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The Influence of Ethanol-Metabolizing Systems on the Intensity of Lipid Peroxidation Processes in the Gastrointestinal Tract of Rats

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Abstract—The effects of some ethanol-metabolizing systems (aldehyde dehydrogenase, catalase, cytochrome P450 2E1) on activation of lipid peroxidation (LPO) processes in the gastrointestinal tract of rats have been studied using inhibitors of these systems. The intensity of LPO processes was evaluated by thiobarbituric acid-reactive substances and chemiluminiscence intensity. It was found, that acetadehyde metabolism plays the major role in the LPO induction in epithelium of the rat gastrointestinal tract.

Keywords: ethanol, gastrointestinal tract, lipid peroxidation.

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INTRODUCTION

According to modern concepts, reactive oxygen species (ROS) contribute to damage of epithelium of the gastrointestinal tract (GIT) in alcohol intoxication [1, 2]. It is known that among three enzymatic systems oxidizing ethanol in the liver, alcohol dehydrogenase, catalase, and microsomal ethanol oxidizing system, the latter one is the most important source of free radical formation [3, 4]. The ethanol-inducible cytochrome P450 2E1 makes the major contribution to ethanol oxidation in liver microsomes. Recently, it has demonstrated that in contrast to the liver, ethanol metabolism in the GIT is significantly lower; nevertheless, the most important enzymatic systems of ethanol metabolism including alcohol dehydrogenase, cytochrome P450 2E1, and catalase have been found there [5]. Thus, ethanol oxidation in GIT mucosa may result in formation of acetaldehyde and free radicals. Domination of the alcohol dehydrogenase and catalase pathways of ethanol oxidation in stomach does not imply active free radical generation. Intestine contains cytochrome P450 2E1 inducible during chronic ethanol administration; among cytochromes P450 this form can generate the highest amounts of free radicals during ethanol oxidation. In large intestine and rectum, high activity of ethanol-metabolizing enzymes (alcohol dehydrogenase, catalase, and microsomal ethanol oxidizing system) and the lowest activity aldehyde dehydrogenase (compared with other parts of the GIT) create favorable conditions for accumulation of toxic and reactive acetaldehyde and aldehyde peroxidation products during alcohol intoxication. Although good evidence exusts that acetaldehyde induces lipid peroxidation (LPO) in vitro and in vivo [6], a role of acetaldehyde in stimulation of peroxidation during alcohol consumption still remains unclear. We have performed experiments for elucidation possible role of some ethanol-metabolizing systems in activation of lipid peroxidation in stomach, small and large intestine, and also in rectum of rats.

MATERIALS AND METHODS

Adult male Wistar CRL:(WI)WUBR rats (200–220 g) were used in experiments. Rats were subdivided into several groups each of which contained 6 animals. Experimental animals were treated with certain inhibitors of ethanol-metabolizing system. The following compounds were used: sodium cyanamide (50 mg/kg, intraperitoneally (i.p.)) for inhibition of aldehyde dehydrogenase, sodium azide (10 mg/kg, i.p.) for inhibition of catalase, and diallyl sulfide (100 mg/kg, i.p.) for inhibition of cytochrome P450 2E1. After certain time intervals required for manifestation of inhibitory effects of these compounds, acute alcohol intoxication was modeled by intragastric administration of 25% ethanol (5 g/kg). Control animals received the same volume of isocaloric solution of glucose. Before this procedure control and experimental rats starved at least 12 h. Euthanasia of animals was performed by cervical dislocation 3 h after ethanol administration. Stomach, parts of small and large intestine, and rectum removed from the body were immediately washed in cold isotonic NaCl solution. Epithelium was obtained by accurate scraping by a scalpel. Concentration of thiobarbituric acid reactive substances

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Table 1. The intensity of lipid peroxidation in the gastrointestinal tract of rats treated with intragastric administration of ethanol (5 g/kg)

Part of GIT	Chemiluminescence, cpm		TBARS, μmol/g of tissue	
	Control	Experiment	Control	Experiment
Stomach	1132 ± 124	2110 ± 141*	57.4 ± 3.8	72.1 ± 7.1
Small intestine	932 ± 119	1955 ± 282*	97.1 ± 6.8	136.5 ± 12.7*
Large intestine	1335 ± 198	1789 ± 74	88.3 ± 10.5	84.3 ± 7.3
Rectum	1299 ± 158	1815 ± 223	83.5 ± 7.0	83.8 ± 11.2

Note: Here and in Tables 2 and 3 data represent arithmetic mean ± SEM; * $p < 0.05$, versus control.

Table 2. The effect of ethanol administration on chemiluminescence in the gastrointestinal tract of rats pretreated with inhibitors of ethanol metabolism

Part of GIT	Chemiluminescence, cpm		
	Ethanol + sodium cyanamide	Ethanol + sodium azide	Ethanol + diallyl sulfide
Stomach	1686 ± 293	2897 ± 293[ab]	3255 ± 693[a]
Small intestine	1482 ± 182[a]	2383 ± 220[a]	2299 ± 263[a]
Large intestine	1043 ± 92[b]	1957 ± 243	3092 ± 461[ab]
Rectum	1328 ± 299	2625 ± 210[ab]	3320 ± 712[ab]

Note: Here and in Table 3: [a] $p < 0.05$ versus control, [b] $p < 0.05$ versus ethanol treated animals.

(TBARS) and chemiluminescence intensity were determined in a transparent solution of the cell extract obtained by centrifugation of homogenates at 5000*g*.

The TBARS content in tissues was determined by the method of Placer at el. [7]. Chemiluminescence intensity was determined using a KHLMITS-01 chemiluminometer. After addition of homogenate and buffer containing 105 mM KCl, 20 mM KH_2PO_4, pH 7.4 (0.2 mL each), the "rapid" burst was induced by adding $FeSO_4$ (final concentration 1×10^{-3} M) and the number of photons were counted during 1 min [8].

Results were analyzed by the method of variation statistics using the Student's *t* criterion to detect statistically significant differences.

RESULTS AND DISCUSSION

Intragastric administration of 25% ethanol (5 g/kg) caused stilumation of LPO processes in gastric and small intestine mucosa as evidenced by increased TBARS content and chemiluminescence in these tissues. In large intestine and rectal mucosa statistically significant changes of these parameters were not observed (Table 1).

Tables 2 and 3 summarize data on the effects of inhibitors of the ethanol-metabolizing systems on LPO intensity in the investigated parts of rat GIT. Administration of the aldehyde dehydrogenase inhibitor sodium cyanamide decreased LPO intensity in gastric mucosa up to control values; in small intestine chemiluminescence remained increased while TBARS concentration normalized.

Administration of the catalase inhibitor sodium azide resulted in activation of free radical processes in gastric mucosa, and also in small intestine and rectal mucosa. In all these parts of the GIT we observed increased chemiluminescence and in gastric mucosa an increased level of TBARS was found.

Administration of the cytochrome P450 2E1 inhibitor diallyl sulfide was accompanied by increased chemiluminescence registered in all the investigated parts of the GIT; however, concentrations of TBARS in small and large intestine and rectum demonstrated statistically significant decrease versus control and ethanol treated animals.

Analysis of these data suggests that aldehyde dehydrogenase makes the major contribution into LPO intensification in rat GIT during acute alcohol intoxication. Inhibition of this enzyme caused minimal changes in the investigated parameters compared with control animals. This is consistent with observations by other researchers reported about the decrease in accumulation of malondialdehyde content in blood and liver of rats after combined administration of ethanol and cyanamide [9]. It is known that combined administration of these compounds results in multifold increase in blood acetaldehyde concentration, which leads to inhibition of ethanol absorption in rat intestine [10]. Such effects are attributed to decreased blood circulation in the intestinal wall induced by high acetaldehyde concentrations stimulating cholinergic

Table 3. The effect of ethanol administration on the content of thiobarbituric acid reactive substances (TBARS) in the gastrointestinal tract of rats pretreated with inhibitors of ethanol metabolism

Part of GIT	TBARS, μmol/g of tissue		
	Ethanol + sodium cyanamide	Ethanol + sodium azide	Ethanol + diallyl sulfide
Stomach	42.7 ± 6.1[b]	70.2 ± 3.4[a]	59.8 ± 6.9
Small intestine	92.9 ± 10.6[b]	122.0 ± 10.8	53.5 ± 7.9[ab]
Large intestine	84.8 ± 5.2	88.5 ± 6.7	53.1 ± 7.8[ab]
Rectum	85.3 ± 1.9	80.5 ± 3.6	44.0 ± 6.4[ab]

nerves [11]. However, it is known that acute alcohol intoxication is accompanied by a decrease of gastric and small intestine motility [12]; this causes alcohol retention in stomach and the upper part of small intestine. Usually, ethanol itself does not stimulate peroxidation and this has been observed in our experiments.

CONCLUSIONS

Thus, based on literature data on LPO activation by in vivo administration of acetaldehyde and results of our experiments with the aldehyde dehydrogenase inhibitor we may conclude that the processes of intracellular acetaldehyde metabolism (rather than acetaldehyde itself) are responsible for ethanol-induced LPO processes in epithelium of the gastrointestinal tract.

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