Development, validation and clinical application of a HPLC-FL method for CYP2D6 phenotyping in South Brazilian breast cancer patients

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A B S T R A C T

Objective: To develop and validate a method for determination of dextromethorphan (DMT) and dextrorphan (DTP) in plasma samples using HPLC-FL and to apply it to CYP2D6 phenotyping of a population from the South of Brazil.

Methods: Samples were prepared by hydrolysis and liquid–liquid extraction. Analysis was conducted in a reversed phase column, with isocratic elution and fluorescence detection. One hundred and forty patients being treated with tamoxifen were given 30 mg of dextromethorphan and their CYP2D6 phenotypes were determined on the basis of [DMT]/[DTP] metabolic ratios in plasma samples collected after 3 h.

Results: Total chromatography running time was 12 min. Precision (CV%) was below 9.7% and accuracy was between 92.1 and 106.9%. The lower limits of quantification were 1 ng mL−1 for DMT and 10 ng mL−1 for DTP. Mean extraction yield of analytes was 86.6%. Mean age of patients was 55.7 years. Phenotype frequencies were as follows: 7.1% poor metabolizers, 13.6% intermediate metabolizers, 77.1% extensive metabolizers and 2.1% ultra-rapid metabolizers. Metabolic ratios for patients on strong (n = 11) and weak (n = 16) CYP2D6 activity inhibitors were different from each other and also different from ratios for patients not taking enzyme inhibitors (n = 113).

Conclusions: A sensitive method for determination of dextromethorphan and its metabolite in plasma samples was developed and successfully applied, providing evidence of the impact that CYP2D6 inhibitors have on the enzyme’s metabolic capacity.

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Introduction

Cytochrome P450 2D6 (CYP2D6) is responsible for oxidative metabolism of approximately 25% of commonly-prescribed drugs. There is a high degree of interindividual variability in the level of activity of this enzyme, primarily caused by genetic polymorphisms [1]. More than 150 different alleles of the CYP2D6 gene are known [2]. The wild type allele is CYP2D6∗1, which codes a complete and functional enzyme, while alleles∗2,∗33 and∗35 have point mutations that do not affect the enzyme’s catalytic properties. Some alleles are associated with absent enzyme activity, such as∗38,∗40,∗42, and∗44, or with reduced activity, such as∗9,∗10,∗17,∗29,∗36,∗37, and∗41 [3].

The CYP2D6 genotype can be classified into four distinct phenotypes, depending on the resulting enzyme’s metabolic activity. An extensive metabolizer (EM) has at least one functional wild allele. An intermediate metabolizer (IM) will have one allele coding for an enzyme with reduced activity and one allele with null activity, while a poor metabolizer (PM) will be homozygous for two alleles with null activity. Finally, an ultra-rapid metabolizer (UM) will be dominant autosomal with a duplicated functional allele [4]. Frequencies and distributions of CYP2D6 alleles and genotypes vary depending on the origin of the population. There is a lack of studies that have investigated the frequency of CYP2D6 polymorphisms in the Brazilian population, which is a highly admixed population originating from three distinct populations; slaves brought from Africa, the indigenous population and the European colonists [5–7].

The importance of CYP2D6 in breast cancer treatment has become a focus of interest in the literature. For more than 30 years, tamoxifen (TAM) has been considered the primary adjuvant endocrine treatment and it has a significant impact on patient survival. Treatment with TAM for 5 years after definitive surgery reduces the disease’s recurrence rate by 41% and the mortality rate by 34% and is linked with a 9.2% absolute reduction in 15-year breast cancer mortality [8]. Tamoxifen's

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antiestrogenic activity is especially attributed to its active metabolite endoxifen, which is metabolically formed mainly by CYP2D6 [9]. Additionally, environmental factors, such as co-administration of drugs that inhibit the enzyme (e.g. fluoxetine and paroxetine) and alterations of liver and/or kidneys functions can cause a given genotype to be expressed as phenotype from a different group [10].

The relationship between the CYP2D6 genotype and clinical outcomes of TAM treatment has been discussed in the literature. Studies have shown a robust association between polymorphisms of the enzyme and use of inhibitors with unfavorable clinical outcomes, including increase in recurrence rates [11] and reduction in survival rates [12]. Recently, studies conducted by the Breast International Group (BIG) and the Tamoxifen, Alone or in Combination (ATAC) group suggested that there is no clinical value in testing for the CYP2D6 genotype [13,14].

It is important to point out that genotyping is only clinically relevant if it is capable of predicting the phenotype. Employing a combination of genetic and environmental factors when evaluating CYP2D6 activity during treatment with TAM is very valuable since it is common for patients to be given drugs concurrently that inhibit the enzyme's catalytic activity [16]. Phenotype analysis is less expensive than genetic assessment, which demands that the correct alleles be chosen for screening, on the basis of the profile of the population.

One established method for determining the metabolic phenotype of CYP2D6 in terms of an activity index is by calculating the metabolic ratio of the native and active metabolite [17,21]. A ddi -

Experimental component

Reagents and materials

Analytical standards for dextromethorphan (DTP) and propranolol were acquired from Sigma (Saint Louis, United States), while the standard for dextromethorphan (DMP) was obtained from Sanofi-Aventis (Paris, France). The reagents monopotassium phosphate, dipotassium phosphate, sodium acetate, sodium hydroxide and tris(hydroxymethyl)aminomethane were obtained from Nuclear (Diadema, Brazil). HPLC grade methyl tert-butyl ether, 85% orthophosphoric acid, methanol and acetonitrile were all obtained from Merck (Darmstadt, Germany). Both 98% triethylamine and β-glucuronidase Helix pomatia (HP-2) 122,700 units mL$^{-1}$ were acquired from Sigma (Saint Louis, United States). The water used was purified in a Pure Lab Ultra system, from Elga Lab Water do Brasil (Cotia, Brazil).

Preparation of reagents and standard solutions

Stock solutions of DMT 0.1 mg mL$^{-1}$ and DTP 1 mg mL$^{-1}$ were made by dissolving the analytical standards in methanol. The working solution was made up from a mixture of the standards, at concentrations of 10 μg mL$^{-1}$ for DMT and 100 μg mL$^{-1}$ for DTP, by diluting stock solutions with methanol. The stock solution of propranolol 1 mg mL$^{-1}$ was prepared by dissolving the analytical standard in methanol. The internal standard propranolol 50 ng mL$^{-1}$ solution was prepared by diluting the stock solution with methanol.

Phosphoric acid 0.1% (v/v) was prepared by diluting 85% orthophosphoric acid with ultrapure water. Tris 0.2 M pH 10 buffer was prepared by dissolving 2.43 g of tris(hydroxymethyl)aminomethane in 100 mL of ultrapure water and duly adjusting pH using 85% orthophosphoric acid. Preparation of β-glucuronidase 1000 units mL$^{-1}$ was by dilution of 815 μL β-glucuronidase H. pomatia (HP-2) 122,700 units mL$^{-1}$ in 100 mL of 0.2 M acetate buffer pH 5.

The mobile phase was prepared by mixing phosphate buffer 0.1 M pH 6.0 and acetoni triol 76:24, v/v with 0.1% triethylamine. The pH 6.0 phosphate buffer was prepared by dissolving 13.8 g of monosodium phosphate and 14.198 g of disodium phosphate in 900 mL of ultrapure water. The pH was adjusted by adding phosphoric acid 85% or 1.5 M sodium hydroxide and then the volume was made up to 1000 mL with ultrapure water. The buffer was filtered through a cellulose acetate membrane filter with 0.45 μm pores (Sartorius, Germany). After mixing the acetonitri le, the mobile phase was gasified in an ultrasonic bath for 5 min.

Chromatography equipment and conditions

A Shimadzu Class VP high performance liquid chromatograph (Kyoto, Japan) comprising a quaternary LC-10AT pump system, an SCL-10A system controller, a DGU-14A degasser, a CTO-10AS column oven, an SIL-10AF autosampler and a fluorescence detector was used. The chromatography system was controlled by Class VP 6.13 SP2 software, also provided by Shimadzu. Separation was conducted in a Hypersil Gold C18 reversed phase column (150 × 4.6 mm, 3 μm) from Thermo Scientific (Waltham, United States), kept at 35 °C throughout analysis. The mobile phase was a mixture of phosphate buffer 0.1 M pH 6.0 and acetoni triol (76:24, v/v) 0.1% triethylamine, with a flow rate of 1 mL min$^{-1}$. Chromatographic analysis lasted 12 min. Chromatograms were acquired at an excitation wavelength of 280 nm and an emission wavelength of 320 nm. Detector sensitivity was set at an initial gain of 32 times and increased to 512 times at 6 min.

Preparation of calibration solutions

Calibration solutions were prepared by diluting the DMT and DTP working solutions in varying volumes of methanol to achieve concentrations of 10 and 100; 50 and 500; 100 and 1000; 200 and 2000; 500 and 5000; 750 and 7500; 1000 and 10,000; and 2500 and 25,000 ng mL$^{-1}$ of DMT and DTP respectively. Plasma calibration solutions were prepared by diluting the calibration solutions 1:10 in plasma free from analytes, in order to obtain samples with concentrations of 1 and 10; 5 and 50; 10 and 100; 20 and 200; 50 and 500; 75 and 750; 100 and 1000; and 250 and 2500 ng mL$^{-1}$, for DMT and DTP respectively.
Sample preparation

Samples were prepared by enzymatic hydrolysis of the conjugated forms of DTP and DMT with glucuronidase, followed by liquid–liquid extraction. Test tubes with threaded lids were filled with a 500 μL plasma sample, followed by 500 μL β-glucuronidase 1000 units mL$^{-1}$, and put in a water bath at 37 °C. After 18 h of incubation, 100 μL of propranolol internal standard 50 ng mL$^{-1}$, 400 μL of buffer Tris pH 10 and 3000 μL of methyl tert-butyl ether were added to the samples. Tubes were mixed for 10 min and centrifuged at 3000 g for 10 min. The organic phase was then transferred to a new test tube with a threaded lid and analytes were re-extracted by adding 200 μL of 0.1% phosphoric acid. After mixing for 15 min, tubes were centrifuged at 3000 g for 15 min. After separation, 150 μL of the aqueous phase was transferred to a microvial and 50 μL was injected into the HPLC-FL system.

Validation of the analytical method

The method was validated as described by Shah et al. [22]. The parameters evaluated were specificity, linearity, sensitivity, precision, accuracy, extraction yield and stability.

Specificity

Six plasma samples free from DMT and DTP and prepared as described under the “Sample preparation” section were analyzed, finding that interferents with the same retention time as analytes were present [22].

Linearity

The linearity of the method was assessed by constructing calibration curves. Calibration solutions with concentrations in the range of 1 to 250 ng mL$^{-1}$ for DMT and 10 to 2500 ng mL$^{-1}$ for DTP were analyzed in sextuplicate. Calibration curves were constructed by calculating the correlations between the calibration solutions' nominal concentrations and the ratios between the areas for the analytes and the areas for the internal standard. The homogeneity of variance of the calibration data was assessed using the F test, with a 95% confidence interval. Curves were fitted using least squares linear regression with a range of weighting factors (1/x, 1/x$^{0.5}$, 1/x$^{2}$, 1/y, 1/y$^{0.5}$, 1/y$^{2}$). Calibration models were evaluated on the basis of their coefficients of correlation (r) and sum percentage relative error ($\sum$%RE), as described by Almeida et al. [23]. Calibration was conducted daily with one replicate of each concentration throughout all experiments of validation and application of the method.

Precision and accuracy

Precision and accuracy were evaluated over 5 days by analyses in triplicate of samples quality control low (QCL) 3 and 30 ng mL$^{-1}$, quality control medium (QCM) 30 and 300 ng mL$^{-1}$ and quality control high (QCH) 90 and 900 ng mL$^{-1}$ for DMT and DTP respectively. Within-assay precision and between-assay precision were calculated by analysis of variance (single-factor ANOVA), with the grouping variable “day” and were expressed as coefficient of variation (CV%). Accuracy was defined as the percentage concentration estimated by the calibration curve in relation to nominal concentration. The criterion for acceptability of precision was a maximum CV of 15% (for both within-assay and between-assay) and acceptable accuracy values were from 85 to 115% of nominal concentrations [22].

Extraction yield

Extraction efficiency was determined over 3 days by comparing the areas of analytes obtained in triplicate analyses of the QCL, QCM and QCH samples with areas for direct injection of metabolic solutions of DMT and DTP at concentrations equivalent to complete extraction. Percentage extraction yield was calculated by assuming that the mean areas for each DMT and DTP solution represented 100%, for the purposes of comparison with the mean areas for the same concentrations for the control plasma samples extracted.

Sensitivity

The method’s lower limit of quantification (LLOQ) was assessed over 3 days by triplicate analyses of the lowest concentration calibration solutions, at concentrations of 1 and 10 ng mL$^{-1}$, for DMT and DTP respectively. The CV% and accuracy of measurements were calculated. The criterion for acceptability was a maximum CV of 20% and accuracy values in the range of 80 to 120% [22].

Stability

In order to assess bench stability of the samples processed under the analytical conditions described, six control samples of DMT and DTP at concentrations of 8.0 and 400 ng mL$^{-1}$ were extracted as described in the “Sample preparation” section. Extracts at each concentration were mixed and injected under conventional conditions analytical at 1-hour intervals, over a 12-hour period. The extracted analytes were defined as stable if variation between the areas measured over 12 h was less than or equal to 10%.

Stability after freezing and thawing cycles was tested with quality control DMT and DTP samples at concentrations of 8; 40 and 400 ng mL$^{-1}$, analyzed before (n = 3) and after 3 freeze–thaw cycles (n = 9). For each freeze–thaw cycle, samples were frozen at −20 °C for 24 h, then thawed and kept at room temperature for 2 h before being prepared. The concentrations of the analytes from each experiment were calculated using the daily calibration curves and variance analyzed using ANOVA. P values < 0.05 were considered statistically significant.

CYP2D6 phenotyping of patients on tamoxifen

This study was approved by the Research Ethics Committee at the Hospital de Clínicas de Porto Alegre under hearing number 240.253. A total of 140 patients on adjuvant hormonal treatment with tamoxifen were enrolled on the study, were provided with a full explanation and signed free and informed consent forms. Exclusion criteria for this study were underlying liver or kidney diseases.

Participants were instructed to fast for 4 h and abstain from alcohol for 48 h prior to the tests. Data on age, weight, body mass index (BMI) and race were recorded, in addition to the time each patient had been on tamoxifen and any drugs they were taking that are CYP2D6 inhibitors. For the phenotyping procedure, each volunteer received a 30 mg oral dose of DMT and 3 h later an 8 mL sample of venous blood was drawn into a tube containing EDTA as anticoagulant. Tubes were centrifuged immediately and plasma was transferred to 2 mL polypropylene tubes, which were stored at −70 °C until analysis.

Concentrations of DMT and DTP were estimated from their calibration curves and then [DMT]/[DTP] metabolic ratios were calculated. CYP2D6 phenotypes were classified into four categories on the basis of the resultant ratios [DMT]/[DTP]: PM ≥ 0.3; 0.3 > IM ≥ 0.03; 0.03 > EM ≥ 0.0003; and UM > 0.0003 [24]. Although these limits were originally defined on the basis of urine sample testing, a high correlation ($r^2 = 0.8736$, $P < 0.0001$) with minor deviation (0.023) has been observed between metabolic ratios from urine and plasma samples [25] and so the same classification is also applicable to the context of this study.

Analysis of data

Initially, a descriptive analysis of the study variables was conducted. The precision and stability validation parameters for the analytes were evaluated using ANOVA. Medians and 25% and 75% percentiles for DTP and DMT levels were determined. The frequency distributions for the CYP2D6 metabolic ratios, expressed as log [DMT]/[DTP], were used to plot a histogram. The normality of the metabolic distribution curve was tested using the D’Agostino–Pearson test. Median DMT and DTP
Fig. 1. CYP2D6 phenotyping chromatograms
A: Control samples: DTP 750 ng mL\(^{-1}\) and DMT 75 ng mL\(^{-1}\).
B: Extensive metabolizer patient sample: DTP 810.4 ng mL\(^{-1}\) and DMT 6.81 ng mL\(^{-1}\); [DMT]/[DTP] = 0.0084.
C: Poor metabolizer patient sample: DTP 59.61 ng mL\(^{-1}\) and DMT 50.7 ng mL\(^{-1}\); [DMT]/[DTP] = 1.176.
concentrations and median metabolic ratios were compared across races, age groups, BMI groups and level of CYP2D6 inhibitor use using the Mann–Whitney test. Analyses were conducted using SPSS version 17.0, and results with \( P < 0.05 \) were considered statistically significant.

### Results and discussion

#### Chromatography, preparation of samples and validation

The chromatography running time was 12 min, and retention times for analytes were 3.4, 7.4 and 10.2 min for DTP, internal standard and DMT, respectively. Fig. 1 shows chromatograms for a control sample for analytes were 3.4, 7.4 and 10.2 min for DTP, internal standard and DMT, respectively. The chromatographic conditions employing a reversed phase column and isocratic elution with fluorescence detection proved capable of selectively separating analytes and endogenous compounds.

Several different methods for detection of DMT and DTP by HPLC-FL have already been described in the literature [17,21,26]. In those studies, chromatography running times ranged from 15 to 33 min, using isocratic elution or mobile phase gradients.

The method developed here was linear for the interval 1 to 250 ng mL\(^{-1}\) DMT and 10 to 2500 ng mL\(^{-1}\) DTP. The calibration data exhibited significant heteroscedasticity and so several weighted regression models were tested, leading to the selection of the reciprocal of concentration (1/x) as the best weighting factor for both analytes, with \( \sum R \) values of 4.47 for DMT and 7.69 for DTP and \( \sum \%RE \) values of 1.5 \times 10\(^{-15}\) for DMT and 7.5 \times 10\(^{-15}\) for DTP, contrasting with the values obtained when unwighted regression was used, giving \( \sum R \) of −24.52 for DMT and −40 for DTP and \( \sum \%RE \) values of 119.59 for DMT and 176.59 for DTP. The calibration curves exhibited acceptable linearity, all with correlation coefficients of \( r > 0.99 \).

The parameters sensitivity, precision and accuracy were all within their limits of acceptability for validation of bioanalytical methods (Table 1). The coefficients of variation (CV%) ranged from 3.4 to 9.7% intra-day and 4.0 to 8.8% inter-day. The method’s accuracy was within the range of 92.1 to 106.9%.

The sample preparation technique based on liquid–liquid extraction and including a re-extraction step provided extracts with a high degree of purity and offered a high yield, greater than 85%. The limits of quantification for analytes were satisfactory for application of the method to clinical samples, at 10 ng mL\(^{-1}\) DTP and 1 ng mL\(^{-1}\) DMT. Afshar et al. proposed a method in which preparation of samples was based on simple precipitation of proteins, but their limit of DMT quantification was 10 ng mL\(^{-1}\), with the result that their method was only able to quantify DMT in 4 of 39 EM patients and could not distinguish between UM, EM and IM phenotypes [17]. Using a modified version of their previously published method, Afshar et al. were able to reduce the limit of quantification to 5 ng mL\(^{-1}\) [20]. However, these conditions were still unable to quantify DMT in any samples from EM patients (\( n = 195 \)). Since the lack of normality in the distribution of metabolic ratios within the EM group suggested that IM patients may overlap into this group, these authors reanalyzed 80 samples chosen at random using a method with a LLOQ of 2 ng mL\(^{-1}\). Despite the improved sensitivity, it was only possible to quantify DMT in four of the 80 samples and for the remainder the metabolic ratio was calculated taking the limit of detection of 1 ng mL\(^{-1}\) as the DMT concentration. As a result, concentrations between the LOD and the LLOQ were disregarded in the calculations and the possibility that this range may had been the determinant in the classification and differentiation of IM, EM and UM phenotypes in their study could not therefore be ruled out.

A study by Lin et al. reported a limit of quantification of 0.27 ng mL\(^{-1}\) for DMT and 0.26 ng mL\(^{-1}\) for DTP utilizing 1 mL of plasma, without a hydrolysis stage, resulting in an upper limit to the calibration curve of 54.3 ng mL\(^{-1}\) for DMT and 51.5 ng mL\(^{-1}\) for DTP [26]. In the present study, with a hydrolysis step, the range of values detectable in samples was considerably wider, with a DMT interval of 1.0 to 251.1 ng mL\(^{-1}\) and a DTP interval of 28.4 to 2349.0 ng mL\(^{-1}\).

The results of the analyte stability tests are shown in Table 2. In the bench stability tests the analytes remained stable for a period of 12 h for both concentrations tested with a maximum variation of 6% in the area (Fig. S1, Supplementary material). It is therefore possible to conduct simultaneous extraction from a number of samples, storing them at room temperature before proceeding to injection. Signs of analyte

### Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Nominal concentration (ng mL(^{-1}))</th>
<th>Precision (CV%)</th>
<th>Accuracy (%)</th>
<th>Extraction yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intra-assay</td>
<td>Inter-assay</td>
<td></td>
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<tr>
<td>Dextromethorphan</td>
<td>LLOQ</td>
<td>8.7</td>
<td>9.3</td>
<td>105.9</td>
</tr>
<tr>
<td></td>
<td>QCL</td>
<td>4.4</td>
<td>5.8</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td>QCM</td>
<td>5.6</td>
<td>7.9</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>QCH</td>
<td>3.4</td>
<td>4.0</td>
<td>98.9</td>
</tr>
<tr>
<td>Dextrophan</td>
<td>LLOQ</td>
<td>15.0</td>
<td>12.1</td>
<td>106.4</td>
</tr>
<tr>
<td></td>
<td>QCL</td>
<td>9.7</td>
<td>8.8</td>
<td>92.1</td>
</tr>
<tr>
<td></td>
<td>QCM</td>
<td>8.1</td>
<td>7.0</td>
<td>97.1</td>
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<tr>
<td></td>
<td>QCH</td>
<td>6.3</td>
<td>6.6</td>
<td>106.9</td>
</tr>
</tbody>
</table>


### Table 2

<table>
<thead>
<tr>
<th>Analyte (ng mL(^{-1}))</th>
<th>Bench stability</th>
<th>Freeze–thaw stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variance of concentration after 12 h (%)</td>
<td>Control concentration after three cycles (n = 3)</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>DTP</td>
<td>4.3</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>3.5</td>
</tr>
<tr>
<td>DMT</td>
<td>6.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4.8</td>
</tr>
</tbody>
</table>
instability were also not detected during the freezing and thawing experiments (P > 0.05).

**Phenotyping of CYP2D6 in patients on tamoxifen**

A total of 140 patients on adjuvant hormonal treatment with tamoxifen were assessed. The sample had mean age of 55.7 ± 10.35 years (28 to 81), mean weight of 69.38 ± 13.63 kg (51 to 116 kg) and mean BMI of 27.0 ± 5.33 kg/m² (18.4 to 46.5 kg/m²), while 114 patients were white, 17 were brown (parda — a Brazilian national census classification) and 9 were black. Twenty-seven patients reported concurrently taking drugs that inhibit CYP2D6, 16 of whom were on weak inhibitors such as venlafaxine, citalopram, chlorpromazine and haloperidol and 11 of whom were on strong inhibitors, such as fluoxetine and bupropion.

The \([\text{DMT}]/[\text{DTP}]\) metabolic ratios were not distributed normally according to the D’Agostino–Pearson test (P < 0.001). Fig. 2 shows a histogram illustrating the frequency distribution of CYP2D6 metabolic ratios, expressed as \(\log_{10} [\text{DMT}]/[\text{DTP}]\), in which the groups PM, IM, EM and UM can be identified.

In samples from three patients, DMT was detected, but at concentrations below the lower limit of quantification and in these cases the metabolic ratio was calculated using a DMT concentration of 1 ng mL\(^{-1}\).

Approximately 20% of the study population exhibited incomplete CYP2D6 metabolism. The participants were classified into phenotype groups by \([\text{DMT}]/[\text{DTP}]\) metabolic ratios as follows: 10 (7.1%) PM, 19 (13.6%) IM, 108 (77.1%) EM and 3 (2.1%) UM. Medians and ranges (P25–P75) for DMT and DTP are given in Table 3. Both DMT and DTP levels and metabolic ratios were statistically different between phenotype groups. The median metabolic ratio for PM (1.490) was 497 times greater than that for EM (0.003), and IM (0.091) was 30 times EM. The magnitude of the difference between PM and EM was similar to that reported by Afshar et al., at 520 times difference between the lowest and highest metabolic ratios (N = 200)[20].

It is known that there is wide variability in the prevalence of non-functional alleles across different races. This study investigated patients from the South of Brazil, which is a region with a history of European immigration and the frequencies of CYP2D6 phenotypes found were similar to the distributions of genotypes found in Caucasian populations at 7 to 11% PM; 10 to 15% IM; 70 to 80% EM and 3 to 5% UM[1].

The percentages of PM reported in previous studies of Brazilian populations have varied in the order of 2.3 to 6.2%[5–7]. In a previous study, we found prevalence rates of CYP2D6 genotypes of 4.1% PM, 4.1% IM, 88.7% EM and 3.1% UM (N = 97)[6]. Jardim et al. found genotype frequencies of 3% PM, 27% IM and 70% EM (N = 30)[5]. Neves et al. assessed CYP2D6 phenotypes using metoprolol metabolic conversion to \(\alpha\)-hydroxymetoprolol as enzyme activity surrogate (N = 130) and did not identify any IM or UM, only PM (n = 3, 2.3%) and EM (n = 127, 97.7%)[7]. The marked differences between the prevalence rates of CYP2D6 phenotypes found in different studies can be attributed to the genetic heterogeneity of the study populations and the contribution of non-genetic factors to enzyme activity, in particular to taking enzyme inhibiting substances.

**Table 3**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
<th>Frequency (%)</th>
<th>Median DTP ng mL(^{-1}) (P25–P75)</th>
<th>Median DMT ng mL(^{-1}) (P25–P75)</th>
<th>Median ([\text{DMT}]/[\text{DTP}]) ratio and ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>10</td>
<td>7.1</td>
<td>60.3(^{a}) (49.3–87.4)</td>
<td>93.4(^{a}) (63.2–113.0)</td>
<td>1.490(^{a}) (0.30–2.31)</td>
</tr>
<tr>
<td>IM</td>
<td>19</td>
<td>13.6</td>
<td>552.0(^{a}) (3543–852.3)</td>
<td>48.1(^{a}) (31.0–66.9)</td>
<td>0.095(^{a}) (0.031–0.177)</td>
</tr>
<tr>
<td>EM</td>
<td>108</td>
<td>77.1</td>
<td>1290.7(^{a}) (1020.1–1597.6)</td>
<td>3.8(^{a}) (2.2–7.1)</td>
<td>0.0005–0.0278</td>
</tr>
<tr>
<td>UM</td>
<td>3</td>
<td>2.1</td>
<td>1990.1(^{a}) (1522.5–1995.3)</td>
<td>&lt;1.0</td>
<td>0.001(^{a}) (0.0001–0.0002)</td>
</tr>
</tbody>
</table>

DMT: dextromethorphan; DTP: dextrorphan; PM: poor metabolizer; IM: intermediate metabolizer; EM: extensive metabolizer; UM: ultra-rapid metabolizer.

The same letter (a, b, c, d) in the column does not differ in Mann-Whitney at 5% significance test.
Many. Moreover, dextromethorphan has shown to be an efficient inhibitor drugs on its activity and may have implications for tamoxifen metabolism in 140 patients on adjuvant therapy with CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clin Pharmacol Ther 2006;80:61–74.


