# CYP2D6遗传多态性对普罗帕酮药动学与药效学影响的研究

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# 中文摘要

细胞色素 P450(cytochrome P450,CYP)是人体内参与内源性物质和外源性物质如药物代谢的重要代谢酶。已知异喹胍羟化酶 CYP2D6 存在着显著的遗传多态性,不同种族和不同个体代谢药物的能力有着较大的差异。普罗帕酮是临床上常用的抗心律失常药物,在体内代谢受 CYP2D6 介导,在药动学和药效学上存在的个体差异是否与 CYP2D6 遗传多态性有关尚有待探讨。因此,进行中国人普罗帕酮药动学与药效学结合 CYP2D6 表型和基因型的研究,将有助于了解普罗帕酮在中国人的体内代谢过程与作用方面存在较大个体差异的原因所在,为相关药物的合理用药提供理论依据。为此,本文进行了以下五个部分的研究。

**第一部分　采用右美沙芬为探针测定健康受试者 CYP2D6 表型**

1. 人尿中右美沙芬及其代谢物的测定　采用反相高效液相色谱法(HPLC)以荧光检测分析人尿中右美沙芬(DM)及其代谢物右啡烷(DX)的浓度。DM 和 DX 的最低检测浓度分别为 0.027 mg·$L^{-1}$和 0.031 mg·$L^{-1}$,平均回收率为 105.3% 和 104.5%,日内、日间相对标准差(RSD)均小于 5%。

2. 中国人 CYP2D6 表型分析　120 名健康中国人口服右美沙芬片剂 20 mg 后留 8 h 尿,用 HPLC 测定尿 DM 和 DX 浓度,并计算代谢比值。结果发现 120 名受试者中有 1 名慢代谢者(PM)(0.8%),在余下的快代谢者(EM)中采用数理统计方法可进一步区分为 76 名(63%)极快代谢者(VEM)和 43 名(36%)中速代谢者(IM)。CYP2D6 表型分析为中国人异喹胍多态性提供了新的信息。

**第二部分　手性药物普罗帕酮对映体的人体药动学研究**

1. 普罗帕酮对映体血浆浓度分析　采用高效液相色谱法加柱前衍生化分析血浆中普罗帕酮对映体浓度，得 *S* -，*R* - 普罗帕酮的最低检测浓度分别为 18.6 μg·$L^{-1}$和 15.7 μg·$L^{-1}$，平均回收率分别为 101.9% 和 102.4%，日内、日间 RSD 均小于 6%。

2. CYP2D6 表型对普罗帕酮对映体药动学的影响　17 名健康受试者(7 名 VEM，9 名 IM 和 1 名 PM)口服单剂量盐酸普罗帕酮 400 mg 后，于 0 ~ 15 h 抽取静脉血，并用 HPLC 测定血浆中普罗帕酮对映体浓度(*S* - PPF 和 *R* - PPF)，计算对映体药动学的参数。结果表明，*S* - PPF 体内代谢速率显著大于 *R* - PPF，具有明显的立体选择性；而且，CYP2D6 表型在普罗帕酮对映体的代谢中起重要作用，IM 组的 *Cl* 只有 VEM 组的一半($P < 0.01$)。CYP2D6 酶活性(lgMR)与普罗帕酮对映体药动学参数($C_{max}$，AUC 和 *Cl*)之间存在着良好的相关性。因此，CYP2D6 表型决定了普罗帕酮对映体的药动学差异，IM 的存在也许与中国人 CYP2D6 活性下降有关。

**第三部分　CYP2D6 抑制剂对右美沙芬表型和普罗帕酮对映体药动学的影响**

1. 氟西汀和特比萘芬对右美沙芬表型的影响　健康年轻受试者 19 名(氟西汀组)和 10 名(特比萘芬组)，男女兼有，肝肾功能正常，不嗜烟酒，参加试验前两周禁用任何药物。受试者两次口服右美沙芬及留尿试验同第一部分，其间服用氟西汀 20 mg 连续 10 天或特比萘芬 250 mg 连续 14 天。19 名口服氟西汀的受试者前后的右美沙芬 MR 值分别为 0.03 ± 0.04 和 0.09 ± 0.07($P < 0.01$)，增加 3 倍，说明氟西汀对 CYP2D6 有一定的抑制作用。10 名受试者连续口服 14 天特比萘芬后，右美沙芬 MR 值从 0.016 ± 0.011 增加到 0.321 ± 0.333($P < 0.01$)，增加总幅度约高达 20

倍，有 4 人从 EM 转变为 PM，说明特比萘芬较氟西汀对右美沙芬代谢的抑制作用更强。另外，氟西汀和特比萘芬对 VEM 的抑制作用均较 IM 更强。

2. 氟西汀对普罗帕酮对映体药动学的影响　9 名健康受试者在应用氟西汀(20 mg/d)10 天前后单剂量口服 400 mg 普罗帕酮，定时抽取静脉血并用 HPLC 测定血浆中普罗帕酮对映体浓度，计算对映体药动学参数。结果表明，普罗帕酮对映体在合用氟西汀之后，$t_{1/2}$，$C_{max}$和 AUC 有显著增加($P<0.01$)，而 $Cl$ 则有明显下降($P<0.05$)。而且氟西汀对 $S$－PPF $C_{max}$增加幅度较 $R$－PPF 小(39% vs 71%，$P<0.05$)，$T_{max}$的延长也仅在 $R$－PPF 有显著差异($P<0.05$)，说明氟西汀不仅造成普罗帕酮对映体代谢的减慢，而且这种作用有立体选择性。

**第四部分　中国人 CYP2D6 基因多态性的分子机制研究**

119 名健康汉族志愿者，男女兼有，肝肾功能正常，不嗜烟酒，右美沙芬表型测定区别为 76 名 VEM、42 名 IM 和 1 名 PM。采取受试者全血，用经改良的酚/氯仿抽提法提取 DNA。采用 PCR－RFLP 法测定受试者 CYP2D6 基因型，PCR 反应特异扩增产物经 1.2% 琼脂糖凝胶电泳分析之后，再经 HphI 酶切反应，最后用 3% 琼脂糖凝胶电泳分析缺陷等位基因 CYP2D6＊10B。

结果表明，CYP2D6＊10B 的基因频率为 58.4%，其中 13 名(10.9%)为野生型(w/w)纯合子，33 名(27.7%)为突变型(m/m)纯合子，其余 73 名(61.3%)为杂合子(m/w)。将右美沙芬表型与基因型作比较，发现 76 名 VEM 中有 63 人(约 83%)为 CYP2D6＊10B 的杂合子，42 名 IM 中高达 29 人(近 70%)是由 CYP2D6＊10B m/m 造成的，而 1 名 PM 亦为 m/m 型。另外，10 名受试者不存在与白种人 PM 相关的 6 种 CYP2D6 缺陷等位基因(CYP2D6＊3，＊4，＊5，＊6，＊7 和＊9)。我们的实验提示，CYP2D6＊10B 等位基因的存在，可在很大程度上解释中国人右美

沙芬氧化代谢酶活性降低的分子机制。

**第五部分 普罗帕酮的临床药效学研究**

1. 普罗帕酮的药效学研究 健康志愿者 10 名，男女各半，所有受试者经右美沙芬表型测定分为 5 名 VEM 和 5 名 IM。受试者口服普罗帕酮 400 mg 后，于给药后 1,2,3,4,6,8 和 15 h 取血并测定心电图指标 PR 间期。血浆中普罗帕酮浓度系采用 HPLC 测得的 *S* - PPF 和 *R* - PPF 浓度之和。采用 CAPP 软件对普罗帕酮血药浓度及 PR 间期延长百分率进行药动 - 药效结合计算，符合一级吸收二房室加效应室模型。实验表明，普罗帕酮的药效学过程符合 Sigmoid $E_{max}$ 模型，效应与时间之间存在着较好的相关性。CYP2D6 表型对药动学和药效学参数亦有影响，IM 组的 AUC 明显高于 VEM 组，而 $Ce_{50}$ IM 组也比 VEM 组大（$P<0.05$）。10 名受试者的平均药效学参数 $K_{e0}$ 为 1.17 $h^{-1}$，$E_{max}$ 为 43.7%，$Ce_{50}$ 为 477.7 $\mu g \cdot L^{-1}$，$\gamma$ 为 1.85。

2. 室性早搏病人口服普罗帕酮达稳态后的药效观察 17 例室性早搏病人（VPC≥1000 次/d），男女不限，肝肾功能、血尿常规检查正常。每位受试者停用所有心脏活性药物 5 个半衰期之后，口服普罗帕酮 150 ~ 200 mg/次，3 次/d 共 7 天，于给药前描记 12 导心电图和做 24 h 动态心电图，在给药 7 天后同上做两种心电图，并抽取用药前和用药后 2 h 血，用 HPLC 测定血浆中的普罗帕酮浓度。所有病例还测定了 CYP2D6 基因型。结果表明，17 例病人室性早搏的总抑制率（VPC%）达 65.3%，PR 间期从用药前的 (0.15 ±0.02)s增加到(0.16 ±0.02)s($P<0.05$)。然而普罗帕酮血浆浓度与 VPC% 和 PR% 等指标相关性不大。CYP2D6 基因型对普罗帕酮血浆浓度和疗效有明显作用，*10/*10 组病人不仅 $C_{max}$ 约 2 倍于 *1/*1 组病人，而且 VPC% 也约 2 倍于后者，说明 CYP2D6 *10B 与右美沙芬中速代谢缺陷高度相关，血药浓度升高与药效增加一致。

**关键词**：细胞色素 P450，异喹胍羟化酶（CYP2D6），遗传（基因）多态性，右美沙芬，氧化代谢，表型，高效液相色谱法（HPLC），普罗帕酮，对映体，立体异构，药动学，抑制剂，基因型，聚合酶链反应（PCR），限制性片段长度多态性（RFLP），药效学，药动－药效结合模型，宅性早搏。

# ABSTRACT

Cytochrome P450 is one of the most important metabolic enzymes, which are responsible for many endogenous and exogenous substances such as drugs in human body. It has been well known that debrisoquine hydroxylase (CYP2D6) exists significant genetic polymorphism with great differences of drug metabolizing ability in different races and individuals. Propafenone, a commonly used antiarrhythmic agent, is given clinically as a racemate. It is biotransformed mainly through CYP2D6 to the active metabolite (5 - OH propafenone). There is great pharmacokinetic and pharmacodynamic variability of propafenone. Whether this variability in Chinese is related to CYP2D6 genetic polymorphism needs to be clarified. Therefor, the purpose of this paper is to determine the effect of CYP2D6 genetic polymorphism on pharmacokinetics and pharmacodynamics of propafenone in Chinese.

**Part I. Determination of CYP2D6 phenotype by using dextromethorphan as a probe drug**

1. Analysis of dextromethorphan (DM) and dextrophan (DX) in human urine: A reverse-phase high performance liquid chromatographic (HPLC) method was established for the determination of DM and DX. The lowest detection levels of DM and DX were 0.027 mg · $L^{-1}$ and 0.031 mg · $L^{-1}$, respectively. The within-day and between-day relative standard deviations (RSD) were all below 5%. The average recoveries of DM and DX were 105.3% and 104.5%, respectively.

2. Phenotyping of CYP2D6 in Chinese subjects: 120 healthy Chinese

subjects took dextromethorphan tablets (20 mg) orally and 8 h urine was collected overnight. The concentrations of DM and DX were assayed by HPLC and molar metabolic ratios (MR) were calculated. The incidence of poor metabolizers (PM) was 0.8% (one in 120 subjects). There were distinct bimodal distributions, which divided extensive metabolizers (EM) into 43 intermediate metabolizers (IM), and 76 very extensive metabolizers (VEM). Dextromethorphan metabolic phenotyping provides a new information for debrisoquine 4 – hydroxylase(CYP2D6) polymorphism in native Chinese.

**Part Ⅱ. Clinical pharmacokinetics of propafenone enantiomers**

1. Analysis of plasma concentrations of propafenone enantiomers: A HPLC method with precolumn derivatization was used to quantitate plasma concentrations of propafenone enantiomers. The lowest detective levels of *S* – propafenone (*S* – PPF) and *R* – propafenone (*R* – PPF) were 18.6 $\mu g \cdot L^{-1}$ and 15.7 $\mu g \cdot L^{-1}$, respectively. The average recoveries of *S* – PPF and *R* – PPF were 101.9% and 102.4%, respectively. The within-day and between-day RSD were all less than 6%.

2. Influences of CYP2D6 phenotypes on pharmacokinetics of propafenone enantiomers: A single dose of propafenone hydrochloride (400 mg) was given orally to 17 healthy Chinese subjects (7 VEM, 9 IM and 1 PM). Plasma concentrations of propafenone were measured by HPLC during 0 ~ 15 h after administration. Pharmacokinetic parameters were calculated thereafter. The results showed that *S* – PPF was less metabolized and had higher plasma concentrations than *R* – PPF in both CYP2D6 phenotypes. Besides, the $t_{1/2}$ of *R* – PPF was larger than that of *S* – PPF in IM, but not in VEM. However, there were significant differences in the metabolism of PPF enanti-

omers between VEM and IM. The $C_{max}$ and AUC of both isomers in the IM group were higher than those in the VEM group ($P < 0.01$). The $Cl$ of PPF enantiomers in IM group were only about half of that in VEM group [(67.3 ± 18.6) vs(124.8 ± 26.1) L · $h^{-1}$ for $S$ – PPF, (90.2 ± 23.6) vs (186.6 ± 70.2) L · $h^{-1}$ for $R$ – PPF, $P < 0.01$]. The $S/R$ ratio of $T_{1/2}$, $C_{max}$, $Cl$ and AUC was not significantly different ($P > 0.05$). The correlation between dextromethorphan MR and pharmacokinetic parameters ($C_{max}$, AUC and $Cl$) were highly significant.

**Part Ⅲ. Influences of CYP2D6 inhibitors on dextromethorphan phenotypes and pharmacokinetics of propafenone enantiomers**

1. Effects of fluoxetine and terbinafine on dextromethorphan: 19 (fluoxetine group) and 10 (terbinafine group) young healthy subjects were recruited, with normal hepatic and kidney functions. All were non-smokers and drug free for at least 2 weeks before and during the study. Fluoxetine hydrochloride 20 mg was given once daily to all 19 subjects for 10 days. Terbinafine hydrochloride 250 mg was given once daily to all 10 subjects for 14 days. Dextromethorphan phenotyping tests were performed before and after pretreatment of CYP2D6 inhibitors. There were significant differences of mean dextromethorphan MR values before and after fluoxetine therapy (0.03 ± 0.04 vs 0.09 ± 0.07, $P < 0.01$), indicating a strong inhibition of the CYP2D6 activity by fluoxetine in Chinese subjects. For 10 subjects treated with terbinafine for 14 days, mean MR values were increased from 0.016 ± 0.011 to 0.321 ± 0.333 ($P < 0.01$) with a 20 fold raise in extent. Four out of 10 subjects were converted to PM of CYP2D6, indicating a stronger inhibitive effect of terbinafine on dextromethorphan metabolism compared to fluoxetine. Besides, the inhibitory effects of fluoxe-

tine and terbinafine on VEM were stronger than on IM.

2. Influence of fluoxetine on pharmacokinetics of propafenone enantiomers: Nine healthy subjects administered fluoxetine hydrochloride 20mg once daily for 10 days. Pharmacokinetics of propafenone enantiomers after a single dose of 400mg propafenone hydrochloride was performed before and after fluoxetine pretreatment. The $t_{1/2}$, $C_{max}$ and $AUC_{0-\infty}$ of two enantiomers after fluoxetine therapy were significantly increased compared to those at baseline ($P < 0.01$), whereas, oral clearance decreased from (75.01 ± 17.69) L/h to (49.36 ± 8.62) L/h for *S* – propafenone ($P = 0.005$) and from (107.62 ± 33.82) L/h to (70.60 ± 12.42) L/h for *R* – propafenone ($P = 0.027$). Besides, fluoxetine increased the peak concentration of *S* – propafenone by 39% and that of *R* – propafenone by 71% ($P < 0.05$). A significant increase of $T_{max}$ was seen only in the *R* – enantiomer, not the *S* – enantiomer of propafenone after fluoxetine therapy. Our results suggest that fluoxetine not only impairs propafenone metabolism significantly, but also raises its effect stereoselectively.

**Part Ⅳ. Molecular mechanism of genetic polymorphism of CYP2D6 in Chinese subjects**

119 healthy HAN volunteers with normal hepatic and kidney functions and both genders were recruited. Dextromethorphan phenotyping showed that there were 76 VEM, 42 IM and one PM of CYP2D6. DNA was extracted from peripheral blood by a modified phenol/chloroforms method. In order to determine CYP2D6 genotype, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were use to analyze CYP2D6 * 10B alleles. The gene frequency of CYP2D6 * 10B was 58.4%, including 13 (10.9%) homozygous wild type (w/w), 33 (27.7%) of homozygous mutant

(m/m), and 73 (61.3%) of heterozygous genotype (m/w). Twenty – nine subjects out of 42 IM (69%) of dextromethorphan were homozygous for CYP2D6 * 10B. One PM subject also showed a m/m genotype. Besides, 10 Chinese subjects were tested and excluded for the presence of any for the six mutant alleles associated with poor metabolism of CYP2D6 in Caucasians. It is concluded that the CYP2D6 * 10B allele containing the $C^{188} \rightarrow T$ mutation is the major cause of CYP2D6 polymorphism in relation to diminished dextromethorphan oxidative capacity in Chinese subjects.

**Part V. Clinical pharmacodynamics of propafenone**

1. Simultaneous modeling of pharmacokinetics and pharmacodynamics of propafenone: Ten healthy volunteers with each gender were recruited. Dextromethorphan phenotyping showed that there were 5 VEM and 5 IM of CYP2D6. After oral administration of 400 mg of propafenone hydrochloride, blood collection and PR interval monitoring were performed at 1,2,3,4,5,6,8 and 15 h. Total propafenone concentrations as a sum of *S* – PPF and *R* – PPF were determined as before. A computer aid pharmacokinetic & pharmacodynamic program (CAPP) was used to simulate plasma concentrations of propafenone and percentage of PR interval prolongation by using a model of first rate, two compartment plus effect compartment. It has been shown that pharmacodynamic course of propafenone accord with sigmoid $E_{max}$ model in Chinese subjects. There were good relationship between drug effect and time. CYP2D6 phenotype also played a role in pharmacokinetic and pharmacodynamics of propafenone. AUC of IM group was significantly higher than that of VEM group. Whereas, $Ce_{50}$ of IM group was also greater than that of VEM group ($P < 0.05$). The average pharmacokinetic parameters in 10 subjects were as following: $K_{co}$

$=1.17\ h^{-1}$, $E_{max}=43.7\ \%$, $Ce_{ss}=477.7\ \mu g \cdot L^{-1}$, $\gamma=1.85$.

2. Pharmacodynamic observation of steady-state propafenone in patients with ventricular premature contractions: 17 patients with ventricular premature contractions (VPC ≥ 1000/d) were recruited from hospitals. They were normal in routine laboratory testing. Every patient was free of cardioactive drugs for at least 5 half-life. The patient administered propafenone hydrochloride 450 ~ 600 mg per day in three divided doses. Twelve-lead cardiogram and Holter monitoring were performed before and after propafenone administration for 7 days. Peak and trough levels of propafenones were drawn after drug treatment for 7 days and were measured by HPLC as before. CYP2D6 genotypes were assayed for each patient. Our result showed that total inhibitory rate of ventricular premature contractions (VPC%) was 65.3%. PR interval prolongation was increased from (0.15 ± 0.02) s to (0.16 ± 0.02) s after drug treatment ($P<0.05$). However, there were no significant relationship between plasma concentrations of propafenone and pharmacodynamic index such as VPC% and PR%. CYP2D6 genotypes played an important role in plasma levels and effect of propafenone. Patients with homozygous mutant of CYP2D6 * 10B not only had a $C_{max}$ two times as high as wild-type, but also had VPC% two times as high as wild-type. It suggests that CYP2D6 * 10B is highly related to IM and elevated plasma level is consistent with better efficiency of propafenone.

**Conclusion**: Genetic polymorphism of CYP2D6 contributes to the variability in pharmacokinetics and pharmacodynamics of propafenone in Chinese. It provides useful information for safe and rational use of propafenone and related drugs clinically.

**KEY WORDS:** Cytochrome P450, debrisoquine hydroxylase (CYP2D6), genetic polymorphism, dextromethorphan, oxidative metabolism, phenotype, high performance liquid chromatography, propafenone, enantiomer, stereoisomerism, pharmacokinetics, inhibitor, genotype, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), pharmacodynamics, simultaneous modeling of pharmacokinetics and pharmacodynamics, ventricular premature contraction.

# 目　录

# 第1章 前言

人肝微粒体中的细胞色素 P450(cytochrome P450, CYP)氧化酶是由许多同工酶(isozyme)组成的重要代谢酶。已发现 30 多种结构与功能相关的超系统基因编码的 P450 氧化酶,在体内除了参与内源性物质如脂肪酸、胆固醇、类固醇激素和花生四烯酸等的合成与分解外,也参与了进入人体内的药物、杀虫剂、毒物和致癌物、致突变剂的代谢。

已知涉及药物代谢比较重要的 P450 氧化酶有 CYP1A2, CYP2D6, CYP2C19 和 CYP3A4 等同工酶,它们负责了约 90% 以上药物的体内代谢。而其中的 CYP2D6(又称异喹胍羟化酶)临床意义最大,因为该酶不仅负责超过 40 种临床重要药物(如抗心律失常药物、抗抑郁药、抗精神病药、抗高血压药及右美沙芬、吗啡等)的体内氧化代谢,而且这些药物的不良反应、相互作用发生率较高也与 CYP2D6 遗传多态性有关。

CYP2D6 存在着显著的遗传多态性,即不同种族或不同个体,因遗传基因上的微小变化导致其表达的酶的活性在质和量上的种族和个体差异,表现为在某一种群中代谢药物的能力呈不连续的多峰曲线分布。已知,CYP2D6 在西方白种人(caucasians)中的慢