BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 399-402

# Inhibition of imipramine *N*-demethylation by fluvoxamine in Chinese young men[1]

XU Zhen-Hua, HUANG Song-Lin, ZHOU Hong-Hao[2]
(*Pharmacogenetics Research Institute, Hu-nan Medical University, Changsha 410078, China*)

**KEY WORDS** imipramine; desipramine; fluvoxamine; drug interactions; pharmacokinetics

**AIM:** To determine effect of fluvoxamine (Flu) on imipramine (Imi) *N*-demethylation in healthy Chinese subjects. **METHODS:** The pharmacokinetic parameters of a single oral dose of Imi (100 mg) were compared before and during 2 wk coadministration of Flu (100 mg·d$^{-1}$). **RESULTS:** Flu resulted in a doubling of $C_{max}$ (112±33 *vs* 62±22 μg·L$^{-1}$, $P<0.01$) and an increase in $AUC_{0-\infty}$ (3.9±1.6 *vs* 1.5±0.6 mg·h·L$^{-1}$, $P<0.01$) of Imi. The $T_{1/2}$ of Imi was prolonged (39±8 *vs* 23±7 h, $P<0.01$) due to a marked reduction in $Cl_0$ of Imi (0.6±0.3 *vs* 1.3±0.6 L·h$^{-1}$·kg$^{-1}$, $P<0.01$). The $C_{max}$ of *N*-demethylated metabolite desipramine (Des) of Imi was decreased (10±2 *vs* 18±5 μg·L$^{-1}$, $P<0.01$) and $AUC_{0-\infty}$ of Des was reduced by 54 % (0.61±0.20 *vs* 1.3±0.5 mg·h·L$^{-1}$, $P<0.01$) during coadministration with Flu. **CONCLUSION:** Flu can markedly inhibit Imi *N*-demethylation in Chinese young men.

Imipramine (Imi), a tricyclic antidepressant, undergoes mainly *N*-demethylation to the active metabolite desipramine (Des) and aromatic hydroxylation to 2-hydroxyimipramine. *N*-demethylation is the most important pathway leading to Des, which is further oxidized to 2-hydroxydesipramine. 2-Hydroxylation of Imi and Des is catalyzed almost entirely by debrisoquine 4-hydroxylase (CYP2D6)[1,2], whereas *N*-demethylation of Imi is catalyzed by other cytochrome isozymes which possibly are mephenytoin 4′-hydroxylase (CYP2C19)[3] and CYP1A2[3,4].

Fluvoxamine (Flu), a selective serotonin reuptake inhibitor, is a potent inhibitor of CYP1A2 in human liver microsomes[5]. Flu, given at therapeutic dosages, increased the plasma levels of concurrently administered Imi, suggesting that CYP1A2 plays an important role in Imi *N*-demethylation *in vivo*[6]. However, the test dose (50 mg) of Imi was not enough to quantify plasma concentrations of metabolically derived Des. A 100-mg dose of Imi was chosen in the present study to obtain definite information on whether Flu inhibits Imi *N*-demethylation. In addition, the CYP2C19 phenotype of each subject was determined using mephenytoin as probe drug in our study to assess the relative contribution of CYP2C19 to Imi *N*-demethylation.

## SUBJECTS AND METHODS

**Subjects** Eight male nonsmoking Chinese Han nationality volunteers aged 21.4±1.2 a and weighing 58.4±7.0 kg, took part in this study. All subjects were in good health according to clinical history, physical examination, and routine laboratory tests. No medications were allowed for at least 2 wk prior to the study. During the study, the subjects were also asked not to consume alcohol and caffeine-containing drinks. Seven of the subjects were extensive metabolizers (EM) of both debrisoquine and mephenytoin. One of the subjects was poor metabolizer (PM) of mephenytoin (S/R ratio=1.12), but EM of debrisoquine. One subject, an EM of both debrisoquine and mephenytoin, was withdrawn because of noncompliance. All subjects gave an informed written consent before enrollment.

**Study design** Each man underwent 2 phases separated by a drug-free interval of 3 wk in a random sequence. In phase 1, each man received 100 mg Imi hydrochloride (Shanghai Huanghe Pharmaceutical Factory) as a single oral dose after an overnight fast. No food was allowed for the following 2 h. Venous blood samples (6 mL) for drug assay were drawn into heparinized tubes at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 h after dosing Imi. Plasma was stored at −20 ℃. In phase 2, each man received 50 mg Flu (Fevarin® Duphar, Weesp, The Netherlands) twice daily for 2 wk. At 7:00 on d 8 of

[1] Supported by grant 92-568 from China Medical Board of New York, USA, and grant 39330230 from National Natural Science Foundation of China
[2] Correspondence to Prof ZHOU Hong-Hao
Received 1995-08-14 Accepted 1996-04-19

treatment with Flu, a single oral dose of Imi (100 mg) was coadministered with 50 mg Flu. Plasma samples were collected as in phase I.

**Sample analysis** Imi and Des in plasma were analyzed by HPLC using α-naphthylamine as the internal standard[7,8]. In brief, a reversed-phase column (150 mm × 4.6 mm ID) packed with 5 μm Spherisorb C18 (Beckman, USA) was used. The eluent consisted of 63 % $KH_2PO_4$ 0.01 mol·$L^{-1}$ containing 0.1 % *n*-butylamine (adjusted to pH 2.3 with concentrated $H_3PO_4$) and 37 % acetonitrile, at a flow rate of 1.5 mL·$min^{-1}$. The wavelength of ultraviolet detection was set at 215 nm. Standard curves (peak height ratio) were linear in the concentration range of 5 – 500 μg·$L^{-1}$ for both Imi and Des. The intra- and interday coefficients of variation were <8.5 %.

**Data analysis** Noncompartmental techniques were used in the pharmacokinetic analyses. The pharmacokinetic parameters were compared by use of paired *t* test.

## RESULTS

During concurrent administration of Flu, plasma concentrations of Imi were considerably higher than those after dosing Imi alone in 6 d (Fig 1, *upper*). Flu caused substantial changes in pharmacokinetic parameters of Imi (Tab 1). In the presence of Flu, both $C_{max}$ and $AUC_{0-\infty}$ of Imi were approximately doubled ($P<0.01$). The $Cl_0$ of Imi was reduced by 60 % ($P<0.01$), accompanied by a 1.7-fold prolongation of Imi $T_{1/2}$ ($P<0.01$) and a 30 % decrease in $V_d$ ($P<0.01$). The $T_{max}$ of Imi seemed to be sligtly delayed by Flu ($P<0.05$), but this had no clinical implication. Meanwhile, Flu resulted in about 50 % decrease in both $C_{max}$ and $AUC_{0-\infty}$ ($P<0.01$) of Des derived metabolically from oral Imi (Tab 2, Fig 1).

**Tab 2. Pharmacokinetic parameters of desipramine after *po* imipramine 100 mg before and during treatment with fluvoxamine. * PM of mephenytoin. [a]$P>0.05$, [c]$P<0.01$ *vs* Imi alone.**

| Man | $C_{max}$/μg·$L^{-1}$ | | $T_{max}$/h | | AUC/μg·h·$L^{-1}$ | |
|---|---|---|---|---|---|---|
| | Imi | Imi + Flu | Imi | Imi + Flu | Imi | Imi + Flu |
| 1* | 14 | 8 | 8 | 8 | 1 017 | 366 |
| 2 | 25 | 16 | 12 | 8 | 1 608 | 939 |
| 3 | 16 | 10 | 6 | 12 | 644 | 412 |
| 4 | 16 | 10 | 8 | 12 | 1 026 | 571 |
| 5 | 19 | 9 | 12 | 12 | 1 496 | 771 |
| 6 | 12 | 7 | 12 | 12 | 1 042 | 434 |
| 7 | 27 | 9 | 8 | 8 | 2 394 | 751 |
| $\bar{x}$ | 18 | 10[c] | 9.4 | 10.3[a] | 1 318 | 607[c] |
| *s* | 5 | 2 | 2.3 | 2.0 | 532 | 202 |

The highest oral clearance of Imi occurred in the *PM* of mephenytoin (Tab 1). To avoid a wrong phenotype of CYP2C19, the subject was tested repeatedly using mephenytoin.

## DISCUSSION

The present study has clearly shown that Flu increases markedly the plasma concentration of concurrently administered Imi by inhibition of Imi *N*-demethylation. The observed Flu-Imi interaction may be attributable to impairment of CYP1A2 activity by Flu[4,5]. However, recently we found that Flu was also a modest inhibitor of CYP2C19 (Xu and Zhou, unpublished), which is partly responsible for Imi *N*-demethylation *in* vivo[3].

**Tab 1. Pharmacokinetic parameters of imipramine (100 mg *po*) before and during treatment with fluvoxamine. * PM of mephenytoin. [b]$P<0.05$, [c]$P<0.01$ *vs* Imi alone.**

| Man | $C_{max}$/μg·$L^{-1}$ | | $T_{max}$/h | | AUC/μg·h·$L^{-1}$ | | $T_{\frac{1}{2}}$/h | | $Cl_0$/L·$h^{-1}$·$kg^{-1}$ | | $V_d$/L·$kg^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Imi | Imi + Flu | Imi | Imi + Flu | Imi | Imi + Flu | Imi | Imi + Flu | Imi | Imi + Flu | Imi | Imi + Flu |
| 1* | 37 | 61 | 2 | 4 | 712 | 1 635 | 12.6 | 25.3 | 2.5 | 1.1 | 44.7 | 39.2 |
| 2 | 53 | 122 | 3 | 4 | 1 696 | 5 364 | 31.9 | 46.8 | 0.9 | 0.3 | 43.1 | 20.0 |
| 3 | 37 | 100 | 3 | 3 | 1 104 | 1 946 | 27.0 | 30.4 | 1.7 | 1.0 | 65.4 | 41.8 |
| 4 | 85 | 103 | 3 | 4 | 1 057 | 3 118 | 15.3 | 37.6 | 1.7 | 0.6 | 38.0 | 31.6 |
| 5 | 90 | 163 | 3 | 3 | 2 257 | 5 608 | 24.4 | 37.7 | 0.7 | 0.3 | 25.2 | 15.6 |
| 6 | 46 | 89 | 4 | 6 | 1 405 | 3 824 | 21.0 | 45.3 | 1.2 | 0.4 | 34.9 | 27.6 |
| 7 | 86 | 149 | 3 | 3 | 2 359 | 5 818 | 31.5 | 50.1 | 0.6 | 0.3 | 29.2 | 18.8 |
| $\bar{x}$ | 62 | 112[c] | 3 | 4[b] | 1 513 | 3 908[c] | 23.4 | 39.0[c] | 1.3 | 0.6[c] | 40.1 | 27.8[c] |
| *s* | 22 | 33 | 0.5 | 1 | 577 | 1 614 | 7.0 | 8.4 | 0.6 | 0.3 | 12.2 | 9.5 |

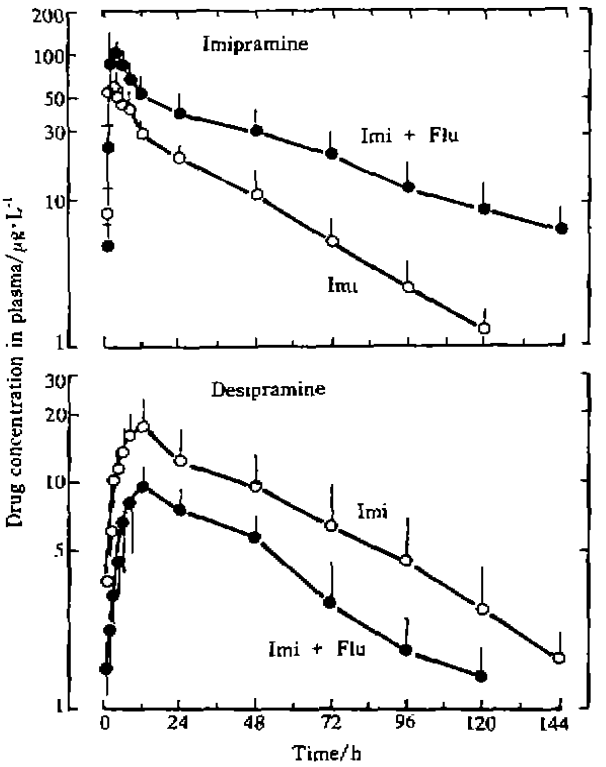


**Fig 1. Imipramine and desipramine concentrations in plasma of 7 men after *po* imipramine 100 mg before and during treatment with fluvoxamine $\bar{x} \pm s$.**

The inhibition of CYP2C19 activity may also explain Flu-Imi interaction. Interestingly, Flu also inhibited Imi *N*-demethylation in one PM of mephenytoin as it did in EMs. Obviously, Flu-Imi interaction in PM of mephenytoin can not be accounted for by inhibition of CYP2C19 since CYP2C19 activity inbornly disappears in PM of mephenytoin. It needs to be explained that the highest oral clearance of Imi occured in the PM of mephenytoin. A possible explanation is that the contribution of CYP2C19 in Imi *N*-demethylation is minor. However, this subject had the highest activity of CYP2D6 among 7 volunteers, which was shown by 0 – 8 h urinary excretion of α-hydroxymetoprolol after *po* metoprolol 100 mg (data not shown). Therefore, the fast hydroxylation of Imi and Des may be another explanation of the higest clearance of Imi in the PM of mephenytoin.

Although the plasma concentration of 2-hydroxyimipramine was not measured in this study, we can postulate CYP2D6-mediated aromatic 2-hydroxylation of Imi is not inhibited by Flu according to the facts: (1) Flu is only a very weak inhibitor of CYP2D6 in human liver microsomes ($K_i$ = 8.2 μM)[9], (2) Flu doesn't affect the disposition of desipramine[6], which is also mainly catalyzed by CYP2D6[1,2].

In summary, Flu can markedly delay the elimination of Imi by inhibition of *N*-demethylation. This is probably based on impairment of CYP1A2 by Flu, which can well explained Flu-Imi interaction in all subjects. Considering only one PM of mephenytoin was enrolled in this study, further studies are required.

**ACKNOWLEDGEMENT** To Dr V Martinelli (University of Cagliari, Italy) for providing us with fluvoxamine.

## REFERENCES

1 Brøsen K, Otton SV, Gram LF. Imipramine demethylation and hydroxylation: Impact of the sparteine oxidation phenotype. Clin Pharmacol Ther 1986; **40**: 543 – 9.

2 Brøsen K, Zeugin T, Meyer UA. Role of P450IID6, the target of the sparteine/debrisoquin oxidation polymorphism, in the metabolism of imipramine. Clin Pharmacol Ther 1991; **49**: 609 – 17

3 Madsen H, Nielsen KK, Brøsen K. Imipramine metabolism in relation to the sparteine and mephenytoin oxidation polymorphisms — a population study. Br J Clin Pharmacol 1995; **39**: 433 – 9.

4 Lemoine A, Gautier JC, Azoulay D, Kiffel L, Belloc C, Guengerich FP, *et al*. Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver. Mol Pharmacol 1993; **43**: 827 – 32

5 Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S Fluvoxamine is a potent inhibitor of cytochrome P4501A2. Biochem Pharmacol 1993; **45**: 1211 – 4.

6 Spina E, Pollicino AM, Avenoso A, Campo GM, Perucca E, Caputi AP. Effect of fluvoxamine on the pharmacokinetics of imipramine and desipramine in healthy subjects. Ther Drug Monit 1993; **15**: 243 – 6.

7 Proelss HF, Lohmann HJ, Miles DG. High-performance liquid-chromatographic simultaneous determination of commonly used tricyclic antidepressants. Clin Chem 1978; **24**: 1948 – 53.

8 Foglia JP, Sorisio D, Perel JM. Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection. J Chromatogr 1991; **572**: 247 – 58.

9 Crewe HK, Lennard MS, Tucker GT, Woods FR, Haddock RE. The effect of selective serotonin re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver

R 96 P R 978.1



microsomes. Br J Clin Pharmacol 1992; 34: 262-5

397-402

## 氟伏沙明抑制中国青年人体内米帕明去甲代谢

许振华，黄松林，周宏灏[1]
(湖南医科大学遗传药理学研究所，长沙 410078，中国)

关键词 米帕明；地昔帕明；氟伏沙明；药物相互作用；药物动力学

目的：研究氟伏沙明(Flu)对健康中国志愿者体内米帕明(Imi)去甲代谢的影响。方法：7名青年志愿者连续口服Flu (100 mg/天) 2周，在服用Flu的第8天受试者口服Imi (100 mg)，将此时Imi及其去甲代谢产物地昔帕明(Des)的药动学参数与Imi单独口服时进行比较。结果：Flu使口服单剂量Imi的体内消除过程发生显著改变，服用Flu导致Imi $C_{max}$和AUC约增高1倍，$T_{1/2}$显著延长，$Cl_0$降低60 %。同时，Imi的主要代谢产物Des的生成减少，表现在$C_{max}$和AUC均明显降低。结论：Flu能明显抑制Imi的去甲基代谢，CYP1A2被抑制可能是Flu和Imi发生相互作用的生化基础。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 402-406

## Doxorubicin cellular pharmacokinetics plays no role in chemosensitizing effect of verapamil on Swiss-3T3 cells

WANG Nan, TANG Zhong-Ming, ZHANG Yang-Pei, DING Qing-Ming
(*Institute of Radiation Medicine, Beijing* 100850, *China*)

**KEY WORDS** doxorubicin; verapamil; drug resistance; pharmacokinetics; fibroblasts; cultured tumor cells

**AIM**: To find whether or not the doxorubicin (Dox) cellular pharmacokinetics plays a role in chemosensitizing effect of verapamil (Ver) on drug sensitive cells. **METHODS**: Cytotoxicity and cellular Dox contents (during accumulation and retention periods) were measured in the absence and presence of verapamil in Swiss-3T3 cells and compared with those in multidrug resistant (MDR) MCF-$7^{Adr}$ cells and drug sensitive MCF-$7^{WT}$ cells. *mdr*-1 mRNA expression in Swiss-3T3 cells was analyzed. **RESULT**: Dox cytotoxicity was enhanced 2.0-fold in Swiss-3T3 cells by Ver (3 μmol·$L^{-1}$) and 3.6-fold in MCF-$7^{Adr}$ cells by Ver (6 μmol·$L^{-1}$), but not in MCF-$7^{WT}$ cells (Ver 6 μmol·$L^{-1}$). Cellular accumulation of equi-effective concentrations of Dox increased at 6-h incubation in the presence of Ver in Swiss-3T3 (1.5-fold) and MCF-$7^{WT}$ cells (2.1-fold) but decreased rapidly in MCF-$7^{Adr}$ cells by 20 % to 50 % compared to that in the absence of Ver. Cellular retention of Dox decreased after 10-min increase in the presence of Ver in Swiss-3T3 cells compared to that in the absence of Ver, that was similar to that in MCF-$7^{WT}$ cells, while the retention was augmented by Ver in MCF-$7^{Adr}$ cells. Slot blot analysis of RNA revealed no *mdr*-1 gene expression in Swiss-3T3 cells. **CONCLUSION**: Changes in cellular accumulation and retention of Dox did not account for the chemosensitizing effect of Ver on Swiss-3T3 cells.

Received 1995-07-03 Accepted 1996-04-24

Multidrug resistance (MDR) was associated with the expression of multidrug resistance genes encoding a family of integral membrane glycoproteins known as P-glycoproteins (Pgp)[1]. These transporting proteins bound various chemotherapeutic agents and pumped them out of the cells. Verapamil (Ver), a well-known resistant modifier or chemosensitizer, competitively bound to Pgp and increased the cellular uptake and cytotoxicity of drugs in a calcium-independent manner[2]. Sensitizing effect of Ver was occasionally noted in drug sensitive cells[3] or non-