行政院國家科學委員會專題研究計畫成果報告

Glutathione 眼用微脂粒製劑的製備及其體外豬眼角膜穿透性之研究

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一、中文摘要:

Glutathione 作為抗白內障藥物時，其療效受限於藥物的輸移欠佳進而影響眼用藥物的生體可用率。在本研究中，含 Glutathione 之微脂粒是採用改良自乙醇注入法之製造技術製備，已負載 Glutathione 之微脂粒，其穿透豬眼角膜之藥物輸移則以體外實驗進行評估，隨後角膜與微脂粒間的交互作用是以熱分析法來檢測。經由上述之實驗設計，體外穿透實驗的結果顯示，微脂粒包覆型藥物的經角膜輸移量顯著高於游離型藥物溶液，由於磷脂質的不同其大小依序是 DSPC>DPPC>DMPC，而且，相較於游離態之藥物溶液，微脂粒有較高的眼角膜內藥物負載量與較緩慢的藥物排除速率，微脂粒包覆能延長藥物在眼睛的留置時間而導致持續的治療效果。為了要傳輸藥物通過眼角膜，又不會造成眼角膜組織明顯的變化，採微脂粒包覆藥物的方式是極佳的選擇。

關鍵詞: Glutathione; 白內障; 經眼角膜輸移; 微脂粒; 熱分析

Abstract

The effectiveness of glutathione as an anticataract agent is limited by poor drug delivery and limited ocular bioavailability. In this study, liposomes containing glutathione have been prepared from different phospholipids using a modification of the ethanol injection technique. The in vitro transcorneal delivery of liposomal glutathione through pig's eyes were studied. Then, the interaction occuring between liposome and pig's cornea was determined by DSC. In the in vitro perfusion studies, the results show that the transcorneal flux of the GSH-loaded liposomes was significantly ($p<0.05$) higher than that of the free drug solution. For the three lipids investigated drug corneal penetration decreased in the order DSPC>DPPC>DMPC. Moreover, the loading of the drug in the cornea with liposome was higher, and that drainage of the liposomal GSH from the cornea was slower than for the solution form. Liposome-encapsulation is able to prolong the residence time of drug in eye and that results in sustained therapeutic effect. To transfer the drug through the cornea did not cause significantly alternation in structure of the tissue and to maintain the pharmacological activity, liposome-encapsulation of the drug is a good choice.

Keywords: Glutathione; Cataract; Transcornea delivery; Liposome; DSC

二.緣由與目的:

Glutathione (GSH) is a tripeptide composed of three amino acids: Cysteine, Glutamic acid and Glycine. In ophthalmology it has a defensive action

against cataract.(1) To achieve effective ophthalmic therapy, an adequate amount of ingredient must be delivered and maintained at its site of action within the eye.(2) The anatomical structure and the protective physiological process of the eye exerts a formidable defense against ophthalmic drug delivery. The most frequently used dosage forms i.e., ophthalmic solutions and suspensions, are compromised in their effectiveness by several limitations leading to poor ocular bioavailability.(3) The process of ocular drug uptake may be modified by the physical properties of the vehicle in which the drug is placed. Because liposomes are vesicle-like structures with a concentric series of alternating compartments of aqueous regions and phospholipid bilayers, they can entrap both lipophilic and hydrophilic compounds.(4,5) Administration of the liposome-encapsulated compounds has proved to be more effective than the same therapeutic regimen of drug solution.(6) The aim of this study therefore was to investigate the effect of liposome-encapsulation on the deposition of glutathione into the cornea of the pig's eye by using in vitro diffusion studies, as well as to try to explain the interaction occurring between liposomes and cornea.

三.結果與討論

Fig.1 shows the flux of drug diffusing through the cornea at different time intervals after the use of GSH-loaded liposome dispersions or a solution of the free drug. The straight line of the curve was defined as the steady-state penetration. Linear regression analysis was used to determine the flux of the drug for each sample. It can be seen that the transcorneal flux of the GSH-loaded liposomes was significantly ($p<0.05$) higher than that of the free drug solution. Ophthalmic liposomes could bring about preferential delivery of the entrapped drug through the cornea for maximum pharmacological action, as well as avoid systemic absorption from the conjunctive sac. For the three lipids investigated drug corneal penetration decreased in the order DSPC>DPPC>DMPC. The cornea was pretreated with liposomal and solution products for 12 hours to reach the steady state of drug loading, then transferred into GBR buffer. Fig. 2 shows the time-course of changes in the amount of drug remaining in the cornea. It can be seen that the loading of the drug in the cornea with liposome was higher and that drainage of the liposomal GSH from the cornea was slower than for the solution form. To elucidate the influence of the liposome on corneal structure, differential thermal analysis (DSC) was used to establish structure-property relationships at the molecular and macromolecular level. The uppermost trace in Fig. 3～5 was obtained from original cornea without treating anything as a control. As shown in Fig. 3, there were no noticeable changes in cornea structure by treating normal saline. However, the major transition of collagen in cornea at 57℃ was absent by treating GSH solution in Fig. 4. The thermograms of cornea treated with DMPC liposome-encapsulated GSH were similar to that treated by normal saline but shifted towards higher temperatures in comparison with the control. The thermograms which were obtained from DPPC and DSPC liposome are all the same to DMPC. The results

indicated that GSH without liposome-encapsulation may cause markedly alternation in cornea. To transfer the drug through the cornea did not cause significantly alternation in structure of the tissue, liposome-encapsulation of the drug is a good choice.

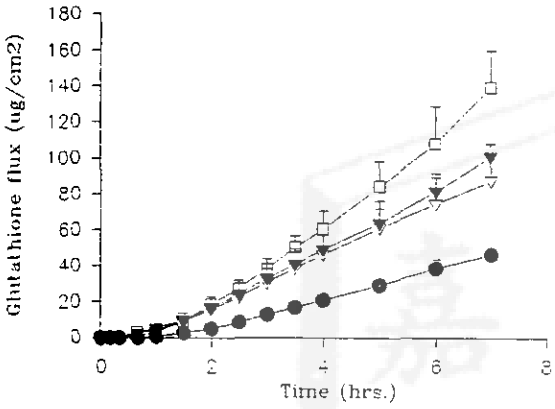

Fig.1 Effect of liposome lipid composition on the diffusion of glutathione through isolated pig cornea: □DSPC; ▼DPPC; ▽DMPC; ●glutathione buffer

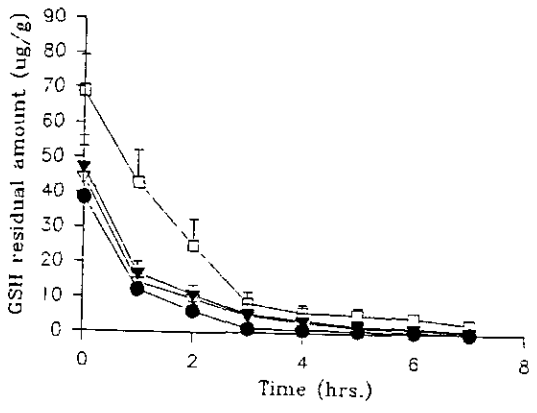

Fig.2 Residual corneal levels of glutathione after pretreatment for 12 h with □DSPC; ▼DPPC; ▽DMPC; ●glutathione buffer, then removal.

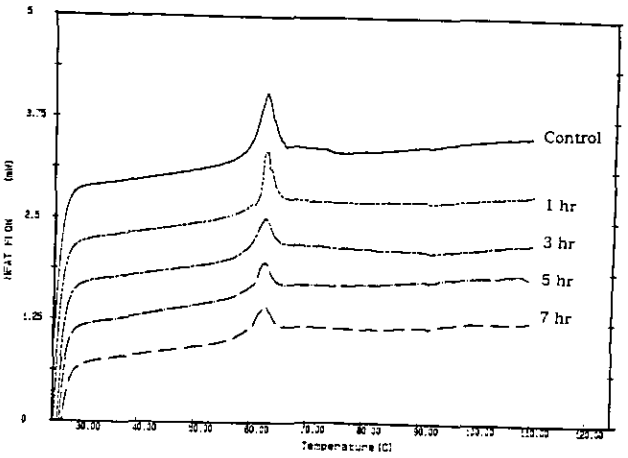

Fig.3 DSC thermograms of pig cornea after treatment with normal saline for various time course.

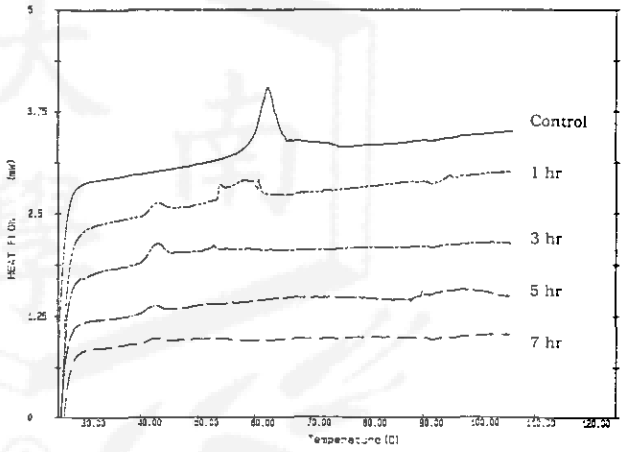

Fig.4 DSC thermograms of pig cornea after treatment with glutathione buffer for various time course.

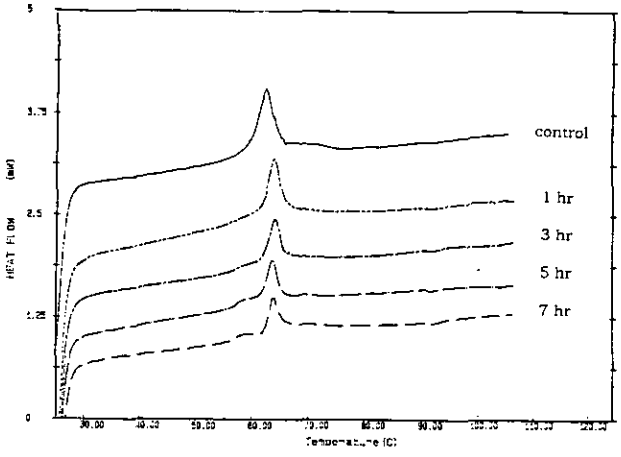

Fig.5 DSC thermograms of pig cornea after treatment with glutathione-DMPC liposome for various time course.

四.計畫成果自評

本研究同時針對藥物輸移與組織影響等雙重考量進行評估，研究成果對市面使用已久之眼用處方，不論是處方改良亦或臨床使用，有革命性的新發現，極具參考價值。

五.參考文獻

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