Stimulation of Mouse Resistance to Bacterial Infection with Muramyl Dipeptide Glycoside

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We studied the capacity of 9 new muramyl dipeptide glycosides to stimulate mouse resistance to experimental sepsis induced by intraperitoneal injection of *Salmonella typhimurium* culture. Preventive intraperitoneal injections of muramyl dipeptide β-glycosides better improved survival of infected animals compared to the original (unmodified) muramyl dipeptide and muramyl dipeptide α-glycosides. The most effective drug muramyl dipeptide β-heptylglycoside injected during sepsis development also reduced animal mortality, decreased bacterial contamination of the viscera, and increased phagocytic activity of peritoneal macrophages in infected animals.

Key Words: *muramyl dipeptide glycosides; Salmonella typhimurium; resistance; bacterial contamination; phagocytosis*

Previous *in vitro* studies showed that β-glycosylation enhanced, while α-glycosylation decreased immunomodulating activity of muramyl dipeptide (MDP) [2,4]. We detected a relationship between biological activity of MDP glycosides and the aglycon nature in *in vitro* test systems [5]. Now we evaluated the effect of aglycon structure and configuration of the glycoside bond of original MDP glycosides on their *in vivo* immunotropic activity in experimental sepsis.

MATERIALS AND METHODS

MDP and its glycosides were synthesized and offered for the trials by Professor A. E. Zemlyakov (V. I. Vernadskii Tavrian National University, Simferopol) [1]. Experiments were carried out with MDP α-heptylglycoside (α-C_7MDP), MDP α-cyclohexylglycoside (α-cycl-MDP), MDP β-butylglycoside (β-C_4MDP), MDP β-heptylglycoside (β-C_7MDP), MDP β-cyclohexylglycoside (β-cycl-MDP), MDP β-adamantylglycoside (β-ada-MDP), MDP β-phenylglycoside (β-cycl-MDP), MDP β-phenethylglycoside (β-phenet-MDP), and MDP β-naphthylglycoside (β-naphth-MDP).

The cells were cultured in RPMI 1640 (Flow Lab) with 5% inactivated FCS (Flow Lab), 2 mM L-glutamine, 10 mM HEPES buffer (Flow Lab), 5×10^{-5} M 2-mercaptoethanol (Serva), and 50 μg/ml gentamicin.

The effect of preventive injection of muramyl dipeptides on the resistance to bacterial infection was evaluated by a modified method [8]. The test preparations dissolved in normal saline were injected intraperitoneally in doses of 0.1, 1, and 10 mg/kg to 6-8-week (CBA×C57Bl/6)F1 mice (Center for Experimental Animal Breeding, Kryukovo). Controls were injected with equivalent volumes of normal saline. Unmodified MDP served as the reference control. Twenty-four hour after injection the control and experimental mice were infected intraperitoneally with *Salmonella typhimurium* culture No. 415 in a dose of 100 LD_{50} (10^4 bacterial corpuscles per animal) estimated in preliminary experiments. The therapeutic effect of β-C_7MDP in sepsis was evaluated after its oral administration in doses of 0.2, 1, and 5 mg/kg 20 min and 24 h after infection. LD_{50} for intraperito-

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neal infection with this *Salmonella* culture and ED_{50} for the test drugs were determined as described elsewhere [7].

Bacterial contamination of the viscera in infected animals was evaluated 48 h postinfection. Blood, liver, spleen, kidney, and urinary bladder specimens were transferred to Petri dishes with meat-peptone agar and incubated at 37°C for 18 h. The index of contamination was estimated as the ratio of positive results to total number of inoculations from different organs and tissues.

Peritoneal macrophages were isolated (48 h after intraperitoneal infection with *S. typhimurium* culture) by washing the abdominal cavity with medium 199 containing 10 U/ml heparin. The cells were incubated with billion suspension of *S. enteritidis* (0.5 ml bacterial suspension/10^6 adhesive cells of peritoneal lavage fluid in 1 ml culture medium) at 37°C for 30 min, after which a smear was made on a slide, dried, and fixed in methanol for 10 min. The smears were stained after Romanowskii—Giemza. The percentage of actively phagocytizing cells (phagocytic number) and the mean number of phagocytosed bacteria per macrophage (phagocytic index) was counted [8].

RESULTS

A single intraperitoneal injection of different MDP glycosides 24 h before infection with *S. typhimurium*

TABLE 1. Effects of MDP Derivatives on Resistance to Intraperitoneal Infection with *S. typhimurium* in a Dose of 100 LD_{50}

Drug	Dose, mg/kg	Survival ($M\pm m$, n=3), %	ED_{50}, mg/kg
Control	—	0	—
MDP (reference control)	200	73.3±5.8	3.93
	20	53.3±5.8	
	2	33.3±5.8	
β-C_4MDP	200	76.7±5.8	5.01
	20	36.7±5.8*	
	2	16.7±15.3	
β-C_7MDP	200	86.7±11.5	2.57
	20	66.7±5.8*	
	2	56.7±5.8*	
α-C_7MDP	200	43.3±20.8	5.33
	20	36.7±5.8*	
	2	30±10	
β-ada-MDP	200	83.3±5.8	2.39
	20	76.7±5.8*	
	2	63.3±5.8*	
β-cycl-MDP	200	76.7±5.8	3.46
	20	60±10	
	2	36.7±5.8	
α-cycl-MDP	200	56.7±5.8*	5.58
	20	46.7±5.8	
	2	6.7±5.8*	
β-phen-MDP	200	83.3±5.8	2.65
	20	66.7±15.3	
	2	56.7±20.8	
β-phenet-MDP	200	73.3±11.5	3.52
	20	66.7±11.5	
	2	26.7±5.8	
β-naphth-MDP	200	76.7±5.8	3.46
	20	60±10	
	2	36.7±5.8	

Note. The table presents typical data of 3 independent experiments. *p<0.05 compared to MDP (reference control).

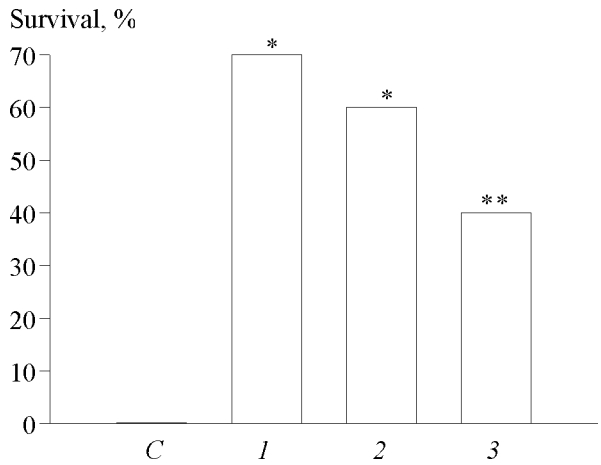

Fig. 1. Effect of oral β-C_7MDP administration during sepsis caused by *S. typhimurium* on survival of experimental animals. Data of 1 representative experiment of 3 independent experiments are presented. *p<0.01, **p<0.05 compared to the control. Here and in Figs. 2 and 3: *C*) normal saline (control); *1*) 0.2 mg/kg β-C_7MDP; *2*) 1 mg/kg β-C_7MDP; *3*) 5 mg/kg β-C_7MDP.

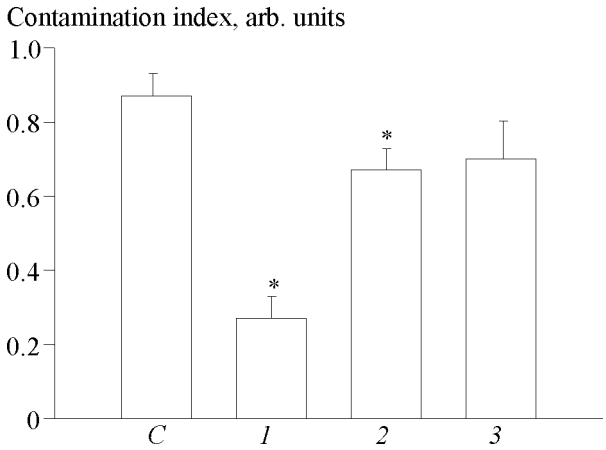

Fig. 2. Effect of oral β-C_7MDP administration during sepsis caused by *S. typhimurium* on contamination of the viscera and biological fluids 48 h postinfection (*M*±*m*, *n*=3). *p<0.001, **p<0.05 compared to the control.

culture had different effects on survival of experimental animals (Table 1). All MDP derivatives reduced mortality of infected mice. α-C_7MDP and α-cycl-MDP exhibited minimum efficiency and in none doses ensured 60% survival of animals (a critical index referring the test substance to immunotropic agents in accordance with methodological recommendations on trials of immunotropic drugs, approved by the Ministry of Health of the Russian Federation) [8]. Unmodified MDP and its β-glycosides ensured 60% survival in at least one of the test doses. The efficiency of β-C_7MDP and β-ada-MDP in doses of 0.1 and 1 mg/kg was higher than that of MDP in the same doses (p<0.05), while β-C_4MDP (1 mg/kg) was inferior to MDP in stimulating animal resistance to bacterial infection. β-phen-MDP in all doses improved survival better than MDP, but the difference from the reference control was insignificant. Other agents little differed from the original glycopeptide by their activity.

These findings confirmed the data obtained in *in vitro* test systems on higher biological activity of MDP β-glycosides in comparison with α-anomers [2,4,5]. The present study and previous reports indicate that β-C_7MDP, β-ada-MDP, and β-phen-MDP are the most promising agents among β-glycosides.

Hence, based on the present findings and previous data on high immunomodulating activity of β-C_7MDP *in vitro* [6,9], we chose this compound for comprehensive studies in order to evaluate the possibility of its clinical application. Despite proven efficiency of intraperitoneal (in the present study), intravenous, and oral administration of β-C_7MDP as a preventive mean in sepsis induced by intraperitoneal injection of *Salmonella* culture [3], the therapeutic activity of this drug under conditions of developing sepsis remained unclear. Therefore, considering previously demonstrated high efficiency of the agent after oral admini-

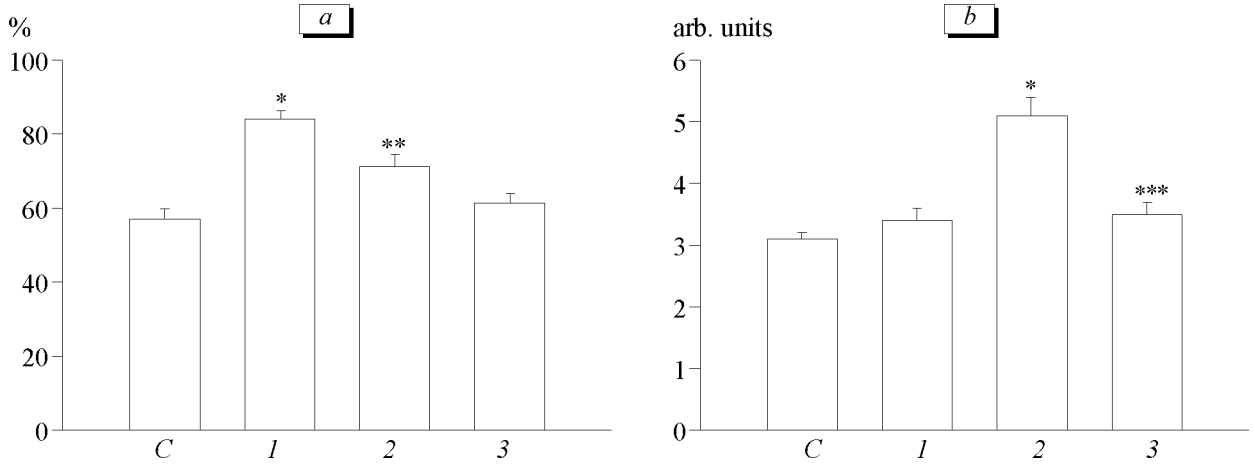

Fig. 3. Effect of oral treatment with β-C_7MDP in sepsis induced by *S. typhimurium* on the phagocytic function of peritoneal macrophages 48 h after infection (*M*±*m*, *n*=3). *a*) phagocytic number, %; *b*) phagocytic index, arb. units. *p<0.001, **p<0.01, ***p<0.05 compared to the control.

stration [3,5] and advantages of this route of β-C_7MDP administration for clinical practice, we evaluated the therapeutic and immunomodulating activity of this glycopeptide in oral treatment of animals with experimental sepsis.

β-C_7MDP in a wide range of doses (from 0.2 to 5 mg/kg) significantly decreased the mortality of animals with experimental sepsis (Fig. 1). The drug produced the maximum effect in a dose of 0.2 mg/kg: 70% infected mice survived ($p<0.01$). Higher doses were less effective.

These data correlated with the data on bacterial contamination of the viscera and biological fluids 48 h postinfection (Fig. 2). β-C_7MDP in a dose of 0.2 mg/kg reduced the contamination index by 79% ($p<0.001$). Higher doses of the drug decreased bacterial contamination less effectively.

β-C_7MDP stimulated the phagocytic activity of peritoneal macrophages, the most important component of nonspecific resistance and specific immune reactions (Fig. 3). The maximum increase in the percentage of actively phagocytizing macrophages (phagocytic number) was observed after administration of 0.2 mg/kg β-C_7MDP, while the maximum increase in the number of phagocytosed bacteria per macrophage (phagocytic index) was observed after administration of 1 mg/kg β-C_7MDP.

Hence, preventive treatment with MDP glycosides decreased the mortality of animals with experimental sepsis (β-glycosides were more effective than α-anomers). Therapy of animals with sepsis with β-C_7MDP increased survival, decreased bacterial contamination of the viscera, and stimulated phagocytic functions of peritoneal macrophages in infected mice.

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