行政院國家科學委員會專題研究計畫　成果報告

用於脂質調節治療之 statins 類藥物經皮輸移研究

計畫類別：個別型計畫
計畫編號：NSC91-2320-B-041-013-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：嘉南藥理科技大學藥學系

計畫主持人：林恆弘

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 92 年 10 月 30 日

行政院國家科學委員會補助專題研究計畫 **■ 成 果 報 告**
□期中進度報告

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計畫類別：■ 個別型計畫　　□ 整合型計畫
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共同主持人：
計畫參與人員：

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執行單位：嘉南藥理科技大學 藥學系

中　華　民　國　　92　年　　10　月　　29　日

中文摘要

Fluvastatin 是第一個合成之 HMG-Co A 還原酶抑制劑，本研究選用 fluvastatin 爲模式藥物來設計用於脂質調節治療之經皮輸藥系統。Fluvastatin 加入幾丁質親水膠體、脂肪醇丙二醇混合基劑與 UCH 基劑來製備軟膏，經檢測軟膏之理化性質與藥物釋離其結果指出，實驗中之軟膏是呈現假塑性流體的特性，且藥物自軟膏釋出速率的快慢依序是幾丁質親水膠體>脂肪醇丙二醇混合基劑>UCH 基劑，藥物的釋出速率與軟膏的黏稠度成反比之關係。藉由熱卡式分析儀之檢測顯示，溶解之 fluvastatin 與所在之軟膏基劑組成分間並無交互作用產生。體外皮膚穿透試驗結果顯示，僅有少量的 fluvastatin 能穿透老鼠皮，界面活性劑可作爲穿皮促進劑來增加該藥之經皮吸收，綜合上述之實驗結果，將有助於發展其他脂質調節的藥物之經皮輸藥系統。

ABSTRACT

Fluvastatin is the first synthetic HMG-Co A reductase inhibitor. This study selected fluvastatin as a model drug to design a transdermal delivery system for lipid-modifying therapy. Fluvastatin was incorporated into chitosan hydrogel，FAPG base and UCH base to prepare ointments. The physicochemical properties of the ointments and the release profile of fluvastatin were investigated. The results indicated that ointments are referred to as pseudoplastic flow and the increase in the drug release rate for the ointments conformed to the following order: chitosan > FAPG > UCH. The release rate of fluvastatin was inversely proportional to ointment viscosity. By differential scanning calorimetry (DSC) measurement, no interaction was found to occur between ointment base and the soluble fluvastatin. In *in vitro* skin perfusion studies, only trace amounts of fluvastatin permeated through the rat skin. Surfactants were used as penetration enhancers to increase the percutaneous absorption of fluvastatin. The above results will be helpful to possible development of the other lipid-regulating drug transdermal delivery systems.

Key words：Fluvastatin； FAPG； chitosan hydrogel； UCH； transdermal delivery； rat skin

INTRODUCTION

Cardiovascular disease is a major cause of death in industrialized countries and places a large burden on society in term of healthcare resources and lost productivity. There are several of known cardiovascular risk factors including abdominal obesity, hypertension, dyslipidaemia, etc. [1-5] Fluvastatin is the first synthetic HMG-Co A reductase inhibitor to be approved for clinical lipid-modifying therapy. [6-9] However, fluvastatin is subject to first-pass metabolism, and plasma half-life of the drug is approximately 30 minutes. The effect of food on the pharmacokinetics of fluvaststin has demonstrated marked reductions in the rate of bioavailability—from 40% to 60%.[10] In order to achieve and maintain an adequate concentration of drug at the side of action for a prolonged period of time so as to improve the therapeutic efficiacy, in this project, we attempt to develop a transdermal delivery system by the use of ointment dosage forms. The type of ointments include chitosan hydrogel、FAPG、UCH and so on. [11-13] In the study, we investigate the effect of variation in the composition and preparation condition of ointments on the viscosity, adhesive of products and drug release. The physicochemical properties of ointments were determined by Cone and Plate viscometer, texture analyser and differential calorimetry. The effect

of ointment physicochemical properties on the release of fluvastatin from ointment base and the percutaneous penetration through rat skin were discussed by *in vitro* studies. In the *in vivo* studies, we will investigate the effect of ointments' composition on the transdermal delivery of fluvastatin in rats. The distribution and elimination kinetics of fluvastatin in rats will be obtained following intravenous and transdermal administration.

MATERIALS AND METHODS

Materials Fluvastatin was purchased from Norvartis Pharma AG (Basle, Switzerland). Chitosan was purchased from Aldrich Chemical Co. (Steinheim, Germany). Lactic acid and polyoxyethylene sorbitan monooleate (Tween 80) was purchased from E. Merck Co. (Darmstadt, Germany). Sodium lauryl sulfate was purchased from Wako Chemical Co. (Osaka, Japan). Ethylenediamine tetraacetic acid disodium salt (EDTA), benzalkonium chloride hydrate and *p* -hydroxy-biphenyl were purchased from Sigma Chemical Chemical Co. (St. Louis, USA). All other chemicals were of analytical grade.

Skin Preparation Rat skins were harvested from male Wistar rats weighing 230 - 270 g. After being sacrificed in a CO_2 chamber, the abdominal hair was removed with electric clippers. A 3 x 3 cm^2 section of denuded skin with a thickness of 0.65 - 0.75 mm was excised immediately before the permeation experiment.

Preparation of Fluvastatin Ointments Fluvastatin ointments were prepared according to the formula in Table 1; chitosan powder was suspended in double distilled water and lactic acid was added while stirring the sample. The other samples were prepared as above by adding 0.3 g EDTA / or 0.2 g absorption enhancers respectively. Fluvastatin was added to ointments to give a concentration of 0.1 %.

Determination of Viscosity Viscosity studies were done in the 24 hours after ointments preparation. By using a Cone and Plate Viscometer (Brookfield Digital Viscometer, Model DV-II), 0.5 g of ointment was placed in the sample cup of the viscometer and allowed to stand for 1 h to reach 37 ℃. To obtain stable display readings, viscosity measurements were made 30 s later.

Differential Scanning Calorimetry (DSC) DSC thermograms were obtained by using a Perkin-Elmer DSC7 differential scanning calorimeter. Sample sizes were in the range of 1.5 ～ 3 mg and were sealed in a volatile type aluminum pan. Thermograms were recorded from 30 to 100 ℃ at a scan rate 5 ℃ / min.

In Vitro Release Experiment Vertical - type diffusion cells were similar to the apparatus of the Franz diffusion assembly.[16] One side of the cell was filled with fluvastatin ointments; this side (donor cell) was separated with the Visking seamless cellulose tubing C-110 membrane from the other side (receptor cell), which was filled with phosphate buffer (pH 7.4, containing 20 % w/w PEG 400). The area available for diffusion was 2.43 cm^2. The receiver compartment was agitated by a magnetic stirrer at 700 rpm. The apparatus was maintained at 37±0.5 ℃ with a water jacket. An aliquot (0.5 ml) of the sample was taken from the receiver compartment at appropriate times, and the concentration of fluvastatin was determined by the spectrophotometer (Hitachi 2000 Hitachi Seisakusho Co., Ltd.) at 236 nm. After each sampling, the same volume of fresh phosphate buffer was added to the receiver compartment to keep the volume constant. The release of Fluvastatin from ointment was recorded continuously for 24 h.

Determination of Permeation through Rat Skin The diffusion cells were used in a way similar to the apparatus of the release experiment. The excised rat skin was used as the membrane for skin permeation experiments. The skin was positioned with the stratum corneum facing the donor cell, and the dermis side was in contact with the receiver compartment. Samples (0.5 ml) were withdrawn from the receiver cell at appropriate time and the concentration of fluvastatin was determined by HPLC.

Measurement of Fluvastatin Concentration in Rat Skin The amount of fluvastatin remaining in the rat skin after the removal of ointments was determined after careful removal of the skin from the diffusion cells and rapid washing several times with distilled water. Then, the skin was weighed and homogenized in 3 ml lactic acid solution (pH 3.0) by means of a tissue homogenizer. The homogenate was centrifuged and the supernatant was filtered, then analyzed by HPLC.

Analytical Methods The amount of fluvastatin in each sample was determined by HPLC. *p* - Hydroxy-biphenyl 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., U.K.); mobile phase pH 5.2 phosphate and citric acid buffer solution, methanol (7 : 3 v/v); UV detector, model L-4000 (Hitachi); wavelength, 236 nm; flow rate, 0.7 ml/min. Peak areas were calculated by using a chromatointegrator, model D-2500 (Hitachi).

Measurement of Apparent Partition Coefficient The degree of fluvastatin partitioning between 1-octanol (oil phase) and pH 4.0 lactic acid aqueous solution (water phase) was determined as follows: 4 ml of fluvastatin aqueous solution (0.10 wt % fluvastatin in pH 4.0 lactic acid solution) and 4 ml of 1-octanol were placed in a glass-stoppered test tube and shaken in a water bath at 37 ℃ for 2 d. The mixture was centrifuged at 3000 rpm for 10 min. The fluvastatin concentration in water was determined by the spectrophotometer at 260 nm.

RESULTS AND DISCUSSION

The rheological properties of ointments are characterized as shown in Fig 1. The viscosity decreases with increase in shear rate and time. The time-dependent behavior of ointments is referred to as pseudoplastic flow and exhibit shear thinning. The *in vitro* release of fluvastatin from ointments is shown in Fig.2. The results indicate that the increase in the drug release rate for the ointments conformed to the following order: chitosan > FAPG > UCH. The mean regression coefficient was calculated and the highest regression coefficient was obtained with the Higuchi model. The release rate was inversely proportional to viscosity of ointments. It has been known that the factors affecting drug release from chitosan ointments involve drug diffusion, hydrogel erosion, swollen and so on. In this work, the release mechanism was concluded to be a complex function of the physicochemical properties of base and was mainly related to the viscosity of ointments rather than the pH value in base. In the *in vitro* skin perfusion studies, after the use of fluvastatin ointment or free drug buffer solution for 48 hours, only trace amount of fluvastatin had permeated through the rat skin. Correlative to the release studies on those results, the transdermal permeation rate is much lower than the release rate of fluvastatin from all of three ointments. Briefly, the permeation of fluvastatin through the skin is the rate-determining step for percutaneous absorption of fluvastatin from ointments. However, it can be seen that fluvastatin cannot permeate through the rat skin in significant amount after application. For the reason,

absorption enhancers such as benzalkonium chloride and sodium lauryl sulfate were applied in order to increase the absorption of fluvastatin. The results show that absorption enhancers exert a significant effect on skin permeation of fluvastatin. The DSC thermogram of ointments, normalized and adjusted to the same baseline, is given in Fig.3. The thermogram obtained according to the ointment base itself was the same as the one from the base containing fluvastatin. DSC measurement showed that there was no interaction between components of ointment and the incorporated drug.

ACKNOWLEDGMENT

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Legend

Fig.1 Rheological properties of UCH ointment containing fluvastatin

Fig.2 Release profile of fluvastatin from various ointments.

Fig.3 DSC thermograms of UCH ointment containing fluvastatin.（upper trace: UCH ointment containing fluvastatin; low trace:UCH base alone）

Fig. 1

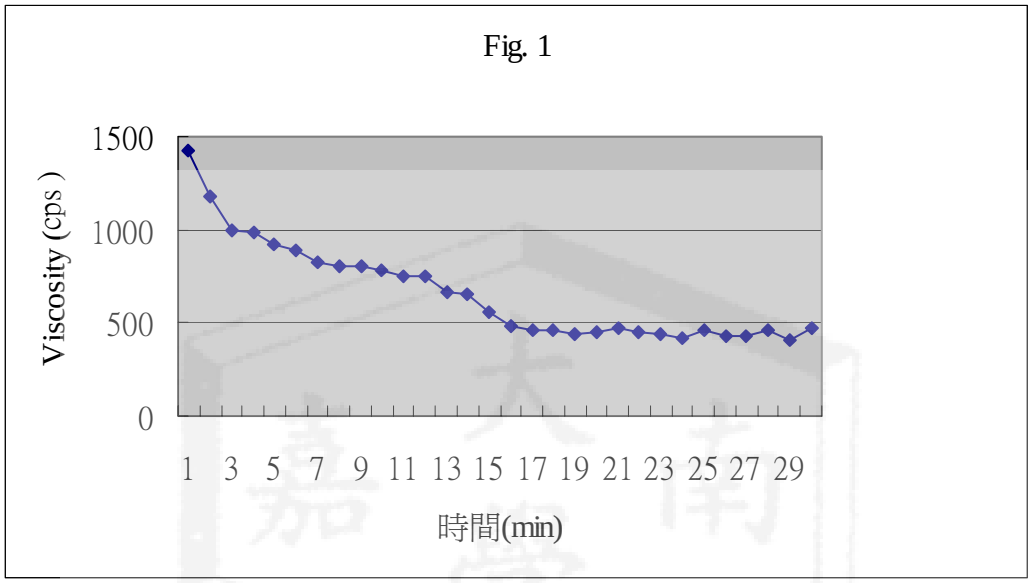
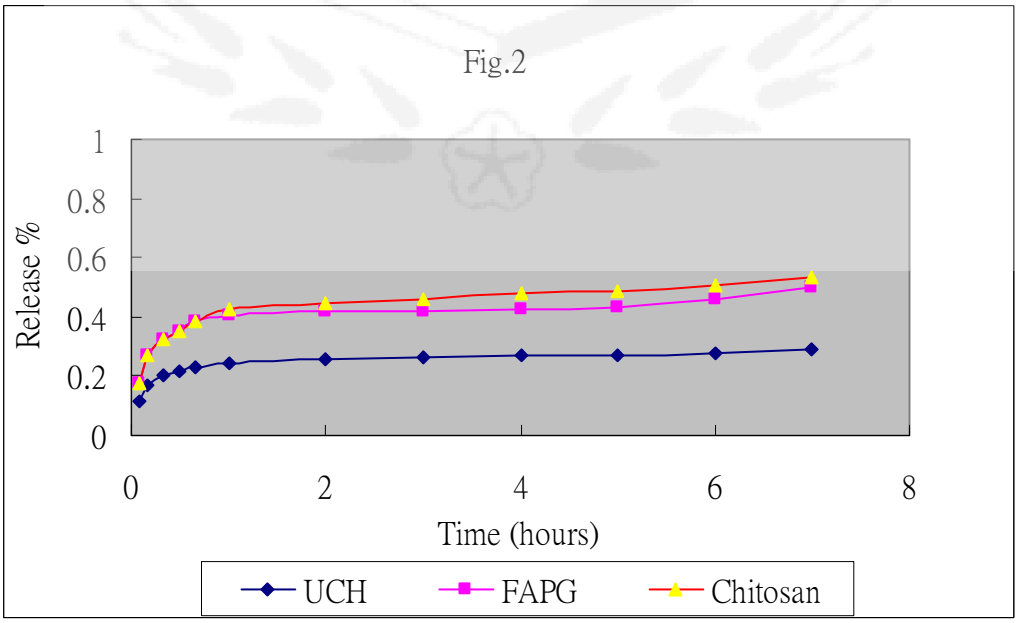
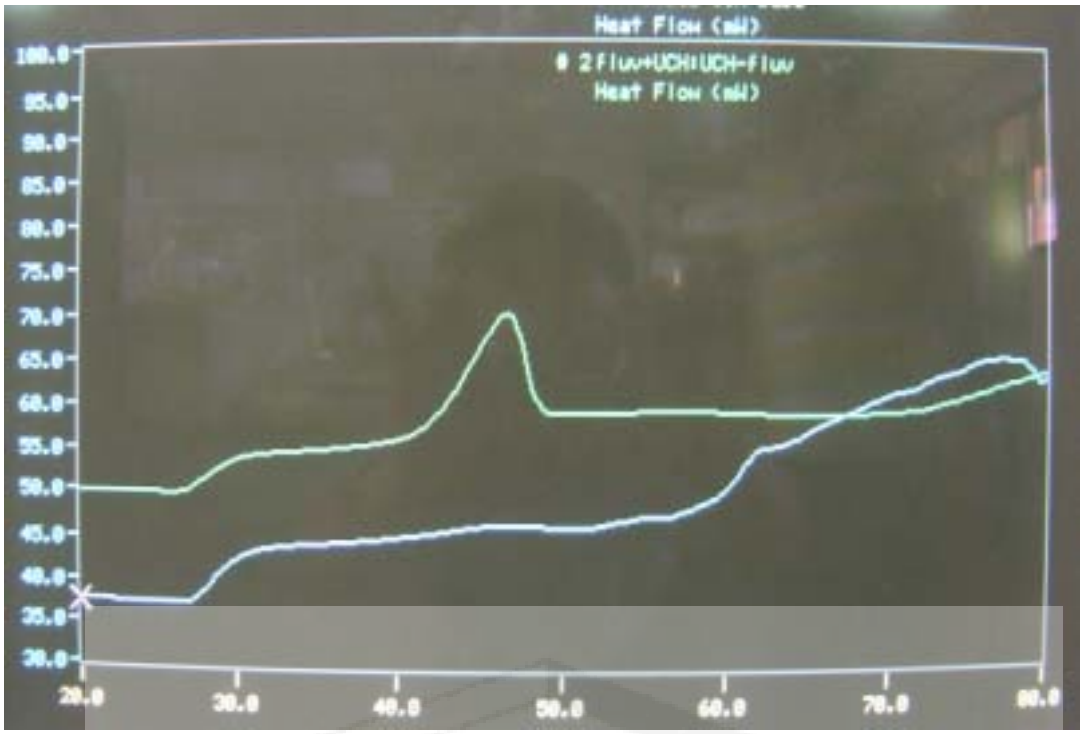


Fig.2





計畫成果自評

本研究依原計畫內容逐步實施，已完成藥物經皮吸收之三種軟膏製劑的體外實驗評估，初步之目標已達成，雖然計畫實施日期已屆滿，惟與本研究相關之議題仍值得再進一步繼續深入探討。目前所獲致的成果除了証實 fluvaststin 經皮吸收之可行性，該研發的相關經驗亦足以作為爾後同類 statin 類藥物開發經皮輸藥系統之研究參考。

可供推廣之研發成果資料表

□ 可申請專利　■ 可技術移轉　日期：92 年 10 月 29 日

國科會補助計畫	計畫名稱：用於脂質調節治療之 statins 類藥物經皮輸移研究 計畫主持人：林恆弘 計畫編號：NSC 91－2320－B－041－013　學門領域：藥學
技術/創作名稱	statins 類藥物經皮吸收軟膏製劑
發明人/創作人	林恆弘
技術說明	中文： 本新式經皮輸藥系統包含 fluvastatin 與軟膏基劑，此研究中成功地發展出藥物釋離與經皮穿透之速率控制技術，同時，軟膏基劑亦提供 fluvastatin 貯存期間較佳之安定性。 英文： A novel transdermal drug delivery system which comprises fluvastatin and ointment base. The technology of this study has been successfully developed to provide rate control over the release and the transdermal permeation of fluvastatin. Moreover, the ointment bases serve the advantage of better stability of fluvastatin during storage.
可利用之產業及可開發之產品	Fluvastatin 經皮吸收軟膏製劑
技術特點	藥物經皮吸收產生全身作用
推廣及運用的價值	避免藥物口服吸收之肝代謝，增加藥物之生體可用率

※ 1.每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

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