行政院國家科學委員會專題研究計畫成果報告

The Preparation of Four Quinolone-loaded Liposomes and Their in Vitro Comparative Study of Ocular Permeation in Pig's Eye

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一.中文摘要:

為了能長時間在作用部位提供並維持適當之藥物濃度，吾等嘗試發展 norfloxacin, enoxacin, ofloxacin 與 ciprofloxacin 供眼部局部投藥之微脂粒藥物處方;為了將藥物覆載於微脂粒,本研究採行兩種製造方式。結果顯示,不論何種 fluoroquinolone 包覆於 DMPC 之脂雙層中,採用乙醇注入法併合冷凍乾燥程序所得之微脂粒,其粒徑與包埋率會高於傳統的薄膜法之微脂粒;在體外角膜擴散實驗中,經微脂粒包覆之藥物其角膜穿透速率遠低於游離態藥物,在四種 fluoroquinolone 藥物中,enoxacin 因油水分配係數較高而有較大之游離態藥物角膜穿透速率。雖然微脂粒包覆 fluoroquinolones 後會降低藥物之眼角膜穿透性,但卻可增加藥物在角膜內之蓄積量。

關鍵字: 微脂粒; 經角膜; 豬眼睛; Fluoroquinolone;

1.Abstract:

In order to provide and maintain an adequate concentration of drug at the side of action for a prolonged period of time, we attempt to develope liposomal drug formulations for topical modes of ocular administration of norfloxacin, enoxacin, ofloxacin and ciprofloxacin. To load these drugs in liposomes, two preparation procedures were carried out. The results showed that no matter which fluoroquinolone for the DMPC bilayer matrix, the liposomes prepared by combining the ethanol injection method with freeze drying had higher particle size and encapsulation capacity than those prepared by the ordinary film method. In the in-vitro corneal perfusion study, the transcorneal flux of the drug-liposomes dispersion were significantly less than that of the drug solution. Among the fluoroquinolones, enoxacin has a higher corneal penetration rate of the free drug solution owing to its higher oil / water partition coefficient. Although liposome-entrapped fluoroquinolones were transferred through the cornea more slowly than the free drug, accumulation in the cornea was greater for the lipospome-entrapped drug.

keywords: Liposome; Transcorneal; Fluoroquinolone; pig's eye;

2.Introduction:

The quinolones are a group of antibacterial agents which have a widespread action against Gram negative microorganisme (1). Substances of this class constitute low soluble heterocyclic carboxylic acids like the well known compounds norfloxacin, ofloxacin, enoxacin and ciprofloxacin. In ophthalmology they are used to treat pyogenic inflammations of the eye (2~5). A major problem in ophthalmic drug delivery is poor ocular bioavailability and the limited rate of corneal absorption (6). The use of antibiotic delivery devices should achieve increased drug concentrations at the site of infection resulting from targeting of antibiotic to the infected tissues (7). Liposomal devices may provide a mean of improving the bioavailability of drugs that are poorly water soluble, like fluoroquinolones (8). We have developed liposomal drug formulations for topical modes of ocular administration of

norfloxacin (9). In the present work, the entrapment of the other fluoroquinolones in lipid vesicles and in vitro comparative study of ocular permeation in pig's eye were studied.

3.Results and Discussion

Particle size and encapsulation efficiency for method A and B are compared in Table 1~4. No matter which fluoroquinolone for the DMPC bilayer matrix, the liposomes prepared by combining the ethanol injection method with freeze drying had higher particle size and encapsulation capacity than those prepared by the ordinary film method. This results were the same as our previous report, i.e., fluoroquinolones which are amphiphilic drugs will dissolve in water and be encapsulated within the aqueous compartment of liposomes prepared by the film method under pK_{al}. However, for lipoosomes prepared through the ethanol injection and freeze-drying processes, these drugs were not only encapsulated within the liposomes cavity but also bound to the lipid bilayer. Thus, the drug encapsulation inside liposomes was increased. When fluoroquinolones were entrapped in liposomes prepared from DPPC or DSPC, the increased chain length of the lipids resulted in a significant increase in particle size but the efficiency of encapsulation was reduced. Fig. 1~3 show the amount of enoxacin, ofloxacin and ciprofloxacin, respectively, diffusion through the cornea of pig's eye at different time intervals after the use of a drug-loaded liposome dispersion or a solution of the free drug in buffer solution. It can be seen that the transcorneal flux of the drug-liposomes dispersion were significantly less than that of the drug solution. Among the fluoroquinolones, enoxacin has a higher corneal penetration rate of the free solution owing to its higher oil / water partition coefficient. Moreover, the liposome prepared from DSPC resulted in a lower corneal penetration rate than those prepared from the

Particle size distribution and encapsulation of norfloxacin in liposomes of various lipid composition and different procedures

Lipid composition	Particle size (nm)	Encapsulation efficiency (μ mol drug/μ mol lipid)
DMPC[a]	250	0.010±0.002
DMPC[b]	1090	0.159±0.007
DPPC[b]	1410	0.149±0.008
DSPC[b]	2230	0.139±0.005

Table 2
Particle size distribution and encapsulation of enoxacin in liposomes of various lipid composition and different procedures

Lipid composition	Particle size (nm)	Encapsulation efficiency (μ mol drug/μ mol lipid)
DMPC[a]	320	0.016±0.005
DMPC[b]	1130	0.136±0.010
DPPC[b]	1230	0.111±0.007
DSPC[b]	1600	0.094±0.009

Table 3
Particle size distribution and encapsulation of ofloxacin in liposomes of various lipid composition and different procedures

Lipid composition	Particle size (nm)	Encapsulation efficiency (μ mol drug/μ mol lipid)
DMPC[a]	340	0.016±0.007
DMPC[b]	679	0.157±0.005
DPPC[b]	1390	0.152±0.007
DSPC[b]	1700	0.135±0.006

Table 4
Particle size distribution and encapsulation of ciprofloxacin in liposomes of various lipid composition and different procedures

Lipid composition	Particle size (nm)	Encapsulation efficiency (μ mol drug/μ mol lipid)
DMPC[a]	280	0.011±0.003
DMPC[b]	860	0.163±0.009
DPPC[b]	1130	0.156±0.010
DSPC[b]	1470	0.142±0.008

a.The liposomes were prepared by method A.
b.The liposomes were prepared by method B.
c.Both methods were carried out at room temperature with an initial drug-lipid ratio of 0.2.

other two lipids. Although liposome-entrapped fluoroquinolones were transferred through the cornea more slowly than the free drug, accumulation in the cornea was greater for the lipospome-entrapped drug.

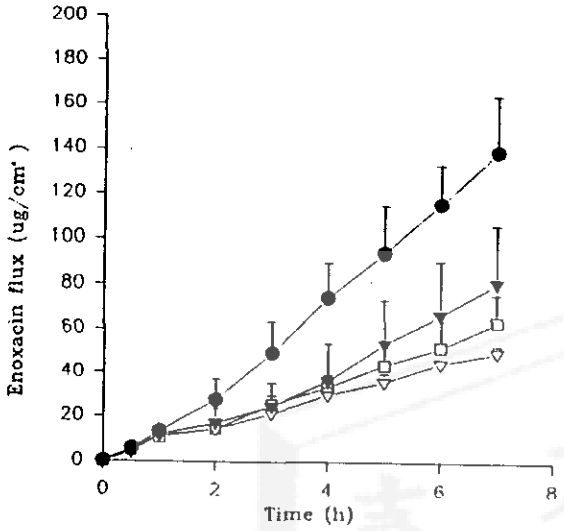

Fig.1. Effect of liposome lipid composition on the diffusion of enoxacin through isolated pig cornea: □ DMPC; ▼ DPPC; ▽ DSPC; ● enoxacin buffer.

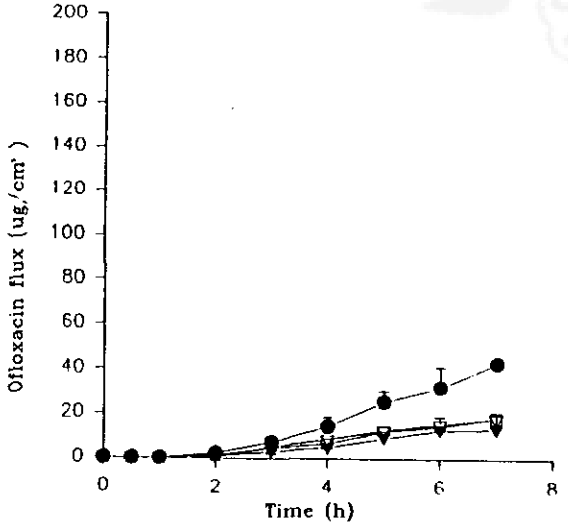

Fig.2. Effect of liposome lipid composition on the diffusion of ofloxacin through isolated pig cornea: □ DMPC; ▼ DPPC; ▽ DSPC; ● enoxacin buffer.

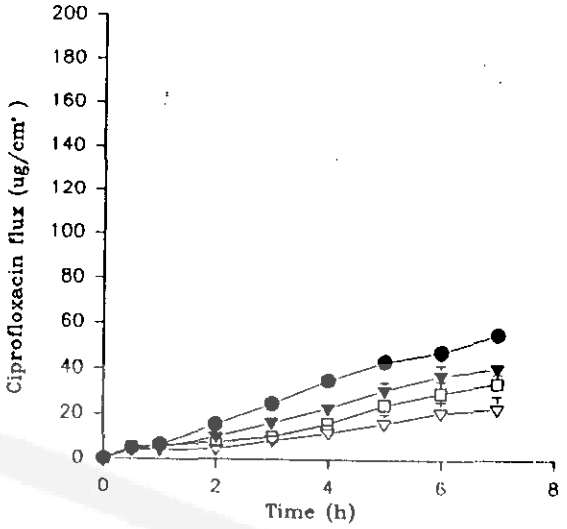

Fig.3. Effect of liposome lipid composition on the diffusion of ciprofloxacin through isolated pig cornea: □ DMPC; ▼ DPPC; ▽ DSPC; ● enoxacin buffer.

4.計畫成果自評

本計畫是針對fluoroquinolone類抗菌劑中norfloxacin以外之enoxacin,ofloxacin及ciprofloxacin等藥物,採行微脂粒包覆並進行豬眼角膜的體外穿透試驗。目前已初步完成此類藥物之微脂粒處方探討,研究之進行與成果大致符合所預期的目標;此計畫之研究成果有助於說明微脂粒覆載fluoroquinolone類藥物在眼部的配達情形,並對爾後發展眼用微脂粒製劑提供了製造與處方體外篩選之依據。

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