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Stereoselective 4-hydroxylation of debrisoquine in Nigerians

The oxidative metabolism of debrisoquine exhibits genetic polymorphism in man such that about 9% of Caucasians have an impaired ability to eliminate the drug by 4-hydroxylation, the major route of metabolism [1, 2]. Using antimodes calculated from bimodal distributions of the $\log_{10}$ debrisoquine/4-hydroxydebrisoquine (D/HD) ratio established in these studies, the incidence of the poor metaboliser (PM) phenotype appears to vary between ethnic groups [3].

As an extension to studies that have shown the beta-adrenoceptor antagonist, metoprolol, to be under similar polymorphic control in the white British population [4, 5], we have recently investigated the role of genetic polymorphism in the metabolism of both metoprolol and debrisoquine in a Nigerian population [6]. Using the Caucasian antimode only one subject, an Asian Indian, was classified as a PM of both D and metoprolol. There was no evidence of bimodality in the frequency distributions of data for black Africans. One explanation for these observations is that the drug/metabolite ratio may not be a sufficiently sensitive experimental index.

4-Hydroxylation of D introduces a chiral centre into the molecule, giving rise to two possible enantiomers. To determine whether HD formation is stereoselective and whether differences in any stereoselectivity exist between phenotypes, the urinary excretion of (*R*)- and (*S*)-HD has recently been studied in panels of PM and extensive metaboliser (EM) subjects [7, 8]. In EMs 4-hydroxylation was found to be almost stereospecific with 98% or more of the HD product being the (*S*)-enantiomer. In PMs metabolism was considerably less stereoselective as 10–36% of HD was in the form of the (*R*)-enantiomer.

The aims of the present study were to characterise the stereoselectivity of HD formation in Nigerians and to determine whether evidence for polymorphic control of this route could be obtained using HD enantiomer composition as an index of enzyme activity.

Methods

One hundred and fourteen unrelated subjects were studied in the Department of Medicine, University College, Ibadan, Nigeria. All were black Africans except one, whose parents were Indian. Detailed information on this population is available elsewhere [6]. After emptying the bladder each volunteer ingested a tablet of debrisoquine hemisulphate (equivalent to 10 mg base). All urine was collected for the next 8 hr and a 20 ml aliquot was stored at −20°. D and total [(*S*)- + (*R*)-] HD were measured by GLC [9]. The enantiomeric composition of HD was assayed using the HPLC method of Meese *et al.* [8]. Briefly, the latter method involved derivatisation with acetylacetone for 72 hr at room temperature followed by separation of the resulting pyrimidines on a "Pirkle" chiral column (25 cm by 4.6 mm i.d. containing 5 μM covalently bound (*R*)-*N*-3,5-dinitrobenzoylphenylglycine). Detection was by fluorescence (λ_{ex} 265 nm, λ_{em} 380 nm) and enantiomer composition was calculated from integrated peak areas. The minimum detectable amount of each enantiomer was 25 pmoles per sample.

Results

In all of the black African subjects there was a high degree of stereoselectivity in the 4-hydroxylation of debrisoquine which favoured the (*S*)-enantiomer. The presence of (*R*)-HD was not detected in 92 of these subjects (Fig. 1) but, based on the limit of detection of the assay, values for the upper limit of (*R*)-HD concentration can be estimated. Thus, the sample possessing the lowest concentration of (*S*)-HD (0.72 nmoles/ml) could theoretically have contained up to 1.7% of (*R*)-HD, with the other samples containing proportionately less (*R*)-HD. In the remaining black African subjects (*R*)-HD constituted between 0.02% and 1.65% of total HD in urine and there was a direct correlation between these values and the D/HD ratio (r_s = 0.87, $P < 0.001$).

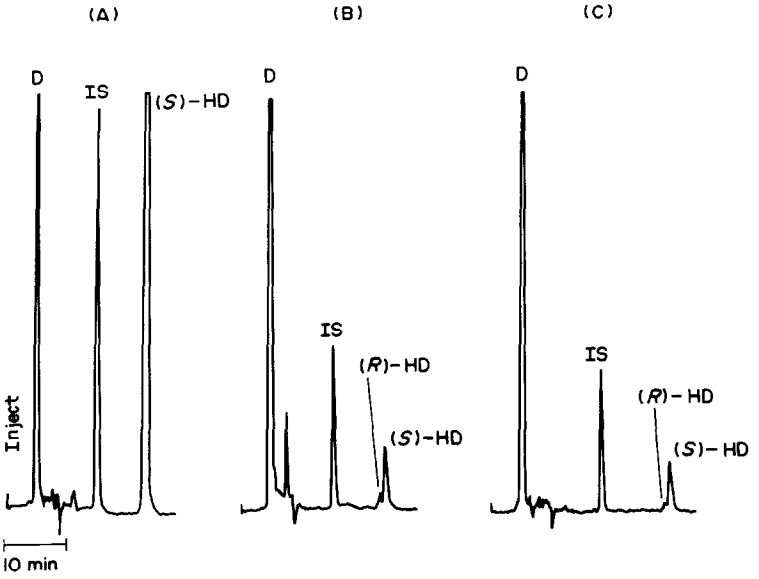

Fig. 1. Chiral column chromatograms of extracts of urine from subjects receiving 10 mg oral doses of debrisoquine. (A) A representative black Nigerian (concentration of (*R*)-HD = not detectable, (*S*)-HD = 4.53 nmoles/ml), (B) an Asian Indian living in Nigeria (concentration of (*R*)-HD = 0.28 nmoles/ml, (*S*)-HD = 1.17 nmoles/ml), and (C) a white British PM (concentration of (*R*)-HD = 0.048 nmoles/ml, (*S*)-HD = 0.351 nmoles/ml). I.S. = Internal standard, 4-hydroxy-4-phenylpiperidine-1-carboxamidinium hemisulphate hemihydrate.

In the urine sample from the Indian subject 19% of total HD was in the form of the (*R*)-enantiomer (Fig. 1). For comparison a chromatogram from the analysis of a Caucasian PM urine sample is also shown (Fig. 1).

Discussion

All of the black Nigerians in this study were similar to Caucasian EMs [7, 8] in their ability to 4-hydroxylate D with a high degree of stereoselectivity. In the majority of subjects only (*S*)-HD was detectable, although it is possible that small amounts of the (*R*)-enantiomer were formed. Nevertheless, in the samples where (*R*)-HD could be measured, stereoselectivity remained high. A greater ability to eliminate D by 4-hydroxylation is associated with increased stereoselectivity. The considerably lower stereoselectivity observed in the Indian subject was characteristic of that observed in Caucasian PMs [7, 8] and this, together with the high D/HD ratio of 60, is compatible with this subject also being of the PM phenotype. The enantiomer data from the present study confirm previous findings using the D/HD ratio [6] in providing no evidence that D metabolism is under polymorphic control in Nigerians. The possible reasons for this have been discussed elsewhere [6].

In summary, a high degree of stereoselectivity was observed for the 4-hydroxylation of D in a black Nigerian population, the (*S*)-metabolite enantiomer being the predominant product. This characteristic is shared with Caucasians of the EM phenotype for debrisoquine.

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DNA recombinant and monoclonal antibody directed methods for determining cytochrome P-450 specificity

We have introduced two new approaches to the analysis of individual cytochrome P-450 function. These new technologies are highly applicable to understanding cytochrome P-450 specificity in respect to both substrate and product formation. These methods engage modern immunochemical technologies and DNA recombinant methods and although we have used them largely to study the product specificity of individual P-450s, they can easily be applied to the study of stereospecific aspects of cytochrome P-450 function and therefore are germane and appropriate to the subject of chirality and cytochrome P-450.

The cytochromes P-450 are a large family of hemoproteins capable of metabolizing xenobiotics, such as drugs, carcinogens and environmental pollutants [1]. It does not exaggerate the situation to suggest that the vast majority of drugs of therapeutic use are substrates for the cytochrome P-450 class of enzymes. In addition, the P-450s metabolize endobiotics, such as steroids, fatty acids and prostaglandins. This group of cytochrome-P-450 enzymes carries out beneficial metabolic activities by detoxification of xenobiotics as well as harmful metabolic conversion of xenobiotics to toxic, mutagenic and carcinogenic forms [1]. There is a multiplicity of cytochrome P-450 forms numbering at least 20–30 and perhaps many more.

The multiplicity and the common properties of the cytochrome P-450 render difficult the separation of different forms of cytochrome P-450, especially the minor forms. This has prevented a full understanding of the role of individual forms of cytochromes P-450 in metabolism, detoxification, and activation of xenobiotic and endobiotic substrates. The complexities and uncertainties of purification have also prevented the defining of individual cyto-