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Prolongation of Anti-Inflammatory Activity of Glucocorticosteroids Encapsulated in Large Oligolamellar Liposomes in Treatment of Arthritis in Rabbits

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Abstract

Background: Liposomes have been shown to be an effective targeted drug delivery system used to decrease side effects of glucocorticosteroids in the treatment of rheumatoid arthritis.

Materials and Results: Experimental arthritis was induced in rabbits by a single intra-articular administration into the knee joint of poly-D-lysine (molecular weight, 175 kDa) and hyaluronic acid (7.5 mg per administration). To determine temperature readings over the joint a standard radiator was used with a temperature of 32^0C. Large oligolamellar liposomes from different phospholipids and and cholesterol containing hydrocortisone acetate in lipid phase and prednisolone hemisuccinate in water phase were used.

Conclusion: Intra-articular administration of the water-soluble prednisolone hemisuccinate (0.125 mg) and the lipid-soluble hydrocortisone acetate (0.125 mg) into the knee joint in the aqueous and lipid phases of large oligolamellar TSL (DPPC + 20 mole % cholesterol) prolongs the anti-inflammatory effect produced by glucocorticoids by 7–8 days compared to 1 day for free glucocorticosteroids at a total dose of 2.5 mg and 2 days for phosphatidylcholine-cholesterol liposomes at a total dose of 0.25 mg in rabbits with aseptic arthritis. **(International Journal of Biomedicine. 2017;7(2):131-134.)**

Key Words: aseptic arthritis • thermosensitive liposomes • glucocorticosteroids • anti-inflammatory activity

Abbreviations

DPPC, dipalmitoylphosphatidylcholine; **HA**, hydrocortisone acetate; **PH**, prednisolone hemisuccinate; **PhC,** phosphatidylcholine **TSL**, thermosensitive liposomes.

Introduction

Since common treatments for rheumatoid arthritis—such as nonsteroidal anti-inflammatory drugs, corticosteroids, disease modifying anti-rheumatic drugs and some biological agents—have proven to be unable to achieve drug-free remission,[1] a number of targeted drug delivery strategies have been developed in order to attenuate side effects to other tissues. These include microemulsions, microspheres, liposomes and others, of which liposomes have been shown to retain the drug in the synovial cavity effectively due to their chemical composition and size.[2,3] In our previous work we have demonstrated prolongation of the anti-inflammatory effect produced by HA encapsulated into the membrane of multilamellar TSL composed of DPPC and cholesterol in rabbits with aseptic arthritis.[4] However, this prolongation was limited to only about 5 days compared to 1 day in the case of intra-articular administration of free hydrocortisone acetate or 2 days for its liposomal form composed of egg lecithin and cholesterol. At the same time, no attempts have been done so far to study the effectiveness of administration of a combination of water-soluble and lipid-soluble glucocorticosteroids entrapped in the aqueous and lipid phases of 1 liposome formulation.

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The study was aimed at evaluating the potential for prolonging the anti-inflammatory activity of glucocorticosteroids encapsulated into TSL composed of DPPC and cholesterol, with the aqueous phase containing water-soluble PH and the lipid phase containing lipid soluble HA.

We compared glucocorticosteroids entrapped in liposomes with free glucocorticosteroids at a 10 times higher dose. Two types of liposomes were compared – egg-phosphatidylcholine (PhC) liposomes with a melting phase transition temperature (T_m) of $+10^0$C and DPPC liposomes with a T_m of 41.5^0C. Adding cholesterol to the liposome membrane decreases the amplitude and increases the T_m range. If liposomes are made from DPPC and 20% mole cholesterol, their membrane is in a metastable state over the temperature range from 37^0C to 47^0C, including the inflammation temperature range.[5]

Methods

Experimental arthritis was induced in rabbits by a single intra-articular administration into the knee joint of poly-D-lysine (molecular weight – 175 kDa) and hyaluronic acid (7.5 mg per administration).[6] There were four groups of rabbits containing 5 animals each. The first group received 0.5 ml saline, the second group received a 0.5 ml mixture of PH (1.25 mg) and HA (1.25 mg), the third group and the fourth received liposomal form containing both glucocorticosteroids at a 10 times lower dose (0.125 mg), the third group receiving egg-PhC liposomes and cholesterol and the fourth group receiving DPPC liposomes and cholesterol. In all groups, the injection was made on the third day after arthritis induction and at the very peak of the inflammatory reaction. Large oligolamellar liposomes were obtained using the reverse-phase technique.[7] Egg-PhC and DPPC purchased from Lipoid, Germany, and cholesterol purchased from Avanti Polar Lipids, Inc., USA, were taken at a ratio of 7:2 (molar), 21 µmol and 6 µmol correspondingly, and placed into a round-bottom flask. Then HA in chloroform and 1µCurie of ^{3}H-HA (specific activity of 48 µCurie/µmol, Izotop, St Petersburg, Russia) were added. The solvent was removed by a rotary evaporator to achieve dryness; the lipid film was cleared of residual chloroform under vacuum by an evaporator (Rotavapor R-114, Buche, Switzerland), dissolved in 3ml of freshly prepared diethyl ether and 9ml of saline containing 5 mg of PH and 1µCu of ^{51}Cr-EDTA (specific activity 100 µCu/ml, Izotop,Russia). The system was processed in a sonicator (UZD-H-1, Sumy, Ukraine) at a frequency of 22 kHz and a power 630 W of at $+4^0$C under argon protection. The obtained reverse emulsion (oil-in-water) was transformed into regular emulsion (water-in-oil) by eliminating the organic solvent using a rotary evaporator (Rotavapor R-114, Buche, Switzerland). Then the liposome emulsion was kept for two hours at 37^0C for egg-PhC and at 50^0C for DPPC and centrifuged for 1 hour at 10000×g using a Sigma 6K10 centrifuge, Germany, to spin down liposomes. Preliminary experiments have shown HA encapsulation into the liposome lipid phase to be 96%–98% as calculated from ^{3}H-HA inclusion into the liposome membrane, while PH encapsulation has been shown to amount to 30%–36% as calculated from ^{51}Cr-EDTA inclusion. The liposome residues were resuspended in the saline to reach HA and PH concentrations of 0.25 mg/ml each. The lipid concentration in samples amounted to 2mg/ml. The liposome samples were used within 1 week. Liposomal preparations were checked for sterility, and whenever possible argon protection was used. The inflammatory reaction in the joint was registered by a thermal camera. To determine temperature readings over the joint a standard radiator (Pergamed, Russia) was used with a temperature of 32^0C. Both hyperthermia severity and hyperthermic area, calculated from negative images using planimetric analysis, were taken into account. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996)

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). The mean (M) and standard error of the mean (SEM) were calculated. The Wilcoxon criterion was used to compare the differences between the paired samples. Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results

A mixture of free HA and PH at a dose of 2.5 mg (1.25 mg per each glucocorticosteroid) caused a statistically significant ($P<0.001$) decrease in temperature over the joint 24 hours after the administration, which came back to baseline 1 day after the decrease (i.e. the anti-inflammatory effect lasted for a little over 1 day) (Fig.1). Glucocorticosteroids encapsulated in the aqueous and lipid phases of PhC/cholesterol liposomes (0.125 mg of each glucocorticosteroid amounting to 0.25 mg) also had an effect 1 day after administration (Fig.1), but their effect was statistically significantly ($P<0.01$) prolonged up to 2 days, the total dose being 10 times less than in free glucocorticosteroids.

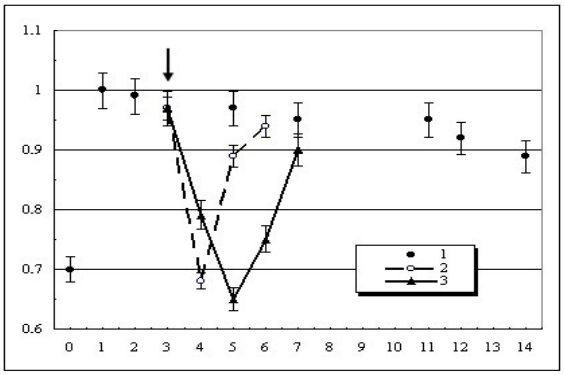

Fig. 1. Anti-inflammatory effect of mixed water-soluble and lipid-soluble free or encapsulated into lecithin-cholestrol liposomes glucocorticoids.

X-line: time after intra-articular administration of poly-D-lysine and hyaluronic acid (days),
Y-line: relative thermometrical index (relative units). The arrow indicates the time of drug administration.
(1) – no treatment; (2) – administration of mixed HA and PH (1.25 mg of each drug amounting to a total of 2.5 mg); (3) liposomes from PhC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline).

The anti-inflammatory effect calculated from both hyperthermia severity and inflammation area yields consistent results (Fig.2). The correlation test showed a strong positive correlation between these parameters: r=+0.88±0.180 (P<0.01). The structure and permeability of liposomes are known to change in the lipid melting phase-transition temperature (T_m) range.[8] This property of lipids is used to increase the rate of drug release from the aqueous phase of the vesicles due to hyperthermia. There are a number of reasons to believe that bringing closer the phase states of the vesicle membrane and the plasma membrane of the target cells, which are synovial cells in this case, may facilitate their interaction.[9]

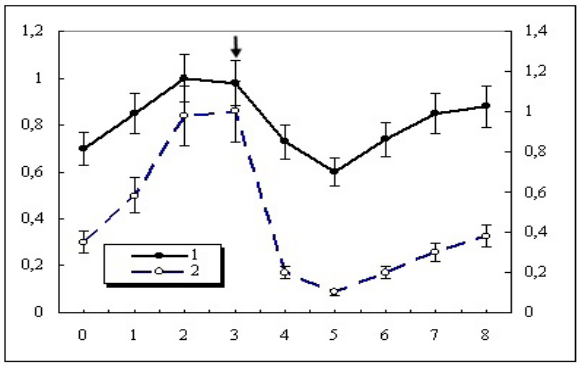

Fig. 2. Hyperthermia severity and hyperthermic area correlation.

X-line - time after arthritis induction (days).Y-line (left) - thermometric index; (right) - inflammation area index (relative units).
(1) - thermometric index after administration of mixed water-soluble and lipid-soluble encapsulated into egg-PhC and 20% mole cholesterol liposomes glucocorticoids; (2) - inflammation area index.

The duration of the anti-inflammatory effect produced by mixed glucocorticosteroids (HA and PH) entrapped in DPPC/cholesterol liposomes proved to be significantly longer, reaching 7–8 days after the drop in temperature (Fig.3).

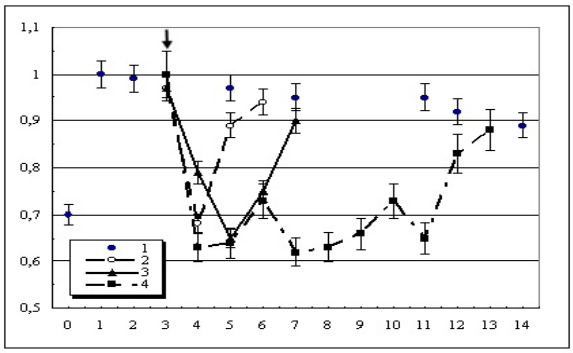

Fig. 3. Prolongation of the anti-inflammatory effect of HA and PH encapsulated in the liposomal membrane with various melting phase transition temperature**.**

X-line: time after intra-articular administration of poly-D-lysine and hyaluronic acid (days),
Y-line: relative thermometrical index (relative units). The arrow indicates the time of drug administration.
(1) - no treatment; (2) - administration of mixed HA and PH (1.25 mg of each drug amounting to a total of 2.5 mg); (3) - liposomes from PhC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline); (4) - liposomes from DPPC and 20% mole cholesterol containing 0.125 mg of HA and 0.125 mg of PH (0.5 mg of lipids in 0.25 ml of saline).

The temperature drop rate over the joint was the same for the both types of liposomes. However, when DPPC/cholesterol liposomes were used, the anti-inflammatory effect lasted for up to 7–8 days after administration.

Discussion

This shows that the prolonged effect of the anti-inflammatory action produced by mixed glucocorticosteroids (HA and PH) entrapped in DPPC/cholesterol liposomes is due to both HA present in the lipid phase and PH present in the aqueous phase of the liposomes. Since HA alone encapsulated in DPPC/cholesterol liposomes yielded a prolongation of only 5 days,[4] the greater prolongation effect can be attributed only to the fact that water soluble PH was also administered. It can also be assumed that when DPPC/cholesterol liposomes come into contact with inflammatory cells within the joint, the effectiveness of inflammatory cell (neutrophils, monocytes) membrane merger depends on the closeness of the phase state of liposome lipids and the inflammatory cells at inflammation temperature. Due to the hypothermic effect of the mixed glucocorticosteroids and the temperature decrease down to 32°C, the vesicles in the joint change to the solid crystalline state so that the period of their utilization increases as they stay there as a depot. However, in this state the water-soluble PH leaves the liposomes along a concentration gradient, which leads to an additional anti-inflammatory effect. The temperature rise peak during the third day after the first temperature drop following the administration of DPPC/cholesterol liposomes is of special importance. We believe that a slight increase of temperature followed by a temperature decrease over a short period of time indicates that the temperature increase in the joints transforms DPPC/cholesterol liposomes once again from the solid crystalline state into liquid crystalline state and HA once again shows an anti-inflammatory effect. This phenomenon clearly demonstrates that the activity of HA encapsulated in DPPC/cholesterol liposomes is temperature related.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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