The CYP2D6 polymorphism in relation to the metabolism of amitriptyline and nortriptyline in the Faroese population

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The metabolisms of amitriptyline (AT) to (E)-10-hydroxyamitriptyline and of nortriptyline (NT) to (E)-10-hydroxynortriptyline are catalysed by CYP2D6.
- A correlation between the sparteine metabolic ratio and the NT/(E)-10-hydroxynortriptyline and the AT/(E)-10-hydroxyamitriptyline ratios, respectively, has been observed in patients in treatment with the same dose of AT or NT.
- The frequency of CYP2D6 poor metabolizers (PMs) is 15% (twofold compared with other Whites) among healthy Faroese.
- This frequency has not been investigated in Faroese patients in AT treatment and the consequences of the CYP2D6 PM phenotype for dose and plasma concentrations of AT and metabolites are not known in these patients.

WHAT THIS STUDY ADDS

- In patients treated with different daily dosages (5–100 mg) of AT a correlation between the sparteine metabolic ratio and the NT/(E)-10-hydroxynortriptyline and AT/(E)-10-hydroxyamitriptyline ratios was observed.
- A high proportion (22%) of CYP2D6 PMs in a Faroese patient group medicated with AT was observed.
- However, similar doses of AT and concentrations of AT and NT were noted in extensive metabolizers and in PMs.

AIM

To determine the frequency of CYP2D6 poor metabolizers (PMs) in a Faroese patient group medicated with amitriptyline (AT) and to investigate plasma concentrations of AT and metabolites in relation to CYP2D6.

METHODS

CYP2D6 phenotype and genotype were determined in 23 Faroese patients treated with AT. Plasma concentrations of AT and metabolites were determined by high-performance liquid chromatography and investigated in relation to CYP2D6 activity.

RESULTS

Of the 23 patients phenotyped and genotyped, five (22%) (95% confidence interval 7.5, 43.7) were CYP2D6 PMs. No difference was found in AT daily dosage between PMs (median 25 mg day⁻¹; range 5–80) and extensive metabolizers (EMs) (median 27.5 mg day⁻¹; range 10–100). The (E)-10-OH-nortriptyline (NT)/dose concentrations were higher in EMs than in PMs and the NT/(E)-10-OH-NT and AT/(E)-10-OH-AT ratios were higher in PMs compared with EMs. The log sparteine metabolic ratio correlated positively with the NT/(E)-10-OH-NT ratio ($r_s = 0.821$; $P < 0.0005$) and the AT/(E)-10-OH-AT ratio ($r_s = 0.605$; $P < 0.006$).

CONCLUSION

A high proportion of CYP2D6 PMs was found in a Faroese patient group medicated with AT. However, similar doses of AT and concentrations of AT and NT were noted in EMs and PMs, probably due to varying doses and indications for AT treatment.

Introduction

For more than 40 years tricyclic antidepressants (TCA) have been used in the treatment of depression [1]. Despite the introduction of newer antidepressants, usually better tolerated and less toxic, TCA are still commonly used [1, 2]. The CYP2D6 polymorphism is an important determinant of TCA metabolism [2], and 7–10% of Europeans are CYP2D6 poor metabolizers (PMs) [3]. We have recently shown an increased occurrence of CYP2D6 PMs (15%) in the Faroese population [4]. Genetic studies of this island population show differences from other White populations [5, 6].

Amitriptyline (AT) is the most widely used TCA [7]. Both AT and its active metabolite nortriptyline (NT) undergo benzylic hydroxylation, largely by CYP2D6 [8–10]. The hydroxylation results in two geometric metabolites, the major being (*E*)-10-OH and the minor (*Z*)-10-OH, each forming two enantiomers [7, 11–13]. The hydroxylated NT metabolites are active and the pharmacological profiles differ between the geometric isomers [10, 14–16]. Furthermore, the hydroxylation of NT by CYP2D6 is highly stereoselective, mainly forming (–)-(*E*)-10-OH-NT [7, 10, 11, 17, 18]. Several studies [8, 13, 19, 20] have shown a significant correlation between total clearance of NT via *E*-10 hydroxylation and the activity of CYP2D6.

The aims of this study were to determine the frequency of CYP2D6 PMs in a Faroese patient group medicated with a CYP2D6 substrate (AT) and to investigate plasma concentrations of AT and metabolites in relation to CYP2D6.

Methods

General practitioners in the Faroe Islands were asked to forward an invitation to their patients receiving TCA. Twenty-three unrelated AT treated patients (14 female and nine male) aged 31–84 years (median 60 years) were included in the study. Their daily dose varied from 5 to 100 mg day^{−1} (median 25 mg day^{−1}) AT (Amitriptyline

'DAK'; Nycomed, Denmark or Saroten®, Lundbeck, Denmark). All 23 patients were CYP2D6 phenotyped and genotyped. In order to phenotype for CYP2D6 the participants were given an oral dose of 100 mg sparteine sulphate pentahydrate (The Central Pharmacy, Odense University Hospital, Odense, Denmark) followed by urine collection for 12 h. The total volume was recorded and 10-ml aliquots were stored at −80°C until analysis. Sparteine, 2,3- and 5,6- dihydrosparteine in urine were assayed by gas chromatography as described previously [21]. For the analysis of sparteine and metabolites the coefficient of variation (CV%) was <15%. The metabolic ratio (MR) was expressed as sparteine to 2,3- and 5,6- dihydrosparteine concentrations. Patients having an MR ≥20 were phenotyped as CYP2D6 PM [22]. Further a 10-ml blood sample was drawn from each patient. DNA was isolated and CYP2D6*3, *4, *6 and *9 genotype analyses were performed as previously described [4].

Under steady-state conditions for AT, plasma levels of AT and relevant metabolites were determined by a modified version of a high-performance liquid chromatography method described previously [23]. The CV% for the AT analysis was <10%. For practical reasons the timing of sampling relative to dosing varied. Plasma concentrations of AT and/or metabolites were determined in 21 of the 23 patients (Table 1). The concentrations were too low to measure in two patients due to low dosages (5 and 10 mg day^{−1}), and these patients were excluded from data analyses concerning AT plasma concentrations.

No patients were taking any substrates known to be CYP2D6 inhibitors. Written, informed consent was obtained from all patients on the basis of verbal and written information in Faroese. The study was approved by the Faroese Ethics Committee.

Statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). All parameters were transformed by natural logarithm to approach a Gaussian distribution before statistical analysis. An unpaired *t*-test was used to analyse differences between PMs and extensive

Table 1

Median, range and statistical inferences of the differences in plasma concentrations of amitriptyline and metabolites in the CYP2D6 extensive (*n* = 17) and poor metabolizers (*n* = 4) among 21 Faroese patients in treatment with amitriptyline

	EM	PM	Geometric mean ratio (PM/EM) (95% CI)	P-value
C _{AT+NT} /dose*	4.4 (1.1–18.2)	6.5 (5.7–10.6)	1.69 (0.75–3.82)	0.191
NT/dose*	1.49 (0.41–5.26)	3.42 (2.22–4.87)	2.21 (0.94–5.20)	0.066
(<i>E</i>)-10-OH-NT/dose*	3.59 (1.61–8.71)	1.00 (0.95–1.94)	0.32 (0.19–0.53)	0.0005
NT/(<i>E</i>)-10-OH-NT	0.34 (0.22–1.05)	3.07 (1.49–5.10)	7.04 (3.92–12.63)	0.0005
AT/dose*†	2.4 (1.20–14.32)	3.89 (1.73–5.74)	1.28 (0.54–3.01)	0.556
(<i>E</i>)-10-OH-AT/dose*‡	0.76 (0.25–1.65)	0.41 (0.16–0.71)	0.56 (0.25–1.22)	0.136
AT/(<i>E</i>)-10-OH-AT§	4.0 (1.7–13.7)	10.7 (5.8–14.1)	2.16 (1.06–4.42)	0.036

*nm mg^{−1} day^{−1}. †EM (*n* = 16), PM (*n* = 4). ‡EM (*n* = 17), PM (*n* = 3). §EM (*n* = 16), PM (*n* = 3). Different *n*'s are used as AT or metabolites were not quantifiable in all samples. EM, Extensive metabolizer; PM, poor metabolizer; NT, nortriptyline; AT, amitriptyline.

metabolizers (EMs). Geometric ratios of means with 95% confidence intervals (CI), calculated by GraphPad Quick-Calcs (GraphPad Software, San Diego, CA, USA), and *P*-values are presented. The correlations presented were tested using the Spearman rank correlations test.

Results

Of the 23 patients, five (22%) (95% CI 7.5, 43.7) were phenotyped as CYP2D6 PMs. These were also characterized as PMs by CYP2D6 genotype analyses, as all had the *CYP2D6**4/*4 genotype. The results from pheno- and genotype analyses were in concordance. Hence, AT administration did not interfere with phenotype assignment with sparteine.

The frequency of CYP2D6 PMs found in this study (22%) was not statistically significantly higher ($P < 1.000$; χ^2 test) than that previously observed among healthy Faroese (15%) [4], but it substantially exceeded the prevalence observed in other populations.

In one of five PMs the AT + NT plasma concentration (454 nM) exceeded the recommended plasma concentrations (AT + NT) during AT treatment (130–325 nM) [24]. No difference was found in AT daily dosage between PMs (median 25 mg day⁻¹; range 5–80) and EMs (median 27.5 mg day⁻¹; range 10–100).

The differences in AT and metabolite concentrations and ratios between PMs and EMs are presented in Table 1.

The median and range of the NT/(E)-10-OH-NT ratio in patients carrying 0 ($n = 4$), one ($n = 7$) or two ($n = 10$) functional CYP2D6 alleles were 3.07 (1.49–5.10), 0.53 (0.27–1.05) and 0.28 (0.22–0.82), respectively. The values for those not carrying any functional allele were statistically significantly higher compared with the ratios for patients with one or two functional alleles. Regarding the AT/(E)-10-OH-AT ratio, the median and range values for patients carrying 0 ($n = 3$), one ($n = 6$) and two ($n = 10$) functional CYP2D6 alleles were 10.69 (5.75–14.10), 4.61 (2.70–9.51) and 3.98 (1.67–13.69), respectively. The ratios differed significantly between patients carrying no functional alleles and patients with two functional alleles.

The log sparteine MR correlated positively with the NT/(E)-10-OH-NT ratio ($r_s = 0.821$; $P < 0.0005$) and positively with the AT/(E)-10-OH-AT ratio ($r_s = 0.605$; $P < 0.006$) (Figure 1).

Discussion

Despite the relatively small number of participants in this study ($n = 23$), an even higher frequency of CYP2D6 PMs was observed (22%) compared with the previous investigation (15%) in healthy Faroese ($n = 309$) [4]. In the light of a previous study of the CYP2C19 polymorphism in the isolated islands of Vanuatu [25], where the frequency of PMs was greatly increased, the isolated geographical position

Figure 1

Scatter plots. (A) Correlation between the log sparteine metabolic ratio (MR) and the steady-state nortriptyline/(E)-10-hydroxynortriptyline ratio in 21 patients treated with 10–100 mg amitriptyline (AT) daily. (B) Correlation between the log sparteine MR and the steady-state amitriptyline/(E)-10-hydroxyamitriptyline ratio in 21 patients treated with 10–100 mg AT daily. (2 functional CYP2D6 alleles, (●); 1 functional CYP2D6 allele, (○); 0 functional CYP2D6 allele, (●))

of the Faroe Islands might represent an interesting factor in the explanation for the high CYP2D6 PM frequency observed.

The high frequency of CYP2D6 PMs presumably results in a large part of the population being at risk of adverse effects when taking CYP2D6 substrates, especially TCA, because of their narrow therapeutic indices [26]. All patients were receiving low dosages (5–100 mg day⁻¹) of AT compared with the recommended daily dosages (150–200 mg day⁻¹ oral). This observation is in concordance with the previous finding that outpatients generally are receiving lower dosages compared with hospitalized patients [27]. In the present study only one PM had an AT + NT plasma concentration exceeding the recommended range. Hence, this study indicates that the remaining PMs are not at any particular risk of side-effects. However, one could speculate that if the patients were receiving recommended daily dosages, the AT + NT plasma concentrations would probably exceed the recommended range in the other PMs also. The very wide range of doses and, to some degree, the low doses used in the patients is probably related to different indications for taking AT, e.g. depression, slight to moderate pain, etc. In a more homogeneous group, e.g. hospitalized AT patients all suffering from major depression, toxic plasma concentrations and side-effects would presumably be expected in PMs.

The correlation between sparteine MR and the NT/(E)-10-OH-NT ratio has been observed in several other studies among different populations and has been confirmed in Faroese patients in treatment with AT. Further, this study has confirmed the correlation between NT hydroxylation to (E)-10-OH-NT and CYP2D6 activity.

In conclusion, we have found a high proportion of CYP2D6 PMs, but similar doses of AT and concentrations of AT and NT in EMs and PMs (Table 1), probably due to varying doses and indications for AT treatment.

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