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NEUTRALIZATION OF THE TOXIC ACTION OF ENDOTOXINS OF GRAM-NEGATIVE BACTERIA BY UNITHIOL AND MAGNESIUM SULFATE

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KEY WORDS: endotoxins; lipid peroxidation; liver; lungs; unithiol

The mechanism of action of endotoxins on the living organism is very complex and has not been adequately studied. One of the results of this action is the formation of free oxygen and hydroxyl radicals and, as a result of this, intensification of lipid peroxidation (LPO), destabilization of the cell membranes, swelling of mitochondria, and so on [1, 5, 8]. It can accordingly be postulated that antioxidants may partially neutralize the action of endotoxins and may prove to be effective agents for the treatment of infectious diseases caused by Gram-negative microorganisms. According to some reports, metallothionein proteins synthesized in the body and the particular feature of which is their high content of sulfhydryl groups and ability to bind ions of certain metals, play an important role in protection of the organism against endotoxins [7]. It was accordingly suggested that drugs carrying sulfhydryl groups and possessing antioxidative properties (in particular, unithiol), could prove to be effective agents protecting the body against the action of endotoxins. The protective action of unithiol and of a combination of unithiol with magnesium sulfate was studied in poisoning caused by breakdown products of *Salmonella typhimurium* and *Shiqella sonnei,* and on the cyclic adenosine monophosphate (cAMP) level during this type of poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 17-20 g. A lysate of the bacteria was prepared by ultrasonic irradiation of a suspension of a 24-h agar culture in the UZDN-1 apparatus (4 min, 44 kHz). The sonicated suspension was centrifuged (15 min, 35,000g), and the supernatant was sterilized by filtration through membrane filters.

LD_{50} of the lysate was determined by probit analysis, the readings being taken after 48 h [2]. The malonic dialdehyde (MDA) concentration was determined by the reaction with 2-thiobarbituric acid and the cAMP was determined with the aid of kits from Amersham International (England) [4]. The results were subjected to statistical analysis by Student's t-test [3].

The plan of the experiments was as follows. Mice were injected intraperitoneally with lysate of *S. Typhimurium* or *Sh. sonnei* in a volume of 0.2 ml. To determine MDA and cAMP, the dose of the lysate was equal to LD_{50}. To study the protective action of unithiol LD_{50} was determined for the experimental and control groups. For this purpose each group was divided into six subgroups each containing five mice. The dose was increased in steps of 1.5.

The mice of the experimental group began to receive unithiol immediately after injection of the lysate. The preparation was injected intramuscularly in a dose of 0.5 mg in a volume of 0.2 ml. Considering that the half-elimination time of unithiol is 2-3 h, the first two injections were given with an interval of 3.5 h. Later, the injections were given every 5 h. Magnesium sulfate was injected twice a day at intervals of 12 h, in a dose of 0.13 mg.

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TABLE 1. Effect of Unithiol and Magnesium Sulfate on MDA Level in Liver of Mice Receiving Injections of *S. typhimurium* lysate (in μmoles/g, M ± m)

Experimental conditions	Time after injection of lysate, h			Lysate not injection
	2	8	24	
Unithiol + magnesium sulfate	4,62±0,43	3,68±0,37	4,10±0,33	3,26±0,35
Unithiol and magnesium sulfate not injected	6,33±0,38**	5,99±0,33**	5,04±0,38	3,82±0,33*

Legend. *) Control, **p < 0.01, ***p < 0.05 compared with control.

TABLE 2. Effect of Unithiol and Magnesium Sulfate on cAMP Level in Liver and Lungs of Mice Receiving Injections of *S. typhimurium* lysate (in pmoles/g, M ± m)

Organ	Experimental conditions	Time after injection of lysate, h			Lysae not injected
		2	8	24	
Liver	Unithiol + magnesium sulfate	271,7±28,9	302,3±26,6	419,4±27,6	366,9±41,1
	Unithiol and magnesium sulfate not injected	193,5±24,8**	233,8±23,3	277,4±24,7***	364,1±41,7*
Lungs	Unithiol + magnesium sulfate	470,5±25,0 ±61,14	488,2±35,0 ±85,74	514,0±39,9 ±102,49	561,2±22,9 ±58,87
	Unithiol and magnesium sulfate not injected	355,0±28,2**	397,2±22,5**	409,3±34,7**	614,3±57,5*

Legend. *) Control, **p < 0.01, ***p < 0.05 compared with control.

TABLE 3. Protective Action of Unithiol and Magnesium Sulfate in Poisoning by *S. typhimurium* and *Sh. sonnei* Endotoxin (M ± m)

Exptl. conditions	Unithiol	Unithiol + magnesium sulfate	Unitiol	Unithiol + magnesium sulfate
Experiment:				
LD_{50}	$4,49 \cdot 10^9$	$7,50 \cdot 10^9$	$3,88 \cdot 10^9$	$6,08 \cdot 10^9$
Log LD_{50}	9,65±0,09	9,88±0,09	9,59±0,09	9,78±0,09
Control				
LD_{50}	$1,07 \cdot 10^9$	$1,23 \cdot 10^9$	$1,04 \cdot 10^9$	$1,21 \cdot 10^9$
Log LD_{50}	9,03±0,11*	9,10±0,16*	9,02±0,14**	9,08±0,14*

Legend. LD_{50} reflects number of microbial cells from which the corresponding dose of lysate was obtained. *p < 0.001, **p < 0.01 compared with experiment.

EXPERIMENTAL RESULTS

As the data in Table 1 show, only 2 h after injection of the lysate there was a sharp increase in LPO in the liver, as revealed by elevation of the MDA level. LPO was at a high level after 8 h, and decreased somewhat after 24 h. Injection of unithiol and magnesium sulfate led to a much smaller increase in LPO. Differences in the MDA levels in this group compared with those in the liver of intact mice were not statistically significant.

Injection of *S. typhimurium* lysate led after 2 h to a significant (approximately twofold) fall in the cAMP level both in the liver and in the lungs (Table 2). The cAMP concentration in the liver later rose gradually, and after 24 h this increase was already statistically significant. However, even after 24 h the cAMP level was lower than in the control. The rise of the cAMP concentration in the lungs was less marked.

Injection of unithiol and magnesium sulfate could not completely prevent the fall of the cAMP level, but the fall was much less in degree. The fall of the cAMP level following injection of endotoxin was probably due to the fact that the free oxygen and hydroxyl radical formation induced by it led to oxidation of unsaturated fatty acids, which are involved in the synthesis of prostaglandins, that realize their action through cAMP.

Successful prevention of LPO activation and also, possibly, of the disturbance of prostaglandin synthesis with the aid of unithiol and magnesium sulfate suggested that these preparations might prove to be effective agents for the treatment of infectious diseases caused by Gram-negative microorganisms. Accordingly the protective action of unithiol and magnesium sulfate were studied in animals poisoned with endotoxins of *S. typhimurium* and *Sh. sonnei.* As the results in Table 3 show, unithiol had a marked protective action. Magnesium sulfate potentiated it.

Thus unithiol, alone and in combination with magnesium sulfate, neutralizes the damaging action of free oxygen and hydroxyl radicals formed during poisoning by breakdown products of gram-negative microoganisms, and protect the affected animals from death.

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MODIFYING EFFECTS OF NEUROTROPIN ON POSTRADIATION DISTURBANCES OF NEUROTRANSMITTER PROCESSES IN CENTERS REGULATING AUTONOMIC FUNCTIONS

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KEY WORDS: neurotransmitters; glucocorticoids; neurotropin; action of radiation; diencephalic syndrome

Previous investigations showed that in the early and relatively late stages after exposure to ionizing radiation in doses insufficient to cause acute radiation sickness, increased activity of the hypothalamo–hypophyseo–adrenocortical system characteristic of stress is observed; this phenomenon is due mainly to changes in the central component of that system [4, 6, 9]. In structures responsible for the regulation of autonomic and somatic functions, activation of both inhibitory and excitatory neurotransmitter processes is observed [1, 8]; disturbance of coordination of the latter, moreover, may lead to changes that constitute the picture of the diencephalic syndrome. Because of the facts described above it was decided to study postradiation changes in neurotransmitter relations in various structures of the brain stem, the parietal zone of the cerebral cortex, and the underlying bioenergetics of these processes.

The aim of this investigation was to study the possibility of correcting disturbances of neurotransmitter relations and the bioenergetics of the brain with the aid of neurotropin (Institute of Bioactive Science, Nippon Zoki, Osaka, Japan), which possesses a broad spectrum of action [11].

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