RELATIONSHIP BETWEEN PROLONGATION OF ANTIINFLAMMATORY ACTIVITY OF HYDROCORTISONE INCORPORATED INTO LIPOSOMES AND THEIR LIPID COMPOSITION IN EXPERIMENTAL ARTHRITIS

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The anti-inflammatory activity of corticosteroids incorporated into the lipid phase of liposomes is known to be appreciably enhanced [6, 9], but the toxicity of the preparations is significantly reduced [4, 8]. The therapeutic effect of intraarticular injection of liposomes with hydrocortisone (HC) is linked with intracellular uptake of vesicles by synovial cells [10]. Injection of HC incorporated into liposomes has been shown to be effective both in the initial acute phase of inflammation and during a flare-up of acute inflammation against the background of chronic arthritis [4, 8].

Prolongation of the anti-inflammatory activity of HC has received much less study, although for intraarticular therapy this parameter is no less important than increased activity, for it allows preparations to be administered in much smaller doses.

This paper gives information on the influence of the lipid composition of liposomes, differing in membrane melting point over a wide range of temperatures (from 23 to 58°C) on the duration of anti-inflammatory activity of HC encapsulated in vesicle membranes.

EXPERIMENTAL METHOD

A model of experimental arthritis was produced in rabbits by a single intraarticular injection of poly-D-lysine (mol. wt. 175,000 daltons) and hyaluronic acid (7.5 mg of each per injection), as in [10]. On the 3rd day after induction of arthritis and at the height of the inflammatory reaction, HC acetate incorporated into liposomes (0.2 mg) or a commercial preparation of HC suspension in a dose of 2.5 mg in a volume of 0.4 ml was injected into the joint. Control animals received injections of 0.4 ml physiological saline at the same time (all groups consisted of five animals). Liposomes were prepared by the emulsion method [3]. For this purpose phospholipids – egg phosphatidylcholine (PC) from Khar'kov Bacterial Preparations Combine, dimyristoyl-PC (DMPC), dipalmitoyl-PC (DPPC), or distearoyl-PC (DSPC) (from Sigma, USA), and cholesterol in the ratio (molar) of 7:2, were introduced into a round-bottomed flask in doses of 21 and 6 μmoles respectively, in chloroform, and HC in chloroform (10 mg) and 1 μCi of [^{3}H]-HC (specific activity 48 Ci/mmole), from Isotop (USSR), were added. The solvent was evaporated to dryness on a rotary vaporizer, the lipid film was freed from traces of chloroform at 5 mm Hg in an exsiccator for 1 h, and it was then emulsified with 2 ml of physiological saline. The emulsification was carried out at 37°C for egg PC and DMPC, at 50°C for DPPC, and at 60°C for DSPC, for 30 min. The microemulsions were kept at the same temperatures for 2 h to allow hydration and sedimentation

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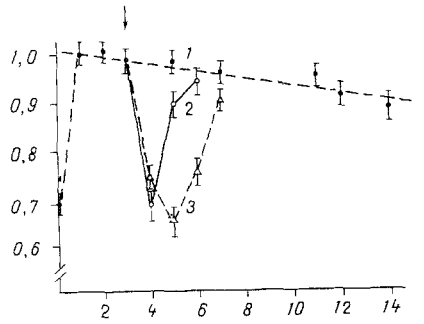

Fig. 1

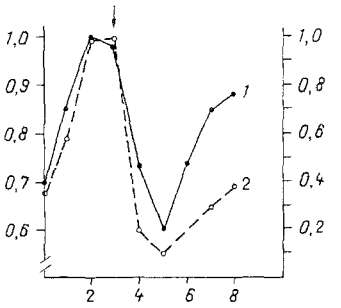

Fig. 2

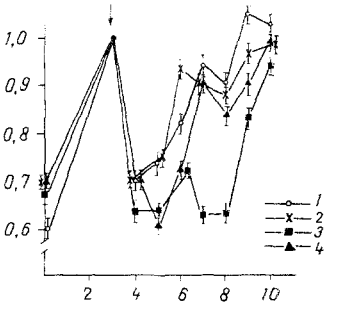

Fig. 3

Fig. 1. Anti-inflammatory effect of hydrocortisone immobilized in liposome membrane. 1) Untreated control; 2) free HC (2.5 mg); 3) HC incorporated into egg PC liposomes (0.2 mg). Here and in Fig. 3, arrow indicates time of addition of preparations. Abscissa, time after intra-articular injection of poly-D-lysine and hyaluronic acid (in days); ordinate, relative thermometric index.

Fig. 2. Correlation between thermometric index and area of hyperthermia. 1) Thermometric index after incorporation of HD into egg PC liposomes; 2) index of area of inflammation. Abscissa, time after induction of arthritis (in days); ordinate (left), thermometric index; (right), index of area of inflammation (in relative units).

Fig. 3. Prolongation of anti-inflammatory effect of HC in liposome membrane with different phase transition temperature. 1) Liposomes from egg PC, 2) from DMPC, 3) DPPC, 4) DSPC. All liposomes contained 20 moles % of cholesterol. Designation of axes of coordinates the same as in Fig. 1.

of the liposomes at 10,000g for 1 h. Preliminary experiments showed that 96-98% of HC is incorporated into the lipid phase of liposomes. Liposome residues were resuspended in physiological saline up to an HC concentration of 0.5 mg/ml (about 2 mg lipids in 1 ml) and they were used in the course of 1 week. The sterility of the liposomal preparations was verified microbiologically, and whenever possible, work with lipids was done under argon.

The inflammatory reaction in the joint was recorded with a thermal imaging system. To determine quantitative values of temperature above the joint, a standard source with temperature of 32°C was used. Both the intensity of hyperthermia and the area of the hyperthermic regions, calculated planimetrically from negatives, were analyzed. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Free HC in a dose of 2.5 mg caused a marked fall of temperature above the joint 24 h after injection, but it reached its initial value 24 h after falling (Fig. 1). HC introduced into the lipid phase of PC of the liposomes (0.2 mg) also induced an effect after 24 h, but the temperature continued to fall and reached a minimum after 48 h (Fig. 1). The effect of prolonging the action of HC in liposomes lasted 2 days, compared with free HC, and in a dose 10 times less than the HC suspension. Antiinflammatory effect, calculated both with respect to the severity of hyperthermia and the area of inflammation, gave results that were in good agreement (Fig. 2). It is well known that in the zone of the phase transition temperature of lipids the structure and permeability of liposomes are changed [1]. This property of lipids is utilized to increase the rate of outflow of therapeutic substances from the aqueous space in vesicles with the aid of hyperthermia [11]. There are definite grounds in support of the view that approximation of the phase state of the vesicle membrane and the plasma membrane of target cells (in this case, synovial cells) can facilitate their interaction [2]. An attempt was made to test this hypothesis by using liposomes formed from lipids with different phase transition temperatures. It will be recalled that the melting points of the lipids used, namely egg PC, DMPC, DPPC, and DSPC, are -10, +23, +41.5, and +58°C respectively.

The most marked effect of prolonging the action of HC was observed when it was incorporated into DPPC liposomes (Fig. 3). The rate of fall of temperature over the joint was the same for all types of liposomes. However, when DPPC liposomes were used the effect lasted until the 5th day and was still detectable even on the 6th day (Fig. 3). An increase in the melting point of the liposomal lipids to 58°C (DSPC), and also a decrease to +23°C (DMPC) or to -10°C (egg PC), reduced the time of prolongation to 1-2 days compared with DPPC liposomes.

The effect of phosphatidic acid in a concentration of 15 moles % in the composition of DPPC liposomes on prolongation of the action of HC was demonstrated in [8]. This prolongation lasted 3-4 days. The authors cited interpret this phenomenon as the effect of coincidence between the melting point of this mixture of lipids and the temperature of inflammation, namely 36°C. Such an interpretation of the results is evidently incorrect because incorporation of phosphatidic acid in the lipid phase of LPPC liposomes leads not to a decrease in phase transition temperature, but to an increase in it to 58-67°C depending on pH of the microemulsion [1, 5]. Incorporation of 20 moles % cholesterol, which was done to increase the stability of the vesicles, is reflected also in the melting point of DPPC and shifts the beginning of phase transition from 41.5 to 37°C [1, 7], i.e., to a temperature that is close to the temperature of inflammation.

It can thus be postulated that the effect of prolongation of the anti-inflammatory action of HC incorporated into DPPC liposomes is due to two circumstances. In the first stage, because of closeness of the phase state of the liposomal lipids and cells at the temperature of inflammation, effective uptake of vesicles by the cells takes place. Later, because of the appearing hypothermic action of HC and the fall of temperature to 32°C, the vesicles taken up are converted into a solid-crystal state, in which their utilization time is considerably lengthened.

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EFFECT OF 1-(CHLOROMETHYL)SILATRANE ON TISSUE BIOCHEMICAL PARAMETERS IN EXPERIMENTAL GASTRIC ULCER

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Silicon has been shown to be an essential trace element for higher animals and man [2]. Among compounds of this element many substances with high biological activity have been found. The silatranes, which stimulate protein and nucleic acid synthesis in cells [1, 12, 14] and also the proliferative-reparative function of connective tissue [1, 8, 12, 14], are particularly important in this respect. It was shown as long ago as in 1975 that certain

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