行政院國家科學委員會補助專題研究計畫成果報告

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※ 從生物藥劑學觀點研究賦型劑 Pluronic 之代謝相關機轉 ※

※ Biopharmaceutical Considerations on CYP3A Related ※

※ Metabolism: Effects of Pluronic ※

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計畫主持人：鄭靜玲

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計畫參與人員：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：嘉南藥理科技大學藥學系

中　華　民　國　九十三　年　十　月　二十九　日

行政院國家科學委員會專題研究計畫成果報告

從生物藥劑學觀點研究賦型劑 Pluronic 之代謝相關機轉

Biopharmaceutical Considerations on CYP3A Related Drug Metabolism: Effects of Pluronic

計劃編號：NSC 92-2320-B-041-006

執行期間： 92 年 8 月 1 日 至 93 年 7 月 31 日

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中文摘要

近年來與 CYP3A 相關的藥物交互作用為國內外研究的熱門主題。過去的研究顯示，各種合成或天然化合物可促進水溶性或脂溶性藥品的口服吸收。近來，賦型劑與藥物間的交互作用因賦型劑改善藥物傳釋系統的性質及其與法規沿革間的密切關係，引起藥學專家的注意。本研究也因此想探討賦型劑與藥物間的交互作用在臨床上的相對應性。前年，膽鹽為我們探討藥物－劑型添加劑間交互作用的第一個探討對象。去年，將全力集中在維生素 E 相關的交互作用上。今後兩年我們選定以 Pluronic®系列的聚何物當作研究對象。

Pluronic®，是一系列構造相似的聚何物。由比例不相同的親水基團(EO)與疏水基團(PO)所組成的基本型(A-B-A)區段(block)共聚物(copolymer)，一般在藥劑上做為非離子性的介面活性劑。可使用在凝膠製劑、w/o 及 o/w 的乳劑、奈米分子的包衣及固體製劑中的結合劑與潤滑劑。近年來因其在藥物(特別是蛋白質及低水溶性藥物)及基因傳釋系統應廣範，在新型賦型劑中特別引人注意。加上臨床上發現，Pluronic®具增加燒燙傷癒合力，並對癌症抗藥性的疏送蛋白具有特別的抑制能力，有關研究更是日益增加。

即令如此，Pluronic®的體內代謝機轉仍不清楚。此點，可能是其一向被視為“惰性“的賦型劑，不被認為需研究代謝機轉的誘因。但由於近年累積的文獻顯示本系列聚合物體內試驗時在肝中的暴露濃度遠高於血液，會受肝內酵素代謝的機率非常大；又其可抑制 Pgp 及 MRP1/2，與 CYP3A 活性相關的可能性大增。顯示此賦型劑之代謝機轉值得更進一步的評估。故今年我們提出 Pluronic®藥品代謝機轉(phase 酵素)的研究專題。第一年我們將使用微粒體作篩選工具，針對 CYP3A(或其他的 CYPs) 進行體外活性篩選找出體內研究的對象；第二年則利用藥物動力學方式進行體內代謝活性評估。

此研究將能使我們對 Pluronic®之代謝機轉能有更進一步的了解，對製劑上選擇賦型劑將有相當的幫助。此初步的實驗將能做為是否進一部執行藥物交互作用之依據。其結果亦有助於瞭解在 Pluronic®製劑上的應用限制。

關鍵詞：Pluronic®、細胞色素、細胞色素 3A、藥物交互作用。

Abstract

Many synthetic or natural compounds can improve the intestinal absorption of hydrophilic and/or lipophilic drugs. Recently, excipient-drug interactions have been increasingly caught attentions of pharmaceutical scientists regarding improvement of drug delivery system and drug regulation policies. This program was developed under this vision to explore its clinical relevance. Following previous years

proposal to investigate bile salts and vitamin E on CYP3A related work, this program we'll focus on Pluronic®.

Pluronic® block copolymers, poloxamers, consist of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a traditional A-B-A structure. This arrangement results in an amphiphilic nature, in which the number of hydrophilic EO and hydrophobic PO units can be altered. The use of Pluronic®-based formulations includes gels, w/o and o/w emulsions, nanoparticles coated by the block copolymer and solid polymer blends. Recently, successful experiences on using these polymers in drug (proteins/poorly soluble drugs) and gene delivery make them attract more attentions. Most importantly, selected Pluronic® polymers also exhibits immune and wound/burn healing ability. As a result, Pluronic® polymers were characterized as "functional excipients". It has been discovered that Pluronic® polymers can inhibit of Pgp and MRP1/MRP2 drug efflux system. Effect of Pluronic® on drug metabolism was only evaluated in MRP close related GSH/GST detoxification system in MDR cells. To our knowledge no other metabolism pathway has been examined until now. With the Pgp/CYP3A cross-substrate specificity and the highly bio-distribution in the liver in mind, we proposed that it is necessary to evaluate the in vitro/in vivo effects of Pluronic® on metabolic activity of cytochrome P450, especially CYP3A. The aim of the present study is to investigate effects of Pluronic® on its possible role of CYP3A drug metabolism. The first year, we'll use microsomal system to evaluate the effect. Due to its multiple binding sites characteristics, CYP3A in vitro probes, delavirdine and testosterone, will be used to evaluate on series of Pluronic® block copolymers. In vivo effects will be followed on the second year by in vivo probe delavirdine.

In terms of clinical/biopharmaceutical benefits, the results of this program will provide valuable insights into whether these polymers can affect CYPs metabolic (mainly CYP3A) activity.

MATERIALS AND METHODS

一、Materials

1、 Purchased from BDH Laboratory Supplies，Poole，England
Acetonitrile (ACN，HPLC grade)
Methanol (MeOH，HPLC grade)

2、 Purchased from J. T. Baker
Magnesium chloride4，6-hydrate，Crystal ($MgCl_2 \cdot 6H_2O$, Lot N18H24)
Sodium phosphate，Monobasic，Monohydrate，Crystal ($KOCO(CHOH)_2COONa \cdot 4H_2O$， Lot N03349)

3、 Purchased from Riedel-deHaën，Germany
di-Sodium hydrogen phosphate-2-hydrate ($Na_2HPO_4 \cdot 2H_2O$，Lot 00770)

4、 Purchased from Karayama Chemical，Japan
Acetic acid (CH_3COOH，Lot A0945)

5、 Sigma，St. Louis，MO，U.S.A.
β-Nicotinamide adenine dinucleotide phosphate， reduced form (β-NADPH，Lot 81K 7059)，Pluronics，Urethane(Ethyl carbamate，Lot 51K 1269)

6、 Purchased from Merck，Darmstadt，F.R. Germany
Sodium acetate (CH_3COONa)

7、 Purchased from Union Chemical Works LTD, Taiwan
Diethyl ether

8、 Purchased from MP Biomedicals INC.，Eschwege，Germany
Ciprofloxacin hydrochloride (Lot. 4913F)

二、Animals

Male Sprague-Dawley rats (250-350 g; obtained from the Animal Breeding Center of

National Cheng Kung University) were maintained on standard laboratory pellets and water *ad libitum*. The study protocol complied with the Institutional Guidelines on Animal Experimentation of National Cheng Kung University.

三、Method for Preparation of Microsomes

本研究目前利用 Male Sprague-Dawley rats 的肝臟進行 microsomes 製備。其使用試劑為：Tris(hydroxymethyl) methylamine(BDH Laboratory Supplies) 、 Sodium acetate (Merck，Germany)、Sucrose (Merck，Germany) 和 Glycerol (關東化學株式會社，Japan)。使用灌流手術方式先將肝臟血液趕出，獲得的肝臟將其剪碎並使用組織均質機(Glas-Col® Terre Haute，U.S.A.)均質之，藉由蔗糖梯度法使用高速離心機(RC-5C ， Sorvall® Instruments) 及超高速離心機 (L7-65 Ultracentrifuge ， Beckman coulter™) 將 microsomes 分離。

四、In vitro Protein Level and Enzyme Activity Evaluation：

1. Lowry method for normalization of total protein level

使用的試劑包括：Sodium hydroxide (Mallinckrodt ， Baker) 、 Folin-ciocalteu's phenol reagent(Fluka)、Kupfer(II)-sulfat-5-hydrate (Riedel-deHaën， Germany)及 Sodium carbonate anhydrous (Riedel- deHaën ， Germany)，其 protein 的定量是使用 Bovine serum albumin (BSA，Sigma)作為標準品，測量在 760 nm 之 UV 吸光。

2. Evaluation of CYP3A in vitro activity by delavirdine and its metabolite desalkyl-delavirdine

用 CYP3A 的典型受質 delavirdine 進行 *in vitro* incubation 試驗，且藉由 HPLC 的分析觀察肝臟代謝活性之變化。其 HPLC 系統包括：自動取樣器(HITACHI L-7200 Auto-sampler) 、 幫浦(HITACHI L-7100 Pump) 、界面控制器(HITACHI D-7000 Interface) 、偵測器(HITACHI L-7420 UV Detector)、管柱(分析：C18 Column 4.6 × 250 mm)和 保護：ODS Guard column、積分系統(D-7000 HPLC System Manager (HSM))。藉由不同總蛋白質含量(50, 100 and 250 μg)、不同藥品濃度的投與(5-500 μM)和不同 incubation time: 0.5, 1, 2 , 4, 7, 10, 15, 30, 45 min 觀察肝臟酵素代謝情形。來確定最後的最適化條件。

3. Delavirdine assay method development

Delavirdine 和 desalkyldelavirdine 在 mcirosomes 及 血漿中濃度之定量將根據我們先前所開發的高壓液相層析方法加以修改。[1,2] 將使用同為 CYP3A 受質之 cisapride 作為內部標準品。

五、Effects of Pluronics on Drug Transporters：

除了針對 CYP3A 之影響之相關實驗外本計劃中亦針對 Pluronics 對 drug transporters 之可能影響做研究。因許多文獻指出 Pluronics 具有調控 MDR 及 MRP 之效果，而影響藥物體內之吸收分佈及排泄，甚至於引起藥物交互作用之可能。於相關文獻中，幾乎所有研究均是以臨床上未使用的 Pluronic P85 及 L61 等聚合物做探討，然而對於其他 Pluronics 卻是討論不多。本計劃中選用 ciprofloxacin 作為 model compound。

Ciprofloxacin 在體內分佈體積甚廣，而經由輸送子之主動運輸可能為其膽汁排除的主要驅動力之一。因此先以文獻上常用於探討多重抗藥性的抑制劑 quinidine，藉由離體穩定灌流大白鼠肝臟的方式，以確認 MDR/MRP 輸送子是否對 ciprofloxacin 的膽汁排除有所影響。於確認 ciprofloxacin 的膽汁排除是受到 MDR/MRP 輸送子的影響

後，進而以離體穩定灌流大白鼠肝臟的方式，分別投與 ciprofloxacin 合併不同濃度 (0.25、0.5 及 0.75%) 之 Pluronic X，以評估其對於 ciprofloxacin 在膽汁分泌的影響。

RESULTS AND DISCUSSION

1. Lowry Method:

To measure the total protein in different batches of microsomes as a normalized basis, Lowry method was employed. The standard curve was depicted here in Fig. 1. Linear concentration range of total protein was between 0.01-0.25 mg/ml (R^2: 0.997, intercept: 0.0063, slope: 1.517).

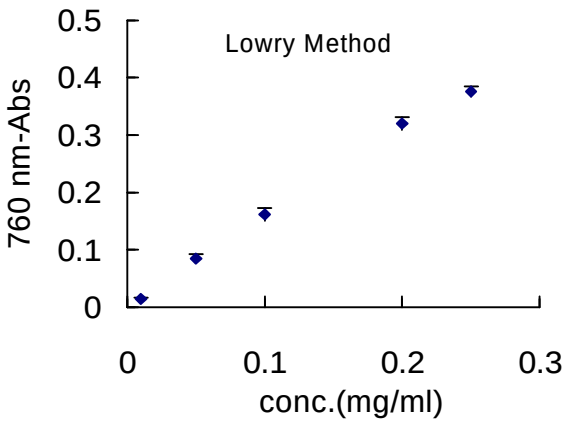


Fig. 1. Lowry method for total protein determination.

2. 評估 CYP3A 體外活性的 delavirdine 微粒體培養法

To quantification the metabolizing activity of CYP3A in microsomes, we first develop a specific assay for delavirdine and dealkyl-delavirdine metabolite, using cisapride as an internal standard. To investigate the metabolic activity of CYP3A on delavirdine, different concentrations of delavirdine will be incubated under specific microsome conditions (total protein 25, 50 and 100 μg). The appropriate protein amount and incubation time were determined to be 100 μg and 4 minutes, respectively (Fig.2).

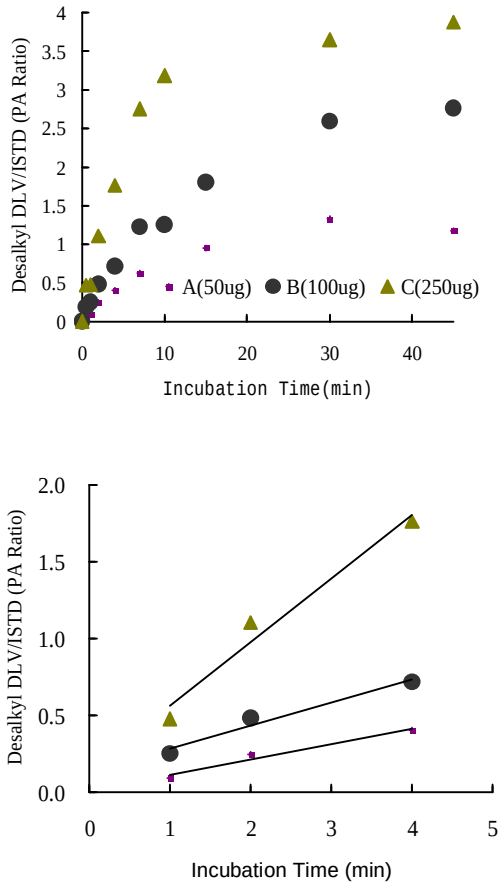


Fig. 2. Formation of desalkyldelavirdine vs time at delavirdine 250 μM and 50, 100, and 250 μg total protein.

Based on the above observations, total protein content 100 μg, delavirdine concentration was 250 μM and incubation time for 4 minutes were selected as the final assay condition.

3. Delavirdine Assay Method Development

HPLC condition:

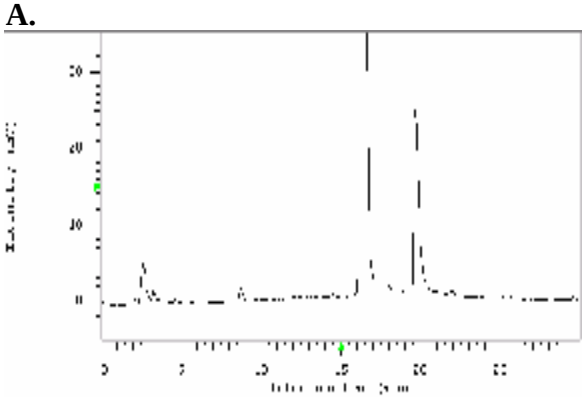
Mobile Phase (A) 50mM phosphate buffer (pH 4.0): acetonitrile = 35:65

Mobile Phase (A) 10mM phosphate buffer (pH 6.0): acetonitrile = 72:28

Time (min)	A (%)	B (%)
0	2	98
2	2	98
15	90	10
25	90	10
27	2	98

Fluorescence detection: Excitation 418 nm, Emission 295 nm; Temperature: ambient; Analytical column: C18; Drug: 250 μM delavirdine, 2 μg/ml cisapride; Retention time: delavirdine: 16.3 min, Cisapride: 19.8 min, desalkyl- delavirdine 8.0 min.

A.



B.

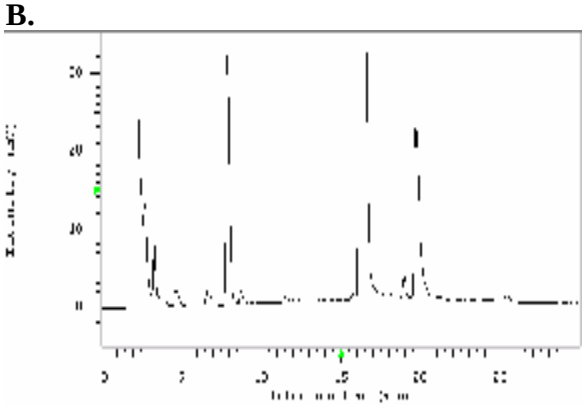


Fig. 4. Chromatograms of delavirdine and desalkyldelavirdine before (A) and 2 minutes after incubation (B).

3. Effects of Pluronics on Drug Transporters

Both quinidine and Pluronic X significant inhibited the biliary secretion of ciprofloxacine in the rat liver as shown in Figure 5. The results suggested that Pluronic X inhibits hepatobiliary MDR/MRP transporters.

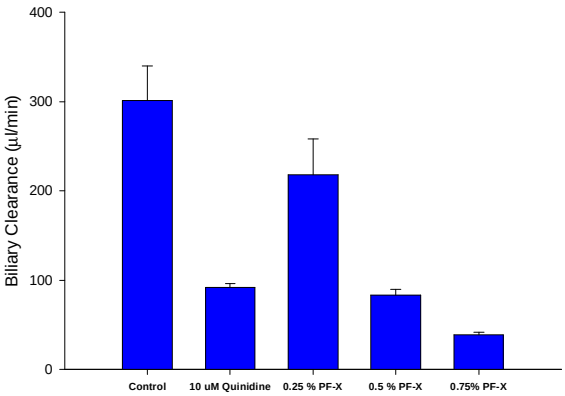


Fig. 5 Effect of quinidine and Pluronic X on the biliary secretion of ciprofloxacin.

CONCLUSION

由上述結果顯示，本實驗室已初步完成 delavirdine 在微粒體系統的定量分析方法，並建立以其評估肝及腸微粒體中 CYP3A 體外代謝活性的系統。此外在肝臟灌流製備試驗中我們首次證明 Pluronic X 亦具有抑制 MDR/MRP transporters 之效果。

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