Cytochrome P450 2D6 Polymorphism in White Lebanese Population

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ABSTRACT

Objective: Genetic, environmental, physiological and pathophysiological factors contribute to the interindividual variability in drug metabolism and response. Among the different cytochromes responsible for drug disposition, cytochrome P450 2D6 (CYP2D6) is a polymorphic enzyme accountable for the clearance of 25% to 30% of medications used including cardiovascular and neuroactive drugs. Severe clinical implications can result from CYP2D6 polymorphism, hence the significance of studying the incidence of different phenotypes in the white Lebanese population.

Methods: A 30-mg dose of dextromethorphan hydrobromide was administered to 156 volunteers. Urine samples were collected 8 hours after dextromethorphan administration then stored at -80°C until analysis for dextromethorphan levels and its metabolites using a sensitive, simple high-performance liquid chromatography assay.

Results: The distribution frequency histogram of CYP2D6 metabolic ratios (MRs) showed a bimodal distribution with a gap between the metabolic ratios of 0.14 and 0.31 corresponding to log MR between -0.85 and -0.51. This gap correlates well with the antimode of MR=0.3 reported by previous studies in white populations. Sixteen subjects were classified as poor metabolizers accounting for 10.25% of the whole population sample with metabolic ratios ranging from 0.31 to 25.77; in contrast, 140 (89.75%) volunteers were found to be extensive metabolizers exhibiting MRs between 0.000439 and 0.139.

Conclusions: The findings demonstrated the presence of a high proportion of CYP2D6 poor metabolizers in the white Lebanese population and hence the sig-

nificance of potential clinical implications in these subjects.

INTRODUCTION

The interindividual variability in drug metabolism and response is extensive and multifactorial. Genetic, environmental, physiological, and pathophysiological factors contribute to this variation. Cytochrome P450 2D6 (CYP2D6) is a polymorphic enzyme responsible for the clearance of 25% to 30% of all clinically used medications and has been associated with the metabolism of over 50 of the 100 best selling drugs in the US including cardiovascular and neuroactive medications.[1-3]

The population is generally divided into two phenotype categories: poor and extensive CYP2D6 metabolizers. Poor metabolizers were found in 5% to 10% and 0% to 1% in whites and Asian populations, respectively,[3,4] and experienced adverse effects after drug ingestion because of decreased enzyme activity and hence accumulation of drug in plasma or in therapeutic failure because lower levels of the active metabolite are obtained. Conversely, the extensive metabolizer phenotype corresponds to the standard metabolic activity of an enzyme and in this category, appropriate therapeutic outcome is achieved with standard drug dosages and minimal or absent toxicity.

Commonly used prototype substrate reactions to characterize cytochrome P450 2D6 activity include bufuralol 1'-hydroxylation, debrisoquin 4-hydroxylation, sparteine oxidation, and dextromethorphan O-demethylation.[5-7] Debrisoquin, sparteine, and bufuralol are not readily available because they are marketed in a limited number of countries. Some have been withdrawn from clinical use because of their associated side effects such as the occurrence of significant cardiovascular adverse effects including orthostatic hypoten-

sion, especially in poor metabolizer subjects.

Dextromethorphan (DM) is the (+isomer of the codeine analog levorphanol; however, unlike the (-)-isomer, it has no analgesic[8] or addictive[9] properties and does not act through opioid receptors. The drug acts centrally to elevate the threshold for coughing[10] and is a constituent of many over-the-counter cough medications. Dextromethorphan exhibits half the potency of codeine and 60 mg dextromethorphan hydrobromide are equivalent to 30 mg codeine phosphate in terms of antitussive activity.[11]

The metabolism of dextromethorphan consists primarily of O-demethylation to dextrorphan, a reaction that is catalyzed by the cytochrome P450 2D6.[12-14] Dextromethorphan is also metabolized to 3-methoxymorphinan and 3-hydroxymorphinan, but these appear to be minor pathways mediated by CYP2D6 and CYP3A3/4, respectively.[12] The metabolites are then further glucoronidated. Once the metabolism of dextromethorphan was established to cosegregate with that of debrisoquin and other prototype substrates of CYP2D6, it was unanimously accepted as an attractive alternative to the other phenotyping probes[15] because of its recognized safety, its worldwide availability, and its specificity to CYP2D6 since no co-inheritance between mephenytoin and dextromethorphan metabolism was found in human subjects. In a study of CYP2D6 polymorphic metabolism in a pediatric population, dextromethorphan was found to be a suitable substrate probe for phenotyping in children since it proved to be innocuous in this population with no occurrence of adverse events after dextromethorphan administration.[16]

Due to the lack of CYP2D6 phenotyping studies in the Lebanese population, as well as the importance of the clinical implications of such polymor-

Table 1. Pharmacokinetic Parameters of Extensive and Poor Metabolizers

	Extensive metabolizers	Poor metabolizers
DM concentration	1.00 ± 0.84	15.29 ± 17.14
DXR concentration	37.12 ± 16.79	14.97 ± 11.50
Total concentration	38.12 ± 17.63	30.26 ± 28.64
MR=DM/DXR	0.0296 ± 0.0224	2.65 ± 6.314

All data shown as mean ± standard deviation.

phisms, a relatively simple, sensitive, and reproducible high-performance liquid chromatography (HPLC) assay, adapted from Bendriss et al,[17] using dextromethorphan as a probe was followed in order to estimate the incidence of CYP2D6 polymorphism in a sample of the Lebanese population.

METHODS
Chemicals and Reagents
Dextromethorphan DM, dextrorphan DOR, 3-methoxymorphinan 3MM, levallorphan (internal standard, IS) and β-glucoronidase (Type IX-A) were purchased from Sigma Chemical Corp. (St. Louis, MO). Sodium acetate, sodium carbonate, hydrochloric acid, glacial acetic acid, and diethylamine were also obtained from Sigma. HPLC-grade dichloromethane, hexane, and acetonitrile were obtained from Romil (Cambridge, England).

The mobile phase consisted of a mixture of distilled water containing 1.5% glacial acetic acid and 0.1% diethylamine, and acetonitrile (75:25, v/v). The mobile phase was degassed under vacuum using a 0.45 μm nylon membrane (Nylon 66, Supelco Inc, Bellifonte, PA) and a Branson 2210 degassing system (Danbury, CT). It was run afterwards through the columns at a flow rate of 1 mL/min at ambient temperature.

Subjects
One hundred and fifty-six volunteers were recruited for the study. All subjects were healthy as determined by interview and medical history. Exclusion criteria were: a positive history for medical illness requiring chronic medication therapy, pregnancy, or history for hypersensitivity reaction to dextromethorphan. Subjects enrolled consisted essentially of pharmacy students at the Lebanese American University (LAU) Byblos Campus, in addition to instructors and employees at LAU and friends.

All subjects were healthy as determined by interview and medical history. Subjects with positive history for a medical illness requiring chronic medication therapy were excluded from the study, in addition to pregnant women and subjects with a history of hypersensitivity reaction to dextromethorphan. After explaining to the volunteers the objectives of the study and potential side effects of the probe drug, informed consent was obtained from all the subjects. The study protocol was approved by the LAU research council. All volunteers were asked to abstain from taking any drugs 1 day before and during the study. Furthermore, they were asked to refrain from alcohol consumption for 4 hours before and during the test.

Dosing and Sample Collection
All volunteers were administered a 30-mg dose of dextromethorphan hydrobromide with a full glass of water. The

Figure 1. Typical chromatogram of an extensive metabolizer subject

probe drug was ingested under supervision to ensure compliance and the time of administration was recorded. Subjects were then asked to collect a urine sample 8 hours after dextromethorphan administration. Upon retrieval, the urine samples were stored at -80°C until analysis. The stability of these samples at 4°C was previously reported to be at least 2 months.[14]

Sample Preparation
The samples were prepared by the method previously described by Bendriss et al with minimal changes.[17] To 500 μL of urine in a 10 mL snap-cap centrifuge tube was added 500 μL of sodium acetate buffer (0.1 M, pH 5), and 200μL β-glucoronidase [Type IX-A, 1263.5 units/200 μL in sodium acetate buffer (0.1 M, pH 5)]. Each sample was gently mixed with a hand vortex mixer, all the tubes were capped and incubated in a water bath at 37°C for 18 hours to ensure complete hydrolysis of conjugated metabolites. After cooling, 500 μL of

sodium carbonate buffer (1 M, pH 9.2) and 200 μL of internal standard (5 μM levallorphan in distilled water) were added. The volume was adjusted to 2 mL with distilled water, and vigorously mixed. Afterwards, 2 mL of a dichloromethane/hexane mixture (95:5, v/v) was added and the eluent was evaporated to dryness under a nitrogen stream.

High-performance Liquid Chromatography Assay
The HPLC system included a Waters 510 pump connected to a rheodyne injector and a Shimadzu C-R6A Chromatopac (Kototo, Japan). It was equipped with a Phenyl Spherisorb Reversible 5 μm column (15 cm × μm 4.6 mm I.D.) serially connected with a Nitrile 5 μm column (25 cm × 4.6 mm I.D.) and a 474 Waters fluorescence detector (Waters, Milford, MA) set at an excitation wavelength of 200 nm.

Accurate volumes of 100 μL of each stock solution (DM, DOR, 3MM) and the internal standard (IS) were injected
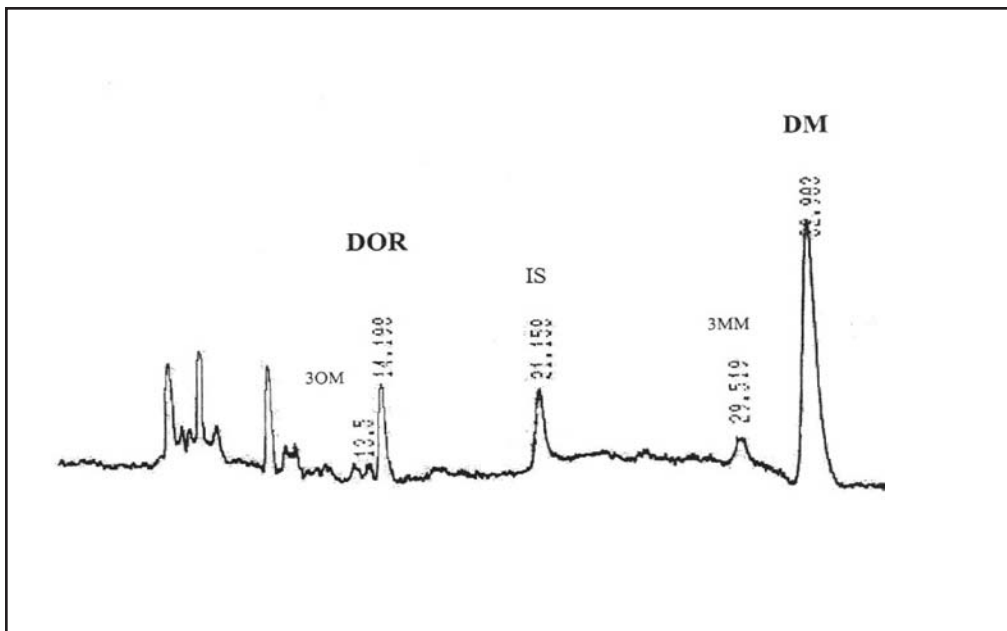
Figure 2. Typical chromatogram of a poor metabolizer subject

separately then as a mixture into the HPLC system at a flow rate of 1 mL/min obtaining retention times of approximately 14, 21.5, 29.5, and 33 minutes for DOR, IS, 3MM, and DM, respectively.

Finally, the extracts obtained from sample preparation were reconstituted with 250 μL of mobile phase and 100 μL of each final sample were injected into the HPLC system at a flow rate of 1 mL/min.

Data Analysis

The oxidative phenotype assignment for the population sample was based on the value of the subject's molar urinary ratio of dextromethorphan to dextrorphan (metabolic ratio) in relation to the antimode. The antimode for dextromethorphan urinary metabolic ratio was previously determined to be equal to 0.3.[14] Subjects with a metabolic ratio greater than or equal to the antimode were classified as poor metabolizers of the CYP2D6 enzyme system. Subjects with a metabolic ratio less than the anti-

mode were identified as being extensive metabolizers: Metabolic Ratio (MR) = Concentration of DM in urine/ Concentration of DOR in urine

Statistical Analysis

Analysis of interindividual variations in the metabolism of dextromethorphan was expressed by computing a histogram with $\log_{10}$ MR on the x-axis and the number of subjects on the y-axis.

The mean dextromethorphan and dextrorphan urinary concentrations in addition to the standard deviations (SD) were computed for extensive and poor metabolizer groups independently. Moreover, the mean metabolic ratios of dextromethorphan to dextrorphan as well as the standard deviations (SD) were calculated in the extensive metabolizer and the poor metabolizer groups separately.

RESULTS

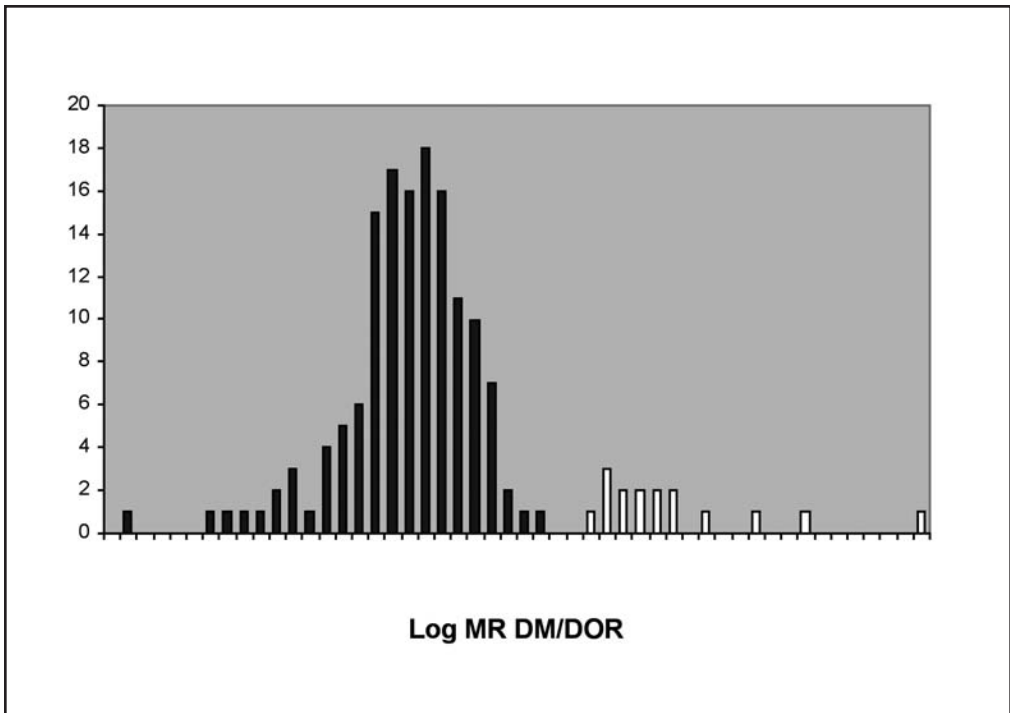One hundred and fifty-six subjects participated in the study. The population

Figure 3. Frequency distribution histogram of CYP2D6 metabolic ratios

sample included 75 male (48%) and 81 female (52%) volunteers between the ages of 18 and 45 years. Individual pharmacokinetic parameters of poor and extensive metabolizers, consisting of the urinary dextromethorphan and dextrorphan concentrations as well as the urinary metabolic ratios along with their means and standard deviations are displayed in Table 1.

Typical chromatograms from the analysis of urine samples of a poor and an extensive metabolizer subject are presented in Figures 1 and 2. Figure 3 shows the semi-logarithmic frequency distribution histogram of metabolic ratio values in all subjects.

A bimodal distribution was found with a gap between the metabolic ratios of 0.14 and 0.31 corresponding to log MR between -0.85 and -0.51. This gap correlates well with the antimode of MR = 0.3 reported by previous studies in white populations.[14] Sixteen subjects

were classified as poor metabolizers accounting for 10.25% of the whole population sample with metabolic ratios ranging from 0.31 to 25.77; in contrast, 140 (89.75%) volunteers were found to be extensive metabolizers according to the aforementioned antimode of 0.3, exhibiting metabolic ratios between 0.000439 and 0.139.

DISCUSSION

Based on the epidemiologic data obtained from this study, it can be stated that the selected sample size was large enough to be significant and represent the white Lebanese population. The sample size required to determine the proportion of the white Lebanese population with the poor metabolizer phenotype while achieving a 95% confidence interval was estimated to be 139 subjects. Therefore, the results based on our sample consisting of 156 volunteers are reliable and can be extrapolated to the

Lebanese population as a whole. The metabolic activity of cytochrome P450 2D6 was found in many studies to be bimodally distributed in Europeans and other white populations.[4] Our study sample also showed a bimodal MR frequency distribution similar to other white populations. Phenotyping investigations in whites have consistently shown that 5% to 10% of the population are poor metabolizers with high metabolic ratios for probe drugs such as debrisoquin, sparteine, and dextromethorphan; in Asians only 0% to 1% of populations display poor metabolizing behavior.[3,4] There is a great variation in prevalence of poor metabolizers in African populations ranging from 0% to 19%, presumably an indication of the ethnic heterogeneity on the continent.[18] In general, black Africans (mostly west, central and southern African people) exhibit a 0% to 2% prevalence of poor metabolizers. In the sample of white Lebanese subjects, 10.25% of the volunteers were classified as poor metabolizers, a frequency lying at the upper range of the reported values for white European populations. This might be explained by the genetic inheritance acquired from the Crusades conquest of the Lebanese territories for over 4 centuries.[19] Besides variation in the proportion of poor metabolizers in different ethnic groups and populations, the mean value of MR in extensive metabolizers also fluctuates between different populations studied. The mean value of metabolic ratios in Asian extensive metabolizers is higher than that in whites, suggesting lower average CYP2D6 activity in Asians.[20] This tendency of Asian populations to have higher metabolic ratios of probe drugs has been termed the "right shift" in the frequency distribution and is also characteristic of most African populations phenotyped. In this study population, there was an apparent tendency toward

the "right shift" of the frequency distribution histogram that was mostly noticeable when the mean metabolic ratio for the extensive metabolizer group was calculated and found to be approximately 0.03. This value is greatly elevated compared to mean MRs of 0.013 and 0.0069 determined in Swiss males and females respectively.[14] Another apparent interethnic difference is the 1000-fold variation in metabolic ratio in white extensive metabolizers with a group of people with very low metabolic ratios. Among white extensive metabolizers, this subgroup of ultrarapid metabolizers exists but this phenomenon has not been observed in Asians and most African populations. Interestingly, Ethiopians and Egyptians have a subgroup of ultrarapid metabolizers.[21,22] This extensive variability in metabolic ratios among extensive metabolizers was also detected in our population sample with metabolic ratios ranging from 0.000439 to 0.139. This might suggest the presence of a subgroup of ultrarapid metabolizers. Genotyping individuals on the lower side of the range seems to be an attractive idea in order to detect any CYP2D6 gene multiplications.

Because of the cosmopolitan origin of the Lebanese population (Phoenicians, Greeks, Romans, Bedouins from Saudi Arabia, Crusades, Turks, and French), the CYP2D6 metabolizer phenotype distribution in Lebanon exhibits variable characteristics inconsistent with any of the populations studied previously. The sample study consisted of a large group of poor metabolizers (10.25%) similar to European populations; in contrast the mean metabolic ratio among extensive metabolizers was higher and shifted toward means observed in Asian or African populations and finally an ultrarapid metabolizer subgroup was delineated but needed confirmation by genotyping. Clinical implications of CYP2D6 polymorphism might be mas-

sive in the Lebanese population, especially with the high frequency of poor metabolizers obtained. In the medical care setting, a large group of patients are treated with psychotropic medications due to the impact of 25 years of war. If the expected incidence of serious or life-threatening adverse effects is approximately 10%, then previous identification of the metabolizer status of patients prior to initiation of therapy might be a beneficial resolution. Antiarrythmics, antidepressants, and antipsychotics are widely used medications with an extremely narrow therapeutic index. Therefore, clinical implications in poor or ultrarapid metabolizers will be significant if these patients are treated with such medications resulting in either increased incidence of serious or fatal adverse effects or, conversely, in therapeutic failure.

In light of the high frequency of poor metabolizer phenotypes obtained in this sample of white Lebanese volunteers, and the possible presence of ultrarapid metabolizers among the Lebanese, further studies are warranted to determine and confirm these results. Genotyping the sample studied as a next step constitutes a justifiable decision in order to detect the common CYP2D6 mutant alleles existing in the white Lebanese population and draw conclusions as to the main origin of the genetic inheritance of CYP2D6 polymorphism and the necessity of CYP2D6 metabolizer status identification prior to initiation of therapy in selected patients.

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