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Enzyme kinetics and inhibition of nimodipine metabolism in human liver microsomes[1]

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KEY WORDS nimodipine; metabolism; cytochrome P-450; liver microsomes; kinetics

ABSTRACT

AIM: To study the enzyme kinetics of nimodipine (NDP) metabolism and the effects of selective cytochrome P-450 (CYP-450) inhibitors on the metabolism of NDP in human liver microsomes *in vitro*. **METHODS**: Microsomes from six individual human liver specimens were used to perform enzyme kinetic studies and the kinetic parameters were estimated by Eadie-Hofstee equation. Various selective CYP-450 inhibitors were used to investigate their effects on the metabolism of NDP and the principal CYP-450 isoform involved in dehydrogenation of dihydropyridine ring of NDP in human liver microsomes. **RESULTS**: There was an important intersubject variability in NDP metabolism in human liver microsomes. For NDP dehydrogenase activity, the K_m value was (36 ± 11) μmol and the V_m value was (17 ± 7) μmol $\cdot g^{-1} \cdot min^{-1}$. The dehydrogenation of dihydropyridine ring of NDP was competitively inhibited by ketoconazole (Ket) and troleandomycin (TAO), and the K_i values for Ket and TAO were 0.59 and 122.2 μmol, respectively. Phenacetin (Pnt), quinidine (Qui), diethyldithiocarbamate (DDC), sulfaphenazole (Sul), and tranylcypromine (Tra) had a little or no inhibitory effects on the dehydrogenation of NDP. **CONCLUSION**: The intersubject variability of NDP pharmacokinetics was attributed to the metabolic polymorphism of NDP in liver. Cytochrome P-4503A (CYP3A) is involved in the dehydrogenation of dihydropyridine ring of NDP.

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INTRODUCTION

Nimodipine (NDP) is a second generation dihydropyridine calcium antagonist with a selective relaxant action on cerebral vessels. Pharmacological studies have shown NDP to be a powerful calcium antagonist increasing cerebral blood flow[1,2]. Clinical studies suggest that it might have a protective anti-ischemic effect in patients with subarachnoid hemorrhage and reduce mortality and improve neurological recovery of patients who have suffered an acute ischemic stroke[3].

It was reported that the oral doses of NDP were almost completely absorbed and underwent extensive first-pass metabolism *in vivo*. NDP is a high hepatic clearance drug[4]. Pharmacokinetic studies on both healthy volunteers and patients reveal that there is an important intersubject variability when NDP is administered orally[5]. Large variability in drug disposition may implicate large variability in clinical effects. The purpose of this study was to study the enzyme kinetics of NDP metabolism and the effects of selective CYP-450 inhibitors on the metabolism of NDP in human liver microsomes *in vitro*.

MATERIALS AND METHODS

Chemicals Nimodipine (NDP) was kindly provided by Tianjing Central Pharmaceutical Factory. Glucose-6-phosphate dehydrogenase, troleandomycin (TAO), quinidine (Qui), diethyldithiocarbamate (DDC), sulfaphenazole (Sul), tranylcypromine (Tra) were purchased from Sigma Chemical Co. Ketoconazole (Ket) and phenacitin (Pnt) were kindly provided by Nanjing Second Pharmaceutical Factory. β-Nicotinamide adenine dinucleotide phosphate (NADP) and glucose-6-phosphate were purchased from Shanghai Lizhudongfeng Biotechnological Co. The dehydrogenated metabolite (DNDP) of dihydropyridine ring of NDP was synthesized by Department of Organic Chemistry, China Pharmaceutical University. All other supplies were of the highest

grades available from standard commercial sources.

Tissue samples and preparation of microsomes The six human liver specimens used in the present study were obtained from Jinling Hospital after the study protocol was approved by the Academic Committee of China Pharmaceutical University. The collection process of liver tissues and its morphologic and biochemical characterization were followed as described elsewhere and microsomes were prepared by differential centrifugation[6]. *Microsomal protein concentration was determined* by the method of Lowry *et al*[7].

Incubation and sample preparation The incubation conditions of the experiment were established and controlled to provide a reproducible and linear rate of the metabolite. The incubation consisted of potassium phosphate buffer 0.1 mol·L^{-1} (pH 7.4), an NADPH-generating system (0.5 mmol·L^{-1} NADP, 5.0 mmol·L^{-1} glucose-6-phosphate, 5.0 mmol·L^{-1} $MgCl_2$, 1000 u·L^{-1} glucose-6-phosphate dehydrogenase), microsomal protein 1.0 g·L^{-1} and various concentrations of NDP with or without inhibitors in a final volume of 1 mL. After incubation at 37 ℃ for 20 min, the reaction was terminated by adding 200 μL of NaOH 2 mol·L^{-1}. NDP and its dehydrogenated metabolite (DNDP) formed in the incubation mixture were determined by HPLC method described previously[8] with minor modifications. The reaction mixtures were extracted with 4 mL of ether-hexane (1:1) and centrifuged at 1000 × *g* for 5 min. The organic fraction was evaporated under a gentle stream of air at 50 ℃. The residue was redissolved in 100 μL of mobile phase and an aliquot (20 μL) was injected onto the Hypersil BDS column (4.6 mm × 250 mm, 5 μm). The HPLC system comprised of an LC-10AT pump and a SPD-10AV UV detector.

Kinetic study The kinetic studies were performed using microsomes from six individual human livers. The concentration of NDP ranged from 6.25 to 200 μmol·L^{-1}.

Inhibition study The effects of various selective CYP inhibitors[9] on the dehydrogenation of dihydropyridine ring of NDP in human liver microsomes were investigated. The inhibitors studied were Pnt, Qui, DDC, Sul, Tra, Ket, and TAO. The concentration of NDP was 25 μmol·L^{-1} and the range of inhibitor concentration was 50 – 400 μmol·L^{-1} for TAO, 2.5 – 20 μmol·L^{-1} for Qui, 12.5 – 100 μmol·L^{-1} for Pnt, Sul, DDC, and Tra, and 0.5 – 5 μmol·L^{-1} for Ket. For the determination of K_i values, the range of NDP concentration was 12.5 – 100 μmol·L^{-1}. The range of inhibitor concentrations was 0.5 – 2 μmol·L^{-1} and 50 – 200 μmol·L^{-1} for Ket and TAO, respectively.

Data analysis The data of kinetic experiments were fitted to Eadie-Hofstee equation to estimate apparent kinetic parameters by linear-square regression analysis. Lineweaver-Burk equation were used to determine K_i values of TAO and Ket. Results were expressed as $\bar{x} \pm s$.

RESULTS

Eadie-Hofstee plots for the NDP dehydrogenase activity in microsomes from six human livers (HL) were linear. The individual kinetic parameters are listed in Tab 1. The mean K_m value was (36 ± 11) μmol and mean V_m value was (17 ± 7) μmol·min^{-1}·g^{-1}. Fig 1 shows a representative plot for NDP dedrogenase activity in human liver 2 (HL2).

The effects of inhibitors or probe substrates on DNDP formation are presented in Fig 2. Ket and TAO,

Tab 1. Kinetic parameters for NDP dehydrogenase activity in human liver microsomes. n = 6 human livers. $\bar{x} \pm s$.

Subject	K_m/μmol	V_m/μmol·g^{-1}·min^{-1}
HL1	40.2	17.2
HL2	36.5	11.9
HL3	24.9	15.1
HL4	31.0	9.6
HL5	55.9	29.9
HL6	28.6	17.3
$\bar{x}$	36	17
s	11	7

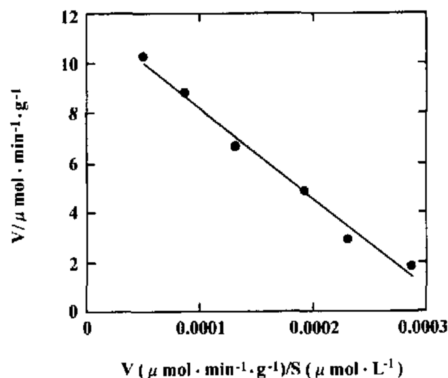

Fig 1. Typical Eadie-Hofstee plot for nimodipine dehydrogenation in human liver microsomes (HL 2). Each point represents the mean of duplicate determinations.

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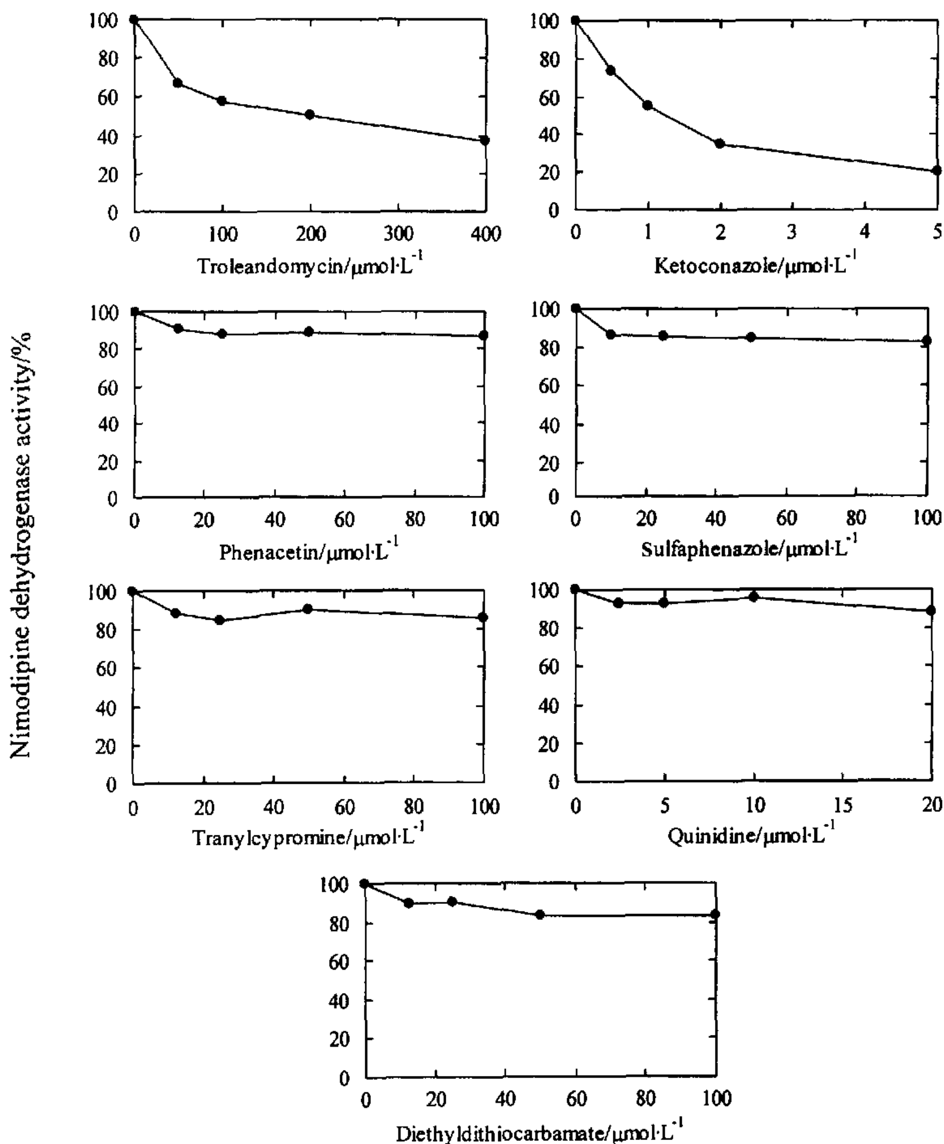

Fig 2. Effects of CYP-selective inhibitors on nimodipine (NDP) dehydrogenase activity in human liver microsomes (HL 2). The effect of each inhibitor was compared with the control values determined by the incubation of NDP (25 μmol·L^{-1}) alone and expressed as the percentage of control values (100 %). Each point represents the mean of duplicate determinations.

Which are specific inhibitors of CYP3A, inhibited the NDP dehydrogenase activity in human liver microsomes while other inhibitors had a little or no inhibitory effect on DNDP formation as shown in Fig 2. Fig 3 shows Lineweaver-Burk plots of NDP dehydrogenase activity in human liver microsomes in the absence or presence of inhibitors. Ket and TAO competitively inhibited the formation of DNDP and lowered the rate of NDP metabolism in human liver mricrosomes. The K_i values for Ket and TAO were 0.59 μmol and 122.2 μmol, respectively (Fig 4).

DISCUSSION

NDP has been shown to be metabolized by three

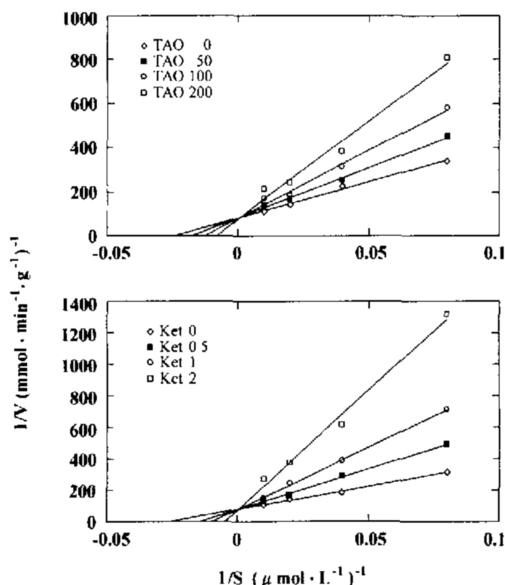

Fig 3. The effects of troleandomycin and ketoconazole on nimodipine dehydrogenase activity in human liver microsomes (HL 2). Each data point represents the mean of duplicate determinations.

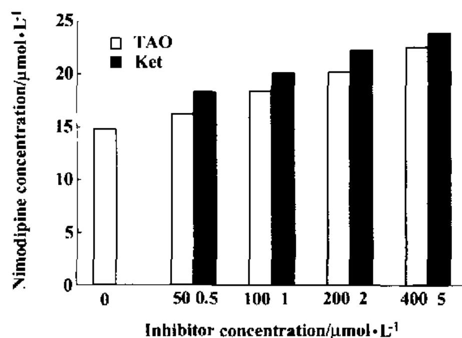

Fig 4. Effects of ketoconazole and troleandomycin on the metabolism of nimodipine (25 μmol·L^{-1}) in human liver microsomes (*HL 2*). *Each data point represents* the mean of duplicate determinations.

major pathways in rats. One of the major metabolite is DNDP, which results from the dehydrogenation of dihydropyridine ring of NDP. This metabolite undergoes futher metabolism to form its corresponding pyridine derivatives[10]. But the enzyme kinetics of nimodipine metabolism and the CYP450 isoform involved in the dehydrogenation of dihydropyridine ring of NDP has not been clarified. The dehydrogenation of dihydropyridine ring of NDP, one of the major metabolic pathways reported in rats were also found in human liver micromsomes. In the previous study, Liu *et al*[11] found that there were large intersubject variability in NDP pharmacokinetics in healthy Chinese subjects. Our results indicated that there was an important intersubject variability in NDP metabolism in human liver microsomes, which suggested that there exists metabolic polymorphism in human liver. The intersubject variability in NDP pharmacokinetics might be attributed to this phenomenon.

Our results showed that Ket and TAO, which are specific inhibitors of CYP3A, could competitively inhibit the formation of DNDP and lower the metabolism rate of NDP while other inhibitors for CYP1A2 (Pnt), CYP2C9 (Sul), CYP2C19 (Tra), CYP2D6 (Qui), and CYP2E (DDC) had a little or no effect on the formation of DNDP. This implicates CYP3A as a major CYP450 isoform involved in the dehydrogenation of dihydropyridine ring of NDP and the above metabolic pathway to be crucial for the elimination of NDP. Pharmacokinetic drug interactions caused by metabolic processes are regarded as the most important factors that affect the concentration of drugs in the body[12]. Therefore, inhibition of above metabolic pathways can lower the rate of NDP metabolism and increase NDP levels in the body, which suggest the possibility of the metabolic interactions of NDP with other therapeutic agents that are inhibitors of CYP3A.

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人肝微粒体中尼莫地平代谢的酶动力学及其抑制[1]

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关键词 尼莫地平; 代谢; 细胞色素 P-450; 肝微粒体; 动力学

目的: 在体外研究人肝微粒体中尼莫地平代谢的酶动力学及选择性的细胞色素 P-450 (CYP450)酶抑制剂对尼莫地平代谢的影响. **方法**: 采用人肝微粒体研究尼莫地平脱氢酶的代谢动力学, 运用 Eadie-Hofstee 方程估算其动力学参数. 在体外运用 CYP450 酶的选择性抑制剂探讨其对尼莫地平代谢的影响及人肝微粒体中参与尼莫地平二氢吡啶环脱氢代谢的 CYP450 酶. **结果**: 尼莫地平在人肝微粒体中的代谢存在较大的个体差异, 尼莫地平脱氢酶的 K_m 为 (36 ± 11) μmol, 其 V_m 为 (17 ± 7) $\mu mol \cdot g^{-1} \cdot min^{-1}$. 酮康唑和三乙酰竹桃霉素竞争性地抑制尼莫地平二氢吡啶环脱氢代谢, 其 K_i 值分别为 0.59 和 122.2 μmol. 非那西丁、奎尼丁、DDC、Sul 和 Tra 对尼莫地平二氢吡啶环脱氢代谢没有明显的影响. **结论**: 尼莫地平在体内的药物动力学个体差异与其在肝中的代谢存在多态性有关. CYP3A 参与了尼莫地平二氢吡啶环脱氢代谢, CYP3A 的抑制剂可能会与尼莫地平发生代谢相互作用.

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