Dextromethorphan (DM) is an antitussive drug that is rapidly absorbed from the gastrointestinal tract (\( k_a = 2.6 \pm 0.9 \, \text{h}^{-1} \)) after oral administration and has a large central volume of distribution (961 l, ranging from 585 to 1,292 l) (ref. 1). DM undergoes rapid and extensive first-pass metabolism mainly through the action of cytochrome P450 2D6 (CYP2D6); the bioavailability of oral DM ranges from 1 to 2% in subjects who are extensive metabolizers (EMs) of CYP2D6 and ~80% in those who are poor metabolizers (PMs).2 Its intrinsic hepatic clearance was ~22 l/h in the EM group and 4,737 l/h in the PM group. 2 In another study, an apparent clearance value of 1,280 ± 483 l/h has been reported for EM subjects.3 The primary steps in the metabolic process include formation of dextrorphan (DO) (mainly by means of CYP2D6) and of 3-methoxymorphinan by means of CYP3A enzymes. DO and 3-methoxymorphinan are further metabolized to 3-hydroxymorphinan by CYP3A4 and CYP2D6, respectively.4,5 The dominant metabolic pathway in the majority of the population is the conversion of DM to DO by CYP2D6, contributing to >80% of DO formation.1,6 The selectivity of DO formation through CYP2D6, in conjunction with its favorable safety features and ready availability, has made DM the probe of choice for CYP2D6 phenotyping.7

CYP2D6 is a highly polymorphic gene with more than 100 known allelic variants (accessed 15 March 2010; http://www.cypalleles.ki.se/cyp2d6.htm). Because the overall disposition of DM is highly dependent on CYP2D6 activity, these polymorphisms are the main source of the wide interindividual variation in the plasma levels attained and the response elicited by DM.8,9 With regard to CYP2D6 substrates such as antidepressants and antipsychotic drugs, such variability may lead to failure of treatment in carriers of alleles encoding very high CYP2D6 activity or may expose individuals who carry alleles that confer low activity, if any, to a high risk of toxicity.10,11 Conversely, with respect to some opioids, such as dihydrocodeine, codeine, and tramadol, in which highly active metabolites are formed by CYP2D6, therapeutic failure may be associated with low-activity genotypes, whereas toxicity may be associated with genotypes that result in high enzymatic activity.12,13

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The pharmacokinetics of dextromethorphan (DM) is markedly influenced by cytochrome P450 2D6 (CYP2D6) enzyme polymorphisms. The aim of this study was to quantify the effects of the CYP2D6*1, *2, and *41 variants on DM metabolism in vivo and to identify other sources of pharmacokinetic variability. Concentrations of DM and dextrorphan (DO) in plasma and urine were evaluated in 36 healthy Caucasian men. These volunteers participated in three clinical studies and received a single oral dose of 30 mg DM-HBr. Data were modeled simultaneously using the population pharmacokinetics NONMEM software. A five-compartment model adequately described the data. The activity levels of the alleles assessed differed significantly. The clearance attributable to an individual CYP2D6*1 copy was 2.5-fold higher as compared with CYP2D6*2 (5,010 vs. 2,020 l/h), whereas the metabolic activity of CYP2D6*41 was very low (85 l/h). Urinary pH was confirmed as a significant covariate for DM renal clearance. These results refine genotype-based predictions of pharmacokinetics for DM and presumably for other CYP2D6 substrates as well.
As a prerequisite to the personalization of therapy with CYP2D6 substrates, various proposals have been made to establish simple, useful systems for predicting the CYP2D6 phenotype on the basis of the CYP2D6 genotype.14

Such predictions have potential pitfalls because they are dependent on the correctness of underlying assumptions. The classic procedure involved dividing populations on the basis of the urinary metabolic ratio of DO to DM and assigning an arbitrary cutoff value, i.e., antimode, for categorization as those who are EMs and those who are PMs.15 Given that subjects with two nonfunctional alleles represent the PM group, it is difficult to predict with precision the phenotype of individuals carrying combinations of functional and reduced-function alleles. Furthermore, wide variability in CYP2D6 activity is observed not only between subjects with different functional genotypes but also—as the rule rather than an exception—among subjects within a genotype group.16 In an attempt at more accurate classification, especially of subjects carrying gene duplications and reduced-function alleles, ultrarapid metabolizer and intermediate metabolizer subgroups have been introduced, creating a polymodal population distribution.17

Another system, known as semiquantitative scoring, has been developed.18 It defines the categories of subjects on the basis of CYP2D6 alleles; a score is assigned to each allele (1 for fully functional alleles, 0.5 for reduced-activity functional alleles, and zero for nonfunctional alleles), and the results are used to calculate “gene doses” as an indicator for phenotype. Another recently devised scoring method, the activity score system, also assigns values to individual CYP2D6 alleles and utilizes their sum as a surrogate to predict phenotype.8 Because the model did not improve after assigning values of 0.25 and 0.75 to alleles perceived to confer slight and substantial reduction in activity, respectively, the simplest model—using the values 1 for fully functional, 0.5 for all reduced activity, and 0 for nonfunctional alleles—was chosen. In an effort to better describe the alleles carrying functional gene duplications such as CYP2D6*1xN or *2xN, the value of the allele was doubled; i.e., a value of 2 was assigned to CYP2D6*1xN. Although these classification systems have improved phenotype prediction, considerable variability within score groups persists. By definition, these scoring systems are valid for nonfunctional alleles such as CYP2D6*3 and *4, but the simplifying assumption that fully functional alleles such as CYP2D6*1 and *2 have identical activity enzymes can be challenged, as is the allocation of a value of 0.5 to every low-activity allele. Indeed, several mechanisms, including differences in splicing and other causes of different expression levels, enzyme stability, and/or substrate-specific enzyme kinetics, may cause net differences in in vivo substrate turnover between individual functional CYP2D6 variants.

There is therefore a need to precisely quantify the in vivo activity associated with CYP2D6 allelic variants and genotypes so as to better predict the phenotype from genotype data. Such information will ultimately be useful for clinical decision making with respect to drug selection and dose adjustment. In the present study, we assessed the activity levels of individual CYP2D6 alleles with regard to the ratio of clearance of DM to DO, using a population pharmacokinetics approach.

RESULTS

A total of 537 plasma samples and 136 urine samples were obtained from 36 healthy Caucasian men after oral administration of a 30-mg DM-HBr dose. The samples were analyzed for DM and DO concentrations. The demographic and CYP2D6 genetic characteristics of these volunteers are given in Tables 1 and 2, respectively.

As expected, we observed large interindividual differences in the time–concentration profiles within the data set, with DM showing the highest variability. The initial concentrations of DM in plasma decreased rapidly in many, but not all, individuals (within the first 5 h after dosing), reflecting the known variability in drug
clearance. DO appeared in the plasma in many individuals earlier than the parent drug did—a finding that supports the importance of first-pass metabolism.

The basic four-compartment model (see Methods section) was not sufficient to describe all the data and resulted in misspecifications, especially with regard to the plasma levels of the parent compound. The addition of a peripheral compartment for DM was associated with a significant drop in the objective function value ($\Delta = -114$). On the basis of individual and population predictions, this model was still unable to account for the early appearance of DO in plasma. Therefore, other models that take the first-pass metabolism into account were tested. These models were simplified stepwise or extended for the purpose of modeling the available data. A hypothetical metabolism compartment was added, which was assumed to be in rapid equilibrium with the central compartment of the parent drug, with all metabolic steps taking place in this compartment. It was assumed that the drug, when administered, goes from the absorption site to the metabolism compartment before reaching the plasma (for specifications, see Appendix). This model was associated with a profound drop in the objective function value ($\Delta = -324$). The inclusion of a lag-time parameter led to a further significant drop in the objective function ($\Delta = -45$).

With respect to identification of covariates, the CYP2D6 genotype had a major impact on the metabolic clearance ($\Delta = -532$), as had been expected. Approximately 55% of the interindividual variability (CV %) in the metabolic clearance of DM, $CL_{23}$, was explained by adding CYP2D6 genotype as a covariate. The inclusion of urine pH value as a covariate in renal clearance of DM also resulted in a significant fall in the objective function value ($\Delta = -105$). The best form of this relationship was the one included in the final model, described by the following empirical equation:

$$\theta_{PV} = \theta_{TV} \times (5.7/UpH)^{\theta s}$$

where $\theta_{PV}$ is the individual value of renal clearance, $\theta_{TV}$ is the population value of renal clearance, the value 5.7 represents a published mean of urine pH in humans, $UpH$ is the measured individual urine pH at each collection period, and $\theta s$ is the shape parameter that explains the change in renal clearance in terms of change in urine pH. Using the nonionized fraction of DM calculated according to the Henderson–Hasselbalch equation as a covariate was inferior as compared with this empirical equation. The inclusion of a factor for the effect of urine pH on renal clearance of DO neither improved the model fit nor led to a decrease in the objective function value (OFV). The model also identified age as an important covariate. Age contributed to interindividual variability in the apparent volume of distribution of DO ($\Delta = -21.9$) and the clearance of DO to other species, $CL_{30}$ ($\Delta = -5.8$). This covariate was modeled according to the relationship:

$$\theta_{PV} = \theta_{TV} \times \text{EXP} \left( \theta_{AGE} \times (\text{AGE} - 27) \right)$$

where $\theta_{PV}$ is the population value of the model parameter, $\theta_{TV}$ is the typical value in an individual of age 27 years (the median age in this study), and $\theta_{AGE}$ is the fractional change in $\theta_{PV}$ per year relative to the age of 27 years. None of the other apparent volumes of distribution, clearance, or intercompartmental clearance parameters was found to be influenced by age in this study. Neither body weight nor height was found to contribute significantly to the model disposition parameters. Plasma data were best fitted when combined additive and proportional error terms were used, whereas urine data were best fitted with proportional error terms. The blueprint of the final model is shown in Figure 1.

Final pharmacokinetic parameter estimates with 95% confidence intervals are shown in Table 3. The apparent metabolic clearance ($CL_{23}$) estimate ranged from 10 to 10,030 l/h in this population. The fraction of the clearance attributable to a CYP2D6*1 copy was, on average, 2.5-fold greater than that for CYP2D6*2. CYP2D6*1-related clearance was lowest: the point estimates per copy were 5,010 l/h, 2,020 l/h, and 85 l/h for CYP2D6*1, *2, and *41, respectively.

Diagnostic plots of the final model are shown in Figure 2, indicating model adequacy. The 5th, 50th, and 95th percentiles from simulated data, based on the final model estimates, are shown in Figure 3. Simulated time courses of plasma concentrations and cumulative amounts excreted in urine for subjects who are homozygous for a given allele (CYP2D6*1/*1, *2/*2, etc.) are shown in Figure 4. The expected values of standard CYP2D6 phenotypic metrics, i.e., metabolic ratios of DM to DO at 3h in plasma and 0–8h in urine with respect to the various alleles, were 0.12 and 0.015 for CYP2D6*1/*1, 0.28 and 0.04 for CYP2D6*2/*2, 5.0 and 0.079 for CYP2D6*41/*41, and 90.9 and

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**Figure 1** Blueprint of the final semimechanistic population model of dextromethorphan (DM) and dextromorphan (DO) in plasma and urine after administration of single oral doses of 30 mg DM hydrobromide. The absorption phase is described with a rate constant ($k_a$) and lag time ($t_{lag}$). $C_p$ and $C_{u}$ are plasma and urine concentrations of DM or DO in the corresponding compartments. $C_{c}$ is the concentration of DM in the hypothetical metabolizing enzyme compartment, which is in rapid equilibration with that in plasma ($C_{p}$). $Q_{s}$ is the intercompartmental clearance between the central compartment of DM and the metabolizing enzyme compartment. $CL_{23}$ is the apparent systemic metabolic clearance of DM to DO, which is the sum of the basic ($CL_{b}$) and CYP2D6-mediated ($CL_{CYP2D6}$) components. $Q_{s}$ is the intercompartmental clearance between central and peripheral compartments of DM, $CL_{30}$ is the renal clearance of DM under the influence of urine pH ($UpH$), $CL_{35}$ represents DO renal clearance, and $CL_{30}$ is the clearance of DO to other species.
Table 3 Pharmacokinetic parameter estimates for the population

| Model parameter | Point estimate | 95% CI |
|-----------------|----------------|--------|
| **Basic pharma-**|                |        |
| cinetic parameters |                |        |
| Absorption rate constant, $k_3$ (h$^{-1}$) | 0.25 | 0.23–0.27 |
| Lag time (h) | 0.31 | 0.30–0.32 |
| Apparent volume of distribution of DM in central compartment, $V_{c,dex}$ (l) | 648 | 493–803 |
| Intercompartmental clearance $Q_1$ (l/h) | 560 | 295–825 |
| Apparent volume of distribution of DM in peripheral compartment $V_{p,dex}$ (l) | 1,560 | 1,092–2,028 |
| Intercompartmental clearance, $Q_2$ (l/h) | 173 | 129–217 |
| Apparent renal clearance of DM, CL$23$ (l/h) | 6.52 | 4.84–8.20 |
| Urine pH effect on DM renal clearance in a man with a standard pH value of 5.7 | 3.93 | 2.74–5.12 |
| **Components of apparent metabolic clearance CL$23$** | | |
| Basic (l/h) | 10.1 | 6.38–13.82 |
| CYP2D6*1 (l/h, per gene copy) | 5,010 | 3,579–6,441 |
| CYP2D6*2 (l/h, per gene copy) | 2,020 | 624–3,416 |
| CYP2D6*41 (l/h, per gene copy) | 85.0 | 63.8–106.2 |
| Apparent volume of distribution of DO Vm, $dor$ (l) | 419 | 237–601 |
| Effect of age on Vm, $dor$ (% change/year) | 0.042 | 0.016–0.068 |
| Apparent renal clearance of DO CL$35$ (l/h) | 45.5 | 34.1–56.9 |
| Apparent clearance of DO to other species CL$30$ (l/h) | 1,260 | 1,061–1,460 |
| Effect of age on CL$30$ (% change/year) | 0.029 | 0.003–0.055 |
| **Between-subject variability (BSV)** | | |
| Plasma | | |
| DM | | |
| Proportional error (% CV) | 30.6 | 1.61$^a$ |
| Additive error (µg/l) | 0.004 | 0.122$^c$ |
| DO | | |
| Proportional error (% CV) | 32.6 | 1.06$^c$ |
| Additive error (µg/l) | 0.006 | 0.18$^c$ |
| Urine | | |
| DM | | |
| Proportional error (% CV) | 56.2 | 3.82$^c$ |
| DO | | |
| Proportional error (% CV) | 44.8 | 2.95$^c$ |

Cl, confidence interval; CV, coefficient of variation; DM, dextromethorphan; DO, dextrorphan.

$^a$See Figure 1 for individual parameters. $^b$Centered around 27 years. $^c$Percentage of relative standard error.

DISCUSSION

The main objective of this study was to quantify the metabolic activity attributable to frequent individual CYP2D6 alleles, using DM as a probe drug. The mean CYP2D6-mediated clearance, CL$23$, is estimated as 15,030 l/h in subjects genotyped as CYP2D6*1/*1x2, 1701 l/h in subjects who carry two CYP2D6*41 alleles, and zero in subjects with genotypes consisting of two nonfunctional alleles. The results suggest that quantitative differences in DM-based phenotyping metrics between CYP2D6 genotypes are similar to the differences in enzyme activity, despite additional processes that have an impact on DM pharmacokinetics (see Table 3).

Per the final model estimates, the activity of the CYP2D6*1 allele with respect to formation of DO in vivo is ~2.5-fold higher than that of the CYP2D6*2 allele. The CYP2D6.2 protein differs from the CYP2D6.1 protein with regard to two amino acids (R296C and S486T) (http://www.cypalleles.ki.se/cyp2d6.htm), whereas there is no evidence that the CYP2D6*2 allele differs from the *1 allele with respect to expression levels. Key substrate binding sites of CYP2D6 include D301, F120, F483, and 30 (l/h), whereas there are no differences with regard to metabolism of intrinsic clearance values (CYP2D6.2:CYP2D6.1) was only 0.20 (refs. 24, 25). The trend was similar for other reactions, although the differences were smaller. The ratios for these various reactions were (CYP2D6.2:CYP2D6.1):DM N-demethylation, 0.77; codeine O-demethylation, 0.35; and fluoxetine N-demethylation, 0.26 (ref. 24). For CYP2D6.2 and CYP2D6.1 expressions in Saccharomyces cerevisiae, the ratios of intrinsic clearance values (CYP2D6.2:CYP2D6.1) were ~0.50 for several progesterone hydroxylation pathways and 0.23 for dopamine formation from p-tyramine. The activity ratios of CYP2D6.2 to CYP2D6.1 expressed in yeast were 0.44 for DM O-demethylation and 0.74 for bufuralol 1′-hydroxylation, whereas there were no differences with regard to metabolism of debrisoquine or metoprolol.27 In a COS-7 expression system, the activity ratios of CYP2D6.2 to CYP2D6.1 were 0.71 and 0.72 for DM O-demethylation and bufuralol hydroxylation, respectively. In summary, on average, CYP2D6.2 is approximately half as active as CYP2D6.1. Whether the reported differences in the activity levels of these substrates in the in vitro assays are real or whether they reflect the notorious problems in reproducibility of in vitro enzyme kinetic parameters remains...
The finding that DM activity in those with the CYP2D6*41 allele is ~2.5-fold higher than in those with CYP2D6*2 suggests that different activity values need to be assigned to these alleles in scoring systems. Both the activity score system\textsuperscript{8} and the semi-quantitative scoring system\textsuperscript{18} assumed similar activity levels for the CYP2D6*1 and *2 alleles. To reflect reduced activity, these systems\textsuperscript{8,18} assigned a value of 0.5 to the CYP2D6*41 allele. On the basis of the results presented here, clearance values associated with CYP2D6*41 are ~60 times lower than those associated with CYP2D6*1. Consequently, scoring values of 1, 0.4, and 0.017 should be assigned to CYP2D6*1, *2, and *41, respectively, in order to more accurately reflect their respective activity levels.

Using the respective final parameter estimates, DM pharmacokinetics for various genotypes was simulated (Figure 4). Similar genotype-specific predictions can be made for other CYP2D6 substrates. Such predictions, however, may need to be modified because previous studies have demonstrated that protein-specific activity of cytochrome P450 enzymes, including CYP2D6, is in part substrate dependent\textsuperscript{11,33,34}.

The final pharmacokinetic model describes the pharmacokinetic profiles of DM and its major metabolite adequately. In this model, the inclusion of the hypothetical metabolism...
compartment was able to explain the finding that DO appears in plasma earlier than DM does. The model presented here shares some features with previously reported models that have been successfully applied to describe first-pass metabolism. The specific features in our model are as follows: (i) activity levels of active alleles of CYP2D6 were estimated separately; (ii) data related to both parent drug and metabolite in urine were included in the model and simultaneously evaluated with plasma data; (iii) the impact of urine pH on DM clearance was quantified; and (iv) the model can be extended to estimate the activity levels of other alleles, such as *9, *10, and *17, in other data sets. These features enlarge the applicability of the model, but its clinical implications remain to be ascertained.

Our findings are in line with published data that assumed a two-compartment model for DM and a one-compartment model for DO. The estimated value of the absorption rate constant (h⁻¹) is ~0.25, which lies between the previously reported values of 2.6 h⁻¹ and 0.1 h⁻¹ (refs. 1,36). The lag time was estimated to be 0.31 h, which also lies between previously reported values, 0.8 and 0.087 h (refs. 1,35). The model estimates for the apparent central and peripheral volumes of distribution of DM in this study are smaller, at 648 and 1,560 l, respectively, as compared with the previously reported values of 961 and 1,951 l. The volume of distribution of DO (419 l) is smaller than the previously reported values of 961 and 1,951 l.1 The estimated quantitation of some DM and DO concentrations in plasma/urine may be explained by the existence of other sources of variability that were not taken into account in the final model. Possible sources could be the interindividual variability in CYP3A activity, incomplete description of the first-pass metabolism (such as the formation of methoxymorphinan from DM), the omission of the metabolic fate of DO, the possible contribution of glucuronidation, interstudy variability, and, finally, the possibility that further sequence variations were present in addition to those covered in the genotype analysis. The interaction between the final model and the study design can be seen from the simulated predictive check plots. Generally, inadequately fitted concentrations form only a negligible fraction of the whole data set.

In conclusion, the final population model adequately described DM pharmacokinetics in healthy Caucasian men. The results confirmed that an individual’s CYP2D6 genotype and, to a lesser degree, urine pH contribute to the variability in pharmacokinetic profiles of DM. The estimated quantitative differences in the metabolic capacity attributable to copies of individual CYP2D6 genes including *1, *2, and *41 suggest that existing scoring systems to predict CYP2D6 activity from CYP2D6 genotype need to be refined. Whether the observed activity differences between functional alleles for DM as a substrate hold for other substrates remains to be assessed.

**METHODS**

**Study design.** The data used in this study were derived from three cocktail interaction studies involving a total of 36 healthy Caucasian men. These features enlarge the applicability of the model, but its clinical implications remain to be ascertained.
male volunteers; n = 15 from study A, n = 10 from study B, and n = 11 from study C. The data were taken from the respective reference periods during which each subject received a single oral dose of 30 mg DM hydrobromide (one capsule of Hustenstiller-riapharm, Ratiopharm, Ulm, Germany). Subjects eligible for inclusion in the analysis were carriers of one, two, or a combination of the following allelic variants: CYP2D6*1, *1xN, *2, *2xN, *3, *4, *4xN, *6, *41, or *41xN. Details of the demographic characteristics of the 36 subjects are shown in Table 1.

Blood samples were collected for quantification of DM and DO in each of the studies. In study A, samples were collected immediately before administration of DM and at 0.17, 0.5, 0.75, 1.0, 1.33, 2.0, 3.15, 4.25, 5.25, 6.0, 8.0, 10.0, 11.95, 14.0, and 24.0 h after dosing. In study B, the time points of blood sample collection were immediately before administration of DM and at 0.17, 0.33, 0.50, 1.0, 1.33, 1.67, 2.0, 2.5, 3.25, 3.95, 4.5, 5.25, 7.00, 10.00, 12.0, 16.0, and 24.0 h after dosing. In study C, the time points of blood sample collection were immediately before administration of DM and at 0.17, 0.33, 0.50, 0.75, 1.00, 1.33, 1.67, 2.0, 2.5, 3.25, 3.95, 4.13, 4.25, 4.5, 4.75, 5.0, 5.5, 6.5, 8.0, 10.0, and 12.0 h after dosing.

In order to determine the metabolic ratio of DM to DO in urine, urine samples were collected periodically, and their volumes and pH values were measured for each collection period. The urine sampling schedules were as follows: in study A, immediately before administration of DM and at 0–2, 2–4, 4–6, 6–8, 8–12, 12–16, and 16–24 h after dosing; in study B, immediately before administration of DM and at 0–6 and 6–12 h after dosing; and in study C, immediately before administration of DM and at 0–2, 2–4, 4–6, 8–12, and 8–12 h after dosing.

Quantification of DM and DO. Plasma and urine samples were analyzed in accordance with a previously published and validated liquid chromatography–tandem mass spectrometry method. Urine samples were treated with β-glucuronidase for cleavage of the DO glucuronides before measurement. The lower limits of quantification were 0.103 ng/ml for DM and 0.101 ng/ml for DO. Precision ranged from 3.2 to 7.8% for DM and from 4.7 to 9.2% for DO, and accuracy was 101.8–102.9% and 97.4–99.5% for DM and DO, respectively.

Genotyping. Genotyping was performed essentially as described previously in detail. Briefly, a 6.6 kb long CYP2D6-specific PCR product was generated and subsequently used as a template to determine the presence of allele-identifying single-nucleotide polymorphisms. Gene duplications and the CYP2D6*5 gene deletion were also determined using long-range PCR.

Population pharmacokinetics modeling. All data related to DM and DO obtained from plasma and urine were analyzed simultaneously, using the nonlinear mixed-effects software in NONMEM, version VI (GloboMax, Hanover, MD). The analysis was performed using the first-order conditional estimation method with interactions (FOCE INTER). Models were specified as a set of differential equations using the ADVAN6 subroutine and were parameterized for disposition kinetics using apparent clearance and volume of distribution terms.

The activity levels of the nonfunctional CYP2D6*4, *3, and *6 and the null enzyme variants were set to zero because these alleles carry inactivating sequence variations (http://www.cypalleles.ki.se/cyp2d6.htm). Clearance of DM in individuals with CYP2D6*null/*null genotypes was assumed to reflect non-CYP2D6-mediated clearance. Therefore, the study evaluated the activity levels of alleles containing CYP2D6*1, *2, and *41 gene copies, which have been recognized as true variants influencing DM metabolism activity in vivo. In addition to CYP2D6 polymorphisms, age, urine pH, and body weight were considered as covariates during model building. An allometric model was tested for the covariate, body weight, whereas exponential and combined models were tested to center the age effect to the mean or the median age values. Different relationships were tested to explore the effect of urine pH on renal clearances of DM and DO. Covariates were included in the model in a forward stepwise manner and backward removal starting with the covariate that resulted in most reduction in the OFV. This procedure was repeated until no significant drop was obtained in OFV.

As a starting point for the model building, a four-compartment open model was tested to describe the entire data set. Each compartment represents each site of measurement (i.e., two compartments each (plasma and urine) for DM data and DO data). A first-order absorption process with and without a lag time was explored. Disposition processes for both substances were assumed to follow linear kinetics. For the purpose of the semimechanistic model, DM was assumed to be converted entirely to DO. This metabolic clearance was described as follows: CLm = CLCYP2D6 + CLDO, where CLm is the total apparent clearance of DM describing the biotransformation of DM to DO, CLCYP2D6 is the basic value for DM metabolic clearance that is not subject to CYP2D6 activity, and CLDO is the metabolic clearance due to CYP2D6 activity. CLCYP2D6 = n1 × CL1 + n2 × CL2 + n41 × CL41, where n1 and CL1 are the number of gene copies observed and metabolic clearance values attributable to the respective gene, respectively. This model was selected as a basic structural model for further model-building activities.

An exponential interindividual variability model was included for all model parameters, forward and backward, step by step interchangeably. Four residual-error models were added, one for each data type (i.e., a separate residual-error model for plasma parent data, another for plasma metabolite data, and so on). Each residual-error model was built up using an additive model, a proportional model, or a combination of both.

Model derivation and justification were guided by (i) the difference in the objective function value (ΔOFV) generated via NONMEM (a ΔOFV of 3.84 (approximate χ2-distribution) for an additional parameter was used to determine the statistical significance (P < 0.05) of the difference between any two models), (ii) visual inspection of goodness-of-fit plots, and (iii) physiological plausibility and statistical precision of model parameter estimates. The final model performance was also justified by simulating plasma and urine concentration–time profiles for 1,000 subjects carrying the observed CYP2D6 allele combinations and visualized using the Active Perl program (version 5.10.0; ActiveState, Vancouver, BC, Canada).

APPENDIX

The differential equations specifying the model shown in Figure 1 for a generic individual are as follows:

\[ \frac{dA}{dt} = -k_{12}A, \]
\[ C = k_{12}A + Q_1C_2/(Q_1 + CL_{23}), \]
\[ \frac{dA_2}{dt} = Q_1C_2 - Q_2C_2 - Q_5C_5 + Q_5C_5 - CL_{24}C_2, \]
\[ \frac{dA_3}{dt} = CL_{23}C_2 - CL_{35}C_3, \]
\[ \frac{dA_4}{dt} = CL_{23}C_2, \]
\[ \frac{dA_5}{dt} = CL_{35}C_5, \]
\[ \frac{dA_6}{dt} = Q_6C_6 - Q_2C_6, \]

The differential equation for the hypothetical enzyme compartment can be written as follows:

\[ \frac{dC}{dt} = k_{12}A + Q_1C_2 - Q_1C_2 - CL_{24}C_2, \]

where \( C \) is the concentration in the metabolizing compartment. The model assumes rapid equilibrium between this compartment and the DM central compartment, i.e., \( dC/\ dt \) is equal to zero,
This equation can be rearranged as follows:

\[ C_i = \frac{(k_2 A_i + Q C_i)}{(Q_i + CL_{23})} C_i \]

The differential expression for DM in the central compartment \((A_2)\) is as follows:

\[ \frac{dA_i}{dt} = Q_i C_i - Q_i C_i - Q_i C_i + Q_i C_i - CL_{24} C_i \]

After substituting the value of \(C_i\) from the previous equation:

\[ \frac{dA_i}{dt} = Q_i \left(\frac{(k_2 A_i + Q C_i)}{(Q_i + CL_{23})}\right) - Q_i C_i - Q_i C_i + Q_i C_i - CL_{24} C_i \]

Similarly, for the DO plasma compartment:

\[ \frac{dA_i}{dt} = CL_{23} C_i - CL_{30} C_i - CL_{35} C_i \]

After substituting the value of \(C_i\):

\[ \frac{dA_i}{dt} = CL_{23} \left(\frac{(k_2 A_i + Q C_i)}{(Q_i + CL_{23})}\right) - CL_{30} C_i - CL_{35} C_i \]

where \(A_i\) and \(C_i\) denote the amount and concentration of the species associated with the 4th compartment in Figure 1.
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