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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※　NADPH cytochrome P450 reductase 及 cytochrome b_5 在　※
※ 細胞色素 3A4 及 3A5 相關之藥物交互作用的角色探討研究 ※
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計畫類別：個別型計畫

計畫編號：NSC89－2320－B－041－012－

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：鄭靜玲

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執行單位：嘉南藥理藥理科技大學藥理學院藥學系

中 華 民 國 90 年 10 月 30 日

行政院國家科學委員會專題研究計畫成果報告

NADPH cytochrome P450 reductase 及 cytochrome b_5 在細胞色素 3A4 及 3A5 相關之藥物交互作用的角色探討研究

Roles of NADPH cytochrome P450 reductase and cytochrome b_5 on CYP3A4 and CYP3A5 related drug-drug interactions

計劃編號：NSC 89-2320-B-041-012

執行期間：89 年 8 月 1 日 至 90 年 7 月 31 日

計劃主持人：鄭靜玲　嘉南藥理藥理科技大學藥理學院藥學系

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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 isoenzymes (如 CYP1A1/2, CYP2D6 及 CYP 2C19 等)，從藥物遺傳學 (pharmacogenetics)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，但也同時使控制其代謝活性的限制步驟之機轉曖昧不清。而影響 CYP3A 催化能力的限制機轉仍待解答。也因此，直接針對 CYP3A 做基因多型性的研究，也許並沒有切入問題之重點。又先前在本人的博士論文研究中曾觀察到：臨床投與一新抗病毒用藥 delavirdine 會抑制 CYP3A (IC50 = 0.89 μM ± 0.88；I_{max} = 73.3 ± 6.8；n=12)。數據顯示具合理的 I_{max} 個體差異，但其 IC50 的最大值與最小值之比竟達 55 倍之多。當時因找不出特別的解釋方法，然推測此 IC50 之不尋常差異可能來自個人的「特異體質」。綜合以上之現象與觀察，此「特異體質」極可能是影響 CYP3A 催化步驟之限制因子影響的表現。我們假設催化循環中必要的 NADPH P450 reductase 或 Cytochrome b_5 為可能的限制步驟酵素，會影響與 CYP3A 有關的藥物交互作用。因此在本研究中，我們首先以老鼠為動物模式探討抗病毒用藥 delavirdine 在體內之動態，希望能藉由此研究與後續實驗來瞭解並釐清影響 CYP3A 藥物交互作用有關表現的可能機轉，並進一步能將此結果應用到新藥開發時的篩選研究。

關鍵詞：CYP3A，抗病毒用藥，delavirdine，藥物交互作用。

二、Abstract

It has been observed that for a CYP3A drug substrate, it exhibits the large variations on drug pharmacokinetic parameters. Inter-racial difference also has been observed for CYP3A drug substrates. For most enzymes, such as, CYP2C19, CYP2D6 and NAT-2, these phenomena can be explained by the genetic polymorphism. However, there are no reports

for a CYP3A4 genetic polymorphism related to its catalytic activity. Evidently, genetic polymorphism alone could not explain this large inter-subject variations associated with CYP3A drug substrates. The reason could be the unidentified rate-limiting step in the CYP3A catalytic mechanism. Many studies indicated that the rate-limiting steps could be associated with NADPH cytochrome P450 reductase or cytochrome b_5. In my Ph.D. thesis work, we have found that a CYP3A substrate and inhibitor, delavirdine (a new anti-viral agent), exhibited an substantial inter-subject difference on their IC50 values of CYP3A activity. This large difference on IC50 values rather than V_{max} values were just opposite to what we expected, since V_{max} should be more environmentally dependent. We could only explained this observation by idiosyncrasy. Reviewing the current researches on CYP3A4, we have the feeling that some factor(s), participating the catalytic mechanism, can modify the system IC50 value that is more associated with the relevant element. It is proposed that the possible factors might be the required enzyme(s) in the catalytic mechanism: NADPH cytochrome P450 reductase and/or cytochrome b_5. Additionally, the ratio of NADPH cytochrome P450 reductase to CYP3A and the ratio of cytochrome b_5 to CYP3A will change the the system metabolizing constant (Km). Thereafter, it will causes the intersubject difference on the inhibition constant (ki).

In this program, we will choose a phospholipid-ionic detergent mixture system as the investigating tool to evaluate the roles of NADPH P450 reductase and cytochrome b_5 on CYP3A related drug-drug interactions. A recent study has shown that CYP3A drug substrates could be categorized into two groups. The major human isoforms of CYP3A are CYP3A4 and CYP3A5. Therefore, we plan to investigate this aspect on each isoform for CYP3A typical substrates of each subgroup for consecutive two years. This program will reveal the mechansim of CYP3A inhibition and the relationship between inhibition constant and the ratio of the investigated coenzymes to CYP3A. It will also show the contribution of these coenzymes to the variations on CYP3A catalytic activity.

Keywords：CYP3A，Antivirus agent，delavirdine，drug-drug interaction。

INTRODUCTION

Delavirdine mesylate is a non-nucleoside reverse transcriptase inhibitor of HIV-1 used in AIDS therapy. *In vitro and in vivo* metabolism of delavirdine has shown that the major metabolic pathway is N-desalkylation, which is mainly catalyzed by CYP3A (Cheng et al., 1997). In this report the plasma and red blood cell (RBC) disposition kinetics of delavirdine in rats was investigated and the RBC/plasma partitioning characteristics of delavirdine explored (Cheng, 2001).

MATERIALS AND METHODS

Materials.

Delavirdine mesylate Biomol (Plymouth Meeting, PA, USA)

Cisapride Sigma (St. Louis, MO, USA)

Disposition Kinetics.

Donor: Sprague-Dawley rats (250~350g).

Dose: Single dose i.v. delavirdine mesylate at 0.45 ± 0.04 mg/kg

Sampling: 0 (pre-dose), 10, 20, 40, 60, 90, 120, 150, 180 and 210 min after administration

Delavirdine Assay. Delavirdine was assayed using HPLC (Cheng et al., 2001).

Pharmacokinetic Analysis. The plasma and RBC concentration-time profiles of delavirdine were analyzed by a one-compartment model using a commercial fitting program (WinNonlin, Prof 2.1, Pharsight Inc).

Data Analysis. All data are presented as means ± S.D. Differences in the pharmacokinetic parameters of delavirdine in plasma and in RBCs were analyzed by paired-t tests. To investigate if there is a correlation between RBC/plasma partition (K_{ep}= C_{RBC}/C_P) and time, and K_{ep} versus plasma concentration, Spearman rank correlation was performed.

RESULTS AND DISCUSSION

The plasma concentration-time profile for delavirdine showed typical monoexponential disposition features with an elimination half-life ($t_{1/2}$) of 36.7 min (Fig. 1). The steady-state volume of distribution (V_{ss}), and clearance (Cl) were 1.22 L/kg and 23.6 mL/min/kg, respectively. The RBC concentration-time curves declined in parallel with that for plasma, albeit at a much lower magnitude (Fig. 1).

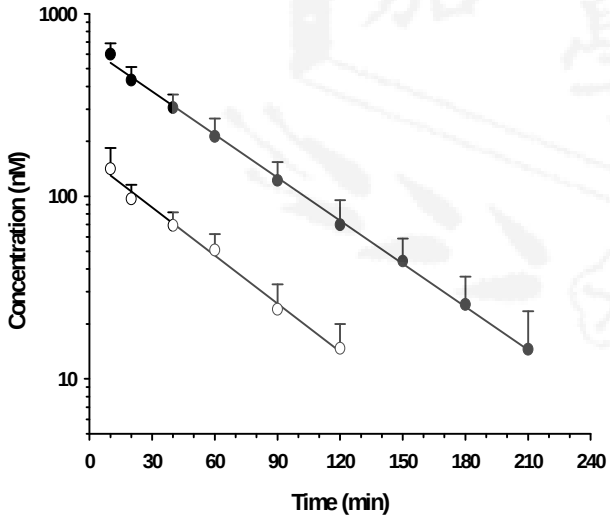

Fig. 1. Plasma (●) and RBC (○) concentration-time profiles of delavirdine in rats after intravenous administration of 0.82 μmol/kg delavirdine mesylate.

The mean value of partition coefficient between RBC and plasma (K_{ep}) for delavirdine was 0.25, and showed no trends over time or concentration-dependency.

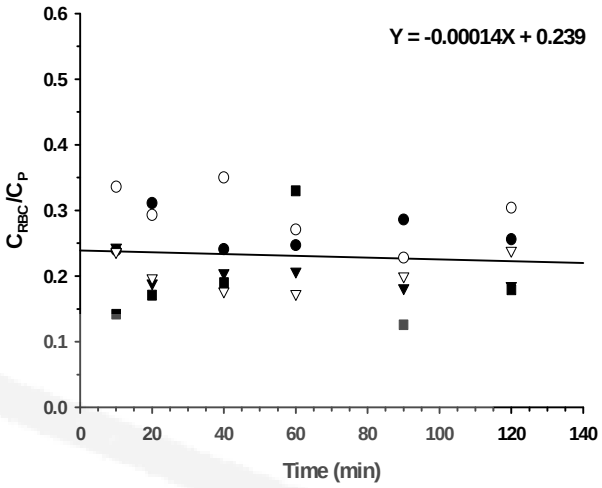

Fig. 2. Time-independent distribution of delavirdine between red blood cells and plasma after intravenous administration. Symbols: rat 1(●), 2 (○), 3 (▼), 4 (▽) and 5(■).

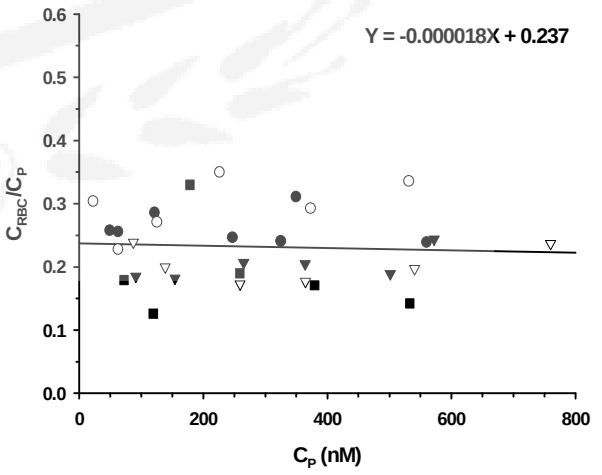

Fig. 3. Concentration-independent distribution of delavirdine between red blood cells and plasma after intravenous administration. Symbols: rat 1(●), 2 (○), 3 (▼), 4 (▽) and 5(■).

CONCLUSION

The results indicated that delavirdine is rapidly and widely distributed into RBCs and extravascular tissues. Although delavirdine binds strongly to plasma protein and partitions into RBC, it is highly extracted and exhibits one-compartmental disposition characteristics in plasma and RBC.

Table 1 Pharmacokinetic Parameters Estimated from Plasma Concentrations after Intravenous Administration of 0.82 μmol/kg Delavirdine in Rats (n=6)

Parameter	Mean	SD	CV (%)
C_0 (μM)	0.68	0.10	14.6
AUC (μM x min)	35.7	6.9	19.3
Cl (mL/min/kg)	23.6	4.8	20.4
V_{ss} (L/kg)	1.22	0.18	15.1
MRT (min)	52.9	8.8	16.6
$t_{1/2}$ (min)	36.7	6.1	16.6
K $(min)^{-1}$	0.019	0.004	20.8

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