CHANGES IN REDUCED GLUTATHIONE LEVEL IN MOUSE LIVER INDUCED BY TYPE A STAPHYLOCOCCAL ENTEROTOXIN AND LIPOPOLYSACCHARIDE

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The study of the mechanisms of action of biologically active macromolecules of pathogenic bacteria is an urgent task in modern microbiology and infectious pathology, the accomplishment of which is essential for the successful conduct of pathogenetic treatment of infectious diseases. Certain microbial exotoxins are known to have the ability to increase the sensitivity of the living organism to the pathogenic action of lipopolysaccharides of Gram-negative bacteria (LPS), a key factor in the development of severe disease, often with a lethal outcome [7]. Besides streptococcal pyrogenic exotoxins, this property is also a feature of toxic staphylococcal proteins, namely enterotoxins and a toxin causing the toxic shock syndrome [2, 3, 5].

The writers previously showed that combined injection of type A staphylococcal enterotoxin (SEA) and *Serratia marcescens* endotoxin (LPS) into mice leads to synergic potentiation of generation of active forms of oxygen by peritoneal phagocytic cells [1]. Under conditions of increased radical formation the ability of the host organism to withstand the toxic action of highly reactive oxygen metabolites is largely determined by the state of the antioxidant systems of the tissues and organs.

One of the main endogenous antioxidants is reduced glutathione [6]. The metabolism of this very important component of the detoxication system during the development of toxic shock has virtually never been studied, and for that reason it was decided to investigate the content of reduced glutathione in the mouse liver under the influence of type A staphylococcal enterotoxin and of a combination of SEA and LPS.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 mice weighing 16-18 g. Animals of each group (4 mice each time) were given an intraperitoneal injection of isotonic phosphate-salt buffer, pH 7.2, and of SEA (Ufa) or prodigiosan (Serratia marcescens endotoxin, LPS). In experiments to study the combined action of the toxins, LPS was injected 4 h after SEA. At various times (from 2 to 24 h) the mice were sacrificed, the liver homogenized, and the concentration of reduced glutathione (GSH) was determined [4]. Protein in the samples was determined quantitatively by Lowry's method.

EXPERIMENTAL RESULTS

The glutathione concentration was first investigated at different times after injection of the enterotoxin. Table 1 shows that as early as 2-4 h after injection of SEA there was a marked fall in the GSH level in the mouse liver, to 47-40% of the control values. The GSH concentration still remained low after 8 h, but thereafter a steady

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TABLE 1. Effect of SEA on Content of Reduced Glutathione in Mouse Liver (M ± m; n = 4)

Time of taking liver, h	Dose of SEA, μg/mouse	GSH concentration, μg/mg protein	Percent of control
Control	PSB	10,765±0,332	100
2	1,0	5,161±0,055	47,9*
4	1,0	4,389±0,186	40,8*
8	1,0	5,327±0,510	49,5*
24	1,0	8,405±0,305	78,0
24	PSB	12,172±2,015	100
	0,1	9,191±0,945	75,5
	0,5	8,225±1,102	67,5
	1,0	8,722±0,891	71,6
	5,0	8,639±1,120	70,9

Legend. PSB) phosphate-salt buffer. *$p < 0.05$ indicates significant differences, here and in Table 2.

TABLE 2. Reduced Glutathione Level in Mouse Liver during Combined Administration of SEA and LPS (M ± m; n = 4)

Time of taking liver, h	Agent injected, μg/mouse	GSH concentration, μg/mg protein	Percent of control
6	PSB	11,786±1,069	100
	SEA (1.0)	6,707±0,514	56,9*
	LPS (50.0)	5,548±0,491	47,1*
	SEA + LPS	3,868±0,085	32,8*
10	PSB	11,427±1,245	100
	SEA (1.0)	7,259±0,819	63,5*
	LPS (20.0)	5,409±0,039	47,3*
	SEA + LPS	4,001±0,095	35,0*
24	PSB	13,745±1,844	100
	SEA (1.0)	10,047±0,820	73,1
	LPS (20.0)	5,849±0,480	42,5*
	SEA + LPS	4,706±0,610	34,1*
6	PSB	10,903±1,233	100
	SEA (0.5)	6,210±0,701	57,0*
	LPS (2.0)	8,777±0,790	80,5
	SEA + LPS	7,894±0,880	72,5

Legend. Doses of SEA and LPS during combined injection were the same as those injected separately.

recovery of the tripeptide level was observed, and after 24 h it averaged 78% of the control. Restoration of the glutathione concentration in the liver took place irrespective of the dose of enterotoxin used: at an SEA concentration of between 0.1 and 5 μg per mouse the glutathione content 24 h later remained virtually at the same level (from 67 to 75%).

Injection of LPS (20-50 μg/mouse) also was accompanied by a fall in the GSH level in the liver (Table 2), but unlike SEA, the endotoxin induced a more prolonged fall of the glutathione concentration, with no tendency toward normalization after 24 h. Small doses of LPS (2 μg) led to only a very small decrease in the glutathione concentration (to 80.5% after 6 h).

We were more interested in the changes in glutathione concentration during combined injection of enterotoxin and LPS, for it is under such conditions that the lethal effect was observed [1]. In mice treated with both SEA and LPS there was an even greater fall in the liver glutathione level – to 32, 35, and 34% after 6, 10, and 24 h respectively (Table 2). It is interesting to note that this fall of the GSH concentration was observed only when LPS was used in concentrations at which a lethal effect was exhibited (20-50 μg/mouse). Injection of low doses of the endotoxin (2 μg/mouse) was not accompanied by such a marked fall of the GSH level.

Under the influence of staphylococcal enterotoxin a fall of the antioxidant potential of the liver thus takes place, and it is aggravated even more by subsequent injection of LPS. The fact that the fall of the glutathione level caused by SEA precedes injection of LPS suggests that this phenomenon plays an important role in the development of hypersensitivity of the animals to the lethal action of endotoxin. To confirm this hypothesis, in the final stage of the investigation experiments were carried out to correct the lethal effect. Animals of the control group received SEA (0.1 μg/mouse), followed 4-h later by LPS (50 μg/mouse). The experimental group (20 mice) received an injection of GSH (20 mg/mouse) simultaneously with SEA. The use of reduced glutathione increased the duration of survival of the mice by 1-2 days and their mortality by 40%, confirming the decisive role of the state of the antioxidant systems in the development of lethal shock due to the combined injection of SEA and LPS.

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