Inhibition of debrisoquine hydroxylation with quinidine in subjects with three or more functional *CYP2D6* genes

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Aims To study whether the CYP2D6 capacity in ultrarapid metabolizers of debrisoquine due to duplication/multiduplication of a functional *CYP2D6* gene, can be 'normalised' by low doses of the CYP2D6 inhibitor quinidine and whether this is dose-dependent.

Methods Five ultrarapid metabolizers of debrisoquine with 3, 4 or 13 functional *CYP2D6* genes were given single oral doses of 5, 10, 20, 40, 80 and 160 mg quinidine. Four hours after quinidine intake, 10 mg debrisoquine was given. Urine was collected for 6 h after debrisoquine administration. Debrisoquine and its 4-hydroxymetabolite were analysed by h.p.l.c. and the debrisoquine metabolic ratio (MR) was calculated.

Results Without quinidine the MR in the ultrarapid metabolizers ranged between 0.01 and 0.07. A dose-effect relationship could be established for quinidine with regard to the inhibitory effect on CYP2D6 activity. To reach an MR of 1–2, subjects with 3 or 4 functional genes required a quinidine dose of about 40 mg, while the sister and brother with 13 functional genes required about 80 mg quinidine. After 160 mg quinidine, the MRs, in the subjects with 3, 3, 4, 13 and 13 functional genes, were 12.6, 10.1, 9.2, 2.4 and 2.2, respectively.

Conclusions A dose-effect relationship could be established for quinidine inhibition of CYP2D6 in ultrarapid metabolizers. The clinical use of low doses of quinidine as an inhibitor of CYP2D6 might be considered in ultrarapid metabolizers taking CYP2D6 metabolized drugs rather than giving increased doses of the drug. Normalizing the metabolic capacity of CYP2D6, by giving a low dose of quinidine, may solve the problem of 'treatment resistance' caused by ultrarapid metabolism.

Keywords: CYP2D6, cytochrome P450, debrisoquine, gene amplification, gene duplication, genotype, phenotype, quinidine

Introduction

Duplication or amplification of a functional *CYP2D6* gene as a cause of increased CYP2D6 activity was originally described in two Swedish families having one and 12 extra copies of the gene, respectively, associated with very low debrisoquine metabolic ratios (MRs) [1]. The frequency of these ultrarapid metabolizers carrying duplicated *CYP2D6* genes has been estimated to be approximately 1–2% in a Swedish Caucasian population [2], 7% among Spaniards [3], 21% in Saudi Arabians [4] and 29% in an Ethiopian population [5].

Ultrarapid metabolizers may develop low plasma concentrations at standard doses and thus require higher than usual doses of drugs metabolized by CYP2D6 to achieve a therapeutic effect, assuming the parent compound is active [6, 7]. Such individuals may also produce increased amounts of pharmacologically active metabolites, e.g. morphine from codeine that could contribute to an increased therapeutic effect as well as leading to side-effects [8].

Quinidine is a frequently prescribed antiarrhythmic drug. The therapeutic oral dose is 0.8–2.0 g day⁻¹ and the therapeutic plasma concentration is 4–12 µmol l⁻¹ [9]. Renal elimination of unchanged drug accounts for 15–40% of ingested dose and 3-hydroxylation and N-oxidation by CYP3A4 account for the remaining part of the elimination [10, 11]. Quinidine is a potent inhibitor of CYP2D6 [12–15]. A dose-effect relationship of

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quinidine within the dose range of 5–80 mg has been found with regard to MR of the CYP2D6 probe drug sparteine [15]. Single doses of 100 mg quinidine to five Chinese and five Caucasian subjects with MRs of debrisoquine between 1.5 and 7, transformed all subjects to phenotypically poor metabolizers (PMs) [16].

The aim of the present study was to investigate if CYP2D6 activity, measured as the urinary debrisoquine MR, in subjects with extremely high metabolic activity due to the presence of extra functional CYP2D6 genes, could be inhibited by quinidine to the same level seen in the majority of extensive metabolizers (EMs). We also studied if a dose–concentration–effect relationship could be established for quinidine with regard to the inhibitory effect on CYP2D6 in these subjects.

Methods

Five healthy Swedish Caucasian subjects with three or more functional CYP2D6 genes and an MR of debrisoquine <0.15 (Table 1) were used for the study. Genotyping for CYP2D6 was performed by allele specific polymerase chain reaction (PCR) for the two common defective CYP2D6 genes, CYP2D6*3 and *4 [17, 18]. EcoRI restriction fragment length polymorphism analysis was used to determine the presence of duplicated or multiduplicated CYP2D6 alleles [1, 2]. The CYP2D6*5 allele with deletion of the entire CYP2D6 gene is also detected by this analysis. The two subjects with 13 copies of CYP2D6 were the brother and sister in the family originally described by Johansson *et al.* [1]. In four out of the five subjects the presence of the CYP2D6*2 allele was confirmed by PCR analysis [2], and the duplicated/multiduplicated functional CYP2D6-gene designated as CYP2D6*2 (Table 1).

All subjects were healthy as assessed by medical history, physical examinations and laboratory indices of cardiac, hepatic and renal function. The study was approved by the ethics committee at Huddinge University Hospital

and performed according to the Declaration of Helsinki. All subjects gave written informed consent.

On separate occasions (time interval between doses was 10–12 days), four subjects were given single oral doses of 5, 10, 20, 40, 80 and 160 mg quinidine sulphate capsules (manufactured by the Production Unit of the National Cooperation of Swedish Pharmacies, 5 mg quinidine sulphate is equivalent to 4.1 mg quinidine) following an overnight fast. The fifth subject with 13 functional CYP2D6 genes only received the 80 and 160 mg doses. Four hours after the quinidine dose, 10 mg debrisoquine (Declinax,[®] F. Hoffmann-La Roche Ltd, Basle, Switzerland), was given. Urine was collected for 6 h after debrisoquine intake. After the 80 and 160 mg quinidine doses, immediately before the administration of debrisoquine, a venous blood sample (10 ml), for the analysis of quinidine concentration, was collected in heparin-treated tubes centrifuged, and plasma separated. Both plasma and urine samples were stored frozen (–20°C) until analysed. Breakfast and lunch were served at 1.5 and 5 h, respectively, after quinidine dosing.

Debrisoquine and 4-hydroxydebrisoquine were determined in urine by high performance liquid chromatography [19] and the debrisoquine MR was calculated as the ratio of the molar concentration of debrisoquine to that of 4-hydroxydebrisoquine. Plasma concentrations of quinidine were determined using a method originally developed for quinine [20]. This method utilizes quinidine as an internal standard for determination of quinine. In the present study, the determination was made in exactly the same way except that quinine was used as an internal standard for determination of quinidine. Limit of quantification (LOQ) was 4.24 nmol l^{–1}. The between assay coefficient of variation (CV) was 8.7% at a concentration of 5.08 nmol l^{–1}. The within assay coefficients of variation (CV) were 6.4% at a concentration of 2.75 µmol l^{–1}, 5.6% at a concentration of 0.34 µmol l^{–1} and 2.9% at a concentration of 4.48 nmol l^{–1}. The standard curves were linear within the concentration range of 4.24 nmol l^{–1} and 20.79 µmol l^{–1}.

Table 1 Demographic data, debrisoquine MR and CYP2D6 genotype in five healthy subjects.

Number of functional CYP2D6 genes	Sex	Age (years)	Weight (kg)	Debrisoquine metabolic ratio	CYP2D6 genotype
3	Female	40	62	0.04	*2 × 2/*1
3	Female	24	60	0.06	*2 × 2/*1†
4	Female	44	81	0.07	*2 × 3/*1
13	Female	27	64	0.02	*2 × 13/*4
13	Male	22	73	0.01	*2 × 13/*4

*2 = CYP2D6*2; *1 = CYP2D6*1; *4 = CYP2D6*4.

†In this subject the identity of the duplicated allele as CYP2D6*2 was not confirmed by PCR, and could thus theoretically have the genotype *1 × 2/*1.

Results

The debrisoquine MR ranged between 0.01 and 0.07 in the five ultrarapid metabolizers without quinidine inhibition, with the lowest MRs of 0.01 and 0.02 in the brother and sister with 13 functional *CYP2D6* genes (Table 1) as previously reported [1, 2].

Increasing doses of quinidine increased the debrisoquine MR in all five subjects (Figure 1). To reach an MR of 1–2, subjects with 3 or 4 functional genes required a quinidine dose of about 40 mg, while the subjects with 13 functional genes required about 80 mg quinidine. After the intake of 160 mg quinidine, the MR increased to 12.6, 10.1, 9.2, 2.4 and 2.2 in the subjects with 3, 3, 4, 13 and 13 functional genes, respectively. There thus seems to be a relationship between the number of functional genes and the MR after the 160-mg quinidine dose.

After 80 mg quinidine, plasma concentrations of this inhibitor, in the subjects with 3, 3, 4, 13 and 13 functional genes, were 0.4, 0.4, 0.3, 0.3 and 0.2 $\mu\text{mol l}^{-1}$ in a blood sample drawn 4 h after drug intake. The corresponding values after the 160 mg dose were 0.8, 0.8, 1.0, 0.7 and 0.6 $\mu\text{mol l}^{-1}$. Quinidine at all given doses was well tolerated. No ECG changes were observed.

Discussion

This study, involving five ultrarapid metabolizers with 3, 4 or 13 copies of a functional *CYP2D6* gene, suggests that a dose of quinidine as low as 20 mg is enough to 'normalise' *CYP2D6* activity in ultrarapid metabolizers, i.e. to transform the metabolic activity to the same level (MR: 0.2–1.2) seen in the majority of EMs [21]. To reach MRs between 1 and 2, quinidine doses of about 40–80 mg were required. Doses of 160 mg or more seem to be required to reach $\text{MR}_s > 12.6$, i.e. to transform ultrarapid metabolizers to phenotypically PMs. Thus, a dose-effect relationship could be established for quinidine with regard to its inhibitory effect on *CYP2D6* in ultrarapid metabolizers. In the study by Bertilsson *et al.* [16], a dose of 100 mg quinidine transformed subjects starting with an MR between 1 and 7 to PMs, indicating that the minimum dose needed to transform EMs to PMs depends on the initial enzyme activity, as reflected by the MR.

Ultrarapid metabolizers may show a poor response or 'therapy resistance' due to inadequate drug plasma concentrations despite normal or high doses of the drug [6, 7]. Such patients may also produce large amounts of metabolite(s), which if pharmacologically active, may contribute to the clinical effects of the parent compound [8]. The metabolites may also produce toxicological effects or undesirable side-effects [8]. Quinidine would

Figure 1 Relationship between the urinary debrisoquine metabolic ratio (MR) and the dose of quinidine. The numerals close to the curves represent the number of functional *CYP2D6* genes in each subject. Debrisoquine MRs without previous quinidine intake are derived from earlier MR determinations [1, 2].

in these cases be expected to normalize the ratio between the parent compound and metabolite(s). Normalization of the metabolic capacity of *CYP2D6*, by use of low dose quinidine, may offer a solution to the problem of inadequate plasma concentrations and lack of clinical effects in ultrarapid metabolizers when treated by *CYP2D6*-metabolized drugs.

All subjects in the present study had a *CYP2D6* genotype with three or more functional *CYP2D6* genes but also with a MR of debrisoquine < 0.15 . However, it is most unusual that Swedes have an MR higher than 0.15 in combination with *CYP2D6* gene duplication/amplification and lack of a *CYP2D6* mutation causing defective metabolism [2] and therefore the data are not biased with respect to a selection of especially low MRs. The MR should be used to guide whether quinidine should be used or not. However, if debrisoquine phenotyping cannot be performed in a patient and there is only access to genotype analysis that shows *CYP2D6* gene duplication/amplification, the probability of a very low MR is high. Accordingly, quinidine could also be given to subjects with two functional *CYP2D6* genes in combination with a low MR as MRs in individuals with two and three functional *CYP2D6* genes overlap.

A similar approach has earlier been tried using fluoxetine and paroxetine, two potent inhibitors of *CYP2D6*, in 12 nonresponding depressed patients in whom low plasma concentrations of desipramine were

determined [22]. After addition of fluoxetine or paroxetine to the treatment regime, therapeutic levels of desipramine were subsequently reached in 10 patients and in 7 of these an adequate clinical response was observed. Whether the response was due to the increased plasma levels of desipramine or to synergistic pharmacodynamic effects of the antidepressants used, cannot be determined. Concomitant use of CYP3A4 inhibitors, such as verapamil, in treatment with immunosuppressive therapy has similarly been suggested to reduce the risk of nephrotoxicity and also to reduce the cost of drugs in transplant recipients [23–25].

The potential pharmacological effects of quinidine itself or in combination with the other drug(s) needs to be considered. For example, the risk of torsades de pointes during quinidine treatment, is estimated to be 1.5% [26], and thus should be considered, especially when used in combination with older antidepressants that are potentially arrhythmogenic. Problems of other potential drug–drug interactions should also be thought about. Treatment with quinidine leads to increased concentrations of digoxin due to inhibition of P-glycoprotein [27]. Potential drug–drug interactions could also involve other enzymes such as CYP3A4. Many other drugs including itraconazole [28] and also grapefruit juice [29] have been reported to decrease quinidine clearance to different degrees by inhibiting CYP3A4 and/or inhibiting the renal secretion of quinidine. Quinidine concentration should therefore be monitored if combination treatment with these drugs is chosen.

There was a tendency towards lower plasma concentrations of quinidine in subjects with a higher number of functional *CYP2D6* genes. Although this observation is based on very few samples and thus not conclusive, it suggests that there may be a relationship between the *CYP2D6* genotype and the metabolism of quinidine. Earlier studies have indicated that although quinidine is a potent inhibitor of CYP2D6 [11–15], it is not a substrate of this enzyme [11, 30–34]. These results are, however, based on data using higher quinidine doses and concentrations than those used in the present study. CYP2D6 is a high-affinity, low capacity enzyme and is saturated at relatively low substrate concentrations. Thus, we cannot exclude that at low quinidine doses/concentrations, CYP2D6 may play a significant role in the metabolism of quinidine, whereas at higher quinidine levels CYP3A4 might become the predominant enzyme [11, 32]. Further studies are required to establish if CYP2D6 is involved in the metabolism of quinidine at low doses and concentrations of the drug. Other possible explanations for the lower plasma levels of quinidine in subjects with a higher number of functional *CYP2D6* genes that need to be considered are the higher activity of CYP3A4 or higher renal clearance of quinidine.

In summary, we have given quinidine in single doses to a limited number of ultrarapid metabolizers of CYP2D6 and have shown a dose–effect relationship for quinidine with respect to CYP2D6 inhibition. The clinical use of low doses of quinidine as an inhibitor of CYP2D6 should be considered in ultrarapid metabolizers rather than giving higher doses of the drug. This may in some cases solve the problem of ‘therapy resistance’, or prevent toxicity when using drugs with toxic metabolites. However, further studies are required to establish if such regimes would be applicable in clinical situations where steady state levels have been reached.

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