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Analysis of Pharmacokinetic Parameters for Assessment of Dextromethorphan Metabolic Phenotypes

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Key Words
Dextromethorphan · Dextrorphan · Metabolic ratio · Pharmacokinetic parameters · Steady state

Abstract
In this study, the metabolic ratios of dextromethorphan to dextrorphan (DM/DX) in plasma were calculated at steady state after administering 2 dosage forms (Medicon® and Detusiv®) of DM with different release rates. The urinary metabolic ratio for each subject was also determined based on the total drug concentration in the urine. An analysis of pharmacokinetic parameters for determining the DM metabolic phenotype was conducted. Results demonstrate that double logarithmic correlations between the metabolic ratios based on pharmacokinetic parameters of either $AUC_{0-\tau,ss}$, $C_{max,ss}$, $C_{min,ss}$, or $C_{ave,ss}$ for Medicon and Detusiv and the urinary metabolic ratios were all significant. Probit plots of the metabolic ratios based on these pharmacokinetic parameters revealed 2 clusters of distribution, representing extensive and intermediate metabolizers. An antimode of 2.0 for total drug based on these pharmacokinetic parameters was determined and correspondingly referred to an antimode of 0.02 for the urinary metabolic ratio to delineate extensive and intermediate metabolizers. This model was also verified to be appropriate when using total plasma concentrations of DM and DX at any time during the period of the dosing interval at steady state to calculate the metabolic ratio for identifying extensive and intermediate metabolizers. Therefore, the metabolic ratio based on the pharmacokinetic parameters of either $AUC_{0-\tau,ss}$, $C_{max,ss}$, $C_{min,ss}$, or $C_{ave,ss}$ and plasma concentrations of DM and DX in a single blood sample at steady state are proposed as an alternative way to identify phenotypes of CYP2D6.

Introduction

Genetic variations in drug metabolism are one of the major causes of interindividual variations in drug effects [17]. Many of these variations have been attributed to polymorphism in cytochrome P450 isoenzymes [5, 14]. Mutations causing changes in genes regulating drug metabolism-catalyzing enzymes have led to genetic polymorphism in populations. Such enzyme deficiencies may

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cause concentration-related side effects in subgroups of a population when certain drugs are administered at their recommended dosages. Therefore, it is important to identify ethnic groups harboring deficient genes and to determine the prevalence of different phenotypes in those subpopulations.

CYP2D6 is one of the most studied polymorphic isoenzymes. A genetic deficiency of CYP2D6 is inherited as an autosomal recessive trait. Racial and ethnic studies of drug metabolism [25] have shown substantial interpopulation differences in the distribution of CYP2D6 polymorphism. CYP2D6 deficiency was observed in 5–10% of Caucasian subjects, but in only 1–2% of Asian subjects [2]. CYP2D6 is responsible for the metabolism of a multitude of drugs, including antiarrhythmics, antidepressants, neuroleptics, opioids, and amphetamines [17].

Biotransformation of dextromethorphan (DM) to dextrorphan (DX) is commonly used as an index reaction for profiling the activity of CYP2D6 both in vivo and in various in vitro systems [3, 12, 21]. It has been reported that DX is formed from DM by microsomes in cDNA-transfected lymphoblastoid cells expressing CYP2C9, -2C19, and -2D6 but not by those expressing CYP1A2, -2E1, or -3A4 [10]. Despite the low in vivo abundance of CYP2D6, this cytochrome was identified as the dominant enzyme mediating DX formation at substrate concentrations below 10 μM. Formation of DX from DM appears to be sufficiently specific to be used as an in vitro or in vivo index reaction for profiling CYP2D6 activity [18]. In vitro formation of DX from DM by liver microsomes is principally mediated by a high-affinity enzyme, with a K_m (substrate concentration producing 1/2 the maximum reaction velocity) of 3–13 μM. Formation of DX from 25 μM DM was strongly inhibited by quinidine, with an IC_{50} (concentration resulting in 50% inhibition) of 0.37 μM; inhibition by sulfaphenazole was approximately 18%, while omeprazole and ketoconazole had minimal effects.

The phenotyping of CYP2D6 involves ingestion of a single oral dose of DM, followed by urine collection for 8–10 h [7, 16, 24]. Urinary DM:DX ratios so obtained have been recognized as a feasible noninvasive method for assessing CYP2D6 activity in vivo. Because urine collection may not be feasible or reliable in patients with renal failure, an alternative method of analyzing saliva samples collected 3 h after taking DM was developed and has been proven to be satisfactory [9]. A salivary metabolic ratio of 14.0 for free compounds concordantly reached the same phenotypic assignment as using a urinary metabolic ratio of 4.0. Usually, a urinary metabolic ratio of 0.3 for total compounds (free plus conjugated compounds) is used to delineate extensive metabolizer and poorer metabolizer phenotypes [10]. Furthermore, the logarithm of urinary metabolic ratios based on free compounds (with an antimode of 4.0) linearly correlates with that of metabolic ratios based on an assay of total compounds (with an antimode of 0.3). In other words, the use of either an antimode of 4.0 for free compounds or an antimode of 0.3 for total compounds does not change the phenotypic assignments.

Salivary analysis requires a larger dose of DM, which can cause more side effects. Technically, the salivary assay is more time consuming and difficult than the urinary assay. Also, some people dislike being asked to collect saliva samples [10]. Overall, the serum assay is more rapid and more accurate than the standard urine approach or salivary assay. Moreover, analysis of serum samples is more convenient than analysis of saliva samples. Therefore, determining DM and metabolites in serum could be advantageous for measuring individual CYP2D6 activities in vivo and thus optimizing the dosing of drugs metabolized by CYP2D6. Accordingly, Köhler et al. [15] reported that the DM/DX ratio ranged from 0.01 to 2.53 in serum and from 0.0007 to 4.252 in urine. Probit analysis of serum ratios revealed a bimodal distribution with an antimode at 0.126. According to this antimode, healthy controls exhibited identical phenotypes and genotypes. Hu et al. [11] also proposed a novel single-point plasma or saliva DM method for determining CYP2D6 activity [20].

In this study, the metabolic ratios of DM/DX in plasma were measured at steady state after administering 2 dosage forms of DM with different release rates. Pharmacokinetic parameters for determination of DM metabolic phenotypes were analyzed. For comparison, the metabolic ratios of DM/DX in urine were also measured and calculated as usual.

Materials and Methods

Drug and Reagents

Medicon® at 15 mg/tablet (lot No. 7902) obtained from Taiwan Shionogi was used as an instant release product. Detusiv® at 60 mg/tablet (lot No. 890524) made by Lotus Pharmaceutical (Taipei, Taiwan, ROC) was used as a sustained release product. DM and DX were purchased from Roche, ICN Biomedicals (Ohio, USA), and the internal standard (betaxolol) from Medochemie (Cyprus). All other reagents used were of reagent grade or better.

Instrumentation

A high-performance liquid chromatographic system equipped with a pump (515 HPLC Pump, Waters, USA) and an autosampler

(717 plus Autosampler, Waters) was used. A 50 × 4.6 mm Cosmosil ODS column with a particle size of 5 μm was employed. The mobile phase consisted of a 0.25% formic acid solution and methanol in the proportion of 60:40 (v/v). The flow rate was set at 0.7 ml/min. The eluent was detected with an LC/MS/MS system (Quattro Ultima, Micromass, Manchester, UK). The data-processing system was controlled by MassLynx computer software (ver. 3.4, Micromass). The LC/MS/MS method was validated. Chromatograms indicated that DM, DX, and betaxolol (the internal standard) were well separated from endogenous substances. The retention times for DM, DX, and betaxolol were 3.06, 1.24, and 3.31 min, respectively. High precision and accuracy with minimal interference and peaks of high symmetry were demonstrated. The coefficients of variation (CVs) of interday and intraday assays for DM in the concentration range of from 0.05 to 100 ng/ml were 2.0–8.9% and 1.9–8.7%, respectively, whereas for DX they were 2.7–9.0% and 1.6–6.9%, respectively. The relative errors of the mean of interday and intraday assays for DM were −3.2 to 6.5% and −5.0 to 3.0%, respectively, whereas those for DX were −5.7 to 5.0% and −8.5 to 4.0%, respectively.

Standard Curve and Sample Preparation

A standard curve in the linear range of from 0.05 to 100 ng/ml was constructed by spiking blank plasma samples (0.5 ml) with required volumes of stock solution containing DM and DX, 50 μl of betaxolol (0.2 ng/ml in 50% methanol), and 50 μl of an NaOH solution (1 *N*). After vortex-mixing thoroughly for 30 s, the mixture was extracted with 4 ml of diethyl ether for 1 min by vortex mixing, and then centrifuged at 3,000 rpm for another 10 min. The supernatant was transferred to another clean glass tube and evaporated under a stream of nitrogen gas until completely dry. Then, 0.4 ml of the mobile phase were added to dissolve the residue, and 20 μl were injected automatically onto the LC/MS/MS system for analysis.

Subjects

The protocol of this study was first approved by the Internal Review Board of Taipei Medical University Hospital. A total of 12 healthy male subjects participated in this study after having signed a consent form. The subjects had a mean ± SD age of 22.8 ± 2.1 years (range 20–28), body weight of 63.9 ± 5.3 kg (range 56.5–74), and height of 173.2 ± 3.5 cm (range 168–179). Subjects with a history of drug allergies or idiosyncrasies, renal or hepatic impairment, or drug or alcohol abuse were excluded. Subjects who had used medications of any kind within 2 weeks of the start or during the study were also excluded.

Drug Administration

The study design is a multiple-dose, 2-treatment, 2-period, 2-sequence crossover with a study duration of 5 continuous days and a washout period of at least 14 days (starting at the end of each period). Subjects were randomly assigned to the 2 dosing sequences. After overnight fasting (at least 10 h), subjects received the first dose of Medicon or Detusiv with 240 ml of water. Treatment A: 2 × 15 mg Medicon tablets (as the reference product) 4 times daily for 5 continuous days at 07.00, 13.00, 19.00, and 01.00 h, as well as at 07.00 h on the sixth day (the final dose); treatment B: 1 × 60 mg Detusiv tablet (as the test product) twice daily for 5 continuous days at 07.00 and 19.00 h as well as at 07.00 h on the sixth day (the final dose). During the last day of each study period, water was allowed ad libitum except for 1 h before and after drug administration. Subjects were served standardized meals no less than 4 h after drug administration. Only standardized meals and beverages at specified times were allowed during the study. Alcohol- or xanthine-containing foods or beverages were prohibited from being consumed for 48 h prior to each study period and until after the last blood sample had been collected. Subjects were confined to the clinical facility for 48 h after each dosing.

Blood Sample Collection and Processing

Blood samples (10 ml each) were drawn at the time of the study beginning date (predose), and at 07.00 h (predose) before dosing (trough concentration) for the first 5 days for both treatments A and B. On the final day (the sixth day), a blood sample (10 ml each) was collected from subjects of treatment A at 07.00 h (predose), and then 0.5, 1.0, 1.33, 1.67, 2.0, 2.33, 2.67, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 23.0, and 36.0 h after dosing. Blood samples (10 ml each) from subjects of treatment B were collected on the sixth day at 07.00 h (predose), and then 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0, 14.0, 24.0, and 36.0 h after dosing. Plasma was separated by centrifugation within 1 h of collection and was stored frozen (for not more than 6 weeks) at −20 °C until being assayed.

Pharmacokinetic Data Analysis

The following parameters were assessed for the 2 treatments: the area under the plasma concentration curves within the dosing interval of τ at steady state ($AUC_{0\text{-}\tau,ss}$); the percent peak-trough fluctuation of plasma concentration (%PTF); the maximum concentration at steady state ($C_{max,ss}$); the minimum plasma concentration at steady state ($C_{min,ss}$); the time to maximum concentration ($T_{max,ss}$) after steady state, and the relative bioavailability and relative total clearance for the profile period (CL/F). All pharmacokinetic variables were calculated by noncompartmental methods. $C_{max,ss}$ and $C_{min,ss}$ were read directly from the data, while $T_{max,ss}$ was determined at the respective blood-sampling times corresponding to $C_{max,ss}$. $AUC_{0\text{-}\tau,ss}$ was calculated according to the linear trapezoidal rule. CL/F is equal to dose/(K_{el} × $AUC_{0\text{-}\tau,ss}$), and the terminal rate constant, K_{el}, was calculated by applying a log-linear regression analysis to at least the last 3 time points. The variable %PTF(τ) was calculated as 100 × $[C_{max,ss} - C_{min,ss}]/C_{ave,ss}$, where $C_{ave,ss} = AUC_{0\text{-}\tau,ss}/\tau$, and τ is 1 dosing interval which was equal to 6 h for treatment A and 12 h for treatment B. The terminal half-life ($T_{1/2}$) is equal to $\ln 2/K_{el}$, and the mean residence time at steady state (MRT_{ss}) is defined as the ratio of $AUMC_{0\text{-}\tau}$ to $AUC_{0\text{-}\tau}$.

Statistical Analysis

Two-way ANOVA was performed with the SAS General Linear Models Procedure at a significance level of 0.05. The test and reference treatments of each study were compared with respect to relevant pharmacokinetic variables using an analysis of variance with subject, treatment, and period effects of the raw data. Point estimates for the mean 'test/reference' ratios of these raw data were calculated. Means and standard deviations of all pharmacokinetic parameters were calculated for both treatments. The individual and mean half-lives for both treatments were reported, and a paired t test was performed with a significance level of 0.05.

Phenotype Analysis

Before the PK (pharmacokinetics) study, the metabolic ratio for DM was determined from the ratio of the molar (μmol) recovery of DM to that of DX in urine collected for 8 h. In brief, after emptying the bladder, each subject received an oral dose of 30 mg DM (Medicon, Shionogi Pharmaceuticals). Urine was collected for 8 h after

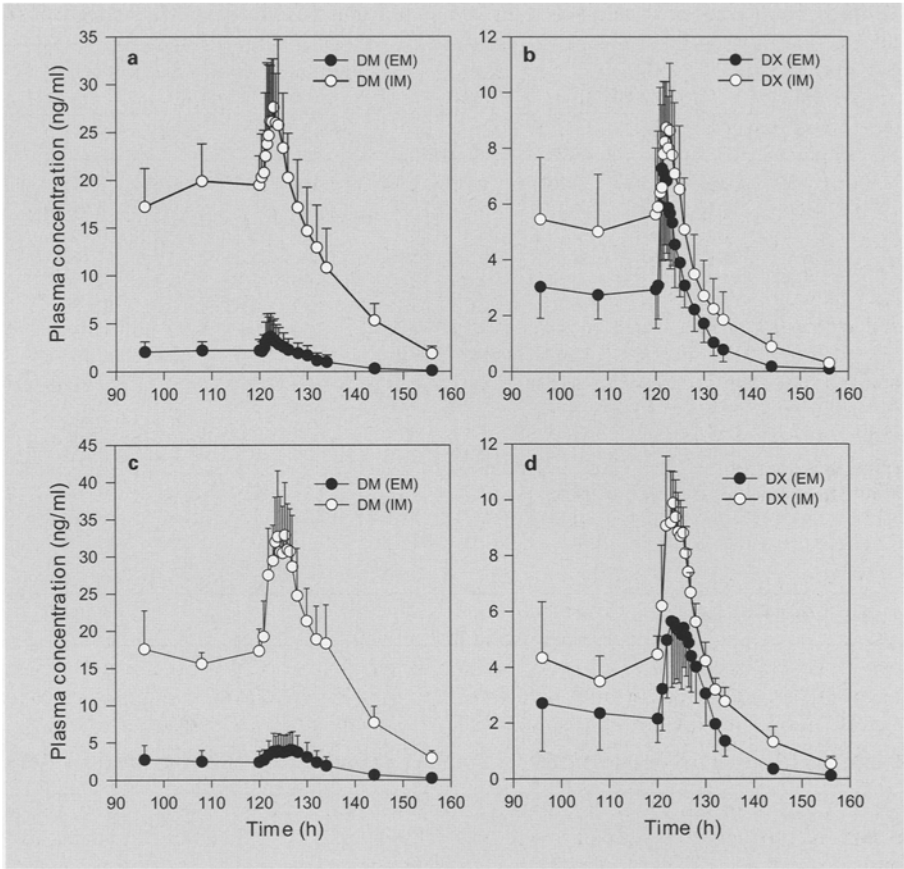


Fig. 1. DM and DX plasma concentration time profiles in 12 volunteers (separated into two groups: EM = extensive metabolizer; IM = intermediate metabolizer) using either Medicon (**a**, **b**) or Detusiv (**c**, **d**) tablets.

administration of DM. Urine volume was measured, and aliquots were stored frozen at –20 °C until being assayed. Urine at 0.5 ml was mixed with 0.5 ml of a β-glucuronidase solution (8,000 U/ml in 0.2 *M* acetate buffer, pH 5), followed by incubation in a water bath at 37 °C overnight (16 h). Samples were then assayed for DM and DX concentrations following the same procedure as that described above for plasma.

Results

Figure 1 displays the mean plasma concentration time profiles of DM and DX in 12 volunteers using either Medicon or Detusiv products. The pivotal pharmacokinetic parameters for DM and DX were correspondingly calculated, and statistical analytical results for the 2 formulations are given in tables 1 and 2, respectively. The mean ± SD ratios of $AUC_{0\text{-}\tau,ss}$, $C_{max,ss}$, and $C_{ave,ss}$ of DM for Medicon to Detusiv were 2.31 ± 0.64, 1.15 ± 0.27, and 1.15 ± 0.32, respectively. They were 1.82 ± 0.57, 0.957 ± 0.349, and 0.910 ± 0.285, respectively, for DX. The relative bioavailability, which was calculated as the ratio of $AUC_{0\text{-}\tau,ss}$ divided by the dose of Detusiv to $AUC_{0\text{-}\tau,ss}$ divided by the dose of Medicon, was 1.15 ± 0.32 for DM and 0.91 ± 0.285 for DX. This indicates that the extent of bioavailability of DM at steady state from these 2 products with different release rates was similar. The conversion of DX from DM at steady state for these 2 formulations was comparable as well, even though the release rate of these 2 formulations greatly differed.

Other pharmacokinetic parameters, such as fluctuation, MRT_{ss}, $T_{max,ss}$, and $T_{1/2}$, between the 2 products are also shown in tables 1 and 2 for DM and DX, respectively. No significant difference was found for $T_{1/2}$ between the 2 products of DM ($p = 0.347$) and DX ($p = 0.218$). However, significant differences in MRT_{ss} and $T_{max,ss}$ were observed for both DM ($p < 0.0001$; $p < 0.0001$) and DX ($p = 0.0005$; $p = 0.0021$, respectively) between these 2 formulations.

Figure 2 illustrates the time change profiles of the average plasma metabolic ratio (closed circles) of DX to DM at steady state in 12 volunteers using either Medicon (treatment A) or Detusiv (treatment B) products. These

Table 1. Pharmacokinetic parameters for DM administered as Medicon (M) and Detusiv (D)

Subject	$AUC_{0-\tau,ss}$ ng/ml·h		$C_{max,ss}$ ng/ml		$C_{min,ss}$ ng/ml		$C_{ave,ss}$ ng/ml		Fluctuation		MRT_{ss}, h		$T_{max,ss}$, h		$T_{1/2}$, h		MR
	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	
1	14.9	24.6	3.47	2.58	1.78	1.53	2.48	2.05	0.680	0.513	2.96	5.87	1.67	6.00	6.47	8.32	0.009
2	100	226	20.7	26.1	15.8	13.9	16.7	18.8	0.293	0.648	3.02	5.84	3.00	4.00	8.95	8.89	0.036
3	172	377	37.4	45.7	21.4	20.3	28.7	31.4	0.557	0.809	3.11	5.92	4.00	4.00	8.81	7.40	0.029
4	167	304	33.4	34.8	22.5	15.9	27.8	25.3	0.392	0.746	2.97	5.68	2.00	3.50	9.21	8.90	0.074
5	15.9	32.6	3.31	3.47	2.48	2.03	2.65	2.72	0.313	0.530	3.00	6.13	2.33	6.50	6.33	6.24	0.006
6	20.0	57.9	4.17	6.23	2.27	3.15	3.33	4.83	0.571	0.638	3.16	5.77	2.67	3.50	6.81	6.62	0.007
7	39.0	80.1	9.43	8.68	4.76	4.44	6.50	6.67	0.719	0.636	2.89	6.05	1.67	7.00	6.55	7.84	0.009
8	128	324	29.0	33.6	18.2	19.2	21.4	27.0	0.506	0.533	3.05	5.81	3.00	6.00	7.41	9.08	0.281
9	15.8	58.7	4.07	5.87	2.07	3.82	2.63	4.89	0.761	0.419	2.92	6.03	2.33	4.00	8.20	6.92	0.013
10	17.1	42.6	3.55	4.75	2.08	3.06	2.84	3.55	0.517	0.476	3.12	5.99	3.00	6.50	7.70	6.77	0.005
11	7.14	19.6	1.78	2.61	1.13	0.65	1.19	1.63	0.546	1.210	2.60	5.06	0.50	1.00	5.67	7.01	0.005
12	6.25	8.05	1.33	0.94	0.91	0.40	1.04	0.67	0.401	0.811	2.87	6.18	1.33	6.00	5.77	9.04	0.001
Mean	58.6	130	12.6	14.6	7.95	7.36	9.77	10.8	0.521	0.664	2.97	5.86	2.29	4.83	7.32	7.75	–
SD	64.4	137	13.6	15.8	8.71	7.61	10.8	11.4	0.152	0.214	0.15	0.29	0.93	1.78	1.25	1.06	–
CV (%)	110	106	108	108	110	103	110	106	29.1	32.2	5.01	5.00	40.7	36.7	17.0	13.6	–
p value	–	–	–	–	–	–	–	–	–	–	<0.0001		0.0005		0.347		–

MR = Metabolic ratio.

Table 2. Pharmacokinetic parameters for DX after administration of DM as Medicon (M) and Detusiv (D)

Subject	$AUC_{0-\tau,ss}$ ng/ml·h		$C_{max,ss}$ ng/ml		$C_{min,ss}$ ng/ml		$C_{ave,ss}$ ng/ml		Fluctuation		MRT_{ss}, h		$T_{max,ss}$, h		$T_{1/2}$, h		MR
	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	
1	32.4	57.0	11.3	6.96	1.89	3.08	5.39	4.75	1.740	0.817	2.84	5.29	1.33	2.00	5.01	5.33	0.009
2	27.5	82.9	6.66	10.8	3.66	4.34	4.58	6.90	0.655	0.936	2.98	5.43	2.33	4.00	9.64	7.27	0.036
3	34.0	64.0	8.19	8.53	3.49	3.59	5.67	5.33	0.829	0.926	3.04	5.42	2.33	3.50	6.91	7.87	0.029
4	52.2	92.6	11.5	12.0	7.28	4.99	8.69	7.72	0.486	0.908	2.77	5.08	1.00	2.00	9.05	9.46	0.074
5	41.3	56.9	9.34	6.92	5.38	2.13	6.88	4.74	0.575	1.010	2.81	6.16	1.33	5.50	5.69	5.74	0.006
6	23.6	54.6	5.85	8.21	1.61	2.05	3.93	4.55	1.080	1.350	3.10	5.02	2.00	3.50	4.85	5.37	0.007
7	44.2	65.7	12.7	9.87	4.59	2.63	7.37	5.48	1.100	1.320	2.77	5.58	1.67	3.00	6.27	8.03	0.009
8	54.2	79.9	11.7	10.2	8.06	4.91	9.04	6.66	0.403	0.795	3.07	5.13	3.00	2.00	8.37	12.3	0.281
9	33.2	61.9	8.40	6.67	2.41	3.42	5.53	5.16	1.080	0.630	2.68	5.51	2.33	5.00	5.32	5.41	0.013
10	18.0	44.6	4.30	5.59	1.77	1.61	3.00	3.71	0.843	1.070	3.31	5.73	3.00	6.50	6.54	5.65	0.005
11	19.9	31.8	5.47	4.92	3.24	1.10	3.32	2.65	0.672	1.440	2.48	5.37	1.33	3.00	6.10	5.91	0.005
12	26.6	21.9	7.48	2.83	2.53	1.16	4.44	1.82	1.120	0.916	2.70	5.74	1.00	5.50	4.11	7.66	0.001
Mean	33.9	59.5	8.57	7.79	3.83	2.92	5.65	4.96	0.882	1.010	2.88	5.46	1.89	3.79	6.49	7.17	–
SD	11.9	20.4	2.76	2.66	2.13	1.37	1.98	1.70	0.369	0.240	0.23	0.32	0.72	1.53	1.73	2.10	–
CV (%)	35.1	34.2	32.2	34.2	55.7	46.9	35.1	34.3	41.9	24.3	7.89	5.91	37.9	40.3	26.7	29.4	–
p value	–	–	–	–	–	–	–	–	–	–	<0.0001		0.0021		0.218		–

MR = Metabolic ratio.

metabolic ratio profiles also demonstrate a similar pattern and extent regardless of the release rate of the formulation administered. It is obvious that there are two groups of plasma metabolic ratios separated by an average line for both formulations at steady state, even though the release rates of these 2 formulations greatly differ. A possible way of determining the phenotype of the activity of CYP2D6 using the plasma metabolic ratio of DM to DX was examined.

The metabolic ratios based on DM and DX concentrations measured in urine of each subject were calculated and are listed in tables 1 and 2 for Medicon and Detusiv,

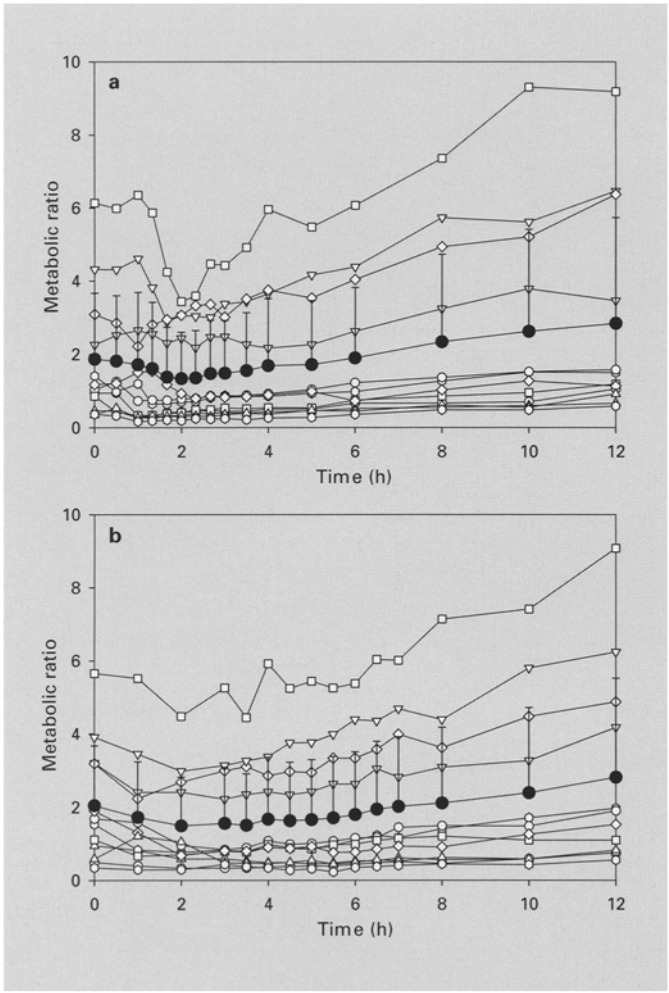
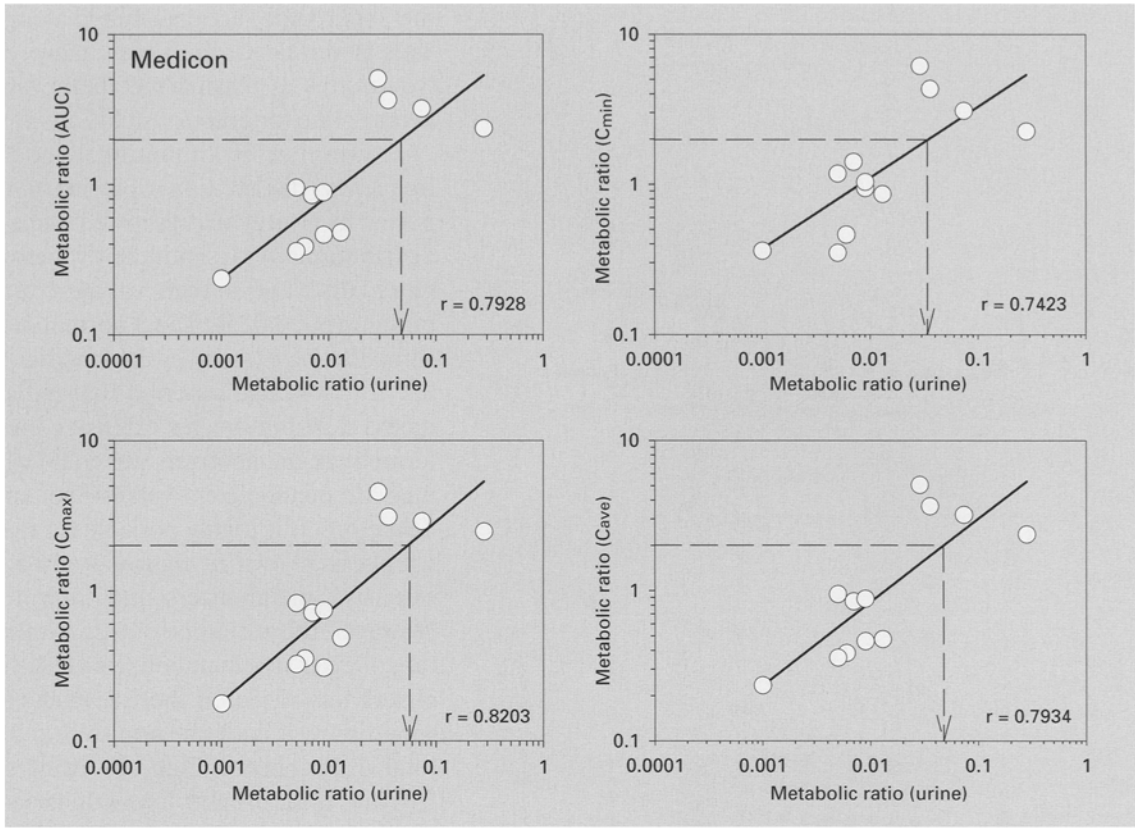


Fig. 2. Time change profiles of metabolic ratios based on plasma concentrations of DM and DX in 12 volunteers using either Medicon (**a**) or Detusiv (**b**).

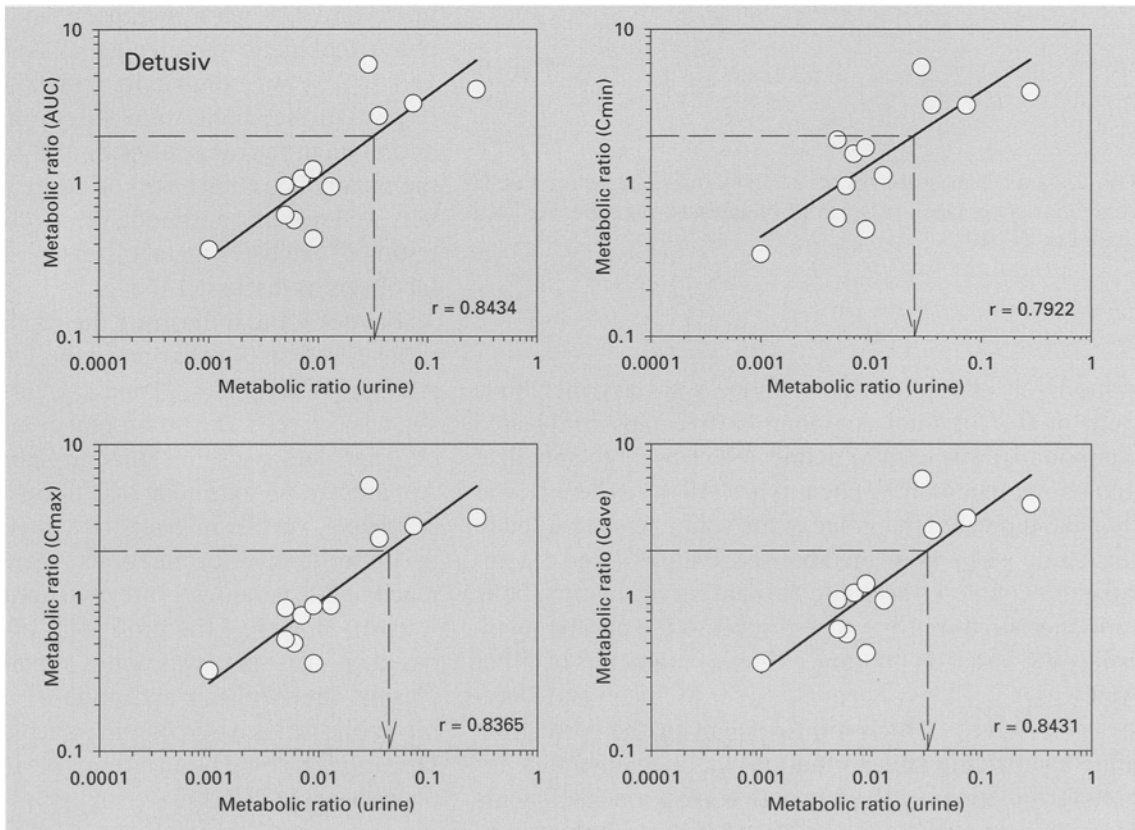
respectively. Based on the criteria, a urinary metabolic ratio of 0.3 for total compounds (free plus conjugated compounds) was used to delineate extensive metabolizer and poorer metabolizer phenotypes. All ratios being lower than 0.3 indicated that none of the volunteers recruited in this study was a poor metabolizer. Figures 4 and 5 compare the double logarithmic correlation of the metabolic ratio measured in urine with respect to the plasma metabolic ratio based on pharmacokinetic parameters of either $AUC_{0\text{-}\tau,ss}$, $C_{max,ss}$, $C_{min,ss}$, or $C_{ave,ss}$ for Medicon and Detusiv, respectively. Much improvement in the correlation after logarithmic transformation was demonstrated for both formulations in all cases with correlation coefficients of greater than 0.75. The results show that 2 clusters of metabolic ratios are distributed along the correlation line. This is similar to the time change profiles, which show two groups of plasma metabolic ratios separated by an average line for both formulations at steady state.

However, a maximum likelihood decomposition of a frequency distribution of urinary metabolic ratios reported by Hou et al. [9] showed that a mixture of 3 normal distributions was significantly better at fitting the observed distribution than was a mixture of 2 normal distributions ($p < 0.025$). The 3 normal distributions were presumed to be extensive, intermediate, and poor metabolizers. It was also reported that the antimode of the predicted distributions for extensive metabolizers versus intermediate metabolizers was 0.25, whereas that for intermediate metabolizers versus poor metabolizers was 4.0. Therefore, it is highly possible for the two clusters or distributions shown in figures 4 and 5 to be delineated as extensive metabolizers and intermediate metabolizers. However, the antimode of 0.25 for the predicted distribution of extensive metabolizers versus intermediate metabolizers was based on the free drug concentration. Correspondingly, it could be adjusted to 0.02143 based on the total drug concentration according to the correlation of free and total urinary DM/DX ratios [$\log(DM/DX_{free}) = 0.897 \times \log(DM/DX_{total}) + 0.895$] [10]. As shown in figures 3 and 4, when a metabolic ratio of 2 (based on the pharmacokinetic parameters of AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, and $C_{ave,ss}$) was drawn to correspondingly obtain the respective metabolic ratio based on the urine data, an antimode in the range of from 0.02 to 0.04 was found for the metabolic ratios based on urine data. This correlates very well with the antimode value predicted for the distribution of extensive metabolizers versus intermediate metabolizers as discussed above.

Figures 5 and 6 illustrate the probit plots of metabolic ratios based on the pharmacokinetic parameters of either $AUC_{0\text{-}\tau,ss}$, $C_{max,ss}$, $C_{min,ss}$, or $C_{ave,ss}$ for Medicon and Detusiv, respectively. The probit plot of metabolic ratios based on urine data is also included in figure 5 for comparison. Apparently, an antimode of 2 to delineate extensive metabolizers versus intermediate metabolizers is appropriate for these 2 formulations regardless of which pharmacokinetic parameters are used to calculate the metabolic ratio. However, the profit plot of the metabolic ratio based on urine data was not as apparent as that based on plasma data with an antimode of 0.02. Therefore, the metabolic ratios based on pharmacokinetic parameters of DM and DX could be an alternative index to delineate the phenotype of CYP2D6.


Medicon
Metabolic ratio (AUC)
r = 0.7928
Metabolic ratio (urine)
Metabolic ratio (Cmin)
r = 0.7423
Metabolic ratio (urine)
Metabolic ratio (Cmax)
r = 0.8203
Metabolic ratio (urine)
Metabolic ratio (Cave)
r = 0.7934
Metabolic ratio (urine)
10
1
0.1
0.0001
0.001
0.01
0.1
1

3


Detusiv
Metabolic ratio (AUC)
r = 0.8434
Metabolic ratio (urine)
Metabolic ratio (Cmin)
r = 0.7922
Metabolic ratio (urine)
Metabolic ratio (Cmax)
r = 0.8365
Metabolic ratio (urine)
Metabolic ratio (Cave)
r = 0.8431
Metabolic ratio (urine)
10
1
0.1
0.0001
0.001
0.01
0.1
1

4

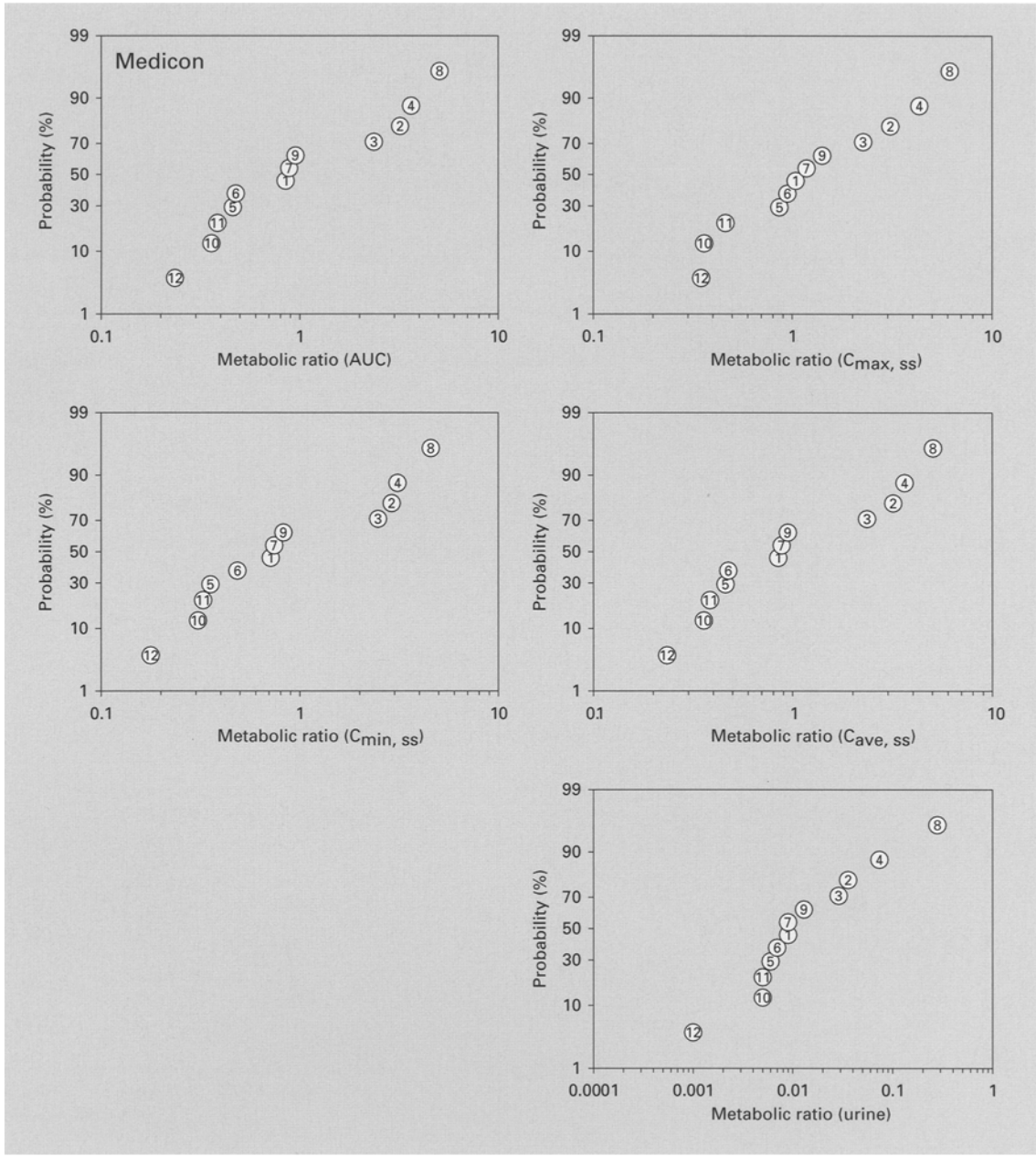


Fig. 5. Probit plots of metabolic ratios based on pharmacokinetic parameters of DM and DX in 12 volunteers using Medicon.

Fig. 3. Double logarithmic correlation between metabolic ratios based on pharmacokinetic parameters of DM and DX in 12 volunteers using Medicon.

Fig. 4. Double logarithmic correlation between metabolic ratios based on pharmacokinetic parameters of DM and DX in 12 volunteers using Detusiv.

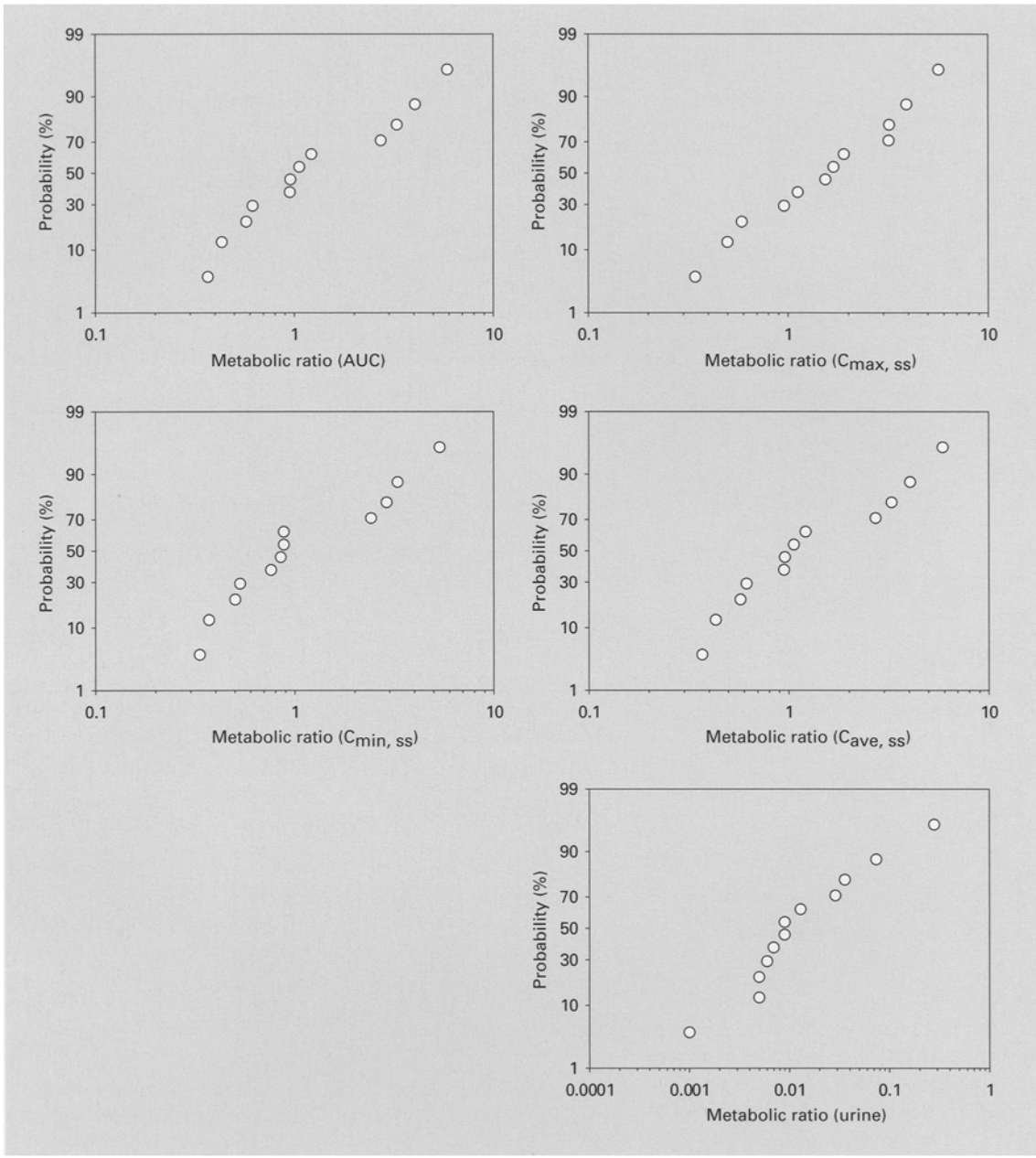


Fig. 6. Probit plots of metabolic ratios based on pharmacokinetic parameters of DM and DX in 12 volunteers using Detusiv.

This result also reveals that the metabolic ratio based on plasma concentrations of DM and DX at these selected time points during the period of the dosing interval (in this case, 12 h) could be an alternative index since the metabolic ratios based on the pharmacokinetic parameters of $C_{max,ss}$, $C_{min,ss}$, and $C_{ave,ss}$ are well qualified for this purpose as discussed above. Figure 7 verifies that 2 clusters of distribution were observed for both Medicon and Detusiv in a double logarithmic correlation of metabolic ratios based on plasma concentrations of DM and DX at each time point versus the urinary metabolic ratio. Therefore, plasma metabolic ratios during the period of the dosing interval at steady state when administering DM formulations with any release rate can be used to identify the phenotype of CYP2D6.

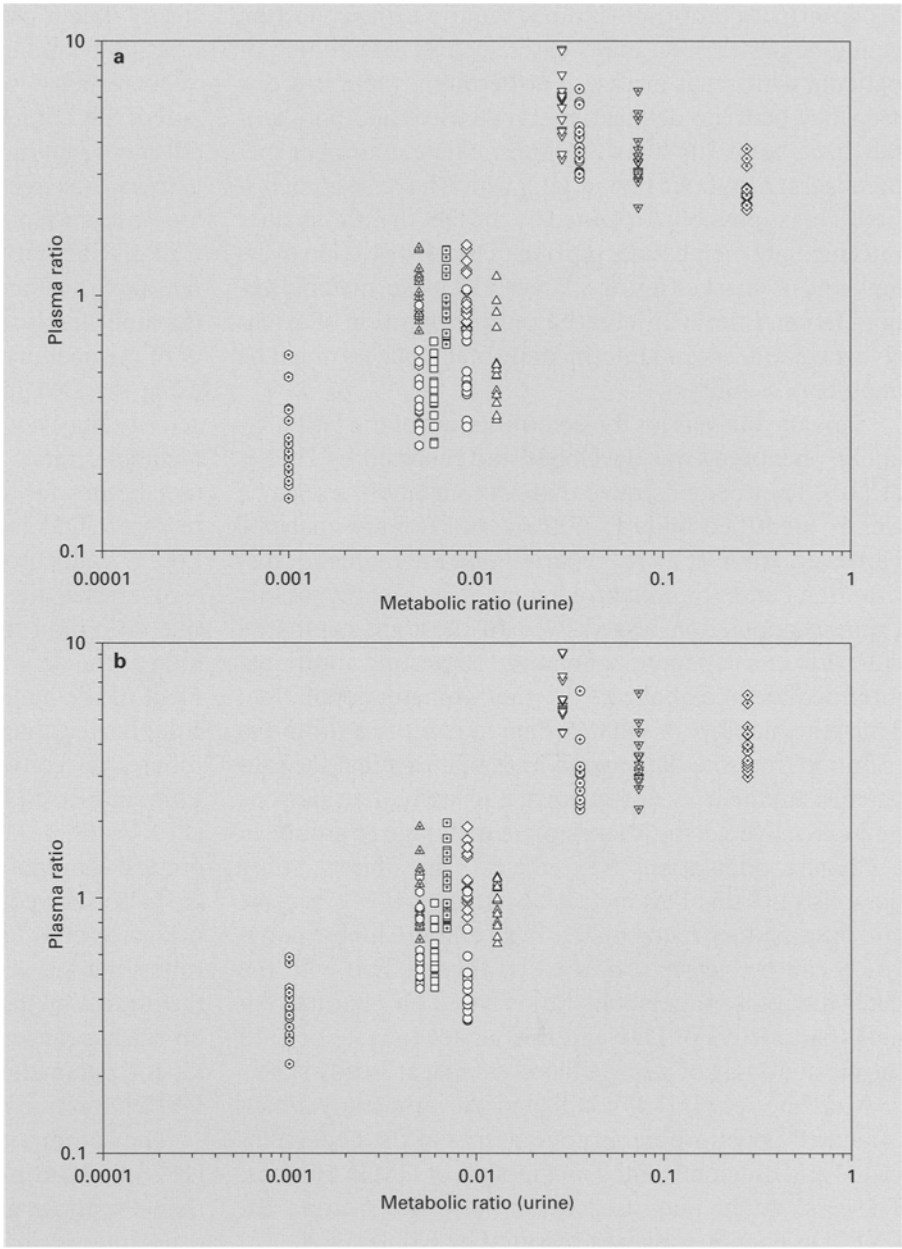


Fig. 7. Double logarithmic correlation between metabolic ratios based on plasma concentrations of DM and DX at each time point in 12 volunteers using either Medicon (**a**) or Detusiv (**b**).

Discussion

Analysis of metabolic ratios based on pharmacokinetic parameters of DM and DX after the administration of 2 formulations with different release rates was conducted in normal volunteers in order to determine their phenotypes. A simple method of calculating the metabolic ratio based on pharmacokinetic parameters of DM and DX at steady state or on the plasma assay of a single blood sample at steady state is proposed. Extensive and intermediate metabolizers in a Chinese population can be clearly identified by this method.

The P450 isoenzyme CYP2D6 is important for the metabolism of DM and the *O*-demethylation of methoxymorphinan to hydroxymorphinan [1, 11]. Microsomal CYP2D6 is genetically determined and has a polymorphic distribution in most populations studied [6]. Determination of the metabolic phenotype involves having subjects take 1 dose of DM orally and then collecting urine for up to 8 h. An alternative method that would take

a shorter period of time and not require urine collection would be desirable in many cases such as for children or patients with renal disease. Furthermore, there is a discrepancy between delineating 2 (extensive and poor metabolizers) and 3 (extensive, intermediate, and poor metabolizers) metabolic phenotypes which have been identified. This is possibly explained by the fact that the smaller intermediate metabolizer portion of the distribution overlaps and is masked by the larger extensive metabolizer population. Ethnic differences in the proportion of extensive metabolizers and intermediate metabolizers might be another possibility.

Salivary analysis for the determination of the DM metabolic phenotype was developed and reported by Hou et al. [10]. They demonstrated that poor metabolizers can be clearly identified by both salivary and urinary analyses. However, Horai et al. [8] reported that the frequency distribution curve for metoprolol metabolic phenotypes in a Chinese population was skewed to the right compared with that in a Japanese population, suggesting additional intermediate metabolizers in the Chinese population. Thus, the inability of salivary data to identify a third distribution (intermediate metabolizers) means that the saliva measurement is not adequate to categorize persons into extensive versus intermediate metabolizer groups in a Chinese population. Nevertheless, the present study demonstrates that DM metabolic phenotypes of extensive and intermediate metabolizers in an ethnic Chinese population can be clearly identified by this method with the determination of metabolic ratios based on pharmacokinetic parameters of DM and DX at steady state or with the plasma assay of a single blood sample at steady state.

Mortimer et al. [19] conducted the first study which evaluated polymorphic serum patterns of the *O*-demethylated and didemethylated metabolites of DM in humans. The first study that used serum instead of urine for CYP2D6 phenotyping was reported by Köhler et al. [15] in which it was demonstrated that all poor metabolizer CYP2D6 genotypes were poor metabolizer phenotypes when serum was analyzed, whereas urine measurements identified 1 patient with an extensive metabolizer genotype as a poor metabolizer phenotype. It is understandable that the ratio calculated from concentrations in urine collected over 8 h is a function of both intrinsic clearance of the precursor to product(s) and the renal clearance of the precursor and the product(s), respectively. Therefore, metabolic ratios based on urinary data potentially provide a flawed index of hepatic enzyme activity in individuals with renal impairment [13]. The present study reveals that carrying out plasma assays of DM and DX at steady state is practical, and they exhibit a stable index of metabolic ratio to the phenotype CYP2D6 isoenzyme.

In response to the concern that in vivo indices are potentially confounded by the effect of renal function, the effect of renal impairment on the assessment of CYP2D6 activity was reexamined from a theoretical viewpoint by Rostami-Hodjegan et al. [22]. They concluded that CYP2D6 activity is compromised in parallel with deterioration of renal function. Since a decrease in enzyme function appears to cancel that in renal function, the possibility of misphenotyping individuals with renal impairment using the DM metabolic ratio as an index of CYP2D6 activity may not be of concern. However, when using a metabolic ratio, it is essential to recover all sequential metabolites formed along the pathway of interest. With respect to DM as a probe for CYP2D6 activity, the sum of DX, DX-glucuronide, and 3-hydroxymorphinan urinary recoveries as the denominator would form a more sensitive ratio than that based only on DX and DX-glucuronide as the denominator. This would make the measurement of the metabolic ratio based on urinary data more complicated than it was before. As to alternative, more robust, yet convenient indices as markers of CYP1A2, Führ and Rost [4] advocated the use of the paraxanthine (17×)/caffeine (137×) ratio in plasma or saliva. The theoretical simulation conducted by Rostami-Hodjegan et al. [23] also showed that the plasma/saliva 17×/137× ratio measured at 5–7 h, as advocated by Führ and Rost [4], is a robust marker of CYP1A2. This supports the potential of the present method which employs metabolic ratios based on plasma concentrations of DM and DX and pharmacokinetic parameters at steady state to serve as an index of CYP2D6 activity.

The serum assay was demonstrated by Köhler et al. [15] and Mortimer et al. [19] to exhibit less variability than the urine assay. It was conducted by determining drug and metabolite concentrations in blood taken only 1 h after drug ingestion of 20 mg DM in the former study, whereas the latter study took blood 2.5 h after administration of 120 mg DM. Metabolite formation in an enzyme reaction is a linear function over time when initial velocities are measured, and the concentration of the metabolite decreases over time because of consumption of substrate. Theoretically, an assay with a short interval between drug administration and sample collection will therefore more appropriately reflect initial velocities than will an assay with a long sampling period. Nevertheless, the metabolic ratio for distinguishing extensive metabolizer and poor metabolizer found in serum in the study conducted by Köhler et al. [15] and others (with an antimode of 0.126 or

0.1, respectively) was found to be lower than that in urine (with an antimode of 0.3). This might have been due to the short time period between drug administration and blood collection. With these metabolic ratios, however, only 2 metabolic phenotypes of extensive and poor metabolizers could be categorized in Caucasian subjects. As described above, this might have been due to the overlap of the smaller intermediate metabolizer portion of the distribution and by it being masked by the larger extensive metabolizers in Caucasian populations. With the present method, metabolic ratios determined by pharmacokinetic parameters or plasma concentrations at steady state showed stable outcomes. Thus, extensive and intermediate metabolizers can be categorized in an ethnic Chinese population. It would be worth using the present method to identify whether or not 2 or 3 metabolic phenotypes exist in Caucasian populations.

In the study by Hun et al. [11], the time point at which metabolic ratios of plasma or saliva concentrations statistically significantly correlated with $AUC_{\tau ss}$ after multiple dose administration is suggested as the sampling point to calculate the metabolic ratios for evaluation of 2D6 activity. As a result, only certain plasma data of DM metabolic ratios could be used for the 2D6 phenotype. Using the extent of correlation with $AUC_{\tau ss}$ as a criterion for determining the best sampling point is simple because, when the steady state has been reached, the drug distribution is in equilibrium among plasma, tissue, saliva, and urine. However, it is unnecessary to have reached equilibrium among plasma, tissue, saliva, and urine to have a metabolic ratio of the plasma concentration which reflects 2D6 activity. As long as a steady state is maintained, metabolic ratios of plasma concentrations at any time point within the dosing interval can accurately reflect 2D6 activity as demonstrated in this study.

The inability to differentiate between extensive and intermediate metabolizers might have been caused by limits of the quantitation of DM and DX in plasma or serum samples, especially for DM measured in extensive metabolizer subjects. For an extensive metabolizer, the plasma concentration of DM in these subjects would be too low to be accurately analyzed. The accuracy and precision of the assay are ultimately dependent on the method selected. If the assay method does not have sufficient accuracy and precision to detect plasma concentrations of DM as low as possible in extensive metabolizers, the frequency distribution of metabolic ratios for extensive and intermediate metabolizers would have a fair chance of overlapping. Plasma concentrations of DM and DX in the present study were assayed using the LC/MS/MS method

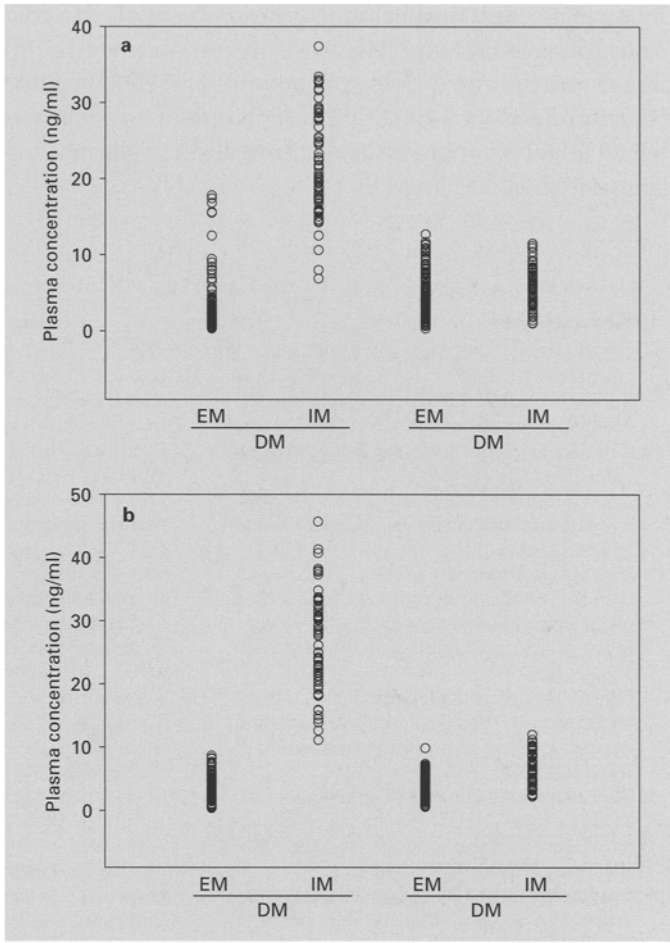


Fig. 8. Distribution plots of plasma levels of DM and DX for extensive (EM) and intermediate (IM) metabolizers after taking either Medicon (**a**) or Detusiv (**b**).

with a limit of quantitation of 0.05 ng/ml, which is 100-fold lower than that of the assay method selected by Köhler et al. [15]. This might have provided a greater chance to differentiate between extensive and intermediate metabolizers in the present study.

We can conclude that the metabolic ratio determined using pharmacokinetic parameters or plasma concentrations of DM and DX during the period of the dosing interval at steady state is an alternative index to identify phenotypes of CYP2D6. An antimode of 2.0 was used to delineate extensive and intermediate metabolizers. Figure 8 further demonstrates the difference in plasma levels between extensive and intermediate metabolizers. This method is simpler and less time consuming. It only requires subjects to take DM formulations with any release rate until the plasma concentration reaches steady state and then a single blood sample is taken any time

following the last dosing for determination of plasma concentrations of DM and DX. Also, an antimode with a different value for identifying phenotypes of CYP2D6 could be determined for population groups with disease statuses which might complicate the determination of plasma concentrations of DM and DX.

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