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PREVALENCE OF CYP2D6 PHENOTYPES IN A YEMENI POPULATION

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ABSTRACT

Due to lack of information on existence and prevalence of CYP2D6 genetic polymorphism in Yemeni population, this study was undertaken to reveal such information in Yemeni males. 70 healthy unrelated Yemeni males participated voluntarily as subjects in this study. They were categorized into two groups including 50 Arabs in one group and 20 Blacks in the other group. Each subject swallowed a standard dose (30 mg) of dextromethorphan (DM; the probe drug of CYP2D6) and slept overnight. The metabolic ratio of the drug to its major metabolite (dextrorphan; DOP) for each subject was determined by HPLC analysis of his urine sample collected 8 hours post-dosing. CYP2D6 phenotyping depended on the antimode ranges, of log metabolic ratio, that separate the phenotypes from each other. These antimodes were determined from the probit plot of log metabolic ratio of subjects in each group. Each subject was then phenotyped as either ultrarapid , extensive (normal), intermediate and poor metabolizer according to the value of his log metabolic ratio. The prevalence of CYP2D6 phenotype in each group was then calculated. It was found that CYP2D6 poor metabolizer phenotype in the Arab and Black subjects were 4% and 10 %, respectively, while the ultrarapid metabolizer phenotype was observed only in black subjects (20 %). These findings reveal the existence of CYP2D6 genetic polymorphism in both Arab and Black Yemeni males . However, pheno/genotype correlation remains to be established.

Key words : CYP2D6, Yemeni , phenotypes , dextromethorphan, metabolic ratio.

INTRODUCTION

The activity of many drugs depends on their interaction with enzymes of the cytochrome P450 (CYP) system. CYP2D6 metabolize approximately 25% of all clinically used medications. This enzyme is the best characterized of the polymorphic CYP isozymes. CYP2D6 polymorphism can be classified into phenotypes according to one of four levels of activity: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM).[1,2] The CYP2D6 alleles table[3] , provided by the Human cytochrome P450 (CYP) alleles nomenclature committee, based on various related studies published in the literature, demonstrates each of these alleles and the associated enzyme activity.[3] There are significant interethnic differences in the distribution of CYP2D6 polymorphism. CYP2D6 deficiency was observed in about **3**-10 % of Caucasians in Europe and North America, 0-19 % of Black populations, 0-1 % of Asians and 1-9 % in Arabs [4-6].

To perform a phenotyping study, an adequate probe drug is administered prior to measurements of these compounds and/or their metabolites in body fluids.[7] Several well-validated CYP2D6 probes are available. However, in most experiments, dextromethorphan (DM) may be preferred due to its wide safety margin, availability and well-characterized metabolic profiles.[8] Phenotyping of CYP2D6 activity ,using DM , may be carried out in human urine , saliva and plasma sample. Limitations in the use of plasma/serum samples are mainly due to the low analyte concentrations; thus, sensitive analytical methods are needed for this purpose. Consequently, the DM urinary metabolic ratio (MR) or its logarithm (log MR) is widely used to quantify CYP2D6 activity in phenotyping trials. [7] To the best of our knowledge no information had been available on CYP2D6 genetic polymorphism and prevalence of CYP2D6 phenotypes in the Yemeni population. Accordingly, this study was undertaken.

MATERIALS AND METHODS

Materials

Standards of dextromethorphan HBr and metoclopramide HCl were kindly provided by Pharmacare Co. (PIC), Yemen. Other materials were purchased and included: dextrorphan tartrate , β-glucuronidase, and acetonitrile (Sigma-Aldrich, Germany); Diethyl ether and sodium hydroxide (Scharlau ,Spain); Sodium acetate, docusate sodium, Ammonium nitrate, water (FLuka Riedel , Switzerland) and Dextro-M syrup (Shiba-Pharma, Yemen, batch No.:

08C22 ; a commercially product containing 15 mg dextromethorphan HBr /5 ml).

Instrumentations

The analytical technique used was the high performance liquid chromatography (HPLC) (LC2000 Jasco- Japan) supplied with a solvent delivery pump (PU 2089), and attached to UV detector(2070) and ChromNAV software. The separation column used was a stainless steel column with 25 cm length and 4.6 mm internal diameter (C18 Analytical Capital limited, UK).

Chromatographic conditions

The chromatographic conditions was based on that recommended by the European pharmacopeia (2004) [9] to test DM related substances. The stationary phase was C18 silica gel with particle size of 5µm, while the mobile phase was a mixture of acetonitrile: Water (60:40) containing 0.65 g ammonium nitrate and 3.11 g docusate sodium per 100 ml of the mixture, with pH adjusted to 2 with glacial acetic acid. The system worked at 25 ^{o}C with a pump pressure of 2000 psi and a flow rate of 1ml / min. Detection was carried out with UV detector at 280 nm. The sample volume injected into the system was 20 µl.

Standard calibration curve

A stock solution was prepared of DM and DOP standards in drug free urine with concentration of 0.08 mg /ml and 2 mg/ml of the two substances respectively. Serial dilution of that solution was made to produce 9 dilute solutions with concentrations ranged from 0.25 – 8 µg/ml for DM and 6.25 – 200 µg/ml for DOP . 0.5 ml of the tested urine was analyzed as described later. The two calibration curves were constructed separately by plotting the analyte peak areas versus its concentrations. The regression equation of each curve was used, thereafter, to determine the analyte concentration in urine samples.

Subjects and clinical procedures

The protocol of this study was approved by the Pharmacy faculty council, Sana'a University. The study population consisted of 70 healthy unrelated Yemeni males categorized into two study groups: 50 Arabs and 20 Blacks. Written informed consent was obtained from each subject. Subjects were asked to take no medication 1 week prior to the study. Each subject swallowed a standard dose of 30 mg dextromethorphan HBr (10 ml of Dextro-M syrup) with 250 ml mineral water and slept overnight. 8 hours postdosing, the subject emptied his

bladder. Urine volume was measured and aliquot of 20 ml was stored till analysis at – 15 ^{o}C for not more than 15 days.

Analysis of urine samples

0.5 ml of the urine sample was mixed with equal amount of β-glucuronidase-buffered solution and left overnight (8hr) at water bath 37^{o}C. The enzyme-buffered solution was prepared by dissolving 20 mg β-glucuronidase(equivalent to 200000 IU of the enzyme) up to 100 ml with 0.1 M sodium acetate buffer pH 5 buffer.[10] 1 ml of the aqueous solution of the internal standard metoclopramide HCl (0.7µg/ml) was added to the sample and filtered through a Millipore filter (0.45 µm).[11] The filtrate was then titrated with 1 M sodium hydroxide to pH 11.5 [12] The analytes were extracted with 4 ml of diethyl ether for 1 minute and then centrifuged at 3000 revolution / minute for 10 minutes.[10] The supernatant was transferred to another clean glass tube and evaporated to yield a dry residue which was then reconstituted with 0.1 ml of the HPLC mobile phase. 20 µl of the obtained solution was then injected into the HPLC system.

Phenotyping

The Metabolic ratio (MR) of each subject was determined as follows: [4]

MR=unchanged DM (µg/ml)/ total DOP (µg/ml).

For each study group, a probit plot of log MR of the subjects was constructed and the antimodes (cut-off) points, that separate the phenotypes, were determined from the plot. [4,13] The plot was constructed on semilog papers with log MR at the horizontal axis and probability % at vertical axis. The probability percent was determined for each subject from the following equation [14] :

Probability % = 100 * (i – 0.5)/ T

Where i was the rank of the subject log MR (from 1-50 for Arabs and from 1-20 for blacks) and T was the total number of the group subjects. The number of subject belonging to each phenotype was determined and multiplied by 100 and divided by the total number of group subjects to yield the prevalence of the phenotype in that group.

Validation

Limit of detection (LOD) and limit of quantitation (LOQ) were determined for both DM and DOP from the calibration curves. [15] Prior to undertaking the clinical study, the absolute

recovery of the two analytes from fresh urine and also their relative recovery from urine stored at– 15 ^{o}C for 15 days were assessed by analyzing urine containing DM and DOP in concentrations ranged from 1- 8 µg/ml for DM and 10-200 µg/ml for DOP. Furthermore, the drug content % in the commercial product intended to be used by the subjects during the clinical study was also estimated by HPLC. Intraday and interday variations (expressed as coefficient of variation C.V %) and accuracy (expressed in terms of relative errors) of DM and DOP quantitation were estimated during all days of urine samples analysis by analyzing three standard urine solutions containing DM (0.4,2 and 8 µg/ml) and DOP (10, 50, 200 µg/ml).

RESULTS AND DISCUSSION

Calibration and validation

The standard calibration curves of DM and DOP in urine were linear with linearity(R^2) ≥0.99 in both curves. LOD and LOQ of DM were 0.08 µg/ml, and 0.24 µg/ml, respectively, while those of DOP were 2.08 µg/ml, and 6.31 µg/ml, respectively. The absolute recovery for DM and DOP from urine were, respectively,(95.5-98.4%) and (95 -97.34%), while the relative recovery, after storage at -15 o C for 15 days, were , respectively, (90.09 – 95.6 %) and (90 - 96.1 %). These findings confirmed the efficiency of urine sample analytical procedures and the stability of the two analyte at the relative storage conditions. The mean drug content % of the commercial product of DM was 100.4% (C.V.: 0.89%). This result assured that all subjects would swallow uniform dose during the clinical study. The relative error of the mean of interday and intraday assay for DM ranged from -12.1 to 14.3 %, while that for DOP ranged from -13.9 to 13 %. The coefficients of variation of intraday and interday assay for DM were 0.09-11.4% and 7.1 %, respectively, whereas those for DOP were 0.03-11.4 %, and 9.9-10.9 %, respectively.

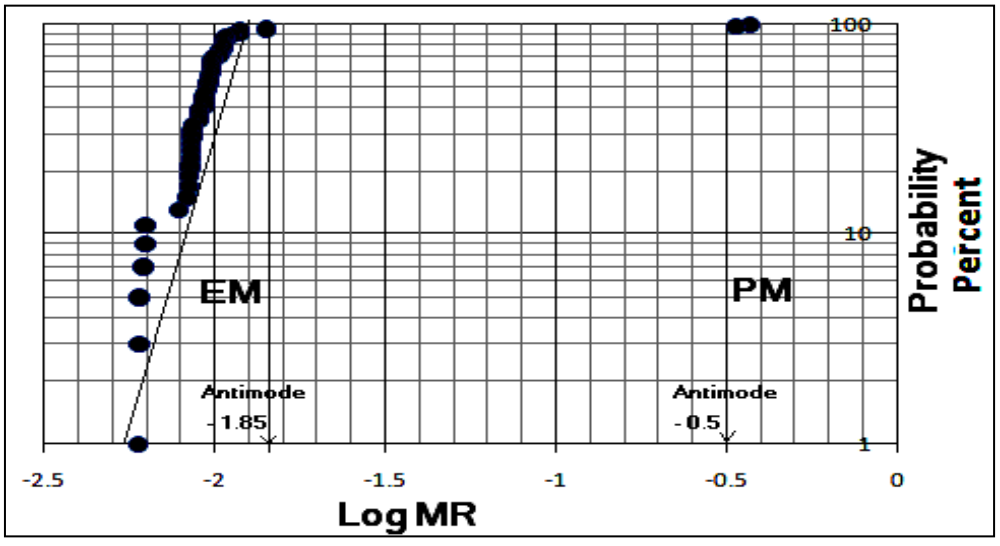
Subjects data

The mean ± SD of age, and weight of Arab subjects, (22.9 ± 2.6 years) , and (60.9 ± 7.5 Kg) , respectively, were not significantly different from those of the Black subjects , (23.7 ± 3 years) and (60.7 ± 6.1 Kg), respectively.

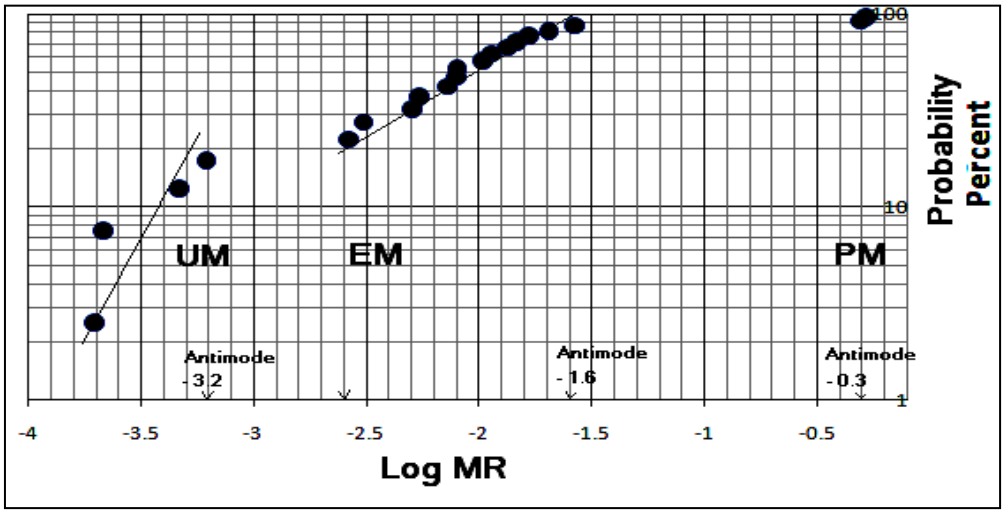
Phenotyping Data

The Probit transformation of log MR in Arab subjects (Fig. 1-a) and that in Black subjects (Fig. 1-b) illustrated bimodal and trimodal distribution profiles, respectively. Accordingly, two phenotypes (EM and PM) and three phenotypes (UM,EM and PM), were distinctively

observed in Arabs and Blacks, respectively. Although no subject in the two group had IM phenotype, this category was presented in the probit plots as a gap between EM and PM phenotypes. The antimodes (cut-off points) of PM, IM and EM phenotypes in Arabs were (log MR ≥ - 0.5), (log MR ≥ -1.85 to < - 0.5) and (log MR < -1.85) , respectively, while those in Blacks were (log MR ≥ -0.3). (log MR ≥ -1.6 to < - 0.3) and (log MR > -3.2 to < -1.6), respectively. The antimode for UM phenotype in blacks was (log MR ≤ 3.2). Within each group, the mean log MR was significantly different among the phenotypes ($P < 0.001$). This finding could be attributed to the different activity level of CYP2D6 enzyme in each phenotype and consequently to the difference in *CYP2D6* alleles that associates with each phenotype. The prevalence of CYP2D6 abnormal phenotypes in Arabs was 4 % (95 % C.l. of 2.4-5.7 %) for PM phenotype, while in Blacks it was was10 % (95 % C.l. of 8.3- 11.7) and 20 % (95 % C.I. of 18.4- 21.7 %) for PM and UM phenotypes, respectively. Furthermore, prevalence of PM phenotype was significantly different between Arabs and Blacks ($P < 0.001$). Comparing with other related populations, PM phenotype prevalence in Yemeni Arabs, was within the range reported in other Arabian populations (1-9 %). Similarly, the PM phenotype prevalence in Yemeni Blacks was within the range observed in Black populations; (0 -19 %) and the prevalence of the UM phenotype in this group was close to that of another Black population; Ethiopian (29 %) who colonized certain parts of Yemen in the ancient times.



(a)



(b)

Fig.1.Probit plot representation of logarithm metabolic ratio in 50 Arab (a) and 20 Black (b) Yemeni males.

CONCLUSION

Based on results obtained in this study it could be concluded that a considerable prevalence (> 1 %) exists for the CYP2D6 PM phenotype in both Arab and Black Yemeni males and also for the UM phenotype in the Black group. Those findings also reveals the existence of *CYP2D6* genetic polymorphism among these two groups of Yemeni population. However, a larger number of Black subjects is still required to obtain more accurate results. Furthermore, the identification of *CYP2D6* alleles that associate with each phenotype in Yemeni males remains to be established.

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