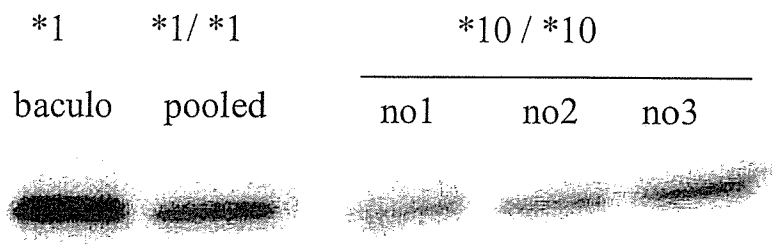

Fig. 7　ウェスタンブロットによる各種ヒト肝ミクロソーム中の CYP2D6 発現量

（左）　0.02pmol CYP2D6*1 バキュロウイルス発現系ミクロソーム (control)

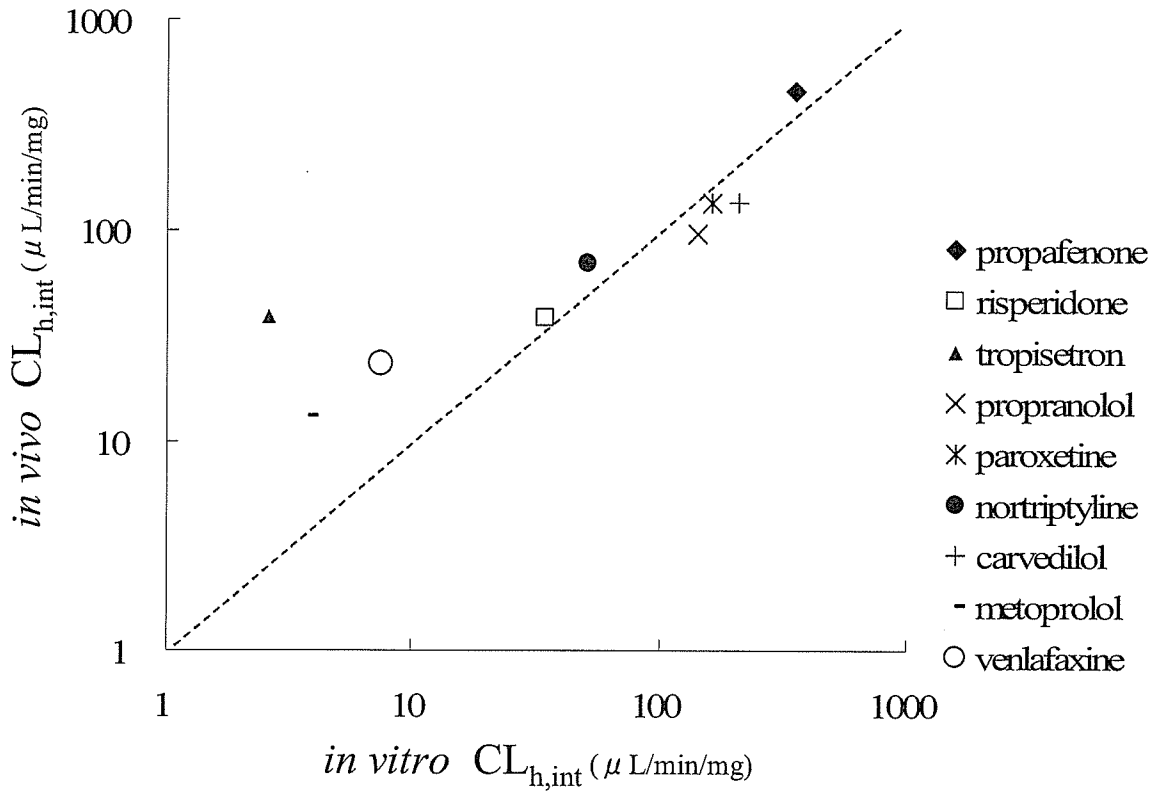

Fig. 8　EM の肝ミクロソームを用いた *in vitro* 代謝実験より求めた肝固有クリアランスと *in vivo* より算出した $CL_{h,int}$※との相関関係

* 経口投与後の AUC 及び血漿中蛋白非結合分率の文献値より算出

資料 2.

The Influence of East Asian Specific Variant, CYP2D6*10, on *in vitro* Formation of Endoxifen, an Active Metabolite of Tamoxifen

Abstract

Tamoxifen (TAM) is widely used in the breast cancer adjuvant therapy, and the most of its efficacy is ascribable to a secondary metabolite, endoxifen (EDX), which is generated from TAM by hepatic cytochrome P450s including CYP2D6. In the present study, we evaluated the *in vitro* metabolizing activity of CYP2D6*10/*10, an East Asian abundant variant with decreasing activity, to form EDX. *N*-Desmethyltamoxifen, a primary metabolite of TAM, was incubated with human liver microsomes from variants of CYP2D6*1/*1 (wild type), *4/*4 (typical genotype of "poor metabolizer" in Caucasians), and *10/*10. The formation rate of EDX by *10/*10 microsome was 23% of the *1/*1 microsome whereas no EDX was produced in the *4/*4 microsome. Based on these data, together with reported data, the steady state plasma concentrations of EDX in *10/*10 and *4/*4 subjects were predicted to be 36% and 17% of wildtype, respectively. Due to the decreased EDX plasma concentration, the relative plasma

antiestrogenic potency during the TAM therapy was estimated to be reduced to 49% in*10/*10 subjects and 34% in *4/*4 subjects, respectively. These predictions indicated that the individualized TAM therapy based on CYP2D6 genotype would be clinically beneficial not only in Caucasians but also in East Asians.

Introduction

Tamoxifen (TAM) is a selective estrogen receptor modulator, which has been widely used for breast cancer around the world since its approval in 1973. Its label dosage is 20-40 mg/day orally, and this drug is administered for generally 5 years.

TAM and its most abundant primary metabolite, *N*-desmethyltamoxifen (NDM), share similar *in vitro* antiestrogenic activities, whereas both of another primary metabolite, 4-hydroxy-tamoxifen (4-OH), and a secondary metabolite, endoxifen (EDX, 4-hydroxy-*N*-desmethyltamoxifen), exert 100-fold higher activity than TAM and NDM (Fig. 1 and Refs. 1 and 2). Considering the lower plasma concentration of 4-OH than EDX, EDX should be the dominating efficacious component in the systemic circulation (2).

It is considered that a hepatic enzyme, CYP2D6, plays a key role in the generation of EDX from TAM (Fig. 1). Particularly, the conversion from NDX to EDX is exclusively mediated by CYP2D6 (2). CYP2D6 is one of the most polymorphic CYP enzymes and its variant frequency is highly dependent on ethnicity. Allele frequencies of inactive variants (*4 and *5) are higher in Caucasians than East Asians, whereas *10 with the decreased activity is East Asian specific. Although CYP2D6*10 homozygote is classified as the intermediate metabolizer (IM) in general, the blood concentration of some drugs were increased markedly in this population (3, 4), which is more evident than Caucasian IMs having genotypes of *1/*4 or *1/*5.

Jin *et al.* (5) have reported that the plasma EDX concentration in Caucasian poor metabolizers of CYP2D6 (PM; *4/*4) was reduced to 26% of the wildtype subjects. Furthermore, Goetz *et al.* (6) have reported that CYP2D6 PM had worse relapse-free time and disease-free survival compared to extensive metabolizers (EM; *1/*1) in the North Central Treatment Group (NCCTG) randomized Phase III trial. In view of these results, in October 2006, the FDA Clinical Pharmacology Subcommittee-Advisory Committee had recommended changes in TAM label so that patients can benefit equally from TAM based on genotype of CYP2D6.

Considering high allele frequency of *10 variants (40-50%) (7, 8), and increasing numbers of breast cancer patients in East Asian countries, there is a serious need to clarify the clinical significance of having the *10 allele(s) in the overall TAM therapy. However, no literature exists on the plasma concentration of EDX or the metabolizing activity to generate EDX in East Asian CYP2D6*10/*10 carriers to our knowledge. Therefore, we evaluated the metabolizing activity of the East Asian specific variant CYP2D6*10 on EDX formation from NDM using *10/*10 human hepatic microsome to predict the influence of the variant on the TAM therapy.

Methods

Reagents

Human liver microsomes of EM (*1/*1, pooled lot from more than 20 subjects) and PM (*4/*4) were purchased from Gentest (Massachusetts, USA). Human liver *10/*10 microsomes from three Chinese subjects were purchased from RILD (Shianghai, China). Tamoxifen, beta-nicotinamide adenine dinucleotide phosphate sodium salt (NADPH), D-glucose 6-phosphate disodium salt hydrate (G6P), and glucose 6-phosphate

dehydrogenase (G6P-DH) were purchased from Sigma (Missouri, USA). 4-Hydroxytamoxifen and *N*-desmethyltamoxifen were purchased from Toronto Research Chemicals (Ontario, Canada). Other reagents of the highest grade available were used. EDX was synthesized from 4-hydroxytamoxifen according to the method of Pelander *et al.* (9).

Incubation of human liver microsome

Microsome incubation was performed to metabolize NDM to EDX. The incubation was initiated by an addition of 0.5 mM NADPH into 10 mL glass tubes with glass lids pooled with 0.1 mM EDTA, 2 mM G6P, 4 mM $MgCl_2$, 1 U/mL G6P-DH, 30 μM NDM, and microsome (0.25-1.0 mg protein/mL). The final volume was 0.3 mL. The glass tubes were incubated for 0 or 10 min at 37°C. The incubation was terminated by an addition of 0.1 mL ice-cold Glycine/Na buffer (pH 11.3). An internal standard (100 nM propranolol in DMSO) and 3 mL of hexane : isopropanol (95:5) were added, shaken for 30 min, and then centrifuged at 3000*g* for 5 min. The upper phase was transferred to a 10 mL glass tube, evaporated to dryness, and resolved in 0.1 mL of the HPLC mobile phase described below.

Quantification of EDX

EDX was quantified by an HPLC method with online derivatization to a fluorescent compound by a photoreactor according to a method by Lee *et al.* (10) with some modifications. The HPLC system was composed of a controller (SCL-10A), pumps (LC-10AD), an auto injector (SIL-10AXL), a degasser (DGU-4A), a column oven (CTO-10A), a fluorometric detector (RF-10A), and an integrator (C-R7Aplus), from Shimadzu (Kyoto, Japan). A UV photoreactor (tube length 5 m, S-3900, Soma Optics,

Tokyo, Japan) was attached prior to the fluorometric detector for postcolumn derivation. The chromatographic separation was carried out on a CN column (YMC-Pack CN, 0.005 mm, 4.6 x 250 mm, YMC, Kyoto, Japan) connecting with a CN guard column (YMC, Kyoto, Japan) by using a mobile phase composed of 0.1 M formic acid (pH 3.0) and acetonitrile (70.5:29.5, v/v) at the flow rate of 1.0 mL/min. The column temperature was set at 40 °C. An aliquot (80 μL) of the sample was injected into the HPLC and the fluorescence of photoreacted derivatives in the effluents was detected at EM 256 nm, EX 380 nm. The detection limit of EDX was 2 nM, and the linearity was confirmed up to 500 nM.

Prediction of the plasma concentration of EDX in CYP2D6*10 carriers

The ratios of the steady-state plasma concentration of EDX in CYP2D6*10/*10 and *1/*10 carriers to that in *1/*1 carriers were predicted by the following two steps. In the first step, the contribution of CYP2D6 in the overall oxidative metabolism of TAM to EDX by various CYPs was estimated from *in vitro* kinetic parameters of the TAM biotransformation in human liver microsome reported by Desta *et al.* (11). The fraction of Substrate A to be transformed to Metabolite B ($F_{A\rightarrow B}$) was estimated by intrinsic clearances (CL_{int}), which were calculated by V_{max}/K_m reported in the literature as follows:

$$F_{A\rightarrow B} = \frac{CL_{int,A\rightarrow B}}{CL_{int,A\rightarrow B} + \sum CL_{int,A\rightarrow Others}} \qquad \text{Eq.1}$$

We used the mean value of the kinetic parameters reported by Desta *et al.* (11), with an exception for $F_{NDM\rightarrow EDX}$. For the determination of $F_{NDM\rightarrow EDX}$, we omitted the value of the lowest CYP2D6 metabolizing activity which may be collected from PM (11). The fractions to EDX from TAM *via* NDM and that *via* 4-OH were calculated by the

following equations, respectively:

$$F_{TAM \rightarrow EDX,NDM} = F_{TAM \rightarrow NDM} \times F_{NDM \rightarrow EDX} \quad \text{Eq.2}$$

$$F_{TAM \rightarrow EDX,4-OH} = F_{TAM \rightarrow 4-OH} \times F_{4-OH \rightarrow EDX} \quad \text{Eq.3}$$

The contribution of CYP2D6 to the overall process from TAM to EDX ($F_{TAM \rightarrow EDX, 2D6}$) was estimated by the following equation considering that the biotransformation from NDM to EDX is exclusively mediated by CYP2D6 (2).

$$F_{TAM \rightarrow EDX,2D6} = \frac{F_{TAM \rightarrow EDX,NDM}}{F_{TAM \rightarrow EDX,NDM} + F_{TAM \rightarrow EDX,4-OH}} \quad \text{Eq.4}$$

In the second step, we predicted the ratios of the steady-state plasma concentration of EDX in CYP2D6*10/*10, *1/*10, PM (assuming *4/*4), and IM (assuming *1/*4) subjects *versus* *1/*1 subjects (R) according to the following equations:

$$R_{*10/*10} = 1 - F_{TAM \rightarrow EDX,2D6} \times (1 - ratio_{*10}) \quad \text{Eq. 5}$$

$$R_{*1/*10} = 1 - F_{TAM \rightarrow EDX,2D6} \times (1 - ratio_{*10}) \times 1/2 \quad \text{Eq. 6}$$

$$R_{PM} = 1 - F_{TAM \rightarrow EDX,2D6} \quad \text{Eq. 7}$$

$$R_{IM} = 1 - F_{TAM \rightarrow EDX,2D6} \times 1/2 \quad \text{Eq. 8}$$

In these equations, "$ratio_{*10}$" refers to *in vitro* EDX formation ratio from NDM by *10/*10 liver microsome divided by that by wild type microsome, which were obtained in the present study.

The steady state plasma concentrations of TAM, NDM, 4OH and EDX in *1/*1 subjects were obtained from results of the clinical research by Jin *et al.* (5). We assumed that the concentrations of TAM, NDM and 4-OH were not altered in *10/*10, *1/*10, PM, and IM subjects. Relative plasma antiestrogenic potencies in *10/*10, *1/*10, PM, and IM subjects during the TAM therapy were calculated as the sum of

products of the estimated plasma concentrations of TAM, NDM, 4-OH and EDX with relative antiestrogenic activities as 1 for TAM and NDM, and 100 for 4-OH and EDX (1, 2).

Results and Discussion

The conversion of TAM to EDX consists of several biological transformation processes mediated by cytochrome P450 enzymes, and the overall process is so slow that the direct measurement *in vitro* is unpractical (Fig. 1). The process includes two pathways; the major pathway is *via* NDM and the minor one is *via* 4-OH (Fig. 1).

It has been reported that CYP2D6 exclusively mediates the transformation of NDM to EDX, while some contribution to a transformation of TAM to 4-OH was also suggested (Fig. 1, Ref. 2). The transformation from TAM to NDM and that from 4-OH to EDX are mediated by CYP3A4 and 3A5 (Fig. 1). In the present study, we hypothesized that the NDM pathway should be abolished completely in the absence of CYP2D6, whereas the contribution of CYP2D6 to the 4-OH pathway is minimal. This hypothesis is supported by the fact that the plasma concentration of EDX decreased markedly in PM of CYP2D6 whereas the concentration of 4-OH was not altered (5). Based on these considerations, we focused our efforts to the conversion of NDM to EDX, in order to evaluate the contribution of CYP2D6 in generation of EDX.

In our experiments, metabolizing velocities for the formation of EDX from NDM were 6.3±2.0 and 26.8 (pmol/min/mg protein, mean±SD) in liver microsomes from CYP2D6*10/*10 carriers (n = 3) and EM (a pooled lot), respectively (Fig. 2). EDX was not detectable by incubating NDM with the human liver microsome from PM. These results suggest that the metabolism to EDX from NDM is exclusively mediated by

CYP2D6 and its activity in *10/*10 carriers is reduced to 23.4% of EM. In general, together with *1/*4 and *1/*5 carriers, *10/*10 carriers are classified as IM. However, by considering the marked decrease in the formation of EDX in *10/*10 microsome, there emerges a need not only in PM but also in *10/*10 carriers to inquire for plasma EDX concentration and clinical outcome of TAM therapy.

From the reanalysis of *in vitro* data reported in Ref. 11, it was estimated that 82% of TAM is metabolized to NDM in the liver and then 17% of NDM is followed by the further transformation to EDX (Fig. 1). In parallel, 6.1% of TAM is metabolized to 4-OH and then 48% of 4-OH is followed by a transformation to EDX (Fig. 1). Consequently, the respective contribution ratios of the NDM and the 4-OH pathways were estimated to be 83 and 17% of the overall generation of EDX.

The result of the prediction of plasma EDX concentrations utilizing *in vitro* and our present results are summarized in Table 1. Considering our results, EDX formation *via* NDM would be reduced in *10/*10 and *1/*10 subjects, and total EDX formation were predicted to be 36% and 68% of control, respectively (Table 1). In the same way, in the PM (inactive variants) and IM (hetero-carrier of inactive variants), the total EDX formation were predicted to be 17% and 59% of control, respectively (Table 1). Concerning the human *in vivo* results, the observed plasma EDX concentration in PM and IM patients taking TAM were reported to be 26% and 55% of EM, respectively (Table 1 and Ref. 5). If we consider the presence of additional metabolic pathway of 4-OH by SULT (エラー! 参照元が見つかりません。), it may be concluded that the clinical data of PM and IM may be satisfactorily predicted by the present analysis method. The present analysis predicted that the concentrations of EDX are in the order of PM < *10/*10 < IM < *1/*10 < EM.

We also simulated the relative plasma antiestrogenic potency during the TAM therapy,

considering plasma concentrations and *in vitro* activities of TAM and its metabolites in PM, IM, *10/*10 and *1/*10 subjects (Fig. 3). The relative potency were calculated to decrease to 49 and 74% in *10/*10 and *1/*10 subjects, and 34 and 67% in PM and IM, respectively (Fig. 3). Although these results need to be quantitatively compared with the actual outcomes of TAM therapy, we could provide the basic information for the individualized therapy of TAM.

Considering the high frequency of *10 allele in the East Asian population, it could be predicted that one fifth of this population, who are *10/*10 carriers, may not be receiving enough benefit from TAM therapy in spite of the long term therapy. The results of the present study suggest that the individualized TAM dosage according to CYP2D6 genotype would be clinically beneficial not only for Caucasians but also for East Asian patients.

In conclusion, our microsome incubation results showed that CYP2D6*10/*10 carriers would have 23% of the metabolizing activity from TAM to EDX compared to EM. Together with the reported data, we estimated that the antiestrogenic potency of TAM therapy would be in the order of PM < *10/*10 < IM < *1/*10 < EM. Considering the high frequency of *10 allele in East Asians, individualized TAM therapy would be clinically beneficial for East Asian patients having CYP2D6 variants with decreasing activity.

References

1. Johnson, M.D., Zuo, H., Lee, K.H., Trebley, J.P., Rae, J.M., Weatherman, R.V., Desta, Z., Flockhart, D.A., and Skaar, T.C.: Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res. Treat.,* 85:151-9 (2004).
2. Stearns, V., Johnson, M.D., Rae, J.M., Morocho, A., Novielli, A., Bhargava, P., Hayes,

D.F., Desta, Z., and Flockhart, D.A: Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J. Natl. Cancer Inst.,* 95:1758-64 (2003).

3. Kim, M.K., Cho, J.Y., Lim, H.S., Hong, K.S., Chung, J.Y., Bae, K.S., Oh, D.S., Shin, S.G., Lee, S.H., Lee, D.H., Min, B., and Jang, I.J.: Effect of the CYP2D6 genotype on the pharmacokinetics of tropisetron in healthy Korean subjects. *Eur. J. Clin. Pharmacol.,* 59:111-6 (2003).
4. Yu, A., Kneller, B.M., Rettie, A.E., and Haining, R.L.: Expression, purification, biochemical characterization, and comparative function of human cytochrome P450 2D6.1, 2D6.2, 2D6.10, and 2D6.17 allelic isoforms. *J. Pharmacol. Exp. Ther.,* 303:1291-300 (2002).
5. Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K.H., Skaar, T., Storniolo, A.M., Li, L., Araba, A., Blanchard, R., Nguyen, A., Ullmer, L., Hayden, J., Lemler, S., Weinshilboum, R.M., Rae, J.M., Hayes, D.F., and Flockhart, D.A.: CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J. Natl. Cancer Inst.,* 97:30-9 (2005).
6. Goetz, M.P., Rae, J.M., Suman, V.J., Safgren, S.L., Ames, M.M., Visscher, D.W., Reynolds, C., Couch, F.J., Lingle, W.L., Flockhart, D.A., Desta, Z., Perez, E.A., and Ingle, J.N.: Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J. Clin. Oncol.,* 23:9312-8 (2005).
7. Tateishi, T., Chida, M., Ariyoshi, N., Mizorogi, Y., Kamataki, T., and Kobayashi, S.: Analysis of the CYP2D6 gene in relation to dextromethorphan O-demethylation capacity in a Japanese population. *Clin. Pharmacol. Ther.,* 65:570-5 (1999).
8. Johansson, I., Oscarson, M., Yue, Q.Y., Bertilsson, L., Sjoqvist, F., and Ingelman-Sundberg, M.: Genetic analysis of the Chinese cytochrome P4502D locus:

characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Mol. Pharmacol.,* 46:452-9 (1994).

9. Pelander, A., Ojanperä, I., and Hase, T. A.: Preparation of N-demethylated drug metabolites for analytical purposes using 1-chloroethyl chloroformate. *Forensic Science International.,* 85:193-198 (1997).
10. Lee, K.H., Ward, B.A., Desta, Z., Flockhart, D.A., and Jones, D.R.: Quantification of tamoxifen and three metabolites in plasma by high-performance liquid chromatography with fluorescence detection: application to a clinical trial. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* 791:245-53 (2003).
11. Desta, Z., Ward, B.A., Soukhova, N.V. and Flockhart, D.A.: Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J. Pharmacol. Exp. Ther.,* 310:1062-75 (2004).
12. Falany, J.L., Pilloff, D.E., Leyh, T.S., and Falany, C.N.: Sulfation of raloxifene and 4-hydroxytamoxifen by human cytosolic sulfotransferases. *Drug. Metab. Dispos.,* 34:361-8 (2006).

Table

Table 1 ----- Predicted EDX plasma concentrations in carriers of CYP2D6*10/*10, *1/*10, PM and IM genotypes during the TAM therapy. Plasma concentrations of EDX were predicted for subjects with different genotypes of CYP2D6 gene. The prediction is based on the data shown in Fig. 2 and the metabolic scheme in Fig. 1. The results are given as the ratio to EM (*1/*1).

Genotype	Plasma concentration of EDX (ratio to EM)	
	Predicted	Observed[$1] (95% confidence interval)
*10/*10	0.36	-
*1/*10	0.68	-
PM[$2]	0.17	0.26 (0.14-0.37, n = 3)
IM[$3]	0.59	0.55 (0.43-0.68, n = 29)

$1: Data from Ref 5.

$1: Combinations of inactive alleles such as *4 and *5 are assumed.

$2: Genotype of *1/*4 or *1/*5 is assumed.

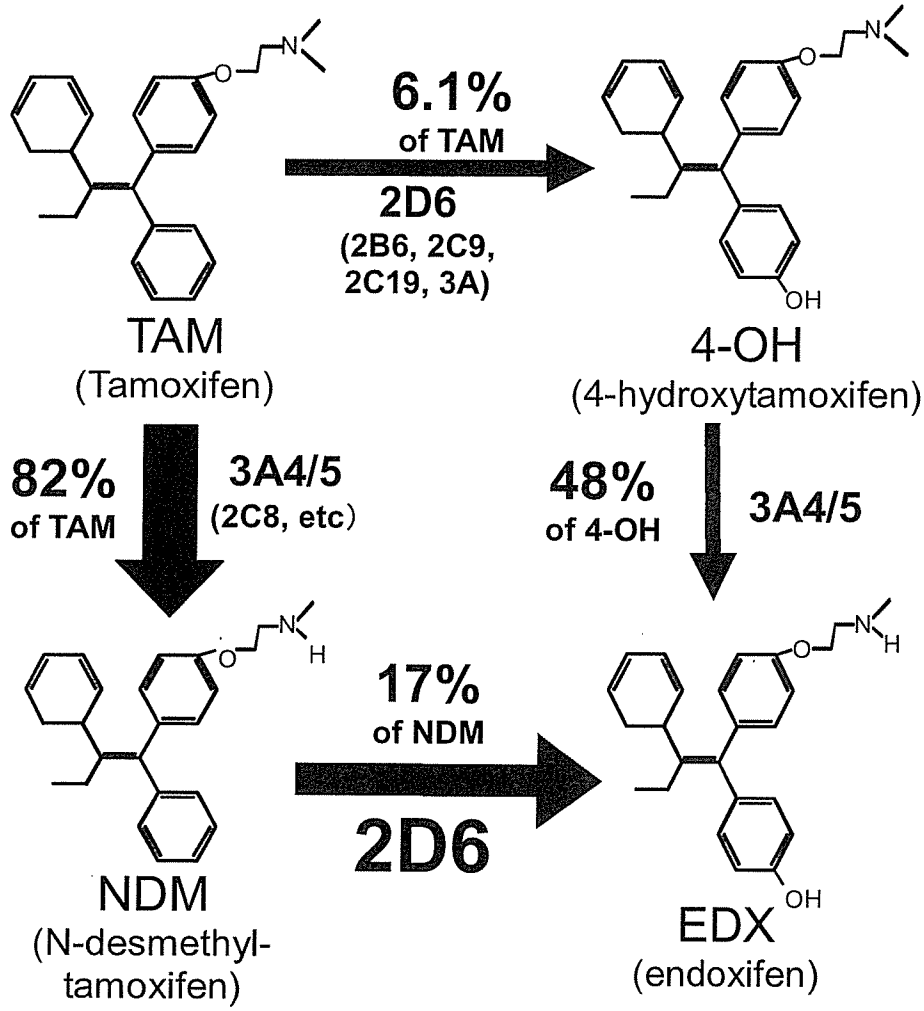

Fig. 1 ----- Collaboration of various CYPs in the metabolism of TAM to EDZ. Metabolic pathway of TAM to EDX is shown. The predicted values for the quantitative contribution of individual steps to the metabolism were calculated based on the literature data (11). See text for the detailed description.

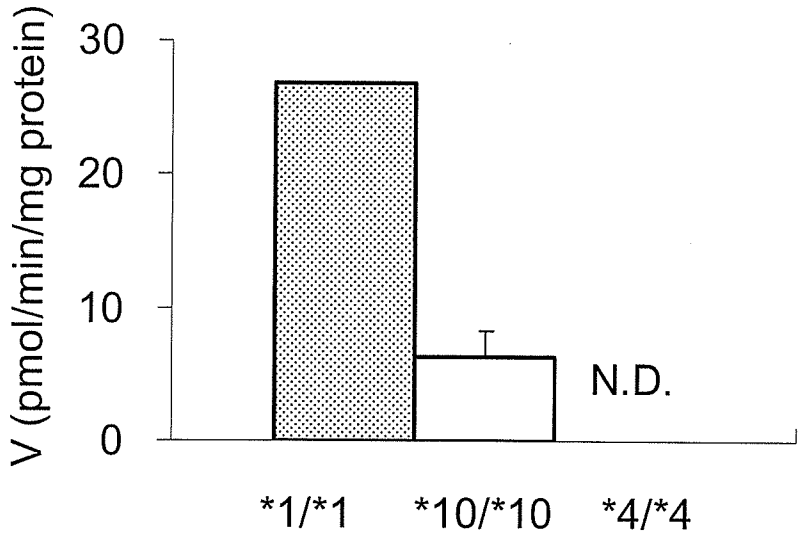

Fig. 2 ----- Metabolizing activities of EDX formation from NDM in human liver microsomes of different CYP2D6 genotypes. NDM (30 μM) was incubated with the human microsomes (0.25-1.0 mg protein / mL) with different genotypes of CYP2D6. For *1/*1, the microsome used was a pooled lot prepared from more than 20 subjects while *4/*4 microsome was prepared from one subject. For *10/*10 microsome, the mean ± SD of three individual lots are shown. All activities were determined by triplicate incubations. ND: not detected.

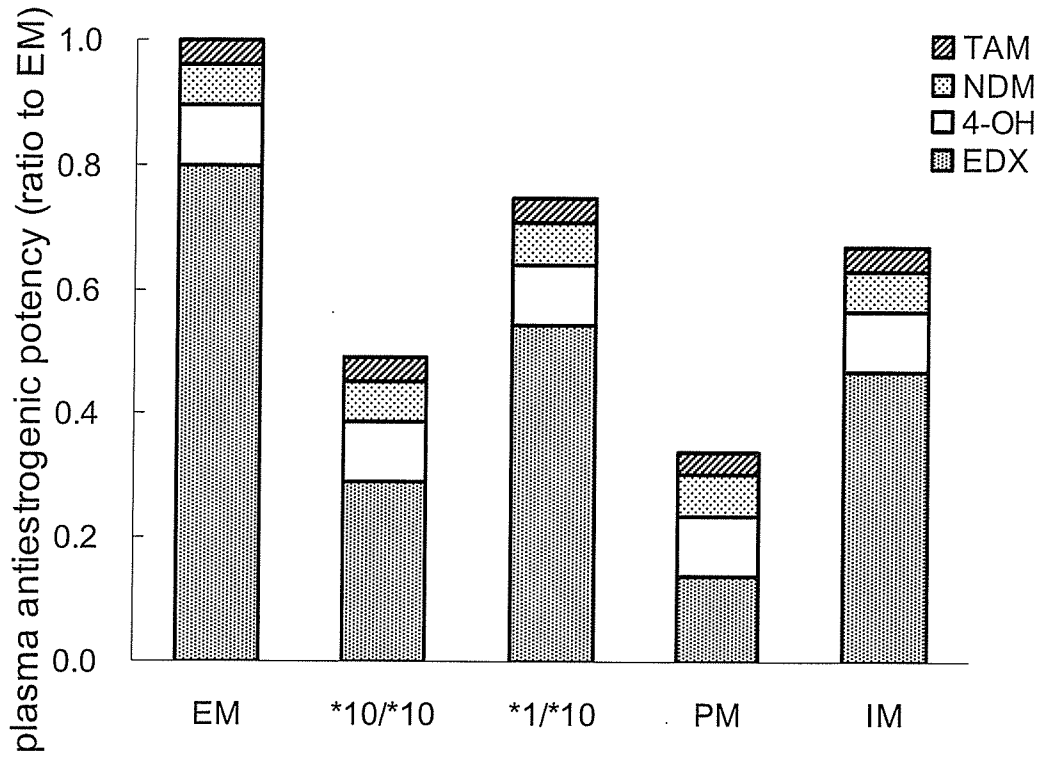

Fig. 3 ----- **Predicted relative plasma antiestrogenic potency of TAM and its three metabolites in different CYP2D6 genotypes.** Relative plasma antiestrogenic potency was calculated by multiplying the predicted plasma concentrations by the *in vitro* antiestrogenic activity of TAM and its subsequent metabolites. Data are presented as a ratio to EM. TAM: tamoxifen, NDM: N-desmethyltamoxifen, 4-OH: 4-hydroxytamoxifen, EDX; endoxifen

資料３.

General Framework for the Quantitative Prediction of CYP3A4-mediated Oral Drug Interactions Based on the AUC Increase by Coadministration of Standard Drugs

Yoshiyuki Ohno, Akihiro Hisaka, and Hiroshi Suzuki

Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo
Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan

(Clinical Pharmacokinetics, in press, 2007.)

ABSTRACT

Background:

Cytochrome P450 (CYP) 3A4 is the most prevalent metabolizing enzyme in the human liver and is also a target for various drug interactions of significant clinical concern. Even although there are numerous reports regarding drug interactions involving CYP3A4, it is far from easy to estimate all potential interactions, since too many drugs are metabolized by CYP3A4. For this reason, a comprehensive framework for the prediction of CYP3A4-mediated drug interactions would be of considerable clinical importance.

Objective:

The objective of this study is to provide a robust and practical method for the prediction

of drug interactions mediated by CYP3A4 using minimal *in vivo* information from drug interaction studies, which are often carried out early in the course of drug development.

Data sources:

The analysis is based on 113 drug interaction studies reported in 78 published articles over the period 1983 to 2006. The articles were used if they contained sufficient information about drug interactions. Information on drug names, doses, and the magnitude of the increase in AUC were collected.

Methods:

The ratio of the contribution of CYP3A4 to oral clearance (CR_{3A4}) was calculated for 14 substrates (midazolam, alprazolam, buspirone, cerivastatin, atorvastatin, cyclosporin, felodipine, lovastatin, nifedipine, nisoldipine, simvastatin, triazolam, zolpidem, and telithromycin) based on plasma AUC increases observed in interaction studies with itraconazole or ketoconazole. Similarly, the time-averaged apparent inhibition ratio of CYP3A4 (IR_{3A4}) was calculated for 18 inhibitors (ketoconazole, voriconazole, itraconazole, telithromycin, clarithromycin, saquinavir, nefazodone, erythromycin, diltiazem, fluconazole, verapamil, cimetidine, ranitidine, roxithromycin, fluvoxamine, azithromycin, gatifloxacin, and fluoxetine) primarily based on AUC increases observed in interaction studies with midazolam. The increases in the AUC of a substrate associated with coadministration of an inhibitor were estimated using the equation $1 / (1 - CR_{3A4} \times IR_{3A4})$ based on pharmacokinetic considerations.

Results:

The proposed method enabled predictions of the AUC increase by interactions with any combination of these substrates and inhibitors (total 251 matches). In order to validate the reliability of the method, the AUC increases in an additional 60 studies

were analyzed. The method successfully predicted AUC increases within 67-150% of the observed increase for 50 studies (83%), and within 50-200% for 57 studies (95%). Midazolam is the most reliable standard substrate for evaluation of the *in vivo* inhibition of CYP3A4. The present analysis suggests that simvastatin, lovastatin and buspirone can be used as alternatives. To evaluate the *in vivo* contribution of CYP3A4, ketoconazole or itraconazole is the selective inhibitor of choice.

Conclusion:

This method is applicable (1) to prioritize clinical trials to investigate drug interactions during the course of drug development, and (2) to predict the clinical significance of unknown drug interactions. If a drug interaction study is carefully designed using appropriate standard drugs, significant interactions involving CYP3A4 will not be missed. In addition, the extent of CYP3A4-mediated interactions between many other drugs can be predicted using the current method.

INTRODUCTION

Cytochrome P450 (CYP) 3A4 is the most prevalent CYP enzyme in the human liver. It accounts for approximately 30% of the total CYP enzymes in hepatic microsomes and is involved in the metabolism of more than 50% of the drugs currently on the market.[1,2] CYP3A4 is also the target enzyme for a number of drug interactions of significant clinical concern. Drug interactions are one of the major sources of adverse events and some have actually led to drug withdrawals in the past.[3-5] Even although there are numerous reports on CYP3A4 drug interactions, it is far from easy to estimate all potential interactions, since too many drugs are metabolized by