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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※ 膽酸及膽鹽與細胞色素 3A 代謝相關藥品交互作用之研究 ※
※ Effects of Bile Acids and Bile Salts on CYP3A related drug-drug ※
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中 華 民 國 九十一 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

膽酸及膽鹽與細胞色素 3A 代謝相關藥品交互作用之研究

Effects of Bile Acids and Bile Salts on CYP3A related drug-drug interactions

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一、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。此乃因為 CYP3A 為肝臟及小腸中含量最豐之細胞色素 P450，且有半數之藥品由此酵素代謝。然而不像其他的細胞色素 P450 iso-enzymes（如 CYP1A1/2, CYP2D6 及 CYP 2C19 等），從藥物遺傳學(pharmaco-genetics)的研究中，已找出許多與藥品活性有直接相關的不同對偶基因，因而可辨認出高危險性的藥品使用患者。相反的，至今雖有許多致力於 CYP3A 基因多型性的研究，然這些研究結果顯示發現的新基因型不能與 CYP3A 的活性直接相關，仍不能解釋為何 CYP3A 的活性具相當大的個體差異。重新檢視細胞色素對藥物的催化步驟，並將 CYP3A 與其他的細胞色素 P450 isoenzymes 做比較後發現：CYP3A 為肝臟及小腸中含量最高之代謝酵素。此點是 CYP3A 被強調其在藥物代謝扮演一重要角色之因。

過去多認為口服劑型中之賦型劑不被吸收，故不會影響藥品的吸收速率。但新的製藥技術與藥品傳輸理論，使賦型劑與藥品間的關係趨向複雜，也使賦型劑對主成分藥劑的影響研究日趨重要。而其中曾有文獻報告膽鹽可能對 CYP3A 的吸收及代謝會有影響。膽鹽是人體內一種內生性的物質，其主要功能是幫助肝臟的膽固醇由膽道排除和幫助小腸脂質的乳化和吸收。於製劑方面，taurohyodeoxycholic acid(THDCA)和 sodium deoxycholic(SDC)可作為賦形劑(excipient)即吸收促進劑(absorption enhancer)。這是利用其具有親水性及親油性基團，似界面活性劑之作用來增進藥品的吸收。於臨床使用方面，長期口服大劑量的 chenodeoxycholic acid(CDCA)和 ursodeoxycholic acid(UDCA)是目前被使用於治療膽結石。而肉類內臟食物也是體內獲得膽鹽的途徑之一。而進來對油性製劑的重視，使膽鹽對藥品吸收的影響評估日趨重要。

因此在本研究中，因此本研究目的在使用膽鹽對老鼠肝臟及腸道進行不同天數的 CYP3A 誘導反應，藉由西方點墨法觀察相對應蛋白質含量的變化並藉由體外微粒體速率試驗評估 CYP3A 體外活性變化，更其憑藉不同途徑投與 CYP3A 的典型受質如 midazolam，當作觀察 CYP3A 體內活性變化之指標。目的在於探討膽鹽對不同器官（腸、肝）之 CYP3A 活性的影響，且希望能進一步了解膽鹽與 CYP3A 相關藥物之間的交互作用並進一步能將此結果應用到臨床給藥的指導及劑型設計的指南。

關鍵詞：CYP3A，膽酸，膽鹽，藥物交互作用。

二、Abstract

Many synthetic or natural compounds can improve the intestinal absorption of hydrophilic and/or lipophilic drugs. Among these compounds, bile salts, fatty acids, and

surfactants have been verified as potent absorption enhancers (Sakai, 1997). Bile acids/bile salts have very important endogenous functions, such as assist cholesterol excreted in the bile and help lipid absorption. They can be obtained from different exogenous ways, excipients, detergent, and medications for gallstone. Therefore, bile acid(s)/salt(s) is interested in this research program. A recent study indicates that 6alpha-hydroxylation of taurochenodeoxycholic acid and lithocholic acid were mediated by CYP3A4 in human microsomes (Araya, 1999). Another study also showed that taurohyodeoxycholic acid (THDCA), tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) could significantly induce hepatic CYP3A activities (about 2-3 fold increase) in rat (Paolini, 1999). Results from this study indicated the possibility of bile acid-CYP3A metabolized drug interactions. For this purpose midazolam, a typical CYP3A substrate, will be selected as a model drug to evaluate this bile acid induce CYP3A activity phenomenum *in vivo*. In other words, the pharmacokinetics of midazolam will be characterized under different pretreatment with bile acid. Besides, in Paolini (1999) study, bile acids were administered via intraperitoneal injection, which is different from oral administration, the common way of gallstone therapy. Hence, the effect of induction by different routs of administration will also be studies. Finally, effect of the hepatic and intestinal levels of CYP3A will also be determined by intravenous and oral administration of midazolam.

Importance of this investigation

The aim of the present study is to investigate effects of the bile acids/salts and CYP3A related drug interactions. Bile acids/salts specifically interested are TDCA, UDCA and TUDCA, drugs used for cholesterol gallstone dissolution. Other bile acids like (1) TDCA, endogenous BA for lipids to be absorbed, and/or (2) SDCA, an endogenous bile salt and an excipient in drugs may also be investigated. Moreover, with different administration routes of bile acids, namely given it intravenously and orally, the degree of bile salts-drug interaction will be evaluated. We also like to evaluate whether the intestinal CYP3A can be induced by these bile acids/salts.

In terms of clinical/practical benefits, the results of the program will not only provide valuable insight into drug-bile salts interactions, but also relevant information on the practical uses of CYP3A drug substrates).

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 (MgCl2．6H2O, Lot N18H24)

Sodium phosphate，Monobasic，Monohydrate，Crystal (KOCO(CHOH)2 COONa．4H2O， Lot N03349)

3、Purchased from Riedel-deHa n，Germany

di-Sodium hydrogen phosphate-2-hydrate (Na2HPO4．2H2O，Lot 00770)

4、Purchasrf from Karayama Chemical，Japan

Acetic acid (CH3CooH，Lot A0945)

5、Sigma，St. Louis，MO，U.S.A.

â-Nicotinamide adenine dinucleotide phosphate，reduced form(â-NADPH，Lot 81K7059)

Urethane(Ethyl carbamate，Lot 51K 1269)

6、Purchasrf from Merck，Darmstadt，F.R. Germany

Sodium acetate (CH3COONa)

7、Purchasrf from Union Chemical Works

LTD, Taiwan
Ethyl ether

二、Method for preparation of microsome
本研究目前利用 Male Sprague-Dawley rats(購自成大醫學院動物中心)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。

三、In vitro activity evaluation：

1. Lowry Method
使用的試劑包括：Sodium hydroxide (Mallinckrodt，Baker)、Folin-ciocalteu's phenol reagent(Fluka)、Kupfer(II)-sulfat-5-hydrate(Riedel-deHa n，Germany)及 Sodium carbonate anhydrous(Riedel-deHa n，Germany)，其 preotein 的定量是使用 Bovine serum albumin (BSA，Sigma)作為標準品，

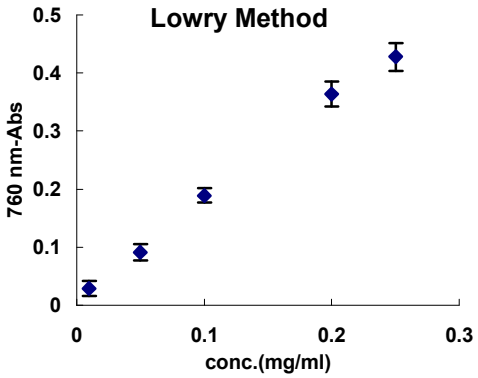
2. Evaluation of CYP3A in vitro activity by midazolam (MDZ)
用 CYP3A 的典型受質 midazolam 進行 *in vitro* incubation 試驗，且藉由 HPLC 的分析觀察肝臟代謝活性之變化。其 HPLC 系統包括：自動取樣器(HITACHI L-7200 Autosampler)、幫浦(HITACHI L-7100 Pump)、界面控制器(HITACHI D-7000 Interface)、偵測器(HITACHI L-7420 UV Detector)、管柱(分析：3ì Ultrasphere ODS Column 4.6×75mm(Beckman coulter™)和保護：ODS Guard column (H5ODS-10C，Hichrom Ltd))、積分系統(D-7000 HPLC System Manager (HSM))。 藉由不同濃度的投與(10 和 100uM)和不同 incubation time:0,0.5,0.75,1,2,3,5,7,9,10,15,20,25,30min 觀察肝臟酵素代謝情形。

3. Evaluation of CYP3A in vitro activity by erythromycin (ERM)
用 CYP3A 的典型受質 erythromycin 進行 *in vitro* incubation 試驗，且藉由加入 Nash 呈色劑反應使其代謝生成之 formaldehyde 呈色(diacetyldihydrolutidine，DDL)，再由 UV 於 412 nm 處分析析光值，進而觀察 CYP3A 代謝活性之變化。

RESULTS AND DISCUSSION

1. Lowry Method:

To measure the total protein in different batches microsome as a normalized basis, lowry method was employed. The standard curve was dipicted here in Fig. 1. Linear concentration range of total protein was between 0.01-0.25mg/ml.



圖一: Lowry method:

R^2:0.997, intercept:0.013, slpoe:1.699

2. 評估 CYP3A 體外活性的 midazolam 微粒體培養法

To investigated the CYP3A metabolic activity of midazolam, different concentrations of MDZ 10-100 uM will be incubated under specific

microsome conditions. The appropriate incubation time was determined to be 5 minutes from Figs 2&3. Similar Plotss can also be obtained when microsomes incubated under 10 uM MDZ (data not shown).

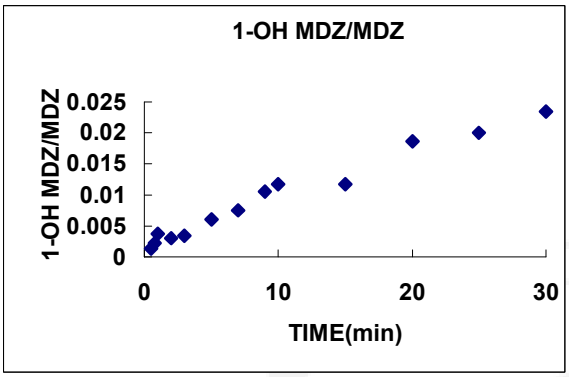


Fig. 2.Formation of 1-OH MDZ vs time at MDZ incubation concentration of 100 uM

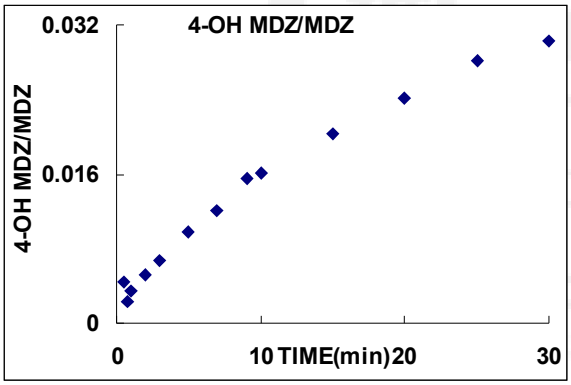


Fig. 3. Formation of 4-OH MDZ vs time at MDZ incubation concentration of 100 uM.

3. 評估 CYP3A 體外活性的 erythromycin 微粒體培養法

To investigated the CYP3A metabolic activity of erythromycin, different concentrations of ERM 50-250 uM will be incubated under specific microsome conditions. The standard curve of DDL was shown in Fig.4. The incubation time remains to be established.

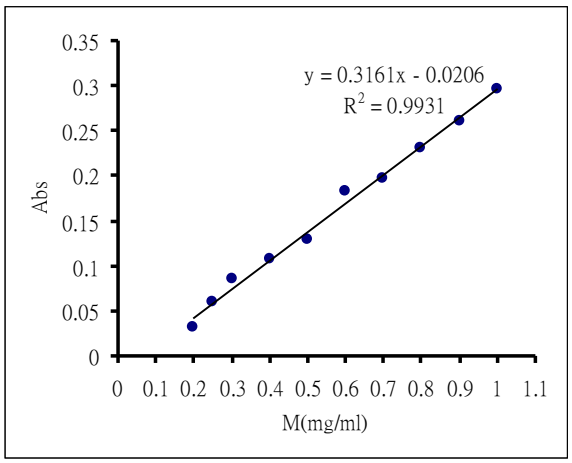


Fig. 4. Calibration of DDL at UV 412 nm (Erythromycin N-deamination Assay)

CONCLUSION

由上述結果顯示，本實驗室已初步完成 MDZ 與 ERM 在微粒體系統的定量分析方法，並建立藉由 midazolam 及 erythromycin 肝及腸微粒體評估 CYP3A 體外代謝活性的系統。未來將針對膽鹽給藥的情況做進一步的體內評估。預試驗結果顯示大量投與膽鹽的情況下，在給予劑量一天後，就觀察到 CYP3A 的活性增加。顯示有急性的酵素誘發反應。而長期的影響仍待進一步的評估。至於相當人類治療膽結石的給藥劑量之長短期對 CYP3A 代謝活性的影響評估，則將在近期內做更進一步評估。

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