ABSTRACT

Objective: The objective of this study was to determine CYP2D6 phenotype in a Javanese and Sundanese healthy subject in Indonesia.

Methods: Ninety unrelated healthy Indonesian subjects from Java and Sunda were studied. Metoprolol was used as phenotyping substrate. A 100 mg oral tablet of metoprolol was administered to all the subjects. Urinary metoprolol and α-hydroxymetoprolol were determined to calculate metoprolol metabolic ratio (MR). Determination of metoprolol and α-hydroxymetoprolol was carried out by high performance liquid chromatography method.

Results: Metoprolol MR varied widely (from 0.08 to 72.75). One subject (1.11%) in the study was classified as poor metabolizer (PM), one subject (1.11%) as ultrarapid metabolizer, and the remaining 88 subjects (97.78%) were classified as extensive metabolizers.

Conclusion: The frequencies of PM for the CYP2D6 phenotype (1.11%) in the Javanese and Sundanese population are in concordance with most results of oxidation metabolizers in other Asian populations.

Keywords: CYP2D6, Javanese and Sundanese, Indonesia, Metoprolol, Phenotype.
Poor metabolizer

Phenotype Study of CYP2D6 using metoprolol

The subjects were not allowed to take any medication or consume alcoholic beverages and drinks/foods containing caffeine for 1 week before administration. The subjects received a single dose of metoprolol tartrate tablets (100 mg) orally with 200 mL of water after overnight fasting before administration of the drug. Urine was collected for 8 h from the time taking metoprolol tablets. Urine samples were stored at −40°C before analysis.

Liquid–liquid extraction was performed using dichloromethane solvent after the addition of sodium hydroxide into the urine. The reversed-phase HPLC method [9] was slightly modified and used for the determination of α-hydroxymetoprolol and metoprolol in urine samples. Separation was carried out in a Purospher® STAR RP-18e LiChroCart® (250×4.6 mm, 5 µm) column with a mobile phase consisting of a mixture of 0.1M KH₂PO₄ solution (pH was adjusted into 3 with orthophosphoric acid)-acetonitrile-methanol (70:15:15, v/v/v) with a flow rate of 1 mL/min and injection volume of 20 µL. The Ultraviolet detector was set at λ 234 nm. Quantification of α-hydroxymetoprolol and metoprolol was performed using internal standard caffeine.

Urinary metoprolol and α-hydroxymetoprolol were determined to calculate metoprolol MR. Based on the MR (or LogMR) value, the phenotype of the test subjects was classified as a PM with MR >12.6 (LogMR >1.1), IM and EM with 0.1<MR<12.6 (−1.0<LogMR<1.1), and UM with MR<0.1 (LogMR<−1.0).

Data analysis

CYP2D6 enzyme activity was determined by determining the ratio of the molar concentration of metoprolol/α-hydroxymetoprolol in the urine. The ratio of metoprolol and α-hydroxymetoprolol in the urine (MR) was calculated by the following equation:

\[
\text{MR} = \frac{[\text{metoprolol}]}{[\alpha\text{-hydroxymetoprolol}]} 
\]

The phenotype was determined by the MR. Subjects with MR >12.6 were classified as PM, subjects with MR 0.1<12.6 were classified as IM and EM, and subjects with MR<0.1 were classified as UM [10].

Table 1: Characteristics of 90 subjects who participated in this study

| Subject characteristics | Number/Value | Percentage (%) |
|-------------------------|--------------|----------------|
| Age (years)             | 19.58±1.47   |                |
| Gender                  |              |                |
| Female                  | 56           | 62.22          |
| Male                    | 34           | 37.78          |
| Tribe                   |              |                |
| Javanese                | 72           | 80             |
| Sundanese               | 18           | 20             |
| Weight (kg)             |              |                |
| Man                     | 57.97±7.64   |                |
| Female                  | 49.48±7.63   |                |
| Height (cm)             |              |                |
| Man                     | 167.21±5.83  |                |
| Female                  | 156.18±5.50  |                |

Table 2: CYP2D6 phenotype data

| Metabolic ratio metoprolol/α-hydroxymetoprolol | Phenotype            | Number of subjects | Percentage (%) |
|-----------------------------------------------|----------------------|--------------------|----------------|
| MR<0.1                                        | Ultra rapid metabolizer | 1                  | 1.11           |
| 0.1<MR<12.6                                   | Extensive metabolizer  | 88                 | 97.78          |
| MR>12.6                                       | Poor metabolizer      | 1                  | 1.11           |
| Total                                         |                       | 90                 | 100            |

MR: Metabolic ratio
The frequencies of PM for the CYP2D6 phenotype (1.11%) in this study are in concordance with most results of oxidation metabolizers in other Asian populations. The prevalence of PM in the Japanese population range from 0.3 to 0.5% in the Malaysian subjects amounted to 3.9% [15], in the Iran subject of at 2.5%. CYP2D6 phenotyping study has been reported using metoprolol substrate or other substrates such as dextromethorphan, debrisoquine, and sparteine with consistent results that the presence of PM is low in Asian populations such as Japan, China, Korea, Malaysia, and Iran. Using debrisoquine as a substrate, PM on Malaysia’s population is 3.9% [15]. Using metoprolol as the substrate, the frequency of PM population in Korea, Japan, and China was 0.5%, 0.7%, and 0%, respectively [16]. The existence of PMs individual in Indonesian subjects (1.11%) is lower than PMs in European and American countries (Gaussian individuals) such as the population of Britain (8.4%) [17], Czech (8.7%), and German (8.7%) [18]. Uruguay (7.3%) [19], and Mexico (10%) [20] and African countries such as Zimbabwe (5%) [21] and Nigeria (3.5%) [22].

The probit plot (Fig. 2) shows a multimodal distribution profile. The plot also clarifies the existence of three phenotypes of the hydroxylation capacity of metoprolol. Fig 1 shows that the mode value of the LogMR is −0.1. The mode values in this Indonesian subjects are shifted to the right when compared to Japanese subjects (mode: 0.7) [22]. This shows that the hydroxylation capacity of Indonesian subjects is stronger than Japanese subjects but is weaker than Chinese subjects.

This polymorphism of CYP2D6 among Javanese had similarity with the polymorphism of CYP2A6 among the same genetic ethnic of Javanese [23]. Genetically, CYP2A6*4 was found higher compared to the occurrence of CYP2A6*1 among smoking and non-smoking subject. The distribution of these allele frequencies was different among those two types of subjects.

The MR data in the CYP2D6 phenotyping study using dextromethorphan as the substrate in the Chinese population (120 people) showed a bimodal distribution and about 36% of subjects were classified as IM [24]. However, the MR value in this phenotyping study involving 90 subjects has not been able to distinguish the extensive and IM.

CONCLUSION

Indonesian healthy subjects have different capacities to metabolize metoprolol through CYP2D6. The frequency of PM of CYP2D6 phenotype (1.11%) in the Javanese and Sundanese is in conformity and comparable to other Asian populations. This study has not been able to identify IM. It needs the additional study using more subjects. Further study was needed to determine the CYP2D6 genotype in Javanese and Sundanese populations. The results of this study might be helpful in patient dose adjusting to achieve the therapeutic goals.

ACKNOWLEDGMENTS

The authors thank the Directorate General of Higher Education Ministry of Research and Higher Education of the Republic of Indonesia and all the participants who have been involved in this study.

CONFLICTS OF INTEREST

All authors have none to declare.

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