Debrisoquine 4-hydroxylation and sulphamethazine *N*-acetylation in patients with schizophrenia and major depression

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Debrisoquine 4-hydroxylation and sulphamethazine *N*-acetylation phenotypes were determined in 115 Czech drug-free in-patients with schizophrenia ($n=64$) or major depressive disorder ($n=51$). These data were compared with a control group of 321 healthy volunteers from the North-East German area of Greifswald. The distribution of debrisoquine hydroxylator phenotypes was almost identical in patients and healthy controls. Thus, there were 8.7% (95% CI 5.4–12.0%) of poor metabolizers (PM) among patients while 8.7% (95% CI 3.6–13.8%) PM among the control group. The prevalences of PM amongst patients with chronic schizophrenia and major depression were 10.9% (95% CI 4.5–21.3%) and 5.9% (95% CI 1.24–16.3%), respectively (χ^2 schizophrenics *vs* control = 0.315, NS; χ^2 depressive patients *vs* control = 0.450, NS). However, within the group of EM patients there was a significant ($P<0.01$) shift towards higher debrisoquine metabolic ratios, reflecting a lower hydroxylation capacity in EM patients compared with EM healthy controls. The proportion of slow acetylators (SA) was 60.0% (95% CI 51.0–68.9%) in the entire group of psychiatric patients and 57.5% (95% CI 52.1–62.9%) in the control group (χ^2 all patients *vs* control = 0.195, NS). Furthermore, there were no significant differences in the prevalence of the SA phenotype between controls and schizophrenics or patients with major depression. Although the results of this modest study were negative, the presence of subtle differences in the metabolic capacity between psychiatric patients and a healthy population cannot be ruled out.

Keywords cytochrome P4502D6 *N*-acetyltransferase schizophrenia major depression metabolic polymorphism pharmacogenetics

Introduction

Inherited traits affecting the activity of drug metabolizing enzymes represent an important pharmacokinetic factor determining interindividual variability in response to psychotropic drugs [1–3]. In particular, genetic polymorphism of the hepatic cytochrome P4502D6 (debrisoquine/sparteine type), may have profound therapeutic and toxicological implications [4, 5], as the majority of tricyclic antidepressants [6, 7] and neuroleptics [1, 8, 9], as well as some selective serotonin reuptake inhibitors [10] are substrates of this cytochrome, and

may cause competitive inhibition of CYP2D6-mediated drug metabolism [1, 11, 12].

Furthermore, it is well known that certain associations may exist between the acetylation (*N*-acetyltransferase, NAT 2) or oxidation (CYP2D6) phenotypes and occurrence of disease states [13, 14]. Genetic association with diseases has focussed mainly on cancer, based on the assumption that poor and extensive metabolizers possess a different potential to activate or inactivate environmental procarcinogens [15]. Certain association between inherited CYP2D6 capacity and early-onset Parkinson's disease [e.g. 16], and between acetylator or

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hydroxylator status and personality characteristics [17, 18] have been reported.

In the present study, the distribution patterns of the debrisoquine 4-hydroxylation and sulphamethazine *N*-acetylation phenotypes were determined within groups of Czech patients with schizophrenia or major depression, with the aim of investigating a possible linkage between the metabolic phenotype and occurrence of psychiatric disorders with probable multifactorial heredity [19].

Methods

Subjects

One hundred and fifteen unrelated Caucasian Czech in-patients (62 M, 63 F; median age 41 years, range 17–76 years) with chronic psychiatric disorders from the Moravian area (Brno) were included in the study. Sixty-four of them were suffering from schizophrenia and 51 from a major depressive disorder. The majority of patients had been previously treated with neuroleptics or antidepressants, but most of them had not taken psychotropics for more than 6 months prior to the phenotyping test. The rest of the group (34 subjects) were seriously ill patients who could only be tested after a 5 day washout. Nevertheless, these subjects were included in the study as they were being treated with drugs which, to our knowledge, have not been reported to have a substantial effect on CYP2D6 or NAT2 activity (lithium carbonate, viloxazine, maprotiline, clobazam, bromazepam, moclobemide 150–300 mg daily) prior to the washout.

Healthy Caucasian volunteers (178 M, 143 F; median age 36.5 years, range 19–80 years) from North-East Germany (Greifswald) were used as a control group. They were phenotyped within the same time period and according to an identical protocol as the psychiatric patients. All 321 subjects were tested for acetylation phenotype, 276 of them for hydroxylation phenotype.

Written informed consent was obtained from all subjects participating in the study, which was approved by the local Ethics Committees in Brno and Greifswald.

Phenotyping protocol

Subjects were phenotyped using debrisoquine (Declinax Hoffmann-La Roche Inc., Germany, 10 mg) and sulphamethazine (Sulphadimidine Tablets, CP Pharmaceuticals LTD, Wrexham, UK, 500 mg) as described elsewhere [20, 21]. The debrisoquine (DB) and 4-hydroxydebrisoquine (OHDB) concentrations in 0–8 h urine samples were determined by h.p.l.c. [22]. Phenotype was assigned using the log molar metabolic ratio (log R) of the DB/OHDB ratio in urine. An antimode of 12.6 (log R: 1.1) was used for CYP2D6 phenotyping [20, 22].

Sulphamethazine (SMZ) and *N*-acetylsulphamethazine (NSMZ) concentrations in serum were measured

photometrically according to the Bratton & Marshall method [23], and the molar acetylation ratio R_s [%] = $NSMZ/(SMZ + NSMZ)$ was calculated. The antimode distinguishing slow (SA) from rapid (RA) acetylators was set to 42% [20].

Statistical analysis

Mean ($\pm$ s.d.) or median (95% confidence intervals) were calculated for each group. Wilcoxon's rank sum test for independent random samples was used to compare patient and control groups, because ratios were not normally distributed in most of the groups. Frequency analysis was performed using the χ^2 -test. Statistical significance was defined as $P \leq 0.05$ in all tests.

Results

Hydroxylation phenotype

The results of debrisoquine hydroxylator phenotyping in groups of patients and healthy volunteers are given in Table 1. The distribution of PM and EM within the entire group of patients was almost identical to that of healthy controls. Thus, 8.7% (95% confidence interval 5.4–12.0%) of patients were PMs, and 8.7% (95% CI 3.6–13.8%) controls were PMs. The prevalences of PM amongst patients with chronic schizophrenia and major depression were 10.9% (95% CI 4.5–21.3%) and 5.9% (95% CI 1.24–16.3%), respectively, which were not significantly different from controls (schizophrenia $\chi^2 = 0.315$, NS, depression $\chi^2 = 0.450$, NS). However, 0–8 h urine ratio was significantly higher in EM patients than in EM healthy controls ($P < 0.01$) which reflected decreased hydroxylation capacity within the patient group.

Table 1 Characteristics of polymorphic debrisoquine hydroxylation in psychiatric patients ($n = 115$) and in a control group of healthy volunteers ($n = 276$)

Group	n	%	Log	R*
<i>Extensive metabolizers</i>				
Healthy volunteers	252	91.3	−0.28	(−1.13–+0.92)
All patients	105	91.3	0.15	(−0.70–+1.08) [†]
Schizophrenia	57	89.1	0.10	(−0.70–+1.04)
Major depression	48	94.1	0.19	(−0.58–+1.08)
<i>Poor metabolizers</i>				
Healthy volunteers	24	8.7	1.72	(1.12–2.24)
All patients	10	8.7	1.17	(1.11–1.31)
Schizophrenia	7	10.9	1.30	(1.11–1.31)
Major depression	3	5.9	1.15	(1.12–1.19)

* Median (95% range). [†] $P < 0.01$, compared with healthy volunteers. χ^2 -test, All patients *vs* healthy volunteers, $\chi^2 = 0.000$, NS

Table 2 Characteristics of polymorphic acetylation in psychiatric patients ($n=115$) and in a control group of healthy volunteers ($n=321$).

Group	n	%	R_s [%]*
<i>Slow acetylators</i>			
Healthy volunteers	185	57.5	23.4 (5.74)
All patients	69	60.0	24.6 (6.06)
Schizophrenia	45	70.3	24.4 (6.59)
Major depression	24	47.1	25.1 (5.36)
<i>Rapid acetylators</i>			
Healthy volunteers	136	42.5	73.4 (9.32)
All patients	46	40.0	74.7 (8.71)
Schizophrenia	19	29.7	74.8 (10.62)
Major depression	27	52.9	74.6 (6.92)

* Mean (s.d.). R_s [%] = $NSMZ/(SMZ + NSMZ)$.

χ^2 -test, All patients vs healthy volunteers, $\chi^2=0.195$, NS.

Acetylation phenotype

The results of acetylator phenotyping are summarized in Table 2. The prevalence of SA within the entire group of patients was 60.0% (95% CI 51.0–68.9%) and in the controls was 57.5% (95% CI 52.1–62.9%; χ^2 all patients vs healthy volunteers = 0.195, NS). Subgrouping according to diagnosis was associated with a slight increase in the prevalence of SA to 70.3% (95% CI 59.1–81.5%) among patients with schizophrenia (χ^2 vs controls = 3.567, $P < 0.10$, NS). In patients with major depression there were 47.1% SA (95% CI 33.4–60.8%, χ^2 vs controls = 1.999, NS).

Four patients with schizophrenia, but none with depression, were both poor metabolizers of debrisoquine and slow acetylators of sulphamethazine.

Discussion

Several studies have been performed in groups of psychiatric patients to investigate the effect of neuroleptic or antidepressive therapy on debrisoquine oxidation phenotype [23–26]. However, few data are available on CYP2D6 activity in drug-free patients with schizophrenia or depression. Peraut *et al.* [28] studied the distribution of CYP2D6 activity in a small group of 16 depressed patients, who were phenotyped at the end of a 5 day washout period, and found that 12.5% were PMs. Brosen *et al.* [29] detected 5.7% of PM among 35 patients with depression and Spina *et al.* [32] showed that there were 9.8% PMs among 41 drug-free schizophrenics and 7.3% in healthy controls. These findings together with the results of our study showing 10.9% PMs in schizophrenia and 5.9% PMs in major depression do not support a genetic linkage between inherited CYP2D6 metabolic capacity and the occurrence of schizophrenia or major depression. Within the EM phenotype in psychiatric patients we found significantly decreased capacity for debrisoquine hydroxylation, although the prevalences of hydroxylator phenotypes

were almost identical in patients and healthy controls. A carry-over effect of previous therapy with various psychotropic drugs may contribute to the higher ratios in EM patients.

Some earlier studies of the distribution of acetylator phenotype have shown a moderate shift towards a higher preponderance of the slow acetylator phenotype in drug-free [31] as well as chlorpromazine-treated schizophrenics [32]. However, patients with neurotic depression did not differ in this respect from healthy controls [33, 34]. In the present study, a marginally increased proportion of slow acetylators was observed in the group of 64 schizophrenics compared with controls. We acknowledge that this is a modest study giving negative results, which do not exclude the presence of small differences in the metabolic capacity between psychiatric patients and a healthy population.

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