行政院國家科學委員會專題研究計畫成果報告

Thermo-Sensitive Liposomes Used in Ocular Delivery: Preparation and Characterization of Norfloxacin Entrapped in Liposomes Bearing Thermo-Responsive Polymeric Hydrogels

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一.中文摘要:

在本研究中,我們製備負載 norfloxacin 且含感溫聚合親水膠體之微脂粒;經由體外藥物釋出試驗與熱分析的檢測結果指出,添加感溫聚合親水膠體在低於室溫下具有安定脂質雙層與減少藥物滲漏的效果, 當溫度由升高 20 ℃至 37 ℃時,僅有微脂粒併合 copoly (NIPAA-AASE)具感溫藥物控釋效果;而且, 在 20 ℃與 37 ℃間,反復升降溫的處理過程下,藥物的釋出並無顯著的差異存在。

關鍵字: 微脂粒; 感溫型; Norfloxacin; 性質描述; 體外實驗

1.Abstract:

In this paper, we prepare and characterize norfloxacin-loaded liposomes bearing thermo-sensitive polymeric hydrogels. Simutaneous in vitro release experiments and DSC measurements indicated that addition of thermo-sensitive hydrogels would stabilize lipid bilayers and reduce leakage below room temperature. The liposomal drug release can only be controlled by liposome bearing copoly (NIPAA-AASE) as the temperature was increased from 20 to 37. Moreover, there were no significant difference among drug release as repeating "on-off" process.

Keywords: Liposomes; Thermo-sensitive; Norfloxacin; Characterization; In vitro

2.Introduction:

Norfloxacin is one of the 4-quinolone antibacterial agents which have a widespread action against Gram(-) microorganisme. In ophthalmology, it is employed for the treatment of most pyogenic inflammations of the eye (1). A major problem in ophthalmic drug delivery is the poor and limited ocular bioavailability (<10% of the applied dose) (2), especially in the limited rate of corneal absorption. It is advantageous for corneal permeation to adjust the pH of the solution to increase the proportion of unionized drug in the instilled dose. However, unionized norfloxacin is very slightly soluble in aqueous solution. Increasing its solubility to change it into ionized form, we found that norfloxacin, then, will not readily permeate the cornea. Thus, the process of ocular drug uptake may be modified by the physical properties of the vehicle in which the drug is placed.

Liposomes are vesicle-like structures with a concentric series of alternating compartments of aqueous spaces and phospholipid bilayers. Consequently liposomes have the ability to entrap both lipophilic and hydrophilic compounds. One of the more recent application is the concept of employing liposomes as drug carriers in ophthalmology (3). Administration of the liposome-encapsulated compounds proved to be more effective than the same therapeutic regimen of drug solution (4).

For this reason, liposomes have gained considerable attention for topical ocular drug delivery.

Recently, with respect to site-specific delivery of drugs. A number of attempts have been made to develop liposomes which can regulate release of drugs responding to various stimuli, such as pH (5). light (6) and temperature (7). Among these stimuli-responsive products, thermo-sensitive liposomes may be the best for medical application. (8)

In the present study, we attempt to encapsulate norfloxacin into liposomes by using a method which combines the ethanol injection method (9) with freeze-drying process to increase the liposome encapsulation efficiency. Simultaneously, thermo-sensitive hydrophilic polymers was used as an additive in ophthalmic solutions of liposomal norfloxacin. Then, we investigate the effect of lipid composition and the additive components on the shape, particle size and encapsulated efficiency of liposomes. Futhermore, the in vitro release of the entrapped norfloxacin from liposomes was studied.

3.Materials and methods

3.1 Materials

Norfloxacin, Dimyristoyl - L - α - phosphatidylcholine (DMPC), Dipalmitoyl - L - α - phosphatidyl-choline (DPPC), Distearoyl - L - α - phosphatidylcholine (DSPC) and N-N-methylenebisacrylamide (NNMAA) were purchased from Sigma Chemical Co.. N-isopropyl acrylamide (NIPAA) and Acrylic acid stearyl ester (AASE) were purchased from TCI Chemical Co.. Acrylamide (AA) and Azobisisobutyronitrile (AIBN) were purchased from WAKO Chemical Co.. All other reagents and solvents were of analytical or equivalent grade.

3.2 Synthesis of copoly(AA-AASE)

Copoly(AA-AASE) was synthesized according to the method of Kono et al. (10) AA, AASE and AIBN were dissolved in dimethylsulfoxide (freshly distilled). The solution was degassed by bubbling with N_2 and then heated to 60 ℃ for 4 h. The polymer was recovered by precipitation with diethylether. The dried polymer was dissolved in water, reprecipitated with methanol, and then dried.

3.3 Synthesis of copoly(NIPAA-AASE)

NIPAA, AASE and AIBN were dissolved in dioxane (freshly distilled). The solution was degassed by bubbling with N_2 and then heated to 60 ℃ for 4 h. The polymer was recovered by precipitation with diethylether. The dried polymer was dissolved in dioxane, reprecipitated with diethylether, and then dried.

3.4 Synthesis of copoly(NNMAA-AASE)

The synthesized method of NNMAA-AASE was the same as that of NIPAA-AASE.

4. The preparation of liposome

Two different procedures were carried out in the preparation of liposome.

Method A Liposomes were prepared by the orthodox ' film method'. The desired amount of lipids in absolute ethanol (the ethanol was removed by rotary evaporation), and was dried to a thin film on the wall of a round-bottomed flask. The lipid film was hydrated with phosphate-buffer saline (pH 5.0) in which Norfloxacin was dissolved. The mixture was gently shaken for 30 min, and then was sonicated in bath-type sonicator (Elma, West Germany) above the phase transition temperature of the lipid materials for 10 min under an atmosphere of nitrogen.

Method B We used a modification of the ethanol injection technique. The norfloxacin was directly added to the lipid ethonal solution. Such a solution was then rapidly injected into the five-fold volume

of a magnetically stirred phosphate-buffer saline. The liposomal dispersions were frozen by a dry ice - acetone bath, and were dried by a freeze dryer (LABCONCO, Kansas, USA). The lyophilized powder was then rehydrated with double-distilled water. Mild vortexing of the flask was performed and was left at room temperature for 30 min before use. The total lipid concentration was adjusted to 9.2 mM.

5. The assay of the entrapped norfloxacin

The norfloxacin-containing liposomes were separated from the untrapped norfloxacin by filtering liposome dispersion with 0.025 um filters (Millipore, MA, USA) under vacuum (5mmHg) and, were washed with phosphate-buffered saline to completely remove the free drug .

The entrapped amount of norfloxacin was determined by the lysis of liposomes dissolved by absolute ethanol. The liposome dispersions were mixed with the equal-volume absolute ethanol to obtain a clear solution. Norfloxacin concentrations were estimated by HPLC method. The entrapped amount of norfloxacin in liposomes was expressed as encapsulation efficiency in μmol norfloxacin per μmol phospholipid.

6. Size distribution determination

Light scattering measurements were performed with a Coulter submicron particle size analyzer (Model N4MD). The liposome preparations were diluted 1:20 with filtered PBS (pH 5.0). The instrument settings used were as follows: temperature 25℃; viscosity 0.01 P; refractive index 1.333; scattering angle 90 ° ; run time 200 sec; range 0～3000nm.

7. Release from liposomes

After the separation of the free drug, the liposome was diluted in PBS 1:100 (pH 5.0). The liposome dispersion was transferred to many glass-top test tubes, and then immersed in a thermostated water bath (at various temperature) with a mild mechanical shaking. At timed intervals, samples were removed from the thermostated bath. Each sample was immediately extruded through filters under vaccum. Norfloxacin concentration was estimated spectrophoto-metrically at 275 nm. Each experiment was carried out three times. The release of norfloxacin from liposomes was recorded continuously for 48 hours.

8. Analytical methods

The amount of norfloxacin in each sample was determined by high-performance liquid chromatography. Pipemidic acid was used as an internal standard. The conditions were as follows: pump, model L-6000 (Hitachi); column, 4.6 x 250 mm Spheris C18 (Phase Separations Ltd., United Kingdom); mobile phase, 0.015M tetrabutyl ammonium iodide solution : acetonitrile (95 : 5 v/v); UV detector, model L-4000 (Hitachi); wavelength, 275nm; flow rate, 1ml/min. Peak areas were calculated by using a chromato-integrator, model D-2500 (Hitachi).

9. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was utilized to establish structure-property relationship of cornea at the molecular level. DSC thermograms were obtained by using a PERKIN-ELMER DSC7 differential scanning calorimeter. Sample sizes of the cornea were in the range of 6 ～9 mg and were sealed in a volatile type aluminum pan. Thermograms were recorded from 20 to 120 ℃ at a scan rate 5 ℃/min.

Results and Discussion

The liposomes bearing thermo-

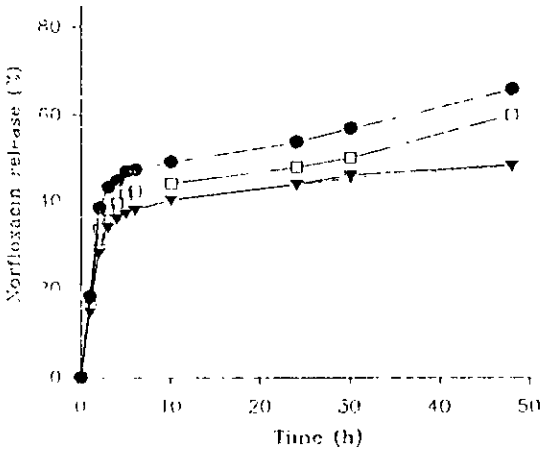

Fig.1. In-vitro release profiles of norfloxacin from DSPC liposome prepared using method B:● without hydrogel; □ NNMAA copolymer; ▼ AA copolymer; ▽ NIPAA copolymer (n=3)

sensitive polymeric hydrogels prepared by method B had a larger mean diameter and greater encapsulation efficiency than those prepared by the ordinary film method A. Fig. 1 shows the influence of thermo-sensitive hydrogels on norfloxacin release of DSPC liposomes. The rate of drug release from liposomes without hydrogels was greater than those containing hydrogels. The release profiles of liposomes prepared from DMPC and DPPC were similar to those for DSPC. In order to examine the interaction between

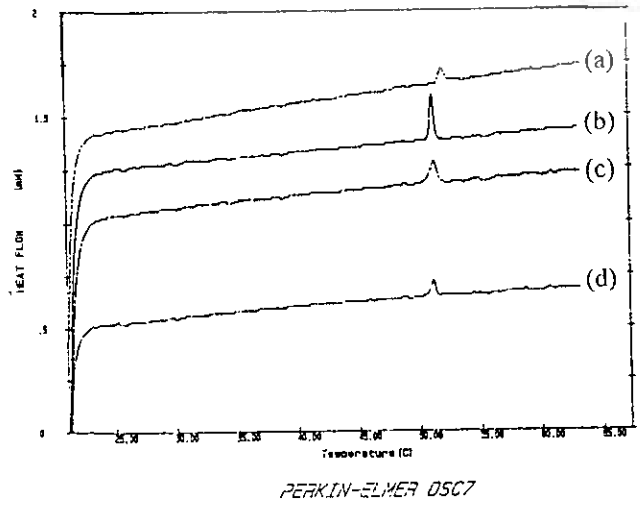

Fig.2. DSC thermograms of liposome prepared by combining with (b)NIPAA copolymer; (c)AA copolymer; (d)NNMAA copolymer and (a)without hydrogel (as control)

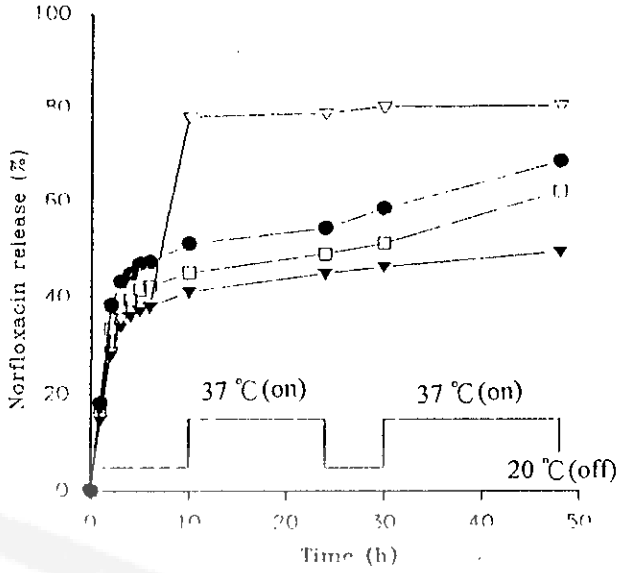

Fig.3. In-vitro release profiles of norfloxacin from DSPC liposome under "on-off" process:● without hydrogel; □ NNMAA copolymer; ▼ AA copolymer; ▽ NIPAA copolymer (n=3)

thermo-sensitive hydrogels and phospholipids, it was investigated by DSC. As shown in Fig. 2, the Tm values of liposomes incorporated with hydrogels shifted markedly towards lower temperatures in comparison with the absent of hydrogels. Therefore, addition of hydrogels would stabilize lipid bilayers and reduce leakage.

Fig. 3 shows the influence of temperature on the drug release. During the experiment, the temperature was change from 20 ℃ to 37 ℃. The drug release can only be controlled by liposome bearing copoly(NIPAA-AASE). Because copoly(NIPAA-AASE) has lower melting point (around 23.07 ℃) than the other copolymers. Moreover, there were no significant difference for drug release as repeating "on-off" (20 ℃ and 37 ℃) process.

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