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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※ PDE5 inhibitors 的相關交互作用影響研究 ※

※ Investigation of Potential Drug Interactions of PDE5 Inhibitors ※

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計畫主持人：鄭靜玲

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行政院國家科學委員會專題研究計畫成果報告
PDE5 inhibitors 的相關交互作用影響研究
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中文摘要

本計劃主要是研究目前在市面上非常熱門的壯陽藥品第五型磷酸二酯酶抑制劑(sildenafil; vardenafil; tadalafil)的相關交互作用評估。由於國情，本類藥品在國內濫用的情況相當普遍，又因為屬於較新上市的藥物產品，相關的藥物動力學及藥品交互作用的文獻，除了第一個上市的 sildenafil(威而剛)有稍微多一些的資訊外，其餘皆鮮有相關報導。在本類藥品普遍濫用情況下，顯然值得進一步去探討相關的藥物交互作用研究，而能對此類藥品的臨床應用與限制有更進一步的瞭解。

第一年計畫成果針對tadalafil的研究方面，我們已完成蔓越莓汁與tadalafil的交互作用試驗。藉由大鼠體內動力學試驗及體外微粒體代謝試驗，我們發現蔓越莓汁與tadalafil併服時，會經由抑制腸道CYP3A活性而增加tadalafil口服的生體可用率，並使其血中濃度顯著增加1.6倍。此部分成果已發表於在日本金澤所舉辦的4th WorldConference on Drug Absorption, Transportand Delivery (WCDATD)學術研討會(2007年6月份)。

而針對vardenafil的研究方面，我們已成功開發了第一個以高效液相層析搭配螢光偵測的分析方法，用來定量vardenafil在血漿與膽汁中的濃度，並成功地應用在研究vardenafil在大鼠體內與肝膽排泄之動力學。此部分成果投稿至Journal of Chromatography A期刊已發表(doi:10.1016/j.chroma.2007.03.077)，其網路版於2007年3月28日刊登。

第二年，我們主要將重點放在三種所選壯陽藥物在不同給藥劑量下的大鼠體內動力學試驗結果，證實sildenafil, tadalafil 與vardenafil 的膽汁排泄皆經由主動運輸驟；其中又以sildenafil的效應最強。因此選定sildenafil為目標藥物並藉由專一性抑制劑的機轉性研究，釐清特定肝臟藥物傳輸子(transporter)在相關交互作用所扮演之角色。結果發現：於離體老鼠肝臟，若給予P-gp、MRP2及OATP抑制劑cyclosporine A (CsA)，會顯著減少sildenafil之膽汁排除，而在灌流液的部分則沒有差異；代謝物部分則發現到灌流液隨CsA濃度增加而呈現上升的趨勢，但在膽汁部分則隨CsA濃度增加而減少。另外，在並用OATP、MRP2抑制劑rifampicin（RIF）時，則發現sildenafil膽汁排除量有明顯增加。代謝物部分則發現其在膽汁濃度有略微下降的趨勢。併用MRP抑制劑probenecid (PBED)則發現Sildenafil的灌流液濃度曲線下面積與膽汁排除量呈現上生的狀況；代謝物部分則發現灌流液代謝物濃度上升，而膽汁代謝物呈不具統計差異的略微下降趨勢。另外，在併用P-gp受質amisulpride (AMI)之結果，與sildenafil併用CsA之結果類似。(部分成果已發表於第五屆海峽化學、生物及材料研討會2008年7月份)

相信此研究的成果，能對於現今國家生技重點健康照護、中草藥製劑開發及健康食品的使用有所助益。

關鍵詞：大鼠、第五型磷酸二酯酶(phosphodiesterase-5, PDE5) 抑制劑、肝膽代謝機轉、藥物交互作用

Abstract
(second year)

Introduction : PDE5 inhibitors are used for the treatment of male erectile dysfunction and pulmonary arterial hypertension. The structures of PDE5 inhibitors are similar to cGMP and they are mainly metabolized by CYP3A and CYP2C9 isozymes in the liver. Currently, the interaction studies of PDE5 inhibitors are focused on the metabolic aspect and none of them has addressed on the transporter interactions. However, recent studies have demonstrated that sildenafil can inhibit the activity of MRP4, MRP5 and OATP. And it has beeen suggested that P-gp, in addition to CYP3A, was responsible for the increase of the concentration of PDE5 inhibitors when concomitant with ritonavir in clinical settings. It is of great interest to investigate the role of drug transporters on the hepatobiliary disposition of PDE5 inhibitors.

Purpose : The aim of this study was to investigate the hepatic disposition of PDE5 inhibitors, and to explore the role of drug transporters on their hepatobiliary transport in the isolated perfused rat liver.

Results : All PDE5 inhibitors showed active biliary secretion during single-pass constant perfusion experiments. As the extent of secretion of sildenafil was greater than that of tadalafil and vardenafil, sildenafil was chosen as the model drug to further explore drug interactions of PDE5 inhibitors. Using the recirculated perfused rat liver preparation, the biliary secretion of sildenafil and its metabolite was found to decrease with increasing concentration of concomitant cyclosporine A, an inhibitor of OATP、MRP2、P-gp. When co-administered with rifampicin, an OATP and MRP2 inhibitor, the perfusate concentration of sildenafil increased slightly, yet the cumulative amount excreted in bile of sildenafil was increased appreciably. In the prescence of the MRPs inhibitor probenecid, both the perfusate concentration and the cumulative amount excreted in bile of sildenafil increased significantly. When concomitant with amisulpride, a P-gp substrate, the biliary secretion of sildenafil and its metabolite was found to decrease slightly.

Conclusion : In this study, we found that all three PDE5 inhibitors underwent active biliary secretion. This study also demonstrated that the disposition of sildenafil was influenced by various inhibitors of membeane transporters. Sildenafil appeared to be a substrate for P-gp, and the role of OATP was limited for hepatic transport of sildenafil. Whether the transporters in sinusolidar membrane are involved in the hepatic disposition of sildenafil or not remains unclear.

Keywords:
PDE5 inhibitors**;** drug-drug interaction**;** P-glycoprotein**;** pharmacokinetics

MATERIALS AND METHODS

一、Materials

1、 Purchased from Sigma，St. Louis，MO，U.S.A.
Acetonitile，Urethane，Probenecid (Lot 118H0534)，Sodium taurocholate

2、 Purchased from Merck，Darmstadt，F.R. Germany
Ammonium acetate，Calcium chloride dihydrate，Magnesium sulfate heptahydrate，Methanol，Potassium chloride，Potassium dihydrogen phosphate，Sodium hydrogen bicarbonate

3、Purchased from Sanofi-Synthelabo，France
Amisulpride

4、Purchased from Lotus Medical Supply Inc. Taiwan，R.O.C.
Cisapride

5、Purchased from YSP Taiwan，R.O.C.
Cyclosporine A (Lot. QR7274)

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

7、 Purchased from Riedel-deHaën，Seelze，Germany
D(+)-Glucose monohydrate，Hydrochloric acid (Lot. 92150)，Potassium dihydrogen phosphate (Lot 21560)，Sodium chloride

8、Purchased from Pharmacia & Upjohn，Michigan，U.S.A
Heparin

9、Purchased from Xiamen Fine Chemical Import & Export Co. Ltd.，Xiamen，China
Rifampicin，Sildenafil

10、 Purchased from Polymed Therapeutics Inc.，Shenzhen，China
Vardenafil·$3H_2O$

11、 Purchased from Virbac Lab，Carros，France
Zoletil 50

二、Animals

Male Sprague-Dawley rats (250-350 g; obtained from the Animal Breeding Center of National Cheng Kung University) or from BioLASCO Co. 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 and the Board of Animal Experimentation of National Cheng Kung University.

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^oC 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 Specific Inhibitors on Rate of Drug Transport

To evaluate the impact of specific inhibitors of the ABC drug efflux pumps and

Oatps, the most profounded effect PDE5 inhibitor on the active hepatobilliary secreation was chosen.

Steady-state infusion. After a 15-min initial stabilization period, 110 nM of digoxin was infused, with or without concomitant of the PDE5 inhibiors with or withoud specific transporter inhibitor (cyclosporine A、rifampicin、probenecid and amisulpride), 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 these inhibitors on the hepatic extraction of PDE5 inhibitors. Also, the bile was collected at 10 minute interval to explore the impact on the biliary transport of PDE5 inhibitors.

RESULTS AND DISCUSSION

1. The steady-state bile availability of PDE5 inhibitors in the isolated perfused rat liver was increased in the order of tadalafil, vardenafil, and sildenafil (Fig. 1) (Table 1).

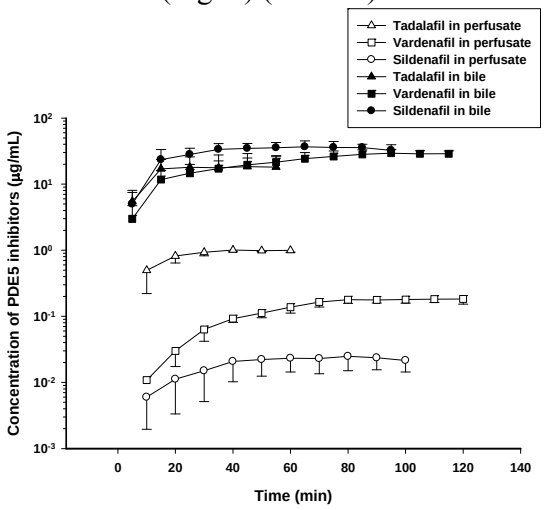

Fig. 1. The concentrations-time profile of PDE5 inhibitors in the isolated perfused rat liver perfusate and bile.

2. The results of coadministration of sildenafil and a selected several transporter inhibitor in the isolated perfused rat liver were summarized in Table 1.

CONCLUSION

All three PDE5 inhibitors underwent active biliary secretion. This study also demonstrated that the disposition of sildenafil was influenced by various inhibitors of membeane transporters. Sildenafil appeared to be a substrate for P-gp, and the role of OATP was limited for hepatic transport of sildenafil. Whether the transporters in sinusolidar membrane are involved in the hepatic disposition of sildenafil or not remains unclear.

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Table 1 Summary of Sildenafil-Specific Transporter inhibitor Interaction

	Perfusate		**Bile**	
	Sildenafil AUC (ng-min/mL)	Metabolite[a.] Concentration (ng/mL)	Sildenafil Ae_{0-60} (μg)	Metabolite Ae_{0-60} (μg)
5 μM CsA	—[b]	↑	↓*	↓*
20 μM CsA	—[b]	↑	↓*	↓*
50μM RIF	—[b]	**N.D.**	↑*	↓[c]
1 mM PBED	↑*	↑	↑*	↓[c]
50 μg/mL AMI	—[b]	↑	↓[c]	↓[c]

* P ＜0.05

a. Over twice increasing from control metabolite in steady state.

b. The difference is less than 10%

c. The difference is higher than 10%, but no significantly difference.