde and glycolic, glyoxylic, and oxalic acids) with sulfhydryl and amino groups of enzymes and inhibition of tissue respiration and oxidative phosphorylation [5,12]. Immunotoxic effects of EG are probably associated with Ca^{2+} binding with oxalic acid in immunocompetent cells, which attenuates activation of T and B lymphocytes due to decreased synthesis of cGMP and cAMP and low production of interleukin-2 by T cells [9,10]. It can not be excluded that acute EG poisoning changes the state of the neuroendocrine system and affects immunocompetent cells, which is confirmed by *in vitro* experiments. Comparison of *in vivo* and *in vitro* effects of EG on the immune system indicates that primary impairment of the T cell-dependent antibody formation in acute EG poisoning is associated with the products of its biotransformation, because *in vitro* damages to T and B lymphocytes caused by EG are practically similar.

Hence, acute EG poisoning impairs NRB, decreases the number of CFU_S, and inhibits humoral and cell immune reactions. More pronounced suppression of T cell-dependent antibody formation is probably related to the effects of products of EG biotransformation. These immune disorders can be prevented with drugs improving NRB, stimulating antibody formation, and activating natural killer and K cells responsible for antibody-dependent cell cytotoxicity.

TABLE 1. Effects of EG (0.8 LD_{50}) on the Immune System (*M*±*m*)

Parameter	Control (n=7-11)	EG (n=7-9)
CFU_S number	9.8±2.1	4.1±1.2
Increase in hindlimb weight, %	31.6±2.6	24.1±2.1
Cytotoxicity, %		
natural	23.1±3.1	12.3±2.8
antibody-dependent	10.3±1.8	5.1±1.7
APC		
to sheep erythrocytes, 10^8	35.3±4.1	17.9±3.3
to Vi-Ag	27.3±3.1	18.4±2.8

Note. All differences from the control are significant at p<0.05.

TABLE 2. *In Vitro* Effects of EG on APC Formation (per 10^6 B cells) by Immunocytes from CBA Mice (*M*±*m*)

Cells incubated with sheep erythrocytes	Control	EG concentration, mM	
		10	100
B	63±7	—	—
B+T	358±36	—	—
B^0+T	—	285±29	211±25*
B+T^0	—	269±31	198±21*

Note. Cells from 7 mice were used in each experimental series. B^0 and T^0: cells preincubated with EG for 1 h before addition of sheep erythrocytes. *p<0.05 compared to the control (B+T).

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