Effect of Liposomal Tetracycline Hydrochloride on Enzymatic Function of the Liver

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Preparation and physicochemical properties of liposome-incorporated tetracycline are described. Comparison of the effects of tetracycline hydrochloride in free and liposome-incorporated forms on enzymatic functions of the liver showed that immobilization of the antibiotic into liposomes protects liver cells from functional disturbances.

Key Words: *antibiotics; liposomes; liposomal form of tetracycline; liver enzymes*

Despite high efficiency of modern antimicrobial preparations, they are the objects of further improvement. Antibiotics often produce highly selective effects on biochemical processes and inhibit a single step in the chain of biochemical reactions [3]. Study of any effective antimicrobial agent should evaluate not only its effect on microbial metabolism, but also the mechanism of its selectivity. To this end, the effects of these compounds on biochemical processes in both microbial and host cells should be analyzed.

After introduction of liposomes into the body, the components of their membranes actively participate in metabolic processes [9].

Accumulation of liposomes in tissues after their repeated administration leads to modification of the phospholipid composition of cell membranes. Changes in physicochemical properties of the cytoplasmic membrane considerably modulate activity of membrane-bound enzymes [2].

Here we compared the effect of free and liposome-immobilized tetracycline hydrochloride on enzymatic activity of the liver.

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MATERIALS AND METHODS

Liposomes for immobilization of antibiotics were prepared by the method of evaporation and phase reversion from a mixture (7:3) of chromatographically pure lecithin (Khar'kov Plant for Bacterial Preparations) and cholesterol (Serva). Tetracycline hydrochloride from Sintez company (Kurgan) was used. This antibiotic was chosen because it is effective in the treatment of infectious diseases [4]. Tetracycline was dissolved in 0.15 M NaCl (pH 7.2) to a final concentration of 150 U/ml. Free antibiotic (not incorporated into liposomes) was separated after 1-h centrifugation at 20,000 rpm on a Ja-21 centrifuge ("Beckman"). The amount of liposome-incorporated antibiotic was determined using microbiological test, lipid oxidation was evaluated by the Klein peroxidation index [10].

The effects of liposome-incorporated (10 mg/ml) and free (10 mg/ml) tetracycline hydrochloride on activity of enzyme systems in the liver were compared; 0.15 M NaCl (pH 7.2) served as the control.

Outbred albino mice weighing 18-20 g (3 groups, 9 animals per group) received single intraperitoneal injection of the test preparations in a volume of 0.3 ml each. The liver was isolated from 3 animals of each group 2, 4, and 24 h postinjection, the samples from each group were pooled, and homogenates were prepared in 0.15 M NaCl (pH 7.2, 1:24).

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Enzyme activity was measured in these homogenates.

Activity of alkaline phosphatase was measured using chromogenic substrate *p*-nitrophenyl phosphate [8], protease activity was determined by the method proposed by Alekseenko [1], ATPase activity was assayed after Umbreit *at al*. with some modifications, and phosphorylase-0 activity was measured by the method of Smirnova [9].

RESULTS

Activity of alkaline phosphatase slightly decreased compared to the control during the first 4 h after administration of free and liposome-incorporated tetracycline (Fig. 1). After 24 h, activity of this enzyme in animals receiving free tetracycline practically returned to the control level, while in animals receiving liposome-incorporated tetracycline it remained unchanged.

Two hours after injection of tetracycline, ATPase activity in experimental and control groups was similar (Fig. 2). After 4 hours, enzyme activity in the liver sharply increased in mice receiving free antibiotic (almost 2-fold compared to the control) and less markedly (by 30% in animals receiving liposomes loaded with tetracycline. After 24 h, ATPase activity in the group treated with free tetracycline remained at high level, while in animals receiving liposome-incorporated drug, enzyme activity returned to the level observed 2 h after injection.

Phosphorylase activity was completely blocked 2 and 4 h after administration of free tetracycline hydrochloride and reduced by 1.6 and 1.4 times after injection of liposome-incorporated drug (Fig. 3). After 24 h, a tendency towards recovery of phosphorylase activity was observed in animals receiving free antibiotic (this parameter was 2-fold lower than in the control). In mice injected with tetracycline in liposomes, enzyme activity decreased.

Two hours after injection of free or liposome-incorporated antibiotic, protease activity was 65 and 68 arb. units and did not differ from the control (65 arb. units). In animals receiving liposome-incorporated tetracycline, activity of this enzyme remained unchanged throughout the experiment (4 and 24 h). Four hours after injection of free antibiotic, activity of this enzyme decreased (to 60 arb. units *vs*. 72 arb. units in the control), while after 24 h this parameter increased (to 80 arb. units *vs*. 72 arb. units in the control).

Thus, the effect of tetracycline on enzyme systems in the liver was most clearly seen for phosphorylase: free antibiotic completely blocked the enzyme during the first 4 h after injection, while

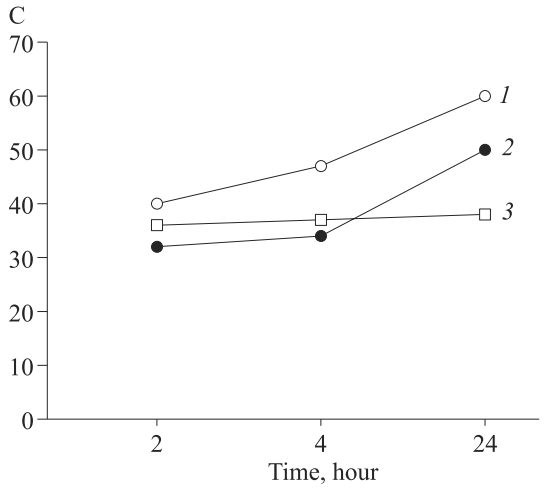

Fig. 1. Dynamics of alkaline phosphatase activity in liver homogenates from albino mice receiving free and liposome-incorporated tetracycline. Here and on Figs. 2, 3: *1*) control, *2*) free tetracycline, *3*) liposome-incorporated tetracycline. C: activity, arb. units ×100.

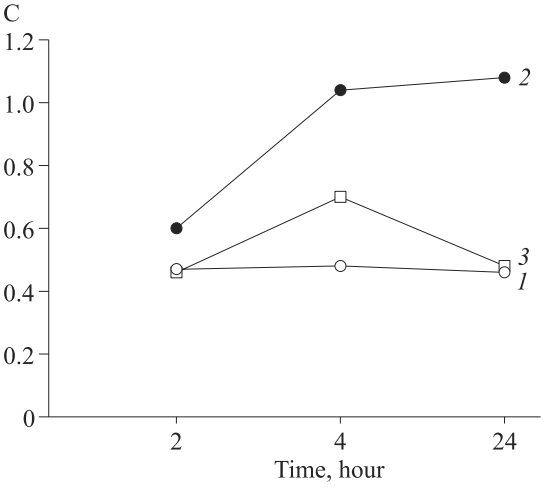

Fig. 2. Dynamics of ATPase activity in liver homogenates from albino mice receiving free and liposome-incorporated tetracycline.

incorporation into liposomes protected tissue enzymes (the inhibitory effect was observed 24 h after injection after degradation of liposomes). Changes in liver protease and phosphatase activities were similar in animals receiving free and liposome-incorporated tetracycline, while ATPase activity increased after injection of tetracycline and this activation was more pronounced under the effect of free antibiotic. This also can be explained by gradual release of the drug from liposomes.

These findings suggest that the effect of free and liposome-incorporated tetracycline on liver enzyme was different. Immobilization of tetracycline hydrochloride in liposomes protected liver cells

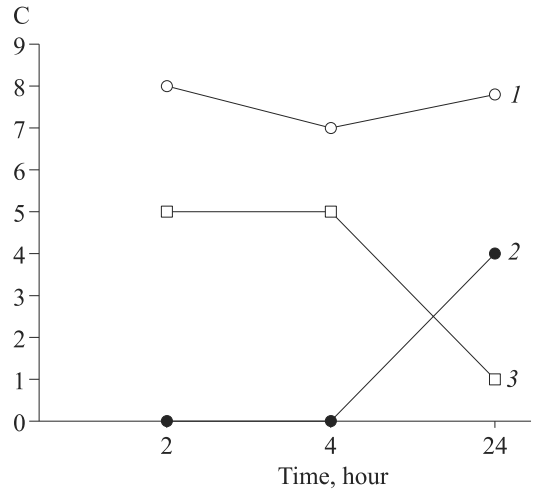

Fig. 3. Dynamics of phosphorylase activity in liver homogenates from albino mice receiving free and liposome-incorporated tetracycline.

from functional disturbances. This can be explained by slow and gradual release of the preparation from phospholipid vesicles due to their disintegration: under these conditions, its concentration did not attain maximum values characteristic of free tetracycline. At the same time, antibacterial activity of the preparation did not decrease. The use of tetracycline and some other preparations in the liposomal form can increase their efficiency in infectious diseases by 2.5-3 times in comparison with usual form [5-7].

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