MICROBIOLOGY AND IMMUNOLOGY

Stimulation of Nonspecific Resistance in Mice by β-Heptylglycoside Muramyl Dipeptide

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The ability of β-heptylglycoside muramyl dipeptide (glymuride) to stimulate nonspecific resistance in mice is studied. Glymuride prevents the death of up to 80% animals intraperitoneally infected with *Salmonella typhi* in a dose of 100 LD_{50}. Glymuride is effective in both oral and intravenous administration. The ED_{50} for these routes of administration are calculated.

Key Words: *β-heptylglycoside muramyl dipeptide; Salmonella typhi; nonspecific resistance*

Search for new effective synthetic derivatives of bacterial muramyl dipeptide (MDP) has been in progress for more than two decades. Several MDP analogs are now clinically tried, some including a Russian-made drug licopide, are used in clinical practice [3,6]. A perspective original muramyl peptide β-heptylglycoside MDP (glymuride) stimulates the main components of the antitumor and antiinfectious immunity in model test systems *in vitro* [4,5,7]. Its effect is superior to that of non-modified MDP and its derivatives.

We investigated the capacity of this drug to stimulate nonspecific antiinfectious immunity and prevent animal death caused by intraperitoneal *Salmonella typhi* infection and determined the ED_{50} for oral and intravenous administration of glymuride.

MATERIALS AND METHODS

β-Heptylglycoside-N-acetylmuramyl-L-alanyl-D-isoglutamin (glymuride) was synthesized as described previously [2]. Experiments were carried out on 6-8-week-old outbred albino mice (Kryukovo Breeding Center of the Russian Academy of Medical Sciences). Nonspecific resistance was studied on a model of peritonitis induced by intraperitoneal injection of *S. typhi*. To this end, a culture of *S. typhi* (Ty_2 4446) suspended in 5% mucin was intraperitoneally injected to mice in a dose of 100 LD_{50} 24 h after glymuride. Glymuride was administered orally and intravenously in doses of 0.2, 2, 20, and 200 μg/mouse. In the control, mice were fed or intravenously injected with 100 μl normal saline 1 day before infection. Animal deaths were recorded during 5 days. Control and experimental groups consisted of 10 animals. ED_{50} was calculated using Kerber's formula [1].

RESULTS

During the first stage, the infective dose (number of bacterial cells) was determined. This dose for intraperitoneal infection was 100 LD_{50} (Table 1). In accordance with these findings, an infective dose of 10^3 bacterial cells/mice was used for inducing peritonitis.

Glymuride improved mouse resistance to bacterial invasion and markedly decreased their mortality (Table 2). The maximum therapeutic effect was observed after oral intake of the drug in a dose of 20 μg/mouse: 80% survival of animals infected with *S. typhi*

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TABLE 1. Titration of *S. typhi* for Determining the Infective Dose

Number of bacteria	Mortality, %					Survival, %
	day 1	day 2	day 3	day 4	day 5	
10^3	80	20	—	—	—	0
10^2	60	20	20	—	—	0
10	40	30	10	—	—	20
1	—	10	—	—	—	90

TABLE 2. Effect of Glymuride on Mouse Resistance to Intraperitoneal Infection with *S. typhi* (10^3 bacterial cells/mouse)

Route of administration, dose, μg		Mortality, % (term, day)					Survival, %
		1	2	3	4	5	
Control (normal saline)							
oral		40	60	—	—	—	0
intravenous		40	30	20	10	—	0
Oral	200	40	20	—	—	—	40
	20	20	—	—	—	—	80
	2	20	—	—	—	—	20
	0.2	40	40	—	—	—	20
Intravenous	200	60	—	—	—	—	40
	20	20	20	—	—	—	60
	2	20	20	—	—	—	60
	0.2	—	40	—	—	—	60

in a dose causing 100% death in the control. This indicates good assimilation of glymuride from the gastrointestinal tract. The range of therapeutic doses for intravenous injection was greater than for oral intake. In doses of 0.2 and 2 μg/mouse, the agent increased animal survival to 60% after intravenous and to 20% after oral administration. Increasing the dose to 200 μg/mouse did not amplify the therapeutic effect in comparison with the dose of 20 μg/mouse irrespective of the administration route.

From these results, ED_{50} calculated for oral and for intravenous administration were 0.75±0.1 and 0.2 mg/kg, respectively. Presumably, the immunomodulating activity of glymuride *in vivo* and good assimilation after oral intake are largely due to its effective penetration through biological membranes, which is explained by a combination of hydro- and lipophilic properties in the glymuride molecule [4,7].

Hence, β-heptylglycoside-MDP is characterized by pronounced immunostimulating activity, which was demonstrated on a model of stimulation of nonspecific resistance in mice. These findings and the results of previous studies of the immunomodulating effect of glymuride *in vitro* [4,5,7] indicate that glymuride is a promising agent for clinical use. Its high efficacy upon oral and intravenous administration extends the probable forms of its application. For instance, this agent is used as an active component of Glymuride, a bioactive food additive. Glymuride was successfully tried at Center for Hygienic Certification at Institute of Nutrition of the Russian Academy of Medical Sciences and can be used for preventing secondary immunodeficiencies and for combined therapy of diseases involving immunity disorders.

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