POLYMORPHIC OXIDATION OF SPARTEINE AND DEBRISOQUINE: RELATED PHARMACOGENETIC ENTITIES

S. ASANTE-POKU¹, D. OFORI-ADJEI², ANDREA , U. GRIESE³, M. EICHELBAUM³, T.K. AND W. KUDZI²

¹Department of Biochemistry, ²The Centre for Tropical Clinical Pharmacology & Therapeutics, University of Ghana Medical School, Accra, Ghana, ³Dr. Margarete Fischer-Bosch-Institut Fur Klinische Pharmakologie, Stuttgart, Germany.

SUMMARY
A randomly selected population of 234 Ghanaian volunteers were phenotyped by administering single oral doses of debrisoquine and sparteine in a cross over study for their ability to 4-hydroxylate debrisoquine and N-oxidize sparteine. The close correlation between urinary metabolic ratios of the two drugs demonstrates that the polymorphic 4-hydroxylation of debrisoquine and N-oxidation of sparteine are related pharmacogenetic entities in Ghanaians. Leucocyte DNA from a subset of subjects were analysed for a number of mutations in the CYP2D6 gene. Genotype was performed using both restriction fragment length polymorphism (RFLP) assay with restriction enzyme XbaI, together with allele-specific amplification polymerase chain reaction assay. Two subjects phenotyped with both sparteine and debrisoquine as poor metabolizers (PMs) were observed to have XbaI-RFLP patterns 44/11.5 kb and showed the 29B mutation.

Many clinically useful drugs are frequently metabolized to therapeutically inactive metabolites by one or more cytochrome P450 forms, the principal conduits for oxidation and elimination of foreign compounds. Inter-individual variability in levels of expression of the P450 enzyme could determine the relative effectiveness of metabolism and efficacy of a particular drug.¹²

Differences in individual handling of drugs could be the result of exposure to environmental or dietary inducers/inhibitors or due to genetic variation in expression. Genetic variability that stably exists at frequencies higher than 1% in a population is commonly referred to as a polymorphism. The debrisoquine/sparteine type polymorphism, a clinically important inherited variation of drug oxidation, probably represents the best studied genetically determined variation in drug metabolism in humans³⁴. It is caused by a deficiency of hepatic cytochrome P450 enzyme P450IID6. Two distinct phenotypes can be defined, the extensive metabolizer (EM) and the poor metabolizer (PM)⁵⁶.

The metabolism of over 25 drugs, some of wide therapeutic use, such as beta-adrenergic blocking agents, anti depressants, antiarrhythmics, neuroleptics and opioids is affected by this polymorphism⁷. The poor metabolizer phenotype inherited as an autosomal recessive trait comprises 5 to 10% of individuals in most Caucasian populations studied⁸. Pronounced inter-ethnic differences in the frequency of the PM phenotype are known to exist. There is a lower reported prevalence of debrisoquine type poor metabolizers among native Chinese⁹, Japanese¹⁰, American blacks¹¹, Saudis¹² and Nigerians.

In a previous phenotyping study of the Ghanaian population where sparteine and/or debrisoquine were used as probe drugs, the frequency of PMs of debrisoquine was reported to be 6% (n= 80)¹⁵. How-
ever, no PMs of sparteine were observed among 154 Ghanaian subjects studied and the close correlation between sparteine and debrisoquine metabolism that exists among Caucasians could not be demonstrated among Ghanaians. We report of consecrating control of debrisoquine and sparteine oxidation in Ghanaian population.

METHODS

Subjects: 234 healthy Ghanaians, 129 men (age 18 to 48 years) and 105 women (age 19 to 48 years) were studied. They were recruited mainly among hospital staff, medical staff, medical students and nurses. The study was approved by the ethical committee of the Ghana Medical School.

Phenotyping: The subjects were phenotyped with both sparteine and debrisoquine with an interval of at least 2 weeks between the two tests. Sparteine was given as Depasan tablets and a single oral dose of 100mg (sparteine sulfate), following an overnight fast. The bladder was emptied before dosing and thereafter all urine passed up to 12 hours was collected. Urine volume was recorded and an aliquot stored at -20°C to await analysis.

Debrisoquine was given as Declinax tablets in a single dose of 10mg by mouth and urine collected for 8 hours.

DNA restriction fragment length polymorphism (RFLP) analysis

In a subgroup of subjects 20ml of blood was collected in ACD tubes and deoxyribonucleic acid (DNA) was isolated from peripheral leukocytes by phenol and chloroform extractions. DNA samples (5-10μg) were digested to completion with Xbal restriction endonuclease and subjected to Southern hybridization with a CYP2D6cDNA clone as previously described.

Allele-specific amplification by polymerase chain reaction (AS/PCR)

DNA samples for AS/PCR were prepared from peripheral leukocytes according to the previously described method of Heim and Meyer. However, except that all PCR reactions were carried out in a total volume of 25μl and in the presence of 0.9mM magnesium chloride. 15μl of each reaction product were analysed on a 1.2% agarose gel.

RESULTS AND DISCUSSION

In 234 healthy Ghanaian subjects the metabolic ratios (MRs) between sparteine and its 2- and 5-dehydro metabolites in urine after a single oral dose were bimodally distributed with an antimode of 1.3 (Figure 1). The antimode observed in our study coincides with the antimode reported in Caucasian populations. Our finding contradicts a recent observation that no poor metabolizers could be observed in Ghanaians (n=154).

Five of the 234 subjects (2.1%) with metabolic ratio MR>20 (Table I) were classified as poor metabolizers in accordance with our antimode and that established for Caucasian populations. The reproducibility of the sparteine MR was assessed by re-challenging 8 subjects (2PMs and 6EMs). The results displayed as a scattergram (Figure 2) show a...
was there a discrepancy in the phenotype assigned for an individual between the two tests.

The five subjects phenotyped as PMs (by thin layer chromatogram and Gas liquid chromatography analysis of 12-hour urine samples) with respect to sparteine N-oxidation were also found to be non-metabolizers of debrisoquine (HPLC analysis of 8-hour urine) Table I.

Two subjects phenotyped as poor metabolizers of sparteine (MR 340.4 and 67.1) seem to be relatively rapid debrisoquine metabolizers (MR 1.1 and 6.0). The two subjects did not consent to be retested in order to confirm the assigned phenotypes.

One subject phenotyped as extensive metabolizer (MR = 1.9) with sparteine but PM (MR = 13.3) with debrisoquine refused to be re-challenged with the test drugs.

When these three subjects are excluded from consideration the present study shows that in the Ghanaian population there is a close relationship between an individual’s ability to 4-hydroxylate debrisoquine and to N-oxidize sparteine.

A sub-group of 22 subjects comprising 2PMs and 20EMS (phenotyped with both sparteine and debrisoquine) had 20ml of blood collected for extraction of genomic DNA. The restriction fragment length polymorphism patterns obtained from the genomic DNA (5-10μg) isolated from peripheral blood and digested with XbaI restriction endonuclease are summarized in Table II.

In our study the 29kb, 44kb and 11.5kb fragments could be detected. The combinations observed were 29/29kb (n=13), 29/44kb (n = 7), 29/11.5kb (n = 1),
Table II: Xbal DNA Restriction Fragment Length Polymorphic Patterns and PCR Analysis of 22 Unrelated Ghana Phenotyped Individuals

<table>
<thead>
<tr>
<th>Subject Code</th>
<th>Metabolic Ratio</th>
<th>Xbal</th>
<th>PCR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SPT</td>
<td>DQ</td>
<td></td>
</tr>
<tr>
<td>GH 1</td>
<td>0.56</td>
<td>0.49</td>
<td>29/29</td>
</tr>
<tr>
<td>GH 4</td>
<td>85.30</td>
<td>40.10</td>
<td>44/11.5</td>
</tr>
<tr>
<td>GH 6</td>
<td>0.17</td>
<td>0.11</td>
<td>44/28</td>
</tr>
<tr>
<td>GH 7</td>
<td>1.54</td>
<td>0.81</td>
<td>29/29/7</td>
</tr>
<tr>
<td>GH 9</td>
<td>0.27</td>
<td>0.48</td>
<td>29/29/7</td>
</tr>
<tr>
<td>GH 10</td>
<td>340.45</td>
<td>1.10</td>
<td>44/28</td>
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<td>1.96</td>
<td>29/29</td>
</tr>
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</tr>
<tr>
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<td>29/29/7</td>
</tr>
<tr>
<td>GH 22</td>
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<td>2.00</td>
<td>44/28/7</td>
</tr>
<tr>
<td>GH 25</td>
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<td>0.30</td>
<td>29/29/7</td>
</tr>
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<td>GH 26</td>
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<td>29/29/7</td>
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<td>1.30</td>
<td>29/11.5/7</td>
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<td>44/28/9</td>
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<td>29/29/7</td>
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<td>2.64</td>
<td>29/29/7</td>
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<td>29/29/7</td>
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<tr>
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</tr>
<tr>
<td>GH 89</td>
<td>1.68</td>
<td>1.52</td>
<td>44/28</td>
</tr>
</tbody>
</table>

restriction fragments of 29kb, 44kb, 11.5kb and 16+9kb have been described (20, 21, 22). The combinations observed and reported are 29/29, 29/44, 29/16+9, 29/11.5, 44/44, 44/16+9, 44/11.5, 11.5/11.5, 29/29+9, 29+9/11.5 and 29/16+9+11.5.

The 44/11.5kb combination has been shown to be predictive of the PM genotype in Caucasians and this was also found to be predictive of PMs in the Ghanaian population (Table II). Our study did not show any homozygous for the 44kb fragment.

DNA sample prepared from peripheral leukocytes were used for amplification by polymerase chain reaction (PCR) and each sample was tested for mutations involving the CYP2D6 gene.

All individuals (n = 13) homozygous for the Xbal-RFLP 29kb were 29wt/29wt, 3 individuals with Xbal 44/29kb were also 29wt/29wt whereas 4 were 29b/29wt. There were no 29b-mutation in the homzygous Xbal 29/29kb individuals but in those with 44/11.5kb (Table II), it seems that in the Ghanaian population the 29b-mutation derives from the Xbal 44kb allele. The two individuals phenotyped PMs produced Xbal-RFLP 44/11.5kb and the 29b mutation in the PCR analysis. The 29a-mutation was not observed in any individual.

and 44/11.5kb (n = 2). In addition a fragment, 7kb, not previously reported was observed. The occurrence of this fragment seems not to be linked to the phenotype.

In Caucasian populations, four polymorphic Xbal

No inconsistency between the genotype and the phenotype for autosomal recessive inheritance was observed both for the RFLPs and the PCR tests. The molecular biology of the CYP2D6 gene in black African subjects versus white subjects is required and such a study is currently underway.
REFERENCES


17. Gaedigk A., Blum M., Gaedigk R., Eichelbaum M., Meyer U.A. Deletion of the entire cytochrome P450CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of


