Lipid Peroxidation during Experimental Cholera Intoxication

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Cholera intoxication in albino mice was induced by intraperitoneal injection of endotoxin in doses of LD_{16}, LD_{25}, and LD_{50} and combination of endo- and enterotoxin in doses equivalent to LD_{25}. Dose-dependent activation of superoxide dismutase, phasic changes in the contents of MDA and conjugated trienes and dienes, and modulatory influence of enterotoxin on catalase activity in the blood were observed during intoxication.

Key Words: *cholera intoxication; lipid peroxidation*

Cholera is extremely dangerous infectious disease that occurs in Russia [9]. Therefore, the pathogenesis of cholera and elaboration of pathogenically substantiated therapeutical methods for the correction of metabolic and functional disorders accompanying this disease are of considerable importance.

Cholera endotoxin (CE) and exotoxin (choleragen) are the major pathogenic factors of *Vibrio cholerae* [2,10]. Endotoxins secreted by gram-negative bacteria have the same lipopolysaccharide structure and produce various effects in the body. These substances display pyrogenic and immunogenic properties and bind to mononuclear and endothelial cells, neutrophils, basophils, mast cells, and platelets. Endotoxins induce the formation of cytokines, produce direct and cytokine-mediated effects on the blood coagulation system, and cause severe microcirculatory disorders [6,12,15].

Cytopathogenic effects of CE in cholera are potentiated by enterotoxin, which binds to enterocytes and is responsible for diarrhea and dehydration syndrome followed by disturbances in the regional blood flow and systemic hemodynamics [3,4,8,10].

In view of this, processes of lipid peroxidation (LPO) can be nonspecifically activated under conditions of circulatory hypoxia typical of cholera and cholera intoxication [7,11,14,15].

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Here we studied LPO and activity of the antioxidant systems during cholera intoxication caused by intraperitoneal administration of CE and enterotoxin.

MATERIALS AND METHODS

Experiments were performed on 350 outbred albino mice with cholera intoxication. Blood levels of intermediate and end products of LPO, malonic dialdehyde (MDA) and conjugated dienes (CD) and trienes (CT), were measured spectrophotometrically [1,13].

Activities of superoxide dismutase (SOD) and catalase in whole blood were estimated [5].

Cholera intoxication of albino mice was induced by intraperitoneal injection of CE in doses of LD_{16}, LD_{25}, and LD_{50} and combined administration of endo- and enterotoxin in doses equivalent to LD_{25}.

CE isolated from fresh reactor culture of *Vibrio cholerae* Inaba 569 B by water-phenol Westphal method, and series 35 enterotoxin obtained from supertoxigenic *Vibrio cholerae* KM-76 M were used. Activity of CE determined by the reaction of hemagglutination inhibition was 1:12,800, and protein content estimated by the Coomassie method was 500 μg/ml. For intraperitoneal administration, LD_{50} of LPS and enterotoxin were 1 and 100 μg/kg/ml physiological saline, respectively. Toxins used in this study were obtained from the Mikrob Russian Research Antiplague Institute.

Experiments were conducted 4 h after intraperitoneal injection of toxins against the background of

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TABLE 1. Activities of Blood Antioxidant Enzymes and LPO Intensity during Cholera Intoxication ($M\pm m$, n=10)

Parameter	Control	Time after CE injection, h		
		4		20
		LD_{16}	LD_{50}	LD_{16}
Catalase activity, IU×10^4/ml	1.86±0.08	2.92±0.14*	3.27±0.19*°	3.15±0.23*
SOD activity, rel. units/ml	258.90±6.07	365.00±10.03*	426.10±9.87*°°°	391.00±11.07*
MDA content, μmol/ml	2.79±0.06	3.50±0.18*	3.28±0.28°°	3.240±0.011*
CD, rel. units/ml	5.77±0.23	3.69±0.08*	4.71±0.08**°	9.07±0.27*+
CT, rel. units/ml	0.35±0.05	—	0.24±0.04**°	1.32±0.16*

Note. *p<0.001 and **p<0.01 compared to the control; +p<0.001 compared to early term postinjection; °p<0.001, °°p<0.02, and °°°p<0.05 compared to the same stage of intoxication induced by CE in a dose of LD_{25}.

severe clinical manifestations of cholera intoxication (adynamia, dyspnea, fever, diarrhea, and death of some animals). Survived animals with clinical manifestations of intoxication were examined 20 h postinjection.

RESULTS

The content of MDA in the blood increased, while the level of CD decreased 4 h after injection of CE in a dose of LD_{16}. CT were absent, and SOD and catalase activities in the whole blood increased (Table 1).

Twenty hours after injection of CE in a dose of LD_{16}, blood contents of MDA, CD, and CT increased, and activities of SOD and catalase were above the control.

Four hours after injection of CE in a dose of LD_{25}, the content of MDA increased (p<0.001), the content of CD remained constant, the amount of CT increased compared to the control (p<0.01), SOD activity was elevated (p<0.001), and catalase activity did not change.

Twenty hours after administration of CE in a dose of LD_{25}, blood content of MDA was above the control (p<0.01), CD decreased compared to that observed during the previous stage of intoxication (p<0.01), CT returned to normal, and activities of SOD and catalase were higher than in the control (p<0.001 and p<0.05, respectively).

The effect of combined administration of CE and enterotoxin was studied in the next experimental series.

Twenty hours after injection of CE and enterotoxin, blood catalase activity sharply increased compared not only to the control (p<0.001), but also to that in animals treated with CE alone (p<0.001). This rise in enzyme activity was accompanied by severe clinical manifestations of cholera intoxication. No modulatory effects of enterotoxin on other LPO parameters were noted. The content of MDA and the activity of SOD remained at high levels similar to those in animals injected with CE alone.

In the next experimental series, activities of SOD and catalase in the blood peaked 4 h after injection of CE in a dose of LD_{50} against the background of severe clinical manifestations of intoxication and death of experimental animals. MDA concentration in the blood did not differ from the control, while the contents of CD and CT were far below the control (Table 1).

Thus, experiments on mice with relatively high resistance to bacterial toxins, including cholera toxins, demonstrated dose-dependent activation of SOD, the key enzyme of the blood antioxidant system. In mice injected with CE in a dose of LD_{50} SOD activity was maximum, and the contents of CD and CT were below the control.

The modulating influence of enterotoxin on biological effects of endotoxin during their simultaneous injection to albino mice manifested as the increase in blood catalase activity.

The increase in MDA content accompanying activation of SOD and catalase attests to relative insufficiency of the antioxidant enzyme system and intensification of LPO during cholera intoxication.

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