NEW BIOMEDICAL TECHNOLOGIES

Effect of Hydrolipophilic Equilibrium of the Muramyl Dipeptide Derivatives on Their Internalization and Interactions in Biological Membranes

O. V. Kalyuzhin and B. B. Fuks

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The interaction between two homologous muramyl dipeptide derivatives (β-heptylglycoside and β-hexadecylglycoside) and model membranes and internalization of these derivatives into K562 human erythroleukemia cells are studied. It is suggested that the difference in the interaction of these homologs with model membranes and in their immunomodulating activities result from different hydrolipophilic balance of these perorations.

Key Words: *muramyl dipeptide derivatives; amphiphility; lipophility; biomembranes*

N-Acetylmuramyl-D-alanyl-D-isoglutamine (MDP) and its derivatives elicit biological effects after internalization into cells and interaction with specific sites [4]. So far, no MDP binding sites were identified on cell surface [5,6,8]. Muramyl dipeptides and its derivatives are lipophilic compounds capable of crossing the plasma membrane via specific and nonspecific (endocytosis) pathways [5].

In this study we compared the internalization of β-heptylglycoside-MDP (C_7H_{15}MDP) and β-hexadecylglycoside-MDP ($C_{16}H_{33}$MDP) and their bindings to model membranes. Previously, we demonstrated that the *in vitro* immunomodulating activities of the peptides differ considerably [3].

MATERIALS AND METHODS

The MDP derivatives were synthesized by Dr. A. E. Zemlaykov (Simferopol State University) [2]. A water/chloroform model membrane was prepared as described elsewhere [7]. Chloroform (0.5 ml), methanol (1 ml) and aqueous solution of MDP derivative labeled with ^{14}C (0.4 ml) were thoroughly mixed in centrifuge vials and left for 1 h at room temperature. The vials were shaken every 5 min. After the addition of chloroform (0.5 ml) and distilled water (0.5 ml), the vials were centrifuged for 5 min at 1000 rpm. Aliquots (10 μl) were withdrawn from each phase of the solution, their radioactivity was measured in an LKB β-spectrometer, and the content of β-heptylglycoside-MDP or β-hexadecylglycoside-MDP was calculated. A cholesterol-phospholipid membrane was prepared by the method [9] by immersing Synpore filtering disks (pore diameter 0.23 mm) into an equimolar mixture of cholesterol and phosphatidylcholine (Bakpreparat, Kharkov) in benzene (100 mg lipids/ml). The membrane (lipid content 2-2.5 mg lipids/cm^2) was placed in a special chamber to divide it into two equal compartments. Distilled water (2 ml) was added to each compartment, and a radiolabeled preparation (20 μl, 20 mg/ml) was added to one of them. The chamber was left at room temperature. Aliquots (10 μl) were withdrawn from each compartment at different periods, their radioactivity was

Laboratory of Cellular Immunopathology and Biotechnology, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow

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measured, and the content of MDP derivative was calculated. At the end of the experiment, the membrane was washed, air-dried, and the amount of incorporated preparation was determined. Internalization of the MDP derivatives into K562 human leukemia cells was studied by the methods [5] with modifications. The preparations and cells were suspended in RPMI-1640 medium (Flow Lab.) containing 5% inactivated fetal calf serum (Flow Lab.), 10 mM HEPES (Flow Lab.), 5×10^{-5} M 2-mercaptoethanol (Serva), and 50 μg/ml gentamicin. The preparations (0.5 ml) and cell suspension (0.5 ml) were incubated in scintillation vials at 37°C in an atmosphere of 95% air/5% CO_2. The final concentrations of preparations and cells in the vial were 20 μM and 10^6 cells/ml. After various periods of incubation cells were sedimented by centrifugation, the supernatant was collected, and its radioactivity was measured in a β-spectrometer. The pellet was lyzed with 1% Triton X-100 (0.5 ml), and its radioactivity was measured.

RESULTS

Using several *in vitro* test systems, we showed that the immunostimulating activity of C_7H_{15}MDP is considerably higher than that of $C_{16}H_{33}$MDP [3]. A relationship between the effects and internalization of these compounds was demonstrated [4]. We think that the differences in immunostimulating activity arise from different hydrolipophilic balances of C_7H_{15}MDP and $C_{16}H_{33}$MDP which determine their interactions with the plasma membrane. The differences in hydrophilic balance can be confirmed by the distribution of these preparations between the water and chloroform phases of a model membrane (Fig. 1). Sixty-four percent of C_7H_{15}MDP radioactivity was recovered in the water phase and 36% in the chloroform phase. In the case of $C_{16}H_{33}$MDP, the distribution was different: 28% in the water phase and 72% in the chloroform phase, which may be due to a longer aliphatic chain of this preparation.

Interesting results were obtained in experiments with the cholesterol-phospholipid membrane dividing two water phases (Fig. 2). The lipophilic preparation $C_{16}H_{33}$MDP was detected in the opposite compartment as early as 1 h after the start of experiment, and its amount increased considerably after 6 h. However, the rate of $C_{16}H_{33}$MDP transport through the membrane was much lower than that for amphiphilic C_7H_{15}MDP. This preparation appeared in the opposite compartment after 24 h, then the rate of its transport increased considerably, and concentrational equilibrium between the phases was reached after 72 h (earlier than that with $C_{16}H_{33}$MDP). A large proportion of $C_{16}H_{33}$MDP was associated with the

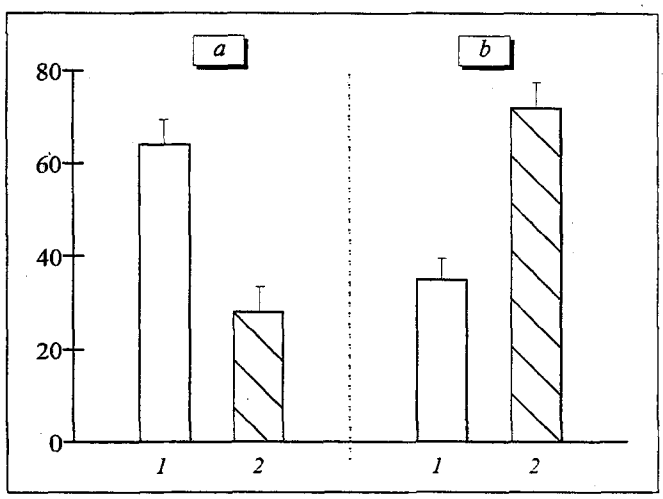

Fig. 1. Distribution of muramyl dipeptide derivatives between the phase of water/chloroform membrane. Ordinate: preparation content in the phase, %. *1*) C_7H_{15}MDP; *2*) $C_{16}H_{33}$MDP; *a*) water; *b*) organic solvent.

membrane: 14.6 ng/cm² *vs.* 3.4 ng/cm² for C_7H_{15}MDP. Presumably, the amphiphilic derivative is incorporated in the lipid membrane more slowly, which accounts for the longer time required to cross the membrane. However, after being incorporated into the membrane, it is readily released into the compartment with a lower concentration.

We also studied internalization of C_7H_{15}MDP and $C_{16}H_{33}$MDP into K562 human leukemic cells. Although this process is determined by physicochemical properties of the immunomodulators, endo- and exocytosis [1], structure of the plasma membrane

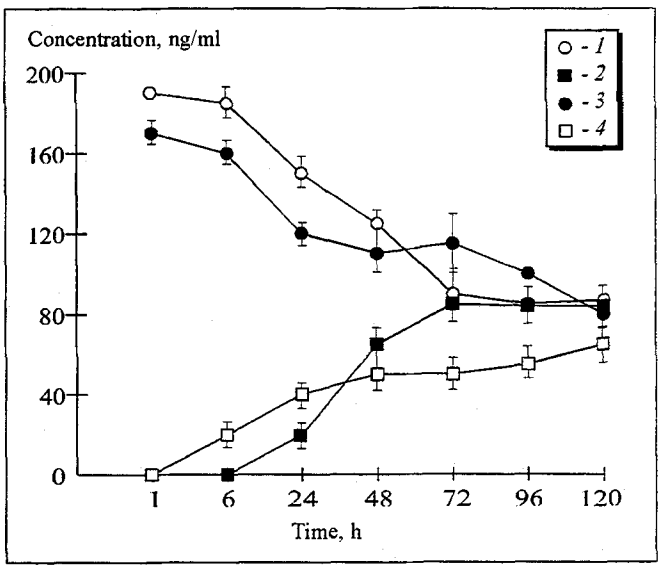

Fig. 2. Time course of muramyl dipeptide derivatives transport via a model cholesterol-phospholipid membrane. *1*, *2*) C_7H_{15}MDP; *3*, *4*) $C_{16}H_{33}$MDP; *1*, *3*) changes in the preparation concentration in the compartment where it was added; *2*, *4*) changes in its concentration in the opposite compartment.

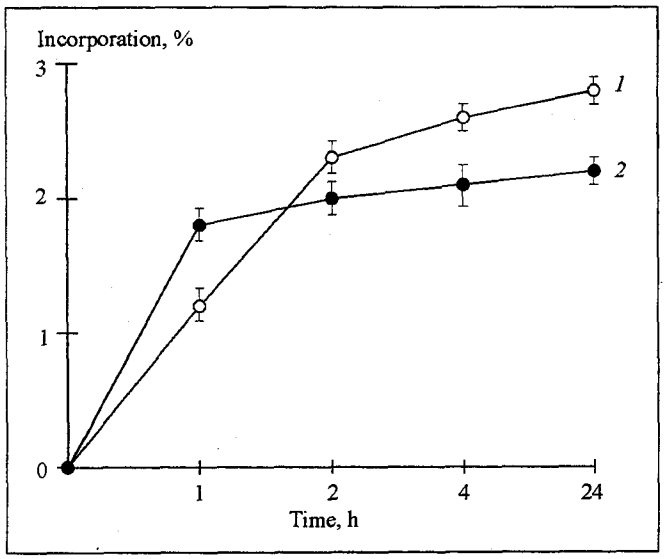

Fig. 3. Internalization of muramyl dipeptide derivatives into K562 cells. *1*) C_7H_{15}MDP; *2*) $C_{16}H_{33}$MDP.

and other factors, the results of these series were consistent with those obtained in experiments with the cholesterol-phospholipid membrane. After 1-h of incubation, the cells were cultured in glutamine-free medium containing radiolabeled preparations. The binding of lipophilic $C_{16}H_{33}$MDP was 50% higher than that of amphiphilic C_7H_{15}MDP ($p<0.05$, Fig. 3). However, the $C_{16}H_{33}$MDP binding gradually decreased and reached a plateau after 2 h of incubation. The internalization of C_7H_{15}MDP increased after 2-4 h of incubation, without reaching a plateau, and after 24 h it was 25% higher than that of $C_{16}H_{33}$MDP ($p<0.05$). Bearing in mind that a considerable proportion of the lipophilic derivative is bound to cell membranes (specifically, to the plasma membrane), the differences between the intracellular contents of these preparations should be greater that the difference between the amounts of bound preparations. After association with the plasma membrane the preparation is internalized by endocytosis, which is probably compensated by exocytosis. The amphiphilic preparation slowly binds to the plasma membrane but is readily released into the cell. Obviously, this provides more effective (compared with $C_{16}H_{33}$MDP) entry of C_7H_{15}MDP into macrophages and lymphocytes with subsequent activation of these cells [3].

From our findings it can be suggested that amphiphilicity of an MDP is the principal factor determining its ability to activate lymphocytes and macrophages.

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