MICROBIOLOGY AND IMMUNOLOGY

Biological Activity of Anomeric Pairs of Lipophilic Glycosides of *N*-Acetylmuramyl-*L*-Alanyl-*D*-Isoglutamine

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We studied the capacity of anomeric pairs of α- and β-dodecyl, α- and β-(1-pentylhexyl), and α- and β-cyclododecyl glycosides of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (muramyl dipeptide) to stimulate the nonspecific resistance of mice to intraperitoneal infection of *Staphylococcus aureus* and *Escherichia coli* cultures. Intraperitoneal pretreatment with the test substances in a wide dose range increased survival of infected animals. No differences were found between the biological effects of α- and β-dodecyl and α- and β-(1-pentylhexyl) glycosides of muramyl dipeptide. An inverse relationship was found between stimulatory activity and dose of α- and β-cyclododecyl glycosides of muramyl dipeptide during sepsis caused by *Staphylococcus aureus*.

Key Words: *muramyl dipeptide; lipophilic α- and β-glycosides; Staphylococcus aureus; Escherichia coli; resistance*

In vitro and *in vivo* studies showed that hydrophilic and amphiphilic glycosides of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (muramyl dipeptide, MDP) with β-glycoside bond surpass α-anomers and unmodified MDP by immunostimulatory activity [4-9,12]. The increase in lipophilicity of glycopeptides can be accompanied by significant changes in the mechanisms of their permeation through the plasmalemma of immunocompetent cells and interaction with surface and intracellular binding sites [4,11,12].

Here we studied the effect of configuration of the glycoside site and structure and nature of aglycones of 3 anomeric pairs of new lipophilic glycosides of MDP on the capacity of these substances to stimulate nonspecific resistance of mice to bacterial infections.

MATERIALS AND METHODS

The synthesis and biological studies were performed with α- and β-dodecyl, α- and β-(1-pentylhexyl), and α- and β-cyclododecyl glycosides of MDM (glycopeptides 1a and 1b, 2a and 2b, and 3a and 3b, respectively; Fig. 1). The methods of synthesis and chemical characteristics of substances were described previously [2]. Unmodified MDP was synthesized as described elsewhere [1] and served as the reference preparation.

Biological activity was tested on the model of sepsis according to methodical recommendations for studying of the immunocompetent effect of pharmacological substances [10]. Experiments were performed on outbred albino mice aging 20-25 days and weighing 12-14 g. The animals were obtained from the Stolbovaya nursery. The test substances

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were dissolved in 0.9% NaCl and injected intraperitoneally (final volume 0.5 ml). Control mice received 0.5 ml 0.9% NaCl. After 24 h the animals were infected with 18-h culture of *S. aureus* (strain Wood 46) or *E. coli* (strain 264). The cultures were grown in 0.2% starvation agar to increase the virulence of microorganisms. To this end, purified Difco agar (200 mg) was suspended in distilled water (100 ml) and heated on a water bath until complete dissolution of agar. Bacterial cultures were added to agar after cooling to 37-39°C. The animals were examined over 10 days. The effectiveness of preparations was estimated from the percent of survived animals. The dose of infectious agents (10^9 and 2×10^7 microbial bodies for *S. aureus* and *E. coli*, respectively) was estimated in preliminary experiments and served as the minimum dose inducing death of 100% animals over the first 3 days.

Glycopeptides in doses of 0.15, 1.5, and 15 mg/kg or 3.75 μg/kg, 75 μg/kg, and 1.5 mg/kg were administered during sepsis induced by *S. aureus* or *E. coli*, respectively.

The logarithm of the substance partition coefficient in *n*-octanol-water biphasic system (ClogP) was calculated by ChemOffice Ultra 9.0 software. This coefficient is used to characterize the hydrolipophilic balance between chemical compounds.

RESULTS

Anomeric pairs of MDP lipophilic glycosides contained linear primary aliphatic alcohol (1a and 1b), symmetrical secondary aliphatic alcohol (2a and 2b), and cycloaliphatic (3a and 3b) alcohols as aglycones. These aglycones are characterized by low lipophilicity: CLogP for these alcohols were 4.95, 4.20, and 4.52, respectively. Conformational mobility of carbohydrate chains and effective surface of aglicons change in the same row, which *a priori* affects the interaction with biological membranes and binding sites. For example, the linear aliphatic chain of aglycone in compounds 1a and 1b is characterized by maximum conformational mobility. Aglycone of glycopeptides 2a and 2b in aqueous solutions can form a double-stranded structure with limited mobility due to hydrophobic interactions. Aglycones in substances 3a and 3b have the most rigid cyclic structure.

The *in vivo* test system for evaluation of the capacity of lipophilic glycopeptides and hydrophilic reference control to stimulate mouse resistance to intraperitoneal infection with bacterial cultures was chosen in such a way that the differences in solubility of glycopeptides in aqueous solutions and probability of aggregation in this test system have lower impact on biological activity compared to *in vitro* conditions or other routes of *in vivo* treatment with glycopeptides.

The test substances had high immunotropic activity to *S. aureus* (Fig. 2). No differences in the immunostimulatory effect were revealed between glycopeptides 1a-1b and 2a-2b in a wide dose range. In contrast to previously described MDP glycosides with alkylalicyclic, alkylaryl, cycloalkyl ethyl, and arylethyl aglycones [3], the test glycopeptides in all doses exhibited 100% protective effect and significantly improved the effect of unmodified MDP. Aglycone lipophilicity probably has a greater effect on protective activity of anomeric pairs (1a and 1b) and (2a and 2b) than glycoside site configuration. However, the dose-effect relationship was opposite for substances 3a and 3b with cyclic aglycone. Increasing the dose of glycopeptide 3a from 0.15 to 15 mg/kg was accompanied by an increase in biological activity. However, biological activity of glycopeptide 3b decreased with increasing the dose.

Significant differences were revealed in the biological effects of 3a and 3b in the maximum and minimum dose. The study of this anomeric pair showed that the optimal dose of MDP β-glycoside increasing the resistance is much lower compared to that of α-anomer.

Taking into account the results of previous series, we studied the protective effect of MDP glyco-

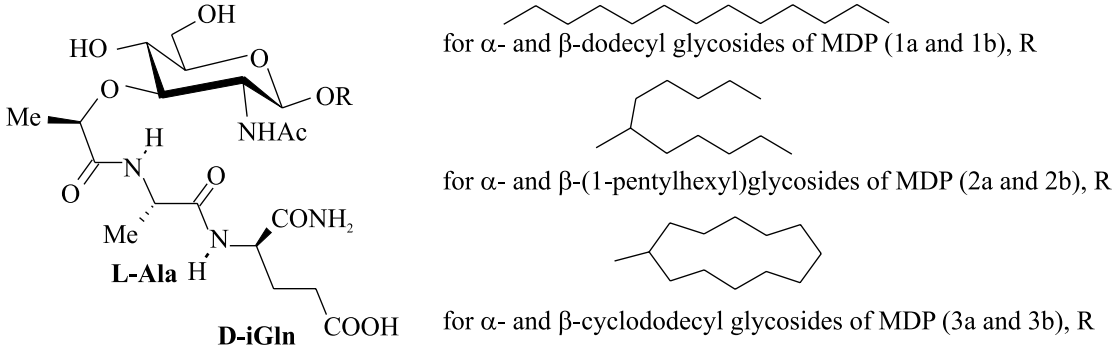

Fig. 1. Structure of lipophilic MDP glycosides.

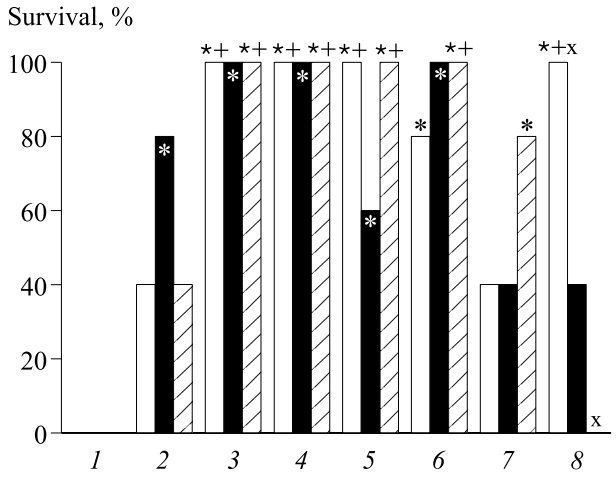

Fig. 2. Effect of lipophilic glycosides of MDP on the resistance to intraperitoneal infection with *S. aureus*. Light bars, 0.15 mg/kg; dark bars, 1.5 mg/kg; and shaded bard, 15 mg/kg. Typical results of 1 of 3 independent experiments. $p<0.05$: *compared to the control; +compared to MDP; ×compared to β-anomer. Here and in Fig. 3: control (*1*); MDP (*2*); 1a (*3*); 1b (*4*); 2a (*5*); 2b (*6*); 3a (*7*); and 3b (*8*).

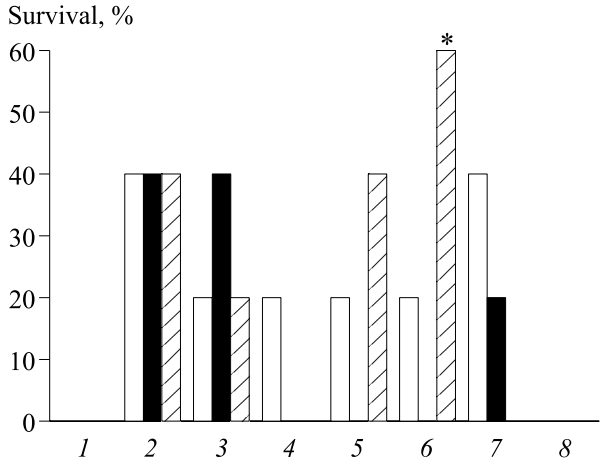

Fig. 3. Effect of lipophilic glycosides of MDP on the resistance to intraperitoneal infection with *E. coli*. Light bars, 3.75 μg/kg; dark bars, 75 μg/kg; and shaded bard, 1.5 mg/kg. Typical results of 1 of 3 independent experiments. *$p<0.05$ compared to the control.

sides in a wide range of low doses on mice with *E. coli* infection. *In vivo* study showed that biological activity of lipophilic glycosides of MDP does not depend on glycoside site configuration and structure (Fig. 3). Substances 1a, 1b, 2a, 2b, and 3a, as well as unmodified MDP, only insignificantly increased the survival of experimental animals. Glycopeptide 3b with cyclic aglycone and α-configuration of the glycoside bond had little effect on the mortality rate of infected mice. The immunostimulatory effect of glycopeptides practically did not depend on the dose. For example, substances 2a and 2b in the minimum and maximum doses tended to increase the resistance of animals. However, these substances in the intermediate dose (75 μg/kg) had no effect on the mortality rate of infected animals. The test substances in various doses and induced endogenous transmitters probably act synergistically with or antagonistically to bacterial products and components (*e.g.*, lipopolysaccharide) under conditions of animal treatment with MDP derivatives and lethal dose of gram-negative bacteria.

Our results show that lipophilic glycosides of MDP increase the survival of animals (mainly to 100%) with experimental sepsis induced by gram-positive bacteria *S. aureus* in the lethal dose. They tended to decrease the mortality rate of animals with *E. coli* sepsis. The configuration of the glycoside bond and structure of aglycones of α- and β-dodecyl (1a and 1b) and α- and β-(1-pentylhexyl) glycosides of MDP (2a and 2b) had little effect on biological activity. An inverse relationship was found between stimulatory activity and dose of anomers 3a and 3b with cyclic aglycone (0.15-15 mg/kg) during sepsis caused by *S. aureus*.

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