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行政院國家科學委員會專題研究計畫成果報告
Enoxacin 微脂粒包覆處方之經皮離子電透入輸藥法研究

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Abstract (英文摘要)

The major purpose of this work was to study the effect of various liposome formulations on transdermal iontophoretic transport of enoxacin through excised rat skin. The electrochemical stability of these liposomes was also evaluated. The enoxacin encapsulation percentage was significantly enhanced after 6 h incubation in electric field, whereas the fusion of enoxacin liposomes was inhibited by application of electric current. The results of iontophoretic transport showed that the permeability of enoxacin released from liposomes was higher compared with that of free drug form. The iontophoretic permeability of enoxacin released from liposomes increased with a decrease in fatty acid chain lengths of phospholipid, which can be due to the various phase transition temperature of phospholipids. Incorporation of charged phospholipid in liposomes resulted in an alteration of transdermal behavior of enoxacin: the iontophoretic permeation as well as enoxacin amount partitioned in skin were greatly reduced after incorporation of stearyl amine in liposomes, which can be attributed to the competitive ion effect. The stratum corneum-based liposomes showed the highest amount of enoxacin partitioned into skin depot. The results of employing cathodal iontophoresis on negative charged liposomes suggested that liposomal vesicles or phospholipids may carry enoxacin into deeper skin strata via follicular route.

Keywords: Enoxacin; Transdermal iontophoresis; Liposome; Stability

1. Introducion (緣由與目的)

Enoxacin is a newly synthesized antimicrobial fluoquinolone structurally related to nalidixic acid. It has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria [1]. However, several drug interactions and adverse drug effects after oral administration have limited its use. Some GI symptons, including anorexia, nausea, vomiting, diarrhea and metallic taste have been reported; the interaction between enoxacin and antiacids containing magnesium or aluminum can result in inhibition of its oral bioavailability [2]. Moreover, the plasma half-life of enoxacin in blood is only 3-6 h [3], suggesting frequent dose is needed. Accordingly, the transdermal drug delivery system (TDDS) may be suitable for enoxacin in order to reduce the adverse effects caused by oral administration and to prolong the therapeutic duration. The feasibility of enoxacin transdermal iontophoretic delivery for systemic and topical application have also been investigated and reported in previous publications [4].

Liposomes are microscopic vesicles consisting of membrane-like phospholipid bilayers. Because of their entrapping ability, liposomes are considered for use as drug-carrying systems. An increasing evidence has shown that the liposome-based drug delivery system is not only suitable for intravenous delivery, but also suitable for topical applications [5]. Recently, the

combination of iontophoresis and liposome technology in drug delivery has been reported [6]: the liposome may act as a drug reservoir to continuously release drug to skin surface; the iontophoresis may pump released drug into dermis for systemic or topical application. The combination of both systems may provide a constant rate of drug input without constraints of traditional diffusion-based transdermal devices such as molecular size and lipophilicity. Nevertheless, in order to optimize this technique, the effect of various liposome formulations on transdermal iontophoretic delivery has to be studied in a systematic way.

There are three major goals in the present study. The first goal is to assess the electrochemical stability of enoxacin and its liposome formulations under application of electric current. The second goal is to study the effect of various liposome formulations on the enoxacin iontophoretic delivery through rat skin. Those liposome formulations include: phospholipids with various chain lengths as well as phospholipids with positive charge, negative charge and neutral charge. Finally, a liposome formulation based on the composition of stratum corneum was prepared to compare the transdermal iontophoretic delivery of enoxacin from phospholipid-based liposome and non phospholipid-based liposome.

2. Results and Discussion (結果與討論)

2.1. Electrochemical stability of liposomes

It is a general phenomenon that pore widening in phospholipid bilayers can be induced by the application of electric field pulses, resulting in the irreversible breakdown of liposomes and leakage of drugs from liposomes [7]. However, the previous study also suggests that electric field may inhibit pore opening and stabilize pores of lipid bilayers in certain conditions [8]. In the present study, the encapsulation percentage of enoxacin was similar with or without applying electric current, and increased in some formulations. Previous report has indicated that the opening and close of liposome pores depends on the forces acting on the pore boundary [8]. In principle, the effective values of the energy (r_{eff}) and of the lateral tension (σ_{eff}) acting on the pore boundary can be either negative or positive depending on the electric fields. If both of them are negative, the pore radius will keep stable equilibrium. In this study, the enoxacin liposomes in the electric field may fit with this condition, contributing to stabilization or sealing of the liposomal pores. However, the mechanism by which this occurs is not completely understood, further studies are needed to explore this mechanism. Another explanation for the observed electrochemical stability of enoxacin liposomes is that in order the pore widening and irreversible breakdown of the membranes to happen, a high enough electric field pulse should be introduced [9]. The 0.5 mA current density used in our study may be too low to induce the rupture of liposomes.

Except for the SC-based liposomes, the vesicle size of enoxacin liposomes with 0.5 mA current treatment was smaller than those without current treatment after 6 h incubation in pH 5 buffer. This indicates that electric field inhibited the fusion of phosphatidylcholines based liposomes. Electric fields can cause motion of the electrolyte as well as the lipid. Moreover, the electric field-induced movement may have a similar effect on vesicle size as sonication [10]; large vesicle liposomes subjected to sonication often reduce vesicle size and form small vesicle liposomes [10,11]. The results are consistent well with the above inferences and previous findings.

The SC liposomes have been used as a model to predict the lipid fluidizing

effect of SC in skin [12,13]. In the present study, SC liposomes showed minor changes either in enoxacin encapsulation percentage or in vesicle size after treatment of electric current. The results suggest that the SC structure of skin may remain intact after transdermal iontophoretic treatment. The results are also in accordance with our previously histological findings that there was no significant change in anatomical structure of SC for rat skin after in vitro transdermal iontophoresis [14].

2.2. Comparison of iontophoretic enoxacin permeation between liposome-encapsulated form and free drug form

It shows that the iontophoretic permeation of enoxacin across rat skin was significantly increased after encapsulation of enoxacin in liposomes. This phenomenon can be well explained by the following inferences and findings: the addition of phosphatidylcholine to dermal dosage forms has been reported to have advantages on the enhancement of transdermal absorption [15]. The liposomes may adsorb and fuse with the surfaces of the skin, which can alter the lipid barrier and result in more permeable structure. It is also shown that mixing of liposomes with intercellular lipid domains in the SC may contribute to the enhancement of skin permeability [16-18]. Moreover, the observed fusion of phospholipids on the skin surface may facilitate accumulation of water in the SC [10,19], which subsequently contribute to the increase of conductivity in skin during iontophoresis and enhancement of drug permeation [14,20].

It shows that the amount of enoxacin partitioned in skin was lower for the enoxacin from DMPC liposomes comparing to that of free drug. The results indicate that the free drug was more easily accumulated whereas the enoxacin from DMPC-based liposomes was not as easily accumulated in the skin. These results are consistent with permeation results since the formation of skin depot can be reduced when total drug permeation increases during iontophoresis [21].

2.3. Transdermal iontophoretic delivery of enoxacin from liposomes with various fatty acid chain lengths

The rate of transdermal drug delivery depends on the rate of drug release from formulation to skin surface and the rate of drug permeate through skin. Both processes can affect rate of drug delivery and the slower one will be the rate-limiting step. In order to identify the predominant rate process, the permeation of enoxacin through skin membrane and cellulose acetate membrane were compared. The permeation study clearly showed that the permeation rate of free enoxacin through skin was dramatically lower than that of enoxacin through cellulose membrane. This results suggest that the rate-limiting of transdermal process for enoxacin was the skin permeation step rather than the drug release step. It showed that the release rate of enoxacin was significantly reduced after encapsulated with liposomes. The rate of enoxacin released from liposomes (especially DPPC and DSPC) approached to the skin permeation rate of enoxacin released from liposomes, demonstrating that the rate-limiting process has gradually shifted to the drug release step.

Since the predominant rate process for the delivery has gradually shifted from permeation step to drug release step, the effect of liposome formulations on drug permeation become increasingly important. It shows that the permeation rate of enoxacin from DMPC liposome is higher than that of DPPC and DSPC based liposomes. This phenomenon can be explained by the phase transition temperature of various phospholipids. The temperature (37℃) used in the skin permeation experiments was higher than

the phase transition temperature of DMPC (23℃) where the hydrocarbon chains are randomly oriented and fluid (liquid crystalline phase); whereas the temperature was below the transition temperature of DPPC (41℃) and DSPC (55℃) where the hydrocarbon chains are fully extended and closely packed (gel phase) [22]. The enoxacin may diffuse more easily through the random hydrocarbon chain than the closed packed gel phase [23]. Accordingly, the DMPC based liposomes showed the highest permeation rate and relatively lower enoxacin permeation rates of DPPC and DSPC liposomes were observed.

2.4. Transdermal iontophoretic delivery of enoxacin from charged liposomes

Since the adsorption of liposomes onto the skin can be due to physical (e.g., electrostatic) forces, the use of charged liposomes may alter this interaction [6]. In this study, stearyl amine and dicetyl phosphate were used as charged components to produce positive and negative liposomes, respectively. The iontophoretic permeation of enoxacin was dramatically reduced after incorporation of stearyl amine in the liposomes. The low enoxacin permeation rate of positive liposomes may be attributed to the competition of stearyl amine and positively charged enoxacin (pka=7.5) for the applied current density. Due to the smaller molecular size of stearyl amine relative to enoxacin, most current density are expected to be carried by stearyl amine released from positive liposomes during iontophoretic delivery [24]. In order to verify this inference, different concentrations of stearyl amine were added in liposomes and a series of iontophoretic permeation experiments were performed. Both the enoxacin permeation rate and amount partitioned in skin decreased following the increase of stearyl amine, which is consistent with competitive ion effect as described previously.

The cathodal iontophoresis for negative liposomes of enoxacin were utilized in the present study to demonstrate how deep liposome may penetrate into the skin. The cathodal iontophoresis for free enoxacin at pH 5 have shown no detectable amounts of enoxacin transported across the skin. It indicates that enoxacin itself with positive charge can not permeate across the skin during cathodal iontophoresis. However, there was low but detectable amounts of positively charged enoxacin observed in receptor after cathodal iontophoresis of enoxacin-encapsulated negative liposomes. Previous reports have shown that neither liposomes nor phospholipid molecules diffuse across intact skin [19]; some reports have indicated that liposomal drug transport into strata may occur via a follicular route [25]. The above experimental results and previous findings both support that the liposomal vesicles or phospholipids may carry enoxacin into deeper skin strata where the receptor sites reside. Moreover, the use of furry rat skin with higher number of hair follicles in the present study may amplify this effect. The results also agree well with the previous study that the liposomes with negative charges are more efficient than other vehicles tested for delivering of drugs through the follicular route [10].

2.5. Transdermal iontophoretic delivery of enoxacin from liposomes with CH and SC

The iontophoretic permeation of enoxacin from DMPC/CH and SC liposomes was lower than that from DMPC liposomes. This results may be attributed to that the CH-incorporated phospholipids are more cohesive and compressible in the electric field and prevents the release of drug from liposomes [26]. Moreover, the incorporation of CH into drug-laden liposomes may hamper the penetration

of lipid vesicles into the skin [27]. Among the formulations studied, It shows that the enoxacin from SC-based liposomes had the highest amount of drug partitioned into the skin reservoir. Previous results has observed that the miscibility of SC liposomes with the lipid layer of skin may facilitates the release of drug from SC liposomes and transport of drug into skin [28]. Moreover, the PA in SC liposomes may act as a penetration enhancer and modify the lipid components of skin [29]. In order to verify this point, an iontophoretic experiment on free enoxacin permeation was performed for the skin pretreated with 0.529 % (w/v) PA (the same dose with the PA concentration in SC liposomes). The results showed that both iontophoretic flux and enoxacin in the skin increased. Although the permeation rate of enoxacin from SC based liposomes was one half of the free drug form after PA pretreatment, this may be due to the presence of CH in SC liposomes preventing the release of enoxacin from liposomes. There was no significant difference ($p > 0.05$, t-test) between the enoxacin amount in skin reservoir of SC liposomes and free drug after PA pretreatment, which suggests PA has a significant effect on the partition of enoxacin in skin reservoir for both free enoxacin and enoxacin from SC liposomes.

Among the liposomes with phosphatidylcholines, DMPC/CH liposomes showed the highest amount of enoxacin in the skin depot. However, no significant difference ($p > 0.05$, ANOVA test) was observed among the enoxacin amount partitioned in skin for DMPC, DPPC, DSPC and DMPC/CH liposomes despite the enoxacin iontophoretic permeation of these formulations were quite different. This can be due to that the skin reservoir has been saturated with enoxacin while administrating phosphatidylcholine-containing liposomes, thus the amount of enoxacin in skin for these liposomes was restricted to be the level of 320-340 mg/g as its maximum capacity.

3. 計劃成果自評

本論文已投稿至 Journal of Controlled Release 並已 revised，基本上於計劃報告書上所條列之試驗均已完成，且亦達到理想中之成果。

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Table 1. Lipid Constituents of the Various Liposomes

No.	Liposome code	Composition (molar ratio)
1	DMPC	DMPC
2	DPPC	DPPC
3	DSPC	DSPC
4	Positive (+)	DMPC : stearyl amine = 9 : 1
5	Negative (−)	DMPC : dicetyl phosphate = 9 : 1
6	DMPC/CH	DMPC : CH = 7 : 3
7	SC (stratum corneum)*	ceramide : CH : PA : CS = 4 : 2.5 : 2.5 : 1*

* The ratio of SC liposome composition is weight ratio.
DMPC, dimyristoyl-L-α-phosphatidylcholine; DPPC, dipalmitoyl-L-α-phosphatidylcholine; DSPC, distearoyl-L-α-phosphatidylcholine; CH, cholesterol. Each value represents the mean± S.D.