**Short Communication**

**The Effects of Drinking and Smoking on the CYP2D6 Metabolic Capacity**

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**ABSTRACT:**

We studied the influence of drinking and smoking habits on CYP2D6 metabolic capacity measured by the use of debrisoquine as a substance test. We did not find any significant differences in the frequency of subjects with CYP2D6 deficiency (poor metabolizers) among four groups of healthy individuals: nonsmokers/non-drinkers, smokers/drinkers, nondrinkers/smokers, and nonsmokers/drinkers. We demonstrated that, among poor metabolizers, alcohol and tobacco consumption was associated with higher metabolic ratios than it was with the control group, but the differences were not statistically significant. Among extensive metabolizers, the lowest metabolic ratio (highest enzyme activity) was detected for nondrinkers/smokers, intermediate values for smokers/drinkers, and the highest metabolic ratio (lowest enzyme activity) for nonsmokers/drinkers. These variations were slight but statistically significant when logarithmic ratio values were applied. These results show that smoking and drinking habits do not need to be taken into account when humans are phenotyped for CYP2D6.

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The polymorphism of CYP2D6 has been described worldwide and CYP2D6 gene and allele nomenclatures have been established (Daly et al., 1996; Nelson et al., 1996). Five to ten percent Caucasian individuals are deficient in this enzyme and defined as poor metabolizers (PMs). These subjects are homozygous for two recessive loss-of-function alleles of the CYP2D6 gene (Meyer et al., 1990; Meyer and Zanger, 1997; Gonzalez et al., 1988). They are inefficient in metabolizing more than 30 clinically used drugs such as antidepressants, neuroleptics, and cardiovascular drugs. A large number of mutations of the CYP2D6 gene has been described (Sachse et al., 1997; Van der Weide and Steijns, 1999) causing the absence of the CYP2D6 protein and resulting in a PM phenotype (Skoda et al., 1988; Kagimoto et al., 1990; Broly et al., 1991). To phenotype subjects for CYP2D6, several probe tests have been proposed. Among them, debrisoquine has been largely used. Subjects who correctly metabolize debrisoquine are called extensive metabolizers (EMs). Most of the EMs present a normal (wild-type) gene. However, some mutations that modify the CYP2D6 metabolic capacity have been found. An example is mutations resulting in several copies of the CYP2D6 gene; the activity level of such subjects is more elevated as the number of copies is increased (Johansson et al., 1993; Griese et al., 1998).

The level of CYP2D6 activity has been demonstrated to be related to the risk of lung and of larynx cancers (Benhamou et al., 1996; Bouchardy et al., 1996). These studies support the importance of measuring the CYP2D6 metabolic ratio (MR) in addition with genotyping.

Our work was designed to study the influence of tobacco and alcohol consumption on CYP2D6 metabolic capacity, because such consumption frequently leads to modified microsomal enzyme activities in the liver.

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**Materials and Methods**

**Sample Population.** We phenotyped 3065 subjects attending the Center for Preventive Medicine at Vandoeuvre-lès-Nancy (East of France) for a health examination (Vincent-Viry et al., 1991). Subjects taking drugs known to inhibit CYP2D6 or taking antihypertensive drugs or pregnant women were excluded before the recruitment. Briefly, the subjects who gave their informed consent for participating in this study were aged 35 to 50 years and were apparently healthy. These subjects completed a detailed standardized questionnaire, especially aimed at lifestyle, including alcohol consumption. The estimates for alcoholic beverages were done in grams per day knowing that 1 liter of red wine = 88 g of pure alcohol; 1 liter of beer = 44 g of pure alcohol; and 1 aperitif = 15 g of pure alcohol. Smoking data were collected with the self-questionnaire used by all the French Centers for Health Check-up supported by the National Health Insurance. The responses were then checked by trained physicians during the standard clinical examination. Tobacco consumption was calculated by using the following conversion factors: 1 cigar = 1 pipe = 3 cigarettes. We found a good relationship between the data given by this self-reported questionnaire and the measurement of serum cotinine concentration, because 95% of the subjects were correctly classified using a 10 μg/liter threshold for the cut-off value between nonsmoker and smoker subjects (data not shown). In a previous study, we found a frequency of 8.2% PMs in the sample population (Vincent-Viry et al., 1991).

Among the 3065 volunteers phenotyped, 418 were selected based on their tobacco and alcohol consumption. EM and PM subjects had the same behavior for alcohol and smoking habits before they were selected for this study. Smokers were subjects who smoked at least 10 cigarettes per day, whereas nonsmokers did not smoke. Alcohol drinkers were individuals who ingested 44 g or more of pure alcohol per day, and nondrinkers claimed abstinence from any alcoholic beverages. A control group of 225 subjects were randomly selected among the nonsmokers and nondrinkers. Consequently, we constituted four groups of subjects: 1) nonsmokers and nonalcohol drinkers (control group); 2) smokers and alcohol drinkers (group 1); 3) nonalcohol drinkers and smokers (group 2); 4) nonsmokers and alcohol drinkers (group 3).
Phenotyping. Phenotyping was assessed using debrisoquine as a probe drug as described earlier (Vincent-Viry et al., 1991). Briefly, it consisted of the ingestion of 22.1 μmol (10 mg) of debrisoquine sulfate (Declinax, molecular mass 452.5 Da; Hoffmann La Roche, Basel, Switzerland), corresponding to 44.2 μmol (7.76 mg) of debrisoquine (molecular mass 175.2 Da). Urine was collected over an 8-h period following the ingestion of the dose. Phenotype was assigned by using the MR of unchanged debrisoquine/4-hydroxydebrisoquine measured in urine by an HPLC analytical procedure (Decolin et al., 1987). Usually, the MR values were transformed into decimal logarithmic values (log MR) to reduce the difference range between extensive and PMs and to improve the performance of the statistical tests. Subjects were classified as PMs (low metabolic capacity) when their MR was calculated according to the Lorentz formula (Lorentz, 1929):

\[
\text{weight (kilograms)} / \text{ideal weight (kilograms)}
\]

Overweight was calculated as the ratio of measured weight (kilograms) to ideal weight (kilograms) × 100. Ideal weight (ID) was calculated according to the Lorentz formula (Lorentz, 1929):

For men, ID (kg) = height (cm) − 100 − \[
\frac{[\text{height (cm) − 150}]}{4}
\]

For women, ID (kg) = height (cm) − 105 − \[
\frac{[\text{height (cm) − 150}]}{4}
\]

Results and Discussion

Table 1 presents the characteristics of the sample population in each of the four groups. Overweight was calculated as the ratio of measured weight (kilograms)/ideal weight (kilograms) × 100. Ideal weight (ID) was calculated according to the Lorentz formula (Lorentz, 1929):

Table 2 shows the results obtained when the effects of alcohol drinking and smoking were examined on the MRs. In PMs, increasing MR were found as following: nonsmokers/nondrinkers (control group) < smokers/drinkers (group 3) < drinkers/smokers (group 1) < nondrinkers/smokers (group 2). The differences between groups when considering log MR were not statistically significant. It seemed, however, that tobacco consumption led to an increase of the MRs corresponding to a decrease of metabolic capacity whereas alcohol consumption appeared to have no effect.

In EMs, the increasing order of MR values was the following: nondrinkers/smokers (group 2) < drinkers/smokers (group 1) < nondrinkers/nonsmokers (control group) < nonsmokers/drinkers (group 3).

Results are expressed as mean ± S.D.

* Groups 1, 2, and 3 were compared with the control group: ns, nonsignificant; * P < .05; ** P < .01.

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristics of the sample population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Overweight (%)</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
</tr>
<tr>
<td>Tobacco (cigarettes/d)</td>
</tr>
<tr>
<td>Men (%)</td>
</tr>
<tr>
<td>PM (%)</td>
</tr>
</tbody>
</table>

People were considered obese when the ratio was >120% and underweight if it was <80%. There were significant differences for mean age in groups 1 and 2, and for overweight in group 1 when results were compared with the control group. The control group comprised essentially women (>80%), and the nonalcohol drinkers/smokers (group 2) the same number of men and women, whereas the two other groups were mainly composed of men. In a previous paper (Vincent-Viry et al., 1991), age, sex, and overweight were demonstrated to have no effect on debrisoquine MR and on the excretion rates of debrisoquine and 4-hydroxydebrisoquine. These results corroborate those of Mahgoub et al. (1977) and Evans et al. (1980) who did not find any influence of age and sex when debrisoquine was used as the probe test. However, Tammenga et al. (1999) found a significant gender difference when humans were phenotyped with dextromethorphan. Because debrisoquine was used in our study, we did not adjust data for these variables, and men and women were pooled together to study the effects of alcohol and tobacco consumption. The frequency of PMs did not significantly vary among groups regardless of their smoking or drinking habits. These results corroborate those of Cholerton et al. (1996) who found no statistically different genotypic frequencies between smokers and nonsmokers.

**TABLE 2**

<p>| Alcohol drinking and smoking effects on the MR values in PMs (A) and in EMs (B) |
|----------------------------------------|----------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Control Group</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) PMs: N = 59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>27</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>0</td>
<td>79.9 ± 33.3</td>
<td>0</td>
</tr>
<tr>
<td>Tobacco (cigarettes/d)</td>
<td>0</td>
<td>27.3 ± 13.5</td>
<td>20.1 ± 6.3</td>
</tr>
<tr>
<td>MR</td>
<td>48.6 ± 34.5</td>
<td>76.7 ± 79.6</td>
<td>79.1 ± 38.8</td>
</tr>
<tr>
<td>Log MR</td>
<td>1.597 ± 0.276</td>
<td>1.721 ± 0.386 (ns)</td>
<td>1.801 ± 0.372 (ns)</td>
</tr>
<tr>
<td>(B) EMs: N = 584</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>198</td>
<td>208</td>
<td>101</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>0</td>
<td>79.5 ± 34.4</td>
<td>0</td>
</tr>
<tr>
<td>Tobacco (cigarettes/d)</td>
<td>0</td>
<td>23.8 ± 10.6</td>
<td>21.8 ± 8.1</td>
</tr>
<tr>
<td>MR</td>
<td>0.79 ± 1.10</td>
<td>0.76 ± 1.06</td>
<td>0.64 ± 0.85</td>
</tr>
<tr>
<td>Log MR</td>
<td>−0.383 ± 0.563</td>
<td>−0.551 ± 0.733*</td>
<td>−0.594 ± 0.730*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D.

* Groups 1, 2, and 3 were compared with the control group: ns, nonsignificant; * P < .05.
3). When log MR were studied, tobacco consumption alone (group 2) or associated with alcohol (group 1) produced a slight but significant decrease corresponding to an increase of metabolic capacity in comparison with the control group (P < .05). This trend was confirmed because a significant negative relationship between the number of cigarettes and log MR was observed (r = −0.203, P = .010): the larger the tobacco consumption, the larger the decrease of log MR was. On the contrary, we did not find any correlation between the amount of alcohol consumed and log MR either in PM or in EM subjects.

The results of this study indicate that in PMs there was no significant association between CYP2D6 metabolic capacity and drinking and smoking behavior, whereas slightly significant relationships were observed in EMs. Smoking has been demonstrated to induce propranolol hydroxylation (Dawson and Vestal, 1982), which cosegregates with debrisoquine. Our results confirm this tendency because mean MR in EM smokers was lower than mean MR found in the control group, but this difference was slight and there was no possibility of patient misclassification. Steiner et al. (1985) reported that smoking did not modify CYP2D6 phenotype. In contrast, when EM subjects ceased smoking (Llerena et al., 1996) mean MR diminished after 1 to 3 months of abstinence, which did not confirm the inducing power of tobacco on CYP2D6.

Debrisoquine metabolism in humans produces minor metabolites such as 5,6,7,8-hydroxydebrisoquine. It has been demonstrated (Idde and Smith, 1979) that these metabolites represented 2.5 to 13.7 and 5.3% of the dose eliminated in EMs and PMs, respectively. We cannot exclude the possibility that these minor pathways could be induced by smoking or alcohol drinking, but these lifestyle habits did not change the CYP2D6 phenotype. Therefore, it is not necessary to take drinking and smoking habits into account when phenotyping humans.

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References


