行政院國家科學委員會專題研究計畫　成果報告

賦型劑對藥品肝膽輸送的機轉研究：Pluronic-X

計畫類別：個別型計畫
計畫編號：NSC94-2320-B-041-004-
執行期間：94 年 08 月 01 日至 95 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：鄭靜玲
共同主持人：周辰熹

報告類型：精簡報告

處理方式：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中　華　民　國 95 年 10 月 31 日

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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※　賦型劑對藥品肝膽輸送的機轉研究：Pluronic-X　※
※　Impact of Excipients on Drug Hepatobilliary Excretion:　※
※　Pluronic-X　※
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計畫類別：☒個別型計畫　□整合型計畫
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計畫主持人：鄭靜玲
共同主持人：周辰熹
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執行單位：嘉南藥理科技大學藥學系

中　華　民　國　九十五　年　十　月　三十一　日

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中文摘要

本計劃主要是研究賦型劑 pluronic®對藥品在肝膽運輸的影響。pluronic®為一系列三區塊型(A-B-A)聚合物的總稱，是一類常用於藥品製劑的非離子型賦型劑。近年來廣用於各類製劑的開發，特別是使用在癌症與愛滋病的藥劑開發與基因製劑的研究。因為癌病與愛滋病用藥在體內的輸送會受到 MDR/MRP 的影響，因而此類賦型劑是否影響 MDR/MRP 輸送子而對藥品輸送有所影響的問題，在近來文獻中亦有所報導，但這些文獻僅著重在小腸與腦膜的影響，至今並無研究 pluronic®對藥品在肝膽輸送的影響報告。

本實驗室在執行前年國科會補助之研究計畫時，卻發現一被文獻報導為對 MDR/MRP 輸送子微影響的 pluronic®-X 賦型劑對藥品 ciprofloxacin 的膽汁排除有顯著的抑制。由此推論此類賦型劑不僅會改變藥物在吸收與分佈的藥動性質，也會影響藥品在膽汁的排除，甚至進而可能會影響藥品的腎臟排除與出現在腸道的再吸收現象。此特殊賦型劑如何影響藥品在肝膽輸送的機轉，顯然值得進一步去探討研究，而能對此賦型劑在製劑的臨床應用與限制有更進一步的瞭解。

由於藥品在肝臟的輸送會受到 OATPs, MDRs and MRPs 等輸送子的影響，典型受質如：digoxin (Mdr1a/1b and Oatp2), doxorubicin (Mdr1a/1b and Mrp2), pravastatin (Oatp2), cisplatin (Mrp2), glyburide (Bsep) and cyclosporin A (Mdr1a/1b)等預計將選用為評估的探針藥品。

計劃中將以大鼠為研究之模式動物，在藥物動力學臨床前試驗階段，為快速瞭解藥品體內藥物動力學特性，大鼠為最常使用的動物模式。在研究藥品代謝相關的交互作用時，亦喜使用此動物模式。實驗分為老鼠體內動力學試驗及原位離體肝臟灌流(Liver perfusion)二部分。

本年度我們提出賦型劑對藥品肝膽輸送的機轉研究，特別是針對從未報導過會抑制體內 ABC 輸送子的 Pluronic-X 來研究，我們相信此研究的成果，將有助於瞭解賦型劑對藥品肝臟排除的機轉，有助於藥品製劑在賦型劑上的選擇，對於現今國家生技重點中草藥製劑開發也將有所助益。

關鍵詞：大鼠、賦型劑 Pluronic®、肝膽分泌、及多重藥品抗藥性輸送子

Abstract

This program is developed to evaluate the impact of Pluronic –X on hepatobiliary secretion of drugs. 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 drugs (i.e., anticancer and antiviral agents) and gene delivery make them attract more attentions. It has been discovered that Pluronic® polymers can inhibit of Pgp and MRP1/MRP2 drug efflux system. This Effect of Pluronic® on drug metabolism was only evaluated in MRP close related GSH/GST detoxification system. However, all these observation were based on studies on cell-lines and have never been evaluated on drug biliary secretion in other group.

Our previous studies demonstrated that a specific hydrophilic Pluronic-X can decrease biliary secretion of ciprofloxacin. This phenomenon could be due to its inhibition effect on Pgp or Mrp related efflux proteins. A surprising finding is that this specific Pluronic block copolymer (Pluronic-X) is hydrophilic. It was suggested to be ineffective on the inhibion of MDR/MRP transporters previously. Apparently to us, it is worth to further investigate the impact of excipients on drug biliary secretion, especially to Pluronic-X.

The aim of the present study is to investigate effects of Pluronic®-X on its possible role of hepatobiliary transporters, selectively (Mdr1a/1b, Mdr2, Mrp2, Bsep, and Oatps). We'll use *in situ* isolated perfused rat liver system to evaluate the inhibition effects of Pluronic-X on specific substrates of the investigated transporter. To evaluate the impact of Pluronic-X on the specific substrate of ABC drug efflux pumps and Oatps, the following typical substrates: digoxin (Mdr1a/1b and Oatp2), doxorubicin (Mdr1a/1b and Mrp2), pravastatin (Oatp2), cisplatin (Mrp2), glyburide (Bsep) and cyclosporin A (Mdr1a/1b), will be chosen. In vivo effects will be followed.

In terms of clinical/biopharmaceutical benefits, the results of this program will be an important new finding for hydrophilic Pluronic block copolymers and will provide valuable insights into whether Pluronic-X can affect hepatobiliary secretion of drugs and which transporters could be involved. It will be helped in selecting excipients in drug formulation.

Keywords: rat, excipients, Pluronic®, hepatobiliary secretion and MDRs/MRPs

MATERIALS AND METHODS

一、Materials

1、 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)

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

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

4、 Sigma，St. Louis，MO，U.S.A.
Pluronics，Urethane (Ethyl carbamate，Lot 51K 1269)，Bovine serum albumin (Lot. 76H0336)，Sodium taurocholate，Quinidine sulfate (Lot. 74H3645)

5、 Purchased from Merck，Darmstadt，F.R. Germany
Calcium chloride dehydrate，Magnesium sulfate heptahydrate，Potassium chloride，Sodium chloride，Sodium hydrogen bicarbonate

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

7、Purchased from Acros Organics，New Jersey，U.S.A
Digoxin (Lot.A012186901)

8、Purchased from ABBOTT，Wiesbaden，Germany
TDx FLx Digoxin Calibrator (Lot. 09560Q100)，TDx FLx Digoxin Control (Lot. 12227Q100)，TDx FLx Digoxin Reagent Pack (Lot. 09723Q100).

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 and was approved by the Board of Animal Experimentation of Chia-Nan University of Pharmacy & Science.

三、Isolated Perfused Rat Liver Preparation

The *in situ* perfused rat liver preparation was similar to that described in previous studies (Chou et al., 1995; Chou, 2000). Under intraperitoneal anesthesia with urethane (1.5g kg^{-1}), the bile duct was cannulated with PE10 (polyethylene tubing, i.d.=0.28mm, o.d.=0.61mm). The portal vein was then rapid cannulated using a 16-gauge (Medicut, o.d. 1.7x45 mm) intravenous catheter placement unit. The liver was perfused (20 mL min^{-1}) in a single-pass mode with Krebs-Henseleit bicarbonate buffer (pH7.4), containing 3 g L^{-1} glucose and saturated with humidified carboxgen gas (O_2: CO_2 = 95%: 5%). The superior vena cava was cannulated through the right atrium without interruption of portal perfusion. The inferior vena cava was ligated above the renal portal vein. All experiments were conducted at 37°C in a temperature-controlled cabinet. Viability of the liver was assessed by its gross appearance, flow recovery, bile production and by monitoring the inflow and outflow perfusate pH throughout the experiment. The wet liver weight was determined immediately after an experiment.

四、Effects of Pluronics on Drug Transporters

To evaluate the impact of Pluronic-X on the specific substrate of ABC drug efflux pumps and Oatps, the typical substrates digoxin (Mdr1a/1b and Oatp2) was chosen.

Steady-state infusion. After a 15-min initial stabilization period, 110 nM of digoxin was infused, with or without concomitant of 0.75% Pluronic-X or 10 μM quinidine, to the isolated rat liver for 40 min. The outflow perfusate samples were collected between 5 to 40 min at 5-min intervals to assess the effect of Pluronic-X or quinidine on the hepatic extraction of digoxin. Also, the bile was collected at 10 minute interval to explore the impact of Pluronic-X and quinidine on the biliary transport of digoxin.

RESULTS AND DISCUSSION

1. The steady-state availability of digoxin in the isolated perfused rat liver was increased by quindine, however, it was not altered in the presence of Pluronic X (Fig. 1) (Table 1).

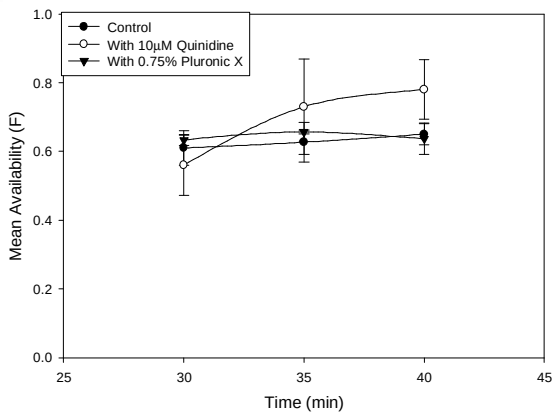

Fig. 1. Effect of concomitant infusion with 10 μM quinidine or 0.75 % Pluronic X on the steady-state availability of digoxin (110 nM) in the isolated perfused rat liver.

2. The biliary excretion of digoxin in the isolated perfused rat liver was decreased significantly by quindine and Pluronic X (Fig. 2) (Table 1).

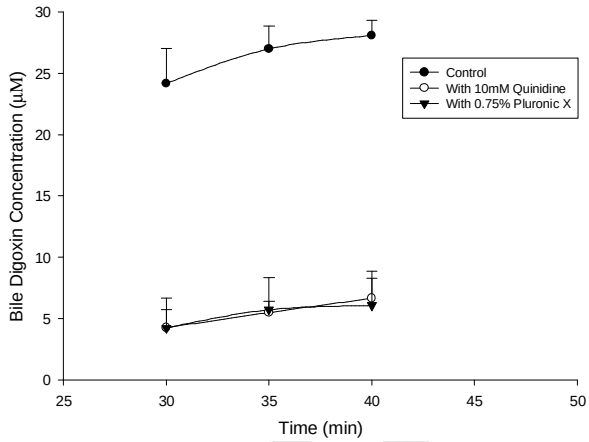

Fig. 2. Effect of concomitant infusion with 10 μM quinidine or 0.75 % Pluronic X on the biliary excretion of digoxin (110 nM) in the isolated perfused rat liver.

CONCLUSION

Both quinidine and Pluronic X significant inhibited the active biliary secretion of digoxin in the isolated perfused rat liver as shown in Figure 2. The results suggested that Pluronic X may inhibit hepatobiliary MDR/MRP transporters.

REFERENCES:

Chou, C.-H., McLachlan, A. J. and Rowland, M.: Membrane permeability and lipophilicity in the isolated perfused rat liver: 5-Ethyl barbituric acid and other compounds. *J. Pharmcol. Exp. Ther.* **1995**, 275, 933-940.

Chou, C.-H.: Uptake and dispersion of metformin in the isolated perfused rat liver. *J. Pharm. Pharmacol.* **2000**, 522, 1011-1016.

Table 1 Pharmacokinetic parameters of digoxin in the isolated perfused rat livers

Digoxin 110 nM	$C_{perfusate, ss}$ (nM)	F	$C_{bile, ss}$ (mM)	$C_{bile/perfusate, ss}$	Cl_{bile} (ml/min)
Control	68 ± 3	0.65 ± 0.03	28.1 ± 1.2	422 ± 25	3.2 ± 0.4
With 10 mM Quinidine	80 ± 18	0.78 ± 0.09	6.6 ± 2.2**	87.7 ± 36.9**	0.7 ± 0.2*
With 0.75% Pluronic X	68 ± 4	0.64 ± 0.05	6.1 ± 2.2**	89.3 ± 32.6**	0.6 ± 0.2**

*** P < 0.05 vs. control group**

**** P < 0.01 vs. control group**