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Note

Liquid chromatographic assay for debrisoquine and 4-hydroxydebrisoquine in urine

BARBARA E. WESTWOOD, PETER J. HARMAN and M. LAURIE MASHFORD*

University of Melbourne Department of Medicine, St Vincent's Hospital, Fitzroy, Victoria 3065 (Australia)

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Debrisoquine, which was first introduced as an antihypertensive agent, has a number of metabolites in man [1]. By far the most important of these is 4-hydroxydebrisoquine, which ranges in amount in urine from 5.4 to 33.4% of the total dose administered [1]. Individuals vary widely in their ability to metabolise debrisoquine and this appears to be genetically determined [2]. Studies of diverse populations have shown that up to 30% of some populations may have defective oxidative ability for this drug [3]. Recently it has been shown that several other drugs which undergo oxidation exhibit similar genetic polymorphism which can be diagnosed by using debrisoquine as a test or marker. These include sparteine, perhexilene and phenformin. As the adverse effects of these drugs are correlated with their blood levels, patients who are slow metabolizers may be more prone to such toxic effects.

The technique for determination of metabolic status involves measurement of the ratio of debrisoquine to 4-hydroxydebrisoquine concentrations in urine after a single 10-mg tablet of debrisoquine. For this purpose, gas—liquid chromatographic (GLC) methods have been developed for the simultaneous determination of debrisoquine and 4-hydroxydebrisoquine in human urine [4, 5]. These are, however, based on cumbersome derivatisation and extraction techniques and are hence complicated and time-consuming if large populations are to be rapidly screened.

We have developed a simple method for the simultaneous determination of debrisoquine and 4-hydroxydebrisoquine in urine which can be used for rapid estimation of oxidation phenotype in population studies.

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EXPERIMENTAL

Materials

Debrisoquine and 4-hydroxydebrisoquine were kindly supplied by Roche (Sydney, Australia). Potassium dihydrogen diphosphate, acetonitrile and benzyl alcohol were analytical grade, obtained from May and Baker (Melbourne, Australia). Water used throughout was redistilled from glass.

Apparatus

The liquid chromatograph consisted of an Altex 112 pump connected to a Perkin-Elmer Model ISS-100 autoinjector. The detector was a Perkin-Elmer LC75 variable-wavelength ultraviolet detector.

Chromatographic details

The mobile phase consisted of 0.008 *M* potassium dihydrogen phosphate buffer (pH 5.0)—acetonitrile (55:45). This was pumped at a flow-rate of 2 ml/min through a Brownlee RP-8 (Santa Clara, CA, U.S.A.) 5-μm, 25 cm × 4.5 mm I.D. column. Detection was by UV spectrophotometry at 208 nm using a setting of 0.08 a.u.f.s.

Sample preparation

Drug-free filtered human urine was spiked with appropriate amounts of debrisoquine and 4-hydroxydebrisoquine using a 100 μg/ml aqueous stock solution to give final concentrations of 1, 2, 5, 10 and 20 μg/ml. These standards were treated by filtration in the same manner as patient samples. Aliquots of 1 ml of urine were passed through Millipore (Milford, MA, U.S.A.) GSWP 02500 (0.22 μm) filters and the resulting filtrate was collected in autoinjector vials. Aliquots of 50 μl were injected onto the column and the peak heights for debrisoquine and 4-hydroxydebrisoquine were measured. Calculation of the concentration of debrisoquine and 4-hydroxydebrisoquine was by external standardisation.

Clinical studies

Healthy volunteers or patients took a single 10-mg tablet of debrisoquine before bed. The subjects voided urine before ingestion of the debrisoquine, and both this sample and all urine for the subsequent 8 h was collected into urine bottles containing 200 μl benzyl alcohol as a preservative. Aliquots were either frozen at −20°C or assayed immediately. The metabolic ratio (MR) was calculated as follows:

$$\text{MR} = \frac{\text{Percentage of dose excreted as debrisoquine}}{\text{Percentage of dose excreted as 4-hydroxydebrisoquine}} \text{ in 0–8 h urine.}$$

RESULTS AND DISCUSSION

Using the procedures discussed above, debrisoquine and 4-hydroxydebrisoquine appear in chromatograms as well shaped peaks with retention times of 5 and 7 min, respectively. Typical chromatograms of a spiked urine sample,

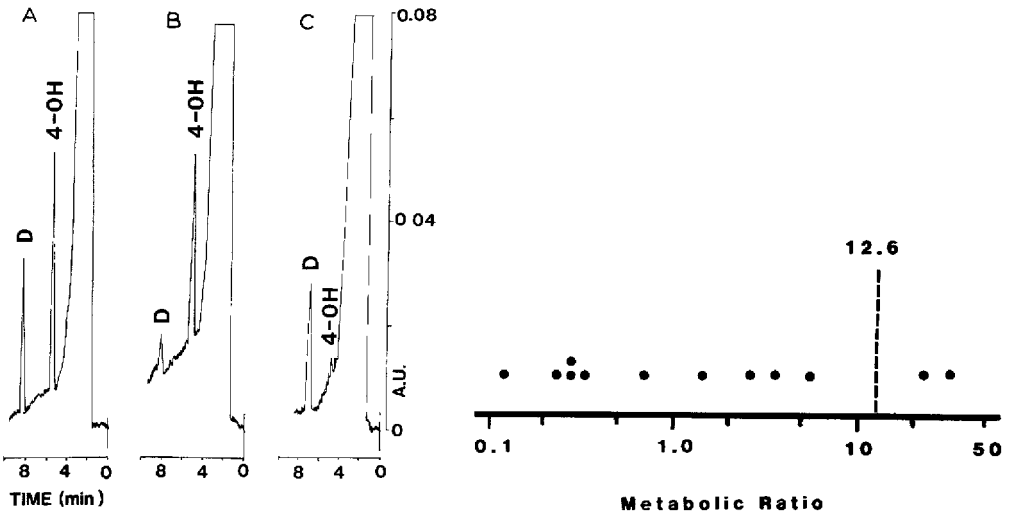

Fig. 1. Typical chromatograms of (A) spiked (5 μg/ml) urine standards, (B) a sample from an extensive metaboliser and (C) a sample from a poor metaboliser. Chromatographic conditions were as described in the text. Concentrations of the samples were 1.21 μg/ml debrisoquine (D) and 3.87 μg/ml 4-hydroxydebrisoquine (4-OH) in the extensive metaboliser, and 4.31 μg/ml D and 0.21 μg/ml 4-OH in the poor metaboliser.

Fig. 2. Metabolic ratios obtained from twelve subjects using this assay technique. Metabolic ratios greater than 12.6 indicate slow hydroxylation.

together with those of an extensive and a poor metaboliser, are shown in Fig. 1. Calibration curves for both compounds were linear up to at least 20 μg/ml and have intercepts which do not differ significantly from zero. The minimum detectable level is 0.1 μg/ml for both debrisoquine and 4-hydroxydebrisoquine and the intra- and inter-assay variation was below 10%.

The detection wavelength was set at 208 nm for both debrisoquine and 4-hydroxydebrisoquine to attain maximum sensitivity. At this wavelength, however, urine contains many interfering peaks. Attempts were made initially to reduce these sources of interference by extraction of the urine using solvents such as diethyl ether, dichloromethane, etc. These methods were unsuccessful however, owing to their selective extraction of debrisoquine. Use of an Extralut column (Merck, Darmstadt, F.R.G.) resulted in poor recovery of both debrisoquine and 4-hydroxydebrisoquine. Interference proved less troublesome in freshly collected urine. Thus bacterial growth may be responsible for many of the interfering peaks. Such problems are minimised by collection into preservative and filtration of the urine using a 0.22-μm filter before chromatography. Neither debrisoquine nor 4-hydroxydebrisoquine was adsorbed by the Millipore filters used but losses occurred with cellulose-based filters. After these precautions are taken the samples are unaffected by storage at −20°C for up to ten weeks.

For the subjects studied the distribution in metabolic ratio is shown in Fig. 2. Two patients out of the twelve showed metabolic ratios in excess of 12.6, the accepted cut-off between fast (MR < 12.6) and slow (MR > 12.6) hydroxylators. The patients investigated were on a variety of medications

including digoxin, perhexilene, metoprolol, bendrofluazide, aspirin and allopurinol, none of which interfered with the assay of debrisoquine and 4-hydroxydebrisoquine. Occasionally, some samples have peaks which do interfere with the assay. These appear to be due to dietary factors. To determine if such peaks are causing interference, a pre-debrisoquine specimen is routinely collected and assayed as a control.

CONCLUSION

This method is rapid, utilizes widely available equipment and is inexpensive. It appears to have considerable advantages over the currently used GLC techniques for the estimation of debrisoquine and its 4-hydroxy metabolite in urine and as such may be of value in screening populations for oxidation phenotype.

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