Urinary bile acid and bile alcohol excretion does not reflect the genetic polymorphism of debrisoquine hydroxylation

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Excretion of the major urinary bile alcohol 27-nor-5 β -cholestane-3 α ,7 α ,12 α ,24,25-pentol, and of cholic, chenodeoxycholic, deoxycholic and lithocholic acid was measured in 24 h urine collections of 10 extensive and seven poor metabolizers of debrisoquine. There was no significant difference of the excretion of these cholesterol metabolites between the two groups, indicating that cholesterol hydroxylation to bile alcohols and bile acids is probably not controlled by the same genes responsible for the 'debrisoquine-type' hydroxylation polymorphism.

Keywords genetic polymorphism bile acid bile alcohol

Introduction

Polymorphism in drug oxidation involving benzylic hydroxylation of debrisoquine (Mahgoub *et al.*, 1977) has been described. An incidence of 10% of poor hydroxylators has also been found among 222 unrelated Swiss subjects using debrisoquine as a test drug (Dick *et al.*, 1982). Evidence has been provided for the co-variation between debrisoquine and sparteine hydroxylation (Bertilsson *et al.*, 1980) and oxidation of the frequently used drugs phenytoin, nortriptyline, phenacetin and perhexiline (Eichelbaum, 1982). Consequently, poor metabolizers are at risk with standard doses of these drugs (Idle *et al.*, 1983).

For the assessment of the drug hydroxylation phenotype the administration of a test drug is necessary. Replacement of such test drugs by an endogenous compound would undoubtedly be desirable. Since many endogenous compounds are known to undergo cytochrome P-450 mediated hydroxylations, putative markers for the polymorphic cytochrome P-450 isoenzyme might exhibit interphenotype differences as well, e.g. fatty acids, prostaglandins, steroid hormones and cholesterol. Since debrisoquine and cholesterol (the precursor of many steroids) are metabolized via cytochrome P-450 dependent reactions, it was of interest to examine whether or not the impaired metabolism of drugs also extends to hydroxylations of cholesterol. There-

fore, in healthy subjects with or without hydroxylation defect for debrisoquine, the urinary excretion of bile acids and bile alcohols (hydroxylated metabolites of cholesterol) was measured.

Methods

Volunteer subjects

Seventeen healthy, non-related subjects (five male, 12 female) were studied, who had their origin in the central and eastern part of Switzerland. The age ranged from 21 to 48 (mean $29.5 \pm$ s.d. 6.2 years). All had given their informed consent for the study. Neither other drugs nor alcohol were allowed 2 days before and during the trial, with the exception of contraceptive steroids.

Phenotyping procedure using debrisoquine

A single tablet of 10 mg of Declinax® , equivalent to 12.8 mg debrisoquine sulphate, was ingested in the fasting state. The bladder was emptied before dosing and thereafter all urine was collected up to 8 h. Urine was analysed for debrisoquine and 4-hydroxydebrisoquine (Dick

et al., 1982) according to a modification of the method of Lennard *et al.* (1977). Urine (1 ml) was incubated for 16 h at 50°C with saturated sodium bicarbonate solution (0.5 ml), methanol (0.5 ml), guanoxan hemisulphate (2 µg; internal standard) and acetylacetone (0.5 ml). The derivatives were then extracted with diethyl ether, back-extracted into hydrochloric acid, and after addition of sodium hydroxide finally extracted into cyclohexane-isoamyl alcohol (19:1, v/v). Three µl of the organic layer was injected into the gas chromatograph equipped with a phosphorus nitrogen sensitive thermionic detector. Separation was achieved with a 1.8 m × 2 mm i.d. glass column packed with 3% XE-60 on Chromosorb W HP. The metabolic ratio was calculated dividing µmol debrisoquine by µmol 4-hydroxydebrisoquine excreted in the 0 to 8 h urine.

Analysis of urinary bile alcohols

Details of the method have been described elsewhere (Karlaganis *et al.*, 1982). Fifty ml of urine of a 24 h collection, to which [4-¹⁴C] testosterone glucuronide (kind gift from PD Dres. H. + S. Matern, University of Freiburg i. Br. FRG) was added (for correction of losses), were extracted with a Sep-Pak C₁₈ cartridge. The extract was passed through a column of SP-Sephadex C25 and a glucuronide fraction was isolated by ion exchange chromatography of Lipidex-DEAP. This fraction was subjected to hydrolysis using *Helix pomatia* intestinal juice (β-glucuronidase). The liberated steroids were extracted with Sep-Pak C₁₈ and filtered through Lipidex-DEAP to obtain a neutral steroid fraction. Trimethylsilyl ethers were prepared and analysed by capillary gas-liquid chromatography.

Analysis of urinary bile acids

[Carbonyl-¹⁴C] taurocholate was added to another 50 ml portion of the 24 h urine collection, which was extracted with Sep-Pak C₁₈. Solvolysis with sulphuric acid in ethyl acetate and alkaline hydrolysis were performed in order to liberate the bile acids from sulphate and glycine/taurine moieties (Almé *et al.*, 1977). Bile acids were isolated on Lipidex-DEAP and methylated with diazomethane. Trimethylsilyl ethers were prepared and analysed by capillary gas-liquid chromatography.

Results

The mean log metabolic ratio debrisoquine/4-hydroxydebrisoquine for the extensive metabo-

lizers was -0.26 (range -0.64 to 0.23), for the poor metabolizers 1.61 (range 1.36 to 1.90), with both populations clearly separated (Table 1). The log₁₀ antimode separating the two populations is 1.10, which was determined in a previous study (Dick *et al.*, 1982). In the extensive metabolizers the excretion of the major urinary bile alcohol 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol ('C₂₆ pentol') ranged from 53 to 226 nmol/mmol creatinine (mean ± s.d. 121 ± 58), in the poor metabolizers from 45 to 116 (mean 79 ± 27). There was no significant difference in the C₂₆ pentol excretion between extensive and poor metabolizers (*P* > 0.1; Mann-Whitney rank sum test). No significant correlation was observed between the C₂₆ pentol excretion and the log metabolic ratio of debrisoquine. The excretion of cholic, chenodeoxycholic, deoxycholic and lithocholic acid (Table 1) was in the same range as published by Almé *et al.* (1977), exhibiting no significant difference between the two groups (*P* > 0.1).

Discussion

Since the hydroxylation of debrisoquine is mediated by microsomal cytochrome P-450 it is of interest to consider the same oxidative steps within the metabolism of cholesterol to bile acids and bile alcohols. A series of hydroxylations in bile acid metabolism in man are microsomal, as shown for the positions 7α and 12α of the ring system and for the positions 24 and 25 of the side chain (Danielsson & Gustafsson, 1981). All these hydroxylations have been shown in systems containing purified cytochrome P-450 and NADPH-cytochrome P-450 reductase from rat and rabbit liver microsomes (Hansson & Wikvall, 1980). Since in an *in vivo* study measurement of each of these oxidative steps in the conversion of cholesterol to bile acids and bile alcohols is not possible, the analysis had to be restricted to urinary end products. Thus, the 7α hydroxylation is reflected by cholic acid, chenodeoxycholic acid and C₂₆ pentol, the 12α hydroxylation by cholic acid, deoxycholic acid and C₂₆ pentol, and the 24 and 25 hydroxylations by C₂₆ pentol. The major urinary bile alcohol C₂₆ pentol was identified previously as 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (Karlaganis *et al.*, 1981). Although hydroxylations in position 7α, 12α, 24 and 25 are microsomal, no relationship between these reactions and the benzylic hydroxylation of debrisoquine could be detected by measuring the urinary excretion of four bile acids and one bile alcohol (Table 1). It is

Table 1 Urinary bile alcohol and bile salt excretion in poor and extensive metabolizers .

Volunteer number	Age (years)	Sex	log MR debrisoquine ^a	Urinary excretion in 24 h (nmol/mmol creatinine)				
				C ₂₆ pentol ^b	Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	Lithocholic acid
Extensive metabolizers of debrisoquine								
1	30	M	-0.64	53	135	38	47	16
2	24	F	-0.51	226	59	112	96	60
3	24	F	-0.39	174	96	37	279	105
4	30	F	-0.38	70	108	60	125	135
5	25	F	-0.32	87	87	74	86	51
6	27	M	-0.30	129	54	72	125	156
7	21	F	-0.24	147	36	47	335	82
8	25	F	-0.076	99	58	32	229	65
9	30	M	-0.0088	168	66	64	113	81
10	26	F	0.23	53	56	81	213	132
Mean			-0.26	121	76	62	165	88
± s.d.			±0.25	±58	±30	±25	±94	±44
Poor metabolizers of debrisoquine								
11	31	M	1.36	116	106	70	253	111
12	48	M	1.38	45	63	74	134	173
13	33	F	1.61	54	40	58	54	159
14	32	F	1.62	83	10	41	137	87
15	33	F	1.66	70	103	50	105	82
16	36	F	1.76	112	12	103	253	110
17	26	F	1.90	71	74	66	95	69
Mean			1.61	79	58	66	147	113
± s.d.			±0.19	±27	±39	±20	±77	±39

^a Log₁₀ metabolic ratio (mol debrisoquine/mol 4-hydroxydebrisoquine excreted in the 0 to 8 h urine)^b 27-Nor-5-cholestane-3,7,12,24,25-pentol

unlikely, therefore, that urinary excretion of C₂₆ pentol, cholic, chenodeoxycholic, deoxycholic or lithocholic acid may serve as an endogenous tracer for the hydroxylation deficiency of the debrisoquine type. Indeed, the present study suggests that cholesterol hydroxylation to bile alcohols and bile acids is probably controlled by genes differing from those responsible for debrisoquine metabolism. The study of Park *et al.* (1982) leads to a similar conclusion. These authors measured the urinary excretion of 6-hydroxycortisol in relation to polymorphic N-oxidation of sparteine. There was no difference in 6-hydroxycortisol excretion between the extensive and poor metabolizers of sparteine, indicating that different forms of cytochrome P-450 are responsible for cortisol 6-hydroxy-

lation and sparteine oxidation. Pharmacogenetic polymorphism in oxidative drug metabolism will have to be compared with other hydroxylation reactions of endobiotics. Such studies may help in defining an endogenous tracer for routine phenotyping.

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