Full Title:

Dextromethorphan to dextrorphan urinary metabolic ratio does not reflect dextromethorphan oral clearance


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Running Title:

Dextromethorphan oral clearance and UMR

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ABSTRACT

Objective. Dextromethorphan urinary metabolic ratio is widely used to determine the CYP2D6 phenotype, but its utility to reflect subtle differences in catalytic activity is unclear. We evaluated the capability of dextromethorphan urinary metabolic ratio to predict dextromethorphan oral clearance as a measure of CYP2D6 activity. Methods. Data from ten healthy extensive metabolizers of CYP2D6 were given 30 mg of dextromethorphan hydrobromide orally on two occasions. Blood and urine samples were collected for 72 hours. Dextromethorphan and dextrorphan were determined in urine by HPLC with fluorescence detection and in serum by liquid chromatography–mass spectrometry. Results. The urinary metabolic ratio was very weakly correlated with dextromethorphan oral clearance (r = 0.24, p = 0.04). In contrast, the dextromethorphan oral clearance was highly correlated with the dextromethorphan to dextrorphan AUC ratio (r = 0.84; p = 0.005) and the 3 hr (r = 0.60; p = 0.003), 4 hr (r = 0.72, p < 0.001), 6 hr (r = 0.67, p < 0.001), and 8 hr (r = 0.74, p < 0.001) dextromethorphan to dextrorphan serum ratios. Assuming an effect size of 30%, the number of volunteers required for cross-over and cross-sectional studies using the urinary metabolic ratio as the CYP2D6 index were calculated to be 56 and 524, respectively, whereas 14 and 60 subjects are needed if oral clearance is employed. Conclusions. Considering the required sample size and the low correlation with oral clearance, urinary metabolic ratio is not recommended as the primary outcome variable in studies requiring the detection of modest changes in CYP2D6 activity.
Cytochrome P-450 2D6 (CYP2D6), a well-characterized polymorphic enzyme, has been shown to be involved in the biotransformation of more than thirty clinically important drugs (Eichelbaum and Gross 1990). The determination of CYP2D6 phenotype is widely used in drug interaction and pharmacogenetic clinical studies. Dextromethorphan (DTM), has been traditionally utilized as a probe to measure in vivo and in vitro CYP2D6 activity (Dayer, Leemann, and Stribeni 1989). The primary enzyme catalyzing the O-demethylation of dextromethorphan is CYP2D6 (Kupfer, Schmid, and Pfaff 1986; Schmid et al. 1985). Although the parent compound and its metabolite concentrations can be determined in different biological fluids (serum, saliva, etc.), their ratio in urine is the most frequently used method to assess CYP2D6 status in vivo. Indeed, the dextromethorphan to dextrorphan urinary metabolic ratio (UMR) is routinely applied to segregate populations into two major phenotypic groups, extensive and poor metabolizers (Hou et al. 1996; Sachse et al. 1997; Tateishi et al. 1999). Although non-invasive and easy to perform, the utility of the urinary metabolic ratio to assess moderate alterations in CYP2D6 activity has not been established (Chladek et al. 2000; Kohler et al. 1997; Tenneze et al. 1999; Yeh et al. 2003).

The aim of this report is to compare DMT/DT urinary metabolic ratio, DMT/DT AUC ratio and DTM oral clearance in order to assess the ability of UMR to determine the activity of CYP2D6 in vivo.

METHODS

These data were obtained in a previous study in which the authors examined the effect of Echinacea purpurea root administration on the in vivo disposition of caffeine,
tolbutamide, dextromethorphan and midazolam, selective probes for CYP1A2, CYP2C9, CYP2D6 and CYP3A, respectively (Gorski et al. 2004). For the purpose of this report, we will focus exclusively on the evaluation of CYP2D6 activity.

**Subjects:** After approval by the Clarian Health Partners, Inc, and Indiana University Purdue University Indianapolis Institutional Review Board and Research Involving Human Subjects Committee of the Food and Drug Administration, 12 volunteers participated in the study conducted at the Indiana University General Clinical Research Center after giving written informed consent. Participants (6 men and 6 women, 31±6 years, 79±10 kg) were nonsmokers and had no significant medical conditions as assessed by medical history, physical examination including electrocardiography, and blood and urine chemistry screens.

**Study design:** In the first phase of the study (part 1) the volunteers received a standard light breakfast in the morning after an overnight fast. One hour later, they received 30 mg of dextromethorphan hydrobromide as part of a cocktail of drugs (Gorski et al. 2004) administered orally with 240 mL of water. Blood samples were collected from an indwelling venous catheter at the following times: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours after drug ingestion. Urine sampling occurred over the following intervals: 0 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, and 60 to 72 hours after oral drug administration. Subjects remained sitting until 4 hours after oral dosing and were allowed to eat 2 hours after dosing. Part 2 of the study took place 5 to 7 days after completing study part 1, the volunteers began a course of Echinacea and on the sixth day of dosing, the study design of part 1 was repeated.
Sample analysis: The urinary concentrations of dextromethorphan and its metabolites were determined in the zero to 24 hour pooled sample by HPLC with fluorescence detection (Jones et al. 1996a; Jones et al. 1996b). The serum concentrations of dextromethorphan and dextrorphan were determined by liquid chromatography–mass spectrometry (Gorski et al. 2004).

Pharmacokinetic analysis: Standard non-compartment pharmacokinetics analyses were used to determine the pharmacokinetic parameters of interest (WinNonlin v. 4.0; Pharsight Corp., Mountain View, Calif). The terminal elimination rate constant (\(\hat{\beta}\)) was determined by log-linear regression. The area under the concentration-time curve (AUC) after oral drug administration was determined by a combination of linear and logarithmic trapezoidal methods with extrapolation to infinity \([\text{AUC} (0-\infty)]\). The oral clearance of DTM was calculated by dividing the oral dose by the DTM AUC.

Statistical analysis: Data were normally distributed after log transformation and were analyzed using paired t-test or by linear regression analysis (SAS/STAT® Software, v. 8.2; SAS Institute Inc., Cary, North Carolina). The comparisons between urinary metabolic ratio and other putative CYP2D6 indices were based on the derived slope estimates of the relationship and their corresponding standard error (SE) and p-values were calculated from a standard normal distribution. In addition, linear mixed models were employed to estimate within-subject and between-subject variations. The total sample size for two-group comparisons was calculated assuming the effect size is 30% change, type I error rate is 5%, and the required power is 80%. All the differences were judged significant when P was less than 0.05.
RESULTS

Eleven out of twelve subjects were classified as extensive metabolizers (EM) (dextromethorphan-to-dextrorphan urinary metabolic ratio ≤ 0.3) (Schmid et al. 1985) and one as poor metabolizer. Urine data for one EM was not available. Echinacea did not significantly alter the dextromethorphan oral clearance in EM (Gorski et al. 2004), and we therefore considered EM dextromethorphan data as replicate determinations and excluded the poor metabolizer data from the analysis. The individual AUC from 0 to infinity, the oral clearance and the DTM/DT UMR for the two study phases are presented in Table 1.

The relationship between the UMR and the oral clearance of DTM and various serum DTM/DT ratios were examined. A poor correlation (r=0.24, p=0.04) between the urinary metabolic ratio and the dextromethorphan oral clearance was observed (Figure 1A). The correlations between the UMR and serum concentration ratios at 3, 4, 6 and 8 hours after DTM administration were also low (0.001, 0.10, 0.21 and 0.19, respectively). The DTM/DT AUC ratio was significantly (r=0.84; p= 0.005) correlated with the dextromethorphan oral clearance (Figure 1B). Likewise, significant correlations were observed between the oral clearance of DTM and the 3 (r=0.60; p=0.003), 4 (r=0.72, p<0.001), 6 (r=0.67, p<0.001), and 8 (r=0.74, p<0.001) hour DTM/DT serum concentration ratios. There was also a high correlation between AUC of DTM and the 3, 4, 6 and 8 hour serum ratios (r=0.84, 0.88, 0.79 and 0.82, respectively).

Intra and inter subject variability for dextromethorphan oral clearance was 15% and 32%, for DTM/DT AUC ratio was 26% and 68%, and for DTM/DT UMR was
35% and 100%, respectively. In comparing the efficiency of the DTM/DT UMR, dextromethorphan oral clearance and DTM/DT AUC ratio to assess CYP2D6 activity, we assumed that an effect size of 30% in the in vivo activity of CYP2D6 would be clinically significant. The sample size required for cross-over and cross-sectional studies using UMR, the AUC ratio or the oral clearance as the outcome measure is 56 and 524, 34 and 250, and 14 and 60, respectively. In contrast to oral clearance, UMR in a 14-subjects cross-over study and a 60-subjects cross-sectional study would only detect an effect size of 74% and 118% in the enzyme activity, respectively.

**DISCUSSION**

The formation of dextrorphan by CYP2D6 is responsible for approximately 97% of the oral clearance of dextromethorphan in EMs (Capon et al. 1996; Gorski et al. 2004). The dextromethorphan urinary metabolic ratio is a commonly used method for quantifying CYP2D6 activity in vivo. However, the capability of the urinary metabolic ratio to predict the oral clearance of dextromethorphan has not been previously evaluated. Nevertheless, the correlation of dextromethorphan to dextrorphan ratios in urine and plasma has been examined in several studies but the results are contradictory. For example, Tenneze et al. showed that the log DTM/DT MR in 24-hour urine correlated with the log DTM/DT MR in plasma 3 hr after DTM administration in CYP2D6 EMs (r = 0.92) (Tenneze et al. 1999). In contrast, the analysis of EM healthy subjects data from Köhler’s study (Kohler et al. 1997) shows a very low correlation between the UMR in 8-hour urine collection and in serum 1 hr after DTM administration (r = 0.19). Chladek et al. found a good correlation between UMR and 3-hour plasma ratio (r=0.88) and Capon
et al. observed a similar correlation between the UMR and partial clearance of DTM to DT ($r^2 = 0.82$) (Capon et al. 1996; Chladek et al. 2000). However, these studies included poor metabolizers in the analysis and the correlations among EMs alone would be significantly lower.

We assessed the capacity of different parameters to reflect dextromethorphan oral clearance and found that the UMR had the lowest performance when compared to the ratio of DTM/DT AUC and other serum concentration ratios. This poor correlation presumably reflects the previously recognized contributions of phenomenon other than CYP2D6 activity to the UMR (Labbe et al. 2000). The phenomena that modulate the UMR, such as urine pH, may also contribute to the increased intra (35% vs. 26%) and intersubject (100% vs. 68%) variability in UMR with respect to DTM/DT AUC ratio.

The poor correlation between dextromethorphan oral clearance and UMR ($r = 0.24$) as compared to DTM/DT AUC ratio ($r = 0.84$) makes the UMR a questionable quantitative measure of in vivo CYP2D6 catalytic activity in EMs. This deficiency precludes the use of the UMR in drug-interaction studies that focus on modest changes in CYP2D6 activity. On the other hand, the UMR remains a suitable index for the identification of poor and extensive metabolizers.

The UMR has been the preferred CYP2D6 phenotyping method over plasma sampling because it is easier and less expensive. Nonetheless, taking into account the required sample sizes, the estimation of DTM CL PO appears to be the most efficient strategy.
REFERENCES


Kohler D., S. Hartter, K. Fuchs, W. Sieghart, and C. Hiemke, 1997. CYP2D6 genotype and phenotyping by determination of dextromethorphan and metabolites in serum of
healthy controls and of patients under psychotropic medication. Pharmacogenetics, 7:453-461.


Footnotes

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Legends

Figure 1.

A. Relationship between the dextromethorphan urinary metabolic ratio and the oral clearance of dextromethorphan in 10 Healthy Volunteers.  B. Relationship between the dextromethorphan to dextrorphan AUC ratio and the oral clearance of dextromethorphan in 11 Healthy Volunteers.  Lines represent the correlation and 95% confidence interval. Symbols reflect individual values.
Table 1. Individual Parameter Estimates for the Disposition of Dextromethorphan Following Oral Dosing on Two Separate Occasions in 11 CYP2D6 Extensive Metabolizers.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Phase I</th>
<th></th>
<th>Phase II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTM AUC (_{0\rightarrow\infty}^a) (µgxhr/L)</td>
<td>CL(_{PO}^b) (L/hr)</td>
<td>UMR(^c)</td>
<td>DTM AUC (_{0\rightarrow\infty}^a) (µgxhr/L)</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>1030.6</td>
<td>0.0075</td>
<td>21.1</td>
</tr>
<tr>
<td>2</td>
<td>21.7</td>
<td>1013.8</td>
<td>0.0019</td>
<td>22.2</td>
</tr>
<tr>
<td>3</td>
<td>36.9</td>
<td>596.2</td>
<td>0.0007</td>
<td>32.7</td>
</tr>
<tr>
<td>4</td>
<td>17.2</td>
<td>1276.2</td>
<td>0.0109</td>
<td>18.6</td>
</tr>
<tr>
<td>5</td>
<td>11.1</td>
<td>1980.3</td>
<td>14.3</td>
<td>1537.2</td>
</tr>
<tr>
<td>6</td>
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<td>1858.7</td>
<td>0.0053</td>
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<tr>
<td>7</td>
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<td>1574.6</td>
<td>0.0042</td>
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</tr>
<tr>
<td>8</td>
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<td>979.6</td>
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</tr>
<tr>
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<td>1439.2</td>
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<tr>
<td>10</td>
<td>15.6</td>
<td>1414.5</td>
<td>0.0011</td>
<td>19.1</td>
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<tr>
<td>11</td>
<td>21.7</td>
<td>1011.8</td>
<td>0.0279</td>
<td>31.5</td>
</tr>
<tr>
<td>Mean (±SD)(^d)</td>
<td>19.0 ± 7.2</td>
<td>1289 ± 414</td>
<td>0.0076 ± 0.0086</td>
<td>19.5 ± 7.3</td>
</tr>
</tbody>
</table>

\(^a\) DTM AUC \(_{0\rightarrow\infty}\): Dextromethorphan Area under the time-concentration curve.

\(^b\) CL\(_{PO}\): Oral clearance.

\(^c\) UMR: Dextromethorphan to dextrorphan urinary metabolic ratio.

\(^d\) Mean of extensive metabolizers only
Table 2. Sample size estimates required to see a 30% change in the oral clearance of dextromethorphan, dextromethorphan to dextrorphan (DTM/DT) serum concentration ratios at 3, 4, 6 and 8 hours, DTM/DT AUC ratio, and the Urinary Metabolic Ratio (UMR) with 80% power.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study Design</th>
<th>Cross Over</th>
<th>Cross Sectional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Clearance</td>
<td>Cross Over</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>DTM/DT serum concentration ratio</td>
<td>Cross Sectional</td>
<td>42</td>
<td>410</td>
</tr>
<tr>
<td>3 hours</td>
<td>Cross Over</td>
<td>42</td>
<td>410</td>
</tr>
<tr>
<td>4 hours</td>
<td>Cross Sectional</td>
<td>50</td>
<td>620</td>
</tr>
<tr>
<td>6 hours</td>
<td>Cross Sectional</td>
<td>46</td>
<td>390</td>
</tr>
<tr>
<td>8 hours</td>
<td>Cross Sectional</td>
<td>26</td>
<td>660</td>
</tr>
<tr>
<td>DTM/DT AUC ratio</td>
<td>Cross Over</td>
<td>34</td>
<td>250</td>
</tr>
<tr>
<td>UMR</td>
<td>Cross Sectional</td>
<td>56</td>
<td>524</td>
</tr>
</tbody>
</table>

DTM, dextromethorphan; DT, dextrorphan; AUC, area under the concentration time curve; UMR, urinary metabolic ratio.
Figure 1B

The graph illustrates the relationship between dextromethorphan oral clearance (L/hr) and dextromethorphan to dextrophen AUC ratio. The correlation coefficient (r) is 0.84, and the p-value is 0.005.