Activity of Some Enzymes in the Liver of Experimental Animals after Treatment with the Liposomal Formulation of Tetracycline and Streptomycin

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A new method for obtaining the liposomal formulation of streptomycin and tetracycline is described in the present work. The physicochemical properties of this formulation were evaluated. We compared the effects of free and liposomal formulations from streptomycin and tetracycline on activity of liver enzymes in albino mice. Immobilization of antibiotics in liposomes was followed by a decrease in the inhibitory effect on protease, alkaline phosphatase, and phosphorylase. The influence of this preparation on ATPase was reduced by 2 times.

Key Words: *antibiotics; tetracycline; streptomycin; liposomes; liver enzymes*

Much recent attention is paid to liposomal preparations of antibiotics. Incorporation of antimicrobial drugs into liposomes helps to reduce their toxicity, lower the therapeutic doses, change their distribution in the organism, and prolong the therapeutic effect [5].

The majority of liposomes are accumulated in the liver and spleen. They interact with cells and modify the physicochemical properties of cell membranes. This is accompanied by significant changes in activity of enzymes located in the plasma membrane (alkaline phosphatase and γ-glutamyltransferase), cytosol (ALT and AST), and mitochondrial matrix (glutamate dehydrogenase) [9]. Changes in enzyme activity are determined by physicochemical properties of lipid vesicles (lipid composition, size, charge of the membrane, and degree of oxidation), dose, and frequency of treatment with liposomal preparations [5].

It should be emphasized that variations in enzyme activity after treatment with free and liposomal forms of antibiotics can serve as a criteria for toxicity of these products [3].

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Here we studied activity of liver enzymes in experimental animals after treatment with liposomal or free form of tetracycline and streptomycin.

MATERIALS AND METHODS

The preparation of liposomes and immobilization of antimicrobial drugs in phospholipid vesicles were performed by the method of phase inversion [8] with chromatographically pure lecithin (Kharkov Factory of Bacterial Preparations) and cholesterol (Serva) in the 7:3 molar ratio. Streptomycin sulfate (Krasnoyarsk Factory "Minmedbioprom") and tetracycline hydrochloride (Kurgan Plant "Sintez") were used as a material for incorporation into liposomes. Liposomal forms of preparations were obtained for each antibiotic. For incorporation of streptomycin sulfate and tetracycline hydrochloride into liposomes, these preparations were dissolved in buffered physiological saline (pH 7.2) to a final concentration of 150 U/ml. The non-incorporated antibiotics were isolated by centrifugation on a Ja-21 centrifuge (Beckman) at 20,000*g* for 1 h. The amount of the antimicrobial drug in liposomes was estimated by the microbiological method [2]. The degree of lipid

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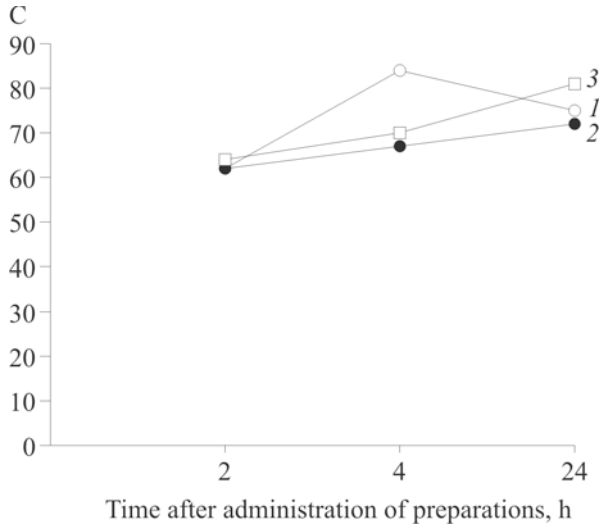

Fig. 1. Protease activity in liver homogenates from albino mice after treatment with free (*3*) and liposomal forms (*2*) of the tetracycline/streptomycin mixture. Here and in Figs. 2 and 3: control (*1*). C, enzyme activity (arb. unit; E×100, where E is extinction).

oxidation was evaluated from the Klein peroxidation index [6].

The effects of free and liposomal forms of antibiotics on liver enzyme activity were studied using the following preparations: mixture of liposomal tetracycline hydrochloride and streptomycin sulfate in a dose of 10 mg/ml (5 mg/ml of each preparation); standard mixture of these agents in the same dose; and 0.15 M NaCl (pH 7.2; control). Outbred albino mice (18-20 g) were divided into 3 groups (9 mice per group). The animals received an intraperitoneal injection of the test preparations in a single dose of 0.3 ml. The liver was excised from 3 animals of each group after 2, 4, and 24 h. Liver samples from animals of the same group were pooled. The homogenates were prepared in 0.15 M NaCl (pH 7.2, ratio 1:24). Enzyme activity (C) was measuread and expressed per sample weight.

We measured activities of alkaline phosphatase (with the chromogenic substrate *p*-nitrophenyl phosphate) [4], protease (method of L. P. Alekseenko) [1], ATPase (method of V. V. Umbreit with modifications) [3], and phosphorylase-0 (method of M. G. Smirnova) [4].

The results were analyzed by Student's *t* test.

RESULTS

The effect of the test preparations on protease activity was detected 4 h after injection. Two hours after injection, enzyme activity in animals of the treatment groups did not differ from the control. Protease activity was suppressed 4 h after treatment with free or liposomal form of the preparation (by 20%). Protease activity in animals receiving the liposomal formulation of antibiotics did not change after 24 h (control level). By contrast, protease activity in mice receiving the mixture of free preparations was 10% higher than in the control.

Figure 2 shows the effect of tetracycline/streptomycin mixture (free and liposomal forms) on phosphatase activity in the liver. Enzyme activity was similarly reduced (by 20%) 2 h after treatment with free and liposomal form of the antibacterial drug. Phosphatase activity also decreased 4 h after administration of free and liposomal form (by 60 and 50%, respectively, compared to the control). By the 24th hour,

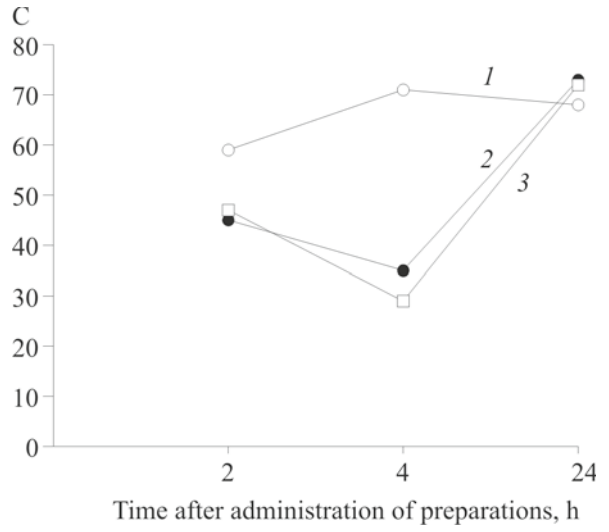

Fig. 2. Alkaline phosphatase activity in liver homogenates from albino mice after treatment with free (*3*) and liposomal forms (*2*) of the tetracycline/streptomycin mixture.

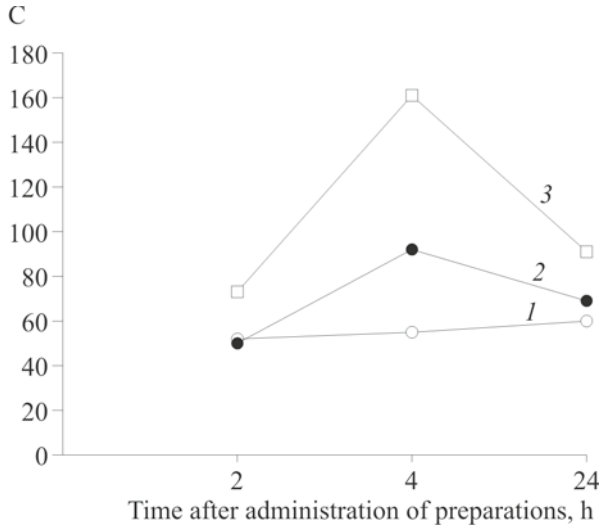

Fig. 3. ATPase activity in liver homogenates from albino mice after treatment with free (*3*) and liposomal forms (*2*) of the tetracycline/streptomycin mixture.

TABLE 1. Phosphorylase Activity (arb. units; E×100, where E is Extinction) in Liver Homogenates from Albino Mice after Treatment with Free and Liposomal Forms of Tetracycline/Streptomycin Mixture (*M*±*m*)

Preparation	Time after administration, h		
	2	4	24
0.15 M NaCl (control)	8±0.1	7±0.2	7.9±0.2
Tetracycline+streptomycin	1±0.1	0	0
Liposomal tetracycline+streptomycin	3±0.2	0.9±0.1	0

phosphatase activity in liver samples from animals of both groups did not differ from the control.

ATPase activity in the liver of albino mice (Fig. 3) was elevated 2 h after treatment with free form of the streptomycin/tetracycline mixture (by 40% compared to control animals receiving the liposomal preparation). By the 4th hour, ATPase activity in the liver of these mice increased up to the level observed after injection of liposomal antibiotics. ATPase activity in animals receiving free form of the test preparations 3-fold surpassed the control. ATPase activity in mice of the treatment groups decreased by the 24th hour, but remained above the control level.

Phosphorylase activity in animals receiving free and liposomal forms of the streptomycin/tetracycline mixture decreased by 8 and 2.7 times, respectively, compared to the control (2 h postinjection; Table 1). Enzyme activity in mice of the treatment groups was completely suppressed by the 4th hour and did not return to normal even after 24 h. It should be emphasized that phosphorylase activity in animals of the control group remained unchanged in various periods.

Antibiotics have an adverse effect on metabolic processes in the macroorganism (at a certain concentration in the medium) [7]. In a complex system of metabolism, even small variations in the course of one reaction can impair other metabolic processes (up to metabolic dysfunction). This work was designed to compare the effects of free and liposomal formulation of streptomycin and tetracycline on activity of liver enzymes in albino mice. Our study showed that immobilization of antibiotics in liposomes is followed by a decrease in the inhibitory effect on protease, alkaline phosphatase, and phosphorylase. The influence of this preparation on ATPase was reduced by 2 times. These effects were mainly observed over the first hours after injection (2 and 4 h). Activity of the enzymes returned to normal in a later period (24 h). The exception was phosphorylase. Phosphorylase activity was completely suppressed during these periods.

We conclude that immobilization of antibiotics into phospholipid vesicles is accompanied by a decrease in the effect of these drugs on liver enzymes. It is probably associated with a gradual release of antibiotics from liposomes. Under these conditions, the concentration of antibacterial drugs in tissue cells does not reach the toxic level.

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